Activation Patterns of Hindlimb Motor Units in the Awake Rat and Their Relation to Motoneuron Intrinsic Properties

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Gorassini, Monica, David J. Bennett, Ole Kiehn, Torsten Eken, and Hans Hultborn. Activation patterns of hindlimb motor units in the awake rat and their relation to motoneuron intrinsic properties. J. Neurophysiol. 82: 709–717, 1999. The activity of hindlimb motor units from the lateral gastrocnemius and tibialis anterior muscles in the awake rat was compared during locomotion and during slow, sinusoidal muscle stretch. The majority of units were activated with high initial frequencies and often commenced firing with an initial doublet or triplet, even when activated by slow muscle stretch. The high firing rates at recruitment occurred without jumps in the firing rates of other concurrently activated units, the firing rate profiles of which were used as a measure of the net synaptic drive onto the motoneuronal pool. This suggested that the sharp recruitment jumps were not due to an abrupt increase in synaptic drive but rather due to intrinsic properties of the motoneuron. In addition, motor units that were activated phasically by the muscle stretch fired more action potentials during muscle shortening than during muscle lengthening, resulting in rightwardly skewed, asymmetrical firing profiles. In contrast, when the same units fired tonically during the imposed muscle stretch, the frequency profiles were modulated symmetrically and no nonlinearities were observed. Tonically firing units were modulated symmetrically throughout a wide range of firing frequencies, and discrete jumps in rate (i.e., bistable firing) were not observed. The sharp recruitment jumps during locomotion and muscle stretch are proposed to have resulted from the additional depolarization produced by the activation of plateau potentials at recruitment. Likewise, the sustained activation of plateaus subsequent to recruitment may have produced the prolonged firing of the motor units during sinusoidal muscle stretch.

INTRODUCTION

The activation patterns of mammalian motoneurons are shaped not only by external synaptic inputs but also by intrinsic conductances (reviewed in Binder et al. 1996). For example, in decerebrate cats, the activation of self-sustained depolarizations (i.e., plateau potentials) has been shown to amplify and prolong the response of motoneurons to either synaptic input or injected current (Conway et al. 1988; Hounsgaard et al. 1988; see Hultborn and Kiehn 1992; Kiehn 1991 for reviews). It recently has been shown that when cat motoneurons are activated naturally with excitatory synaptic input from muscle stretch (as compared with intracellular current injection), plateau potential activation occurs near the threshold for action potential generation (Bennett et al. 1998a). Thus when a motoneuron is recruited from rest, the simultaneously activated plateau can produce an abrupt increase in the membrane potential at the time of recruitment, resulting in high initial firing rates, in addition to producing self-sustained firing.

In recordings of hindlimb motor units in the awake rat during unrestrained walking, the majority of hindlimb motor units were recruited with high initial firing rates, often with an initial doublet or triplet (Gorassini et al. 1995). The question arose as to whether the high initial rates during walking were the result of a strong and abrupt locomotor synaptic drive or alternatively, from the activation of plateau potentials as described above. To test this, we compared the recruitment pattern of rat motor units during more gradual synaptic activation by applying slow, sinusoidal muscle stretch to the whole leg of the awake animal. We hypothesized that the motor units still would be recruited abruptly to a high rate (due to plateaus), even though they were being activated by a slow, gradual muscle stretch.

As mentioned earlier, plateau potentials also produce hysteretic or self-sustained firing, i.e., firing that persists for longer than the duration of the synaptic input (decerebrate cat: Conway et al. 1988; Hounsgaard et al. 1988; intact rat: Eken and Kiehn 1989). With respect to slow sinusoidal muscle stretch, it has been shown that cat motoneurons will discharge for a longer period during muscle shortening than during muscle lengthening (i.e., hysteretic firing), as a result of sustained plateau potential activation (Bennett et al. 1998a). We wanted to investigate if motor units in the awake rat also exhibited hysteretic firing profiles during sinusoidal muscle stretch in a similar manner to cat motoneurons with respect to plateau potential activation.

Motor unit activity was recorded in muscles containing a high percentage of fast-twitch muscle fibers (tibialis anterior, TA and lateral gastrocnemius, LG) (Close 1967; Tóthsey de Zepetnek et al. 1992). As such, the mean firing rates of the motor units during muscle stretch were quite high, ranging from 30 to 80 Hz. Because the firing rate of a motor unit is a reflection of the underlying membrane potential of the parent motoneuron, it is convenient that rat motor units fire at such high rates as this will give good temporal resolution (i.e., a high sampling rate) of the underlying membrane potential. This is in comparison with cat or human motor units, which discharge at much lower frequencies (10–30 Hz) (cat: Grillner and Udo 1971; Hoffer et al. 1987; human: DeLuca et al. 1982; Jones and Bawa 1995). Because of this, recordings from rat motor units, as compared with cat and human, may give us a better resolution to investigate motor unit activity resulting from activation of intrinsic conductances of the motoneuron.
Parts of this paper have been presented in abstract form (Gorassini et al. 1995).

**METHODS**

Data were obtained from 10 adult male Wistar rats ranging in weight from 270 to 390 g. Fine intramuscular electrodes were implanted in the TA (n = 7), or LG (n = 10) muscles. In four rats, two fine intramuscular electrodes were implanted in the LG muscle to record from different populations of motor units. In all rats, gross electromyographic (EMG) electrodes were implanted in flexor (TA) and extensor (LG) muscles. Experiments were approved by the local ethics committee in Copenhagen.

**Implant procedures and EMG electrodes**

Animals were anesthetized with either equithesin (4.0 mg/100 g ip), fentanyl-fluanisone (0.25 mg/100 g sc) or pentobarbital sodium (4.5 mg/100 g ip). Maintenance doses for all three were roughly one-eighth the induction dose. During recovery, the rats were kept isolated and warmed by radiant heat.

The fine intramuscular electrodes used for recording single motor units (micro-EMG electrodes) were fashioned similar to that described in Eken and Kiehn (1989) and Eken (1998). Briefly, a 25-mm length of 50-μm platinum/iridium (Pt/Ir) wire (Advent, PT671711) was soldered onto a 25-cm length of multistranded stainless steel Cooner Wire (AS632). The solder joint was insulated by carefully stretching the teflon insulation of the Cooner Wire over and past the solder joint by 4–5 mm and sealing the end with a silicone adhesive (Dow Corning Medical Adhesive, Silicone Type A). Three of these soldered wires were used to make a single micro-EMG electrode. The three wires were held together by a 5-mm piece of silicone tubing (0.5 mm diam, Dow Corning) that was stretched over the Pt/Ir-Cooner Wire solder joints. The fine Pt/Ir wires then were twisted together to form a tight Pt/Ir electrode bundle that was cut to a distance of 15 mm from the solder junction. This left the three tips of the Pt/Ir wires de-insulated (i.e., producing 3 50-μm recording surfaces) and separated from one another by ≈25 μm (i.e., twice the thickness of the insulation of each wire).

To reduce muscle damage, the Pt/Ir electrode bundle was inserted into the muscle without the use of a hypodermic needle (Eken 1998). To do this, a small hole was cut into the overlying fascia, and the connective tissue between the superficial muscle fibers was separated. The Pt/Ir electrode bundle was gently pushed rostrally into the muscle between the muscle fibers, so that the tip was situated 5–10 mm in from the entry point. The remaining Pt/Ir electrode bundle lying outside the muscle was spring loaded by bending it back 180° into a U-shape and suturing the Cooner Wire portion onto the fascia of adjacent muscles. This arrangement allowed for greater stability in recording position because the electrode bundle could move with the muscle fibers as they shortened during muscle contractions.

The bipolar gross-EMG electrodes were made from the same Pt/Ir and Cooner wire configuration as the micro-EMG electrodes except that the end of the Pt/Ir wire was bent back into a hook and de-insulated by 3–4 mm. The larger recording surface allowed for a larger sample of motor units to be recorded from to obtain an estimate of the overall activity of the motoneuron pool of the muscle. The Pt/Ir wire was implanted into the muscle by inserting the hooked end into a curved 25-gauge needle that was inserted into and poked out from the muscle. Once the electrode tip was estimated to be in the proper position, the needle was removed while holding onto the EMG wire. Each gross-EMG wire was inserted on either side of the micro-EMG electrode, separated by ≈1 cm, to record from the same population of motor units. The gross-EMG wires were also spring loaded and sutured to the surrounding fascia. A ground, consisting of a Cooner Wire de-insulated by 2 cm also was sutured to the fascia of the lower leg.

All wires were led under the skin and soldered to a back pack that consisted of two female telephone jacks (12 recording channels) glued to a rubber matting. The solder joints and exposed wires were covered with silastic. The back pack was fixed to the animal by threading sutures through the axial muscles, ligaments and skin of the back and tying them onto the rubber matting. The sutures were housed in small diameter intravenous tubing to prevent the holding sutures from cutting the skin. With this arrangement, the back pack was stable for ≈2 wk of recording, approximately the same duration of time that the micro-EMG electrodes recorded discrete motor unit activity.

**Data recording and analysis**

Similar to Eken and Kiehn (1989) and Eken (1998), the rats were placed in a large glass aquarium during the recording sessions. The wire leads from the back pack were connected to a swivel, preventing coiling of the wires as the animal circled the aquarium. The single unit micro-EMG was recorded differentially, using the pair combination that gave the best spike discrimination (i.e., from the 3 Pt/Ir wires there were 3 possible pair combinations). The amplified, single-unit EMG was stored on two channels, one in which the signal was band-pass filtered at 25 Hz to 10 kHz and the other at 800 Hz to 10 kHz with a four-pole Kron-lute filter. The gross-EMG was band-pass filtered at 25 Hz to 3 kHz. Signals were digitized on-line with a Masscomp EF12M A/D (A/D) converter, with the raw, unrestricted gross-EMG sampled at 5 kHz and the single-unit EMG at 20 kHz.

Data were analyzed using custom-designed software on a Masscomp 5400 computer (Concurrent Computer). The single motor unit action potentials (MUAPs) were selected off-line by first setting a voltage trigger. Each selected MUAP then was inspected by eye, verifying that the discriminated potentials were of similar shape and belonging to the same motor unit. Once all units were selected, each successive waveform was superimposed to compare the shape of each MUAP and to examine if and how it changed throughout the recording period. The gross-EMG signals were rectified digitally and filtered (33 Hz low-pass) off-line (after analogue filtering, see preceding text).

**Muscle stretch**

Stretches to the hindlimb were applied by the experimenter gently rocking the rat’s body back and forth while it sat quietly. The movement of the experimenter’s hand was guided by attaching a string from the hand to a linear motor (Cambridge Instr. 310B) that was set to apply sinusoidal movements at a frequency of ≈1 Hz. The force that the experimenter pulled on the string was monitored to ensure that the movement of the hand followed the motor. In some rats, it was possible to attach the string from the motor directly to the animal’s back pack to produce the rocking movements.

The input/output properties of the motor units were studied by recording the frequency response of the motor units to sinusoidal muscle stretch. We assumed that the main excitatory drive to the motor units during muscle stretch followed a similar activation profile as the primary muscle spindle (Ia) afferents. To estimate the Ia spindle drive (i.e., input), the length output of the motor was passed through a linear spindle model where Rate = 0.1 * velocity + length (Matthews and Stein 1969). The predicted spindle discharge was plotted against the firing frequency of the motor unit to estimate the input/output properties of the motor unit. The model we used was derived from recordings of primary spindle discharge in the cat, and we are assuming that muscle spindles in the rat have a similar length and velocity sensitivity.

**RESULTS**

During locomotion, very high firing rates were reached when the motor units were recruited, i.e., units immediately jumped to high rates of discharge at the onset of a locomotor...
burst (see Figs. 1 and 2) (Gorassini et al. 1995). The sharp frequency jumps at recruitment may have been due to sharp increases in synaptic drive from the locomotor network or alternatively, intrinsic conductances activated at recruitment level may have contributed to the high initial firing rates (see Introduction). For example, extra spikes arising out of the afterdepolarization (Fulton and Walton 1986; Granit et al. 1963) or the rapid depolarization from a synaptic activation of a plateau potential at recruitment (Bennett et al. 1998a) may have produced the high initial rates.

Evidence to support an intrinsic mechanism comes from simultaneous recordings of motor unit pairs that were recorded during walking (n = 7 unit pairs from 5 rats). Figure 1 displays an example from two LG units. The first unit of the pair exhibited a gradual increase in discharge rate (see trace 3) as the second unit (trace 4) was recruited with a high initial rate. Note that there was not a jump in the firing rate of the first unit when the second unit was recruited. This suggested that the abrupt recruitment of the second unit was not due to an abrupt increase in synaptic drive but rather due to an intrinsic property of the motoneuron. In all unit pairs (7/7), the firing frequency of the first unit recruited either remained constant or gradually increased as the second unit was recruited with a high initial rate except when both units were recruited simultaneously. The analysis described in the preceding text, however, does not rule out the possibility that the LG motoneuron pool was activated by several separate locomotor drives to the individual motor units, i.e., that there was compartmentalization of the LG motoneuron pool (Pratt and Loeb 1991).

Firing patterns during sinusoidal muscle stretch

To study this further, we compared the initial activation patterns of single motor units to sinusoidal muscle stretch to determine whether gradually increasing excitatory synaptic input also could produce high initial frequency jumps at recruitment (6 TA units, 5 LG units). We used sinusoidal muscle stretch because it produces smooth, symmetrical firing rate modulations of muscle spindle afferents (Hulliger et al. 1977), especially in the intact animal where the background level of static fusimotor drive is relatively high (Prochazka 1989). Stretches to the hindlimb were applied by the experimenter gently rocking the rat’s body back and forth while it sat quietly. The movement of the experimenter’s hand was guided by attaching a string from the hand to a linear motor (see METHODS).

Figure 2A shows the phasic stretch activation of a TA motor unit. Each time the unit was recruited by the stretch, it jumped to a relatively high-frequency of discharge, and quite often the unit was recruited with an initial doublet. This suggested that a graded excitatory drive also could recruit the units to a high initial discharge rate. We are assuming, of course, that the majority of the excitatory drive to the motor unit came from the stretch activation of muscle spindle afferents since the activation of the motor unit was time-locked to the muscle stretch. Nine of the 11 units recorded were recruited from rest by the muscle stretch (phasic activation), and the average recruitment frequency of the TA motor units was 79.9 ± 34.0 (SD) Hz (range = 20–182 Hz, n = 45 stretch cycles) and for LG motor units 49.1 ± 28.7 Hz (range = 16–120 Hz, n = 43 stretch cycles).

The discharge pattern of the TA motor unit from Fig. 2A is also shown in Fig. 2B for comparison during a bout of locomotion. Note that the mean firing rates are much higher during locomotion, suggesting that the synaptic drive to the unit during muscle stretch was much lower than during walking.

![FIG. 1. Activity of 2 simultaneously recorded lateral gastrocnemius (LG) units during a single step. After recruitment, the firing rate of unit 1 gradually increased until unit 2 was recruited to a high rate of discharge. No abrupt increase in the firing rate of unit 1 was observed when unit 2 was recruited. Rectified and smoothed gross electromyogram (EMG) of TA and LG are shown in traces 1 and 2, respectively. Superimposed motor unit action potentials (MUAPs) of the motor units are placed to the right of the frequency graph (as for all subsequent figures).](image1)

![FIG. 2. Comparison of recruitment pattern of TA motor unit during sinusoidal muscle stretch (A) and walking (B). A: despite more gradual excitation by muscle stretch, the unit was still recruited to high frequencies, at times with an initial doublet (see frequency graph, trace 2). Rectified and smoothed gross-EMG is shown in trace 1; length trace not available. B: activity of same TA motor unit as in A but during a bout of walking. Note the higher doublet and mean frequencies reached.](image2)
Importantly, jumps to a high rate in discharge only occurred when the units were activated from rest. This is shown in Fig. 3, which compares the response profiles of an LG motor unit to muscle stretch when it was activated from rest (phasic activation: first 8 cycles) to when the unit fired continuously during the stretch (tonic activation: last 8 cycles). When the unit was activated from rest, the instantaneous frequency was always higher for the first interval than for the last few intervals in a burst, producing “asymmetrical” firing rate profiles. Around the ninth stretch cycle, the unit began to fire tonically, probably as a result of an increase in descending drive since the rocking movements of the rat were kept as constant as possible. When the unit was activated tonically, the firing frequencies at the beginning and end of a stretch were the same, with the peak firing rates now occurring near the point of maximum stretch. These firing rate profiles were classified as being “symmetrical.” Note that the firing rates at the beginning of a stretch during tonic activation were well below the initial frequencies during phasic activation (the horizontal line marks the average initial frequency reached when the unit was activated from rest), especially on the 10th stretch cycle (see ↔). A switch from a phasic to tonic or tonic to phasic discharge profile was seen in 4 of the 11 motor units recorded.

The switch from phasic to tonic firing of the motor unit in Fig. 3 was probably due to an increase in the descending synaptic drive to the motoneuron pool as evidenced by the higher mean and peak frequencies reached during the tonic activation periods. The switch from an asymmetrical to symmetrical firing rate profile, however, is more difficult to explain as being caused by a simple increase in synaptic input alone (see Self-sustained firing).

As mentioned above, abrupt jumps in rate were only seen during recruitment. For example, the same unit shown in Fig. 3 was recruited to a high discharge rate (80 Hz, frequency clipped at top) when the animal made a quick postural adjustment (see Fig. 4). The unit’s mean rate then slowly decreased in a ramp-like fashion and was modulated cyclically by the muscle stretch. In the last eight stretch cycles, the initial frequencies decreased to well below the average initial frequency (i.e., ~30 Hz) when the same unit was activated phasically from rest (the horizontal line marks the average initial firing rate when the unit was phasically activated in Fig. 3). Note also that as the unit’s mean discharge rate slowly decreased, there were no abrupt jumps to a lower firing rate, i.e., there were no indications of bistable firing (cf. Eken and Kiehn 1989) (see Lack of bistable firing during locomotion and muscle stretch). The firing profile of the unit was still symmetrical as it was modulated throughout the large range of firing frequencies even at its minimal firing rate of ~15 Hz in the last few stretch cycles. Abrupt jumps in firing from one rate to another were not seen in this study when units gradually increased or decreased their firing through a wide range of discharge frequencies (10–80 Hz), similar to the LG unit in Fig. 4.

**Self-sustained firing of motor units**

The discrepancy in the two firing profiles shown in Fig. 3 (asymmetrical vs. symmetrical) can be explained if one assumes that the rat motor units behaved in a similar manner to cat motoneurons with respect to the activation of plateau potentials (Bennett et al. 1998a). For example, it has been shown that when stretch-mediated synaptic input recruited a motoneuron from rest and a plateau potential was simultaneously activated, the plateau acted to boost the recruitment of the motoneuron. This resulted in a high initial firing rate. In addition, when the plateau remained activated for a few seconds during muscle shortening, it prolonged the duration of the motoneuron discharge. That is, a smoothly graded, symmetrical input profile resulted in an asymmetrical, rightwardly skewed output profile (see Fig. 6 and 9 in Bennett et al. 1998a). On the contrary, when the same muscle stretch activated the plateau tonically, the response to subsequent muscle stretches was not further amplified by the plateau and the motoneuron then responded to this input in a linear manner, i.e., a smoothly graded, symmetrical input profile resulted in a smoothly graded, symmetrical output profile.

During sinusoidal muscle stretch, similar changes from a smoothly graded symmetrical to a rightwardly skewed asymmetrical firing profile also were observed in the rat motor units (e.g., unit 1 in Fig. 5). As commonly was observed for all units

![Image of Fig. 3](https://example.com/image3.png)

**Fig. 3.** Comparison of LG motor unit when phasically and tonically activated by muscle stretch (length output of motor guiding experimenter’s hand is shown in trace 1). When the unit was activated from rest (1st 8 stretch cycles), the instantaneous frequency was always higher for the 1st interval than for the last few intervals in a burst, producing “asymmetrical” firing rate profiles, in comparison with the symmetrical pattern that was produced when the unit fired tonically (last 7 stretches). ——, average initial firing rate of unit during its phasic activation period.

![Image of Fig. 4](https://example.com/image4.png)

**Fig. 4.** Sinusoidal modulation of the same LG motor unit from Fig. 3. Unit was recruited to a high rate of discharge and its mean rate slowly decreased to its minimum firing level. ——, average initial firing rate (~30 Hz) when the unit was activated from rest in Fig. 3. Note that when the unit was cyclically modulated throughout the large range of firing frequencies, no discrete jumps in rate (i.e., bistable firing) occurred. Initial shape of the MUAP changed from the previous figure, but the unit was still identifiable from the linked potential that was associated with the waveform.
that fired tonically during the imposed muscle stretch (6/6 units), the firing frequency profile of unit 1 in Fig. 5A (trace 3) was smoothly modulated with the length signal (trace 1). As described in the preceding text, the unit’s frequency profile during this tonic activation period should reflect the underlying synaptic drive, i.e., the discharge profile of the Ia spindle afferents (assuming that this was the primary source of the modulatory synaptic input). Note that similar to what would be expected for primary spindle afferent discharge, the smoothed frequency profile of unit 1 (dashed line, trace 2) was slightly phase advanced to the length (trace 1: the solid vertical line denotes the time of peak muscle length). When the spindle discharge was actually predicted from the length signal using a linear spindle model (Matthews and Stein 1969) (see METHODS), there was a close overlap between the smoothed frequency profile of the motor unit (dashed line, trace 2) and the predicted Ia spindle input (solid line, trace 2). The linear input/output relationship for these two parameters is shown for one stretch cycle (*) in Fig. 5C and is what would be predicted from a unit with tonic plateau activation.

In contrast, during the phasic activation of unit 1 in Fig. 5B, the response profiles were not as well related to the gradual muscle stretch. For example, unit 1 was recruited with a doublet, and it fired more MUAPs during muscle shortening than during muscle lengthening, giving rise to a rightwardly skewed, asymmetrical firing profile. If the motor unit was behaving linearly in response to the symmetrically graded synaptic input, it would have stopped firing at the same frequency it started at (after the doublet), but instead it continued to fire at slowly decreasing rates of discharge (see hatched area in second stretch cycle of Fig. 5B). Prolonged firing during muscle shortening was observed in seven of nine units that were activated phasically during muscle stretch. The duration of prolonged activity ranged from 0 to 400 ms, i.e., 0–40% of the entire stretch cycle. The other two phasic units recorded only discharged briefly at the peak of the muscle stretch.

With respect to the estimated synaptic input, unit 1 in Fig. 5B also fired for longer than expected. For example, the solid horizontal line in the second stretch cycle indicates the level of predicted Ia input that recruited the unit from rest. If the unit was behaving linearly, then it should have stopped firing when the same level of synaptic input was reached (i.e., at the 2nd dashed vertical line). The unit, however, continued to fire for another ~300 ms, well below the estimated synaptic recruitment level. This suggested that the prolonged discharges seen during this period were not due solely to synaptic activation but may very well have resulted from a residual depolarization produced by a plateau potential to produce self-sustained firing. This resultant nonlinear or “hysteretic” behavior is depicted clearly in Fig. 5D where the firing during decreasing synaptic drive (solid symbols) continues well beyond the level of synaptic input that first recruited the unit (see firing above arrow).

The change from a symmetrical tonic, to an asymmetrical phasic firing pattern of unit 1 was probably not due to a change in the pattern of the stretch-mediated synaptic drive. Rather it may have resulted from a slight decrease in tonic, background synaptic drive or alternatively, from an inactivation of the plateau potential as seen for high-threshold motoneurons in the cat (see Fig. 9 in Bennett et al. 1998a; Lee and Heckman 1997), though either interpretation does not change the preceding argument. Evidence to support the fact that the pattern of synaptic input remained unchanged in the two stretching episodes was provided by a third, low-threshold unit that was tonically active throughout Fig. 5 (not shown). Its frequency profile was used as a measure of the average synaptic drive onto the TA motoneuron pool (Crone et al. 1988; Eken and Kiehn 1989), assuming that it received the same stretch-mediated synaptic inputs as unit 1.

The averaged firing profile of this unit (unit 3, Fig. 6A) was compared during the tonic (○) and phasic (●) activation periods of unit 1 in Fig. 5, A and B, respectively. In both cases, the shape of the firing frequency profile remained sinusoidal, verifying that the synaptic input was similar in both stretching episodes. This suggests that the transition from the tonic to phasic firing pattern of unit 1 was not produced by a change from a symmetrical to an asymmetrical synaptic input. Likewise, the overlay of the averaged frequency profile of unit 1 (see Fig. 6B) during its tonic (○, Fig. 5A) and phasic (●, Fig. 5B) activation periods illustrates that modulation depth and mean firing rate were the same during the period of peak
phenomenon. For example, the gradual increase in activity of rat motoneurons in the awake animal also show a warm-up (Bennett et al. 1998b). Here we present indirect evidence that repeated activation can be mediated by an activity-dependent facilitation of plateau potentials. This effect is called "warm up" and was described in spinal interneurons and motoneurons (Russo and Hounsgaard 1994; Svirskis and Hounsgaard 1997).

Warmed-up. Activity dependent increases in motor unit discharge also were observed in three other units from different rats.

**DISCUSSION**

Effects of intrinsic properties (plateaus) on motor unit discharge

Recruitment with high initial firing frequencies. A key finding in this study is that LG and TA motor units that were activated from rest, either during walking or from muscle stretch, were recruited to high firing frequencies. Although we did not measure the synaptic inputs directly (e.g., the discharge from locomotor networks or stretch-activated afferents), the firing profiles of other concurrent, tonically activated units were used as a functional indication of the input to the motoneuron pool (Crone et al. 1988; DeLuca and Erim 1994; Eken and Kiehn 1989). The firing profiles of these tonically active "control" units did not exhibit abrupt increases in rate as other units were recruited to high rates of discharge. It is likely, therefore, that the high rates at recruitment, both during walking and muscle stretch, were a result of intrinsic conductions activated at the time of motoneuron recruitment. This is consistent with the recent finding that when plateau potentials are activated near the threshold for action potential generation, the plateau can produce an abrupt increase in the membrane potential at the time of recruitment, resulting in high initial firing rates (Bennett et al. 1998a).

Similar to rat motor units recorded in this study, human motor units are recruited to relatively high rates of discharge (≥9–13 Hz) during slowly incrementing muscle contractions (DeLuca et al. 1982). They are not, however, as likely to initiate firing with a doublet or triplet. This is probably due to the fact that human motor units fire at frequencies that are 5–10 times slower than rat motor units and as such are not as responsive to rapid changes in the underlying membrane potential of the parent motoneuron. For example, a brief overshoot in membrane potential (~80 ms in duration) can occur in cat motoneurons when a plateau is simultaneously activated at the time of cell recruitment, resulting in an initial doublet or triplet discharge (Bennett et al. 1998a). It may be that the slower human motor units are not influenced by such fast transient events or alternatively, human motoneurons do not exhibit such overshoots in membrane potential at recruitment.  

**ASYMMETRICAL FIRING PROFILES.** The asymmetrical firing profiles of phasically activated motor units during sinusoidal muscle stretch also may have been due to the activation of plateau potentials. Assuming that the plateau was initiated with a threshold that was at or below the level for cell firing (as seen for cat motoneurons), then we would expect the plateau to be activated at recruitment and remain activated until after or when the motoneuron stops firing (see Bennett et al. 1998a; Kiehn and Eken 1997 for discussion). Thus in response to a smoothly graded input profile, as occurs during sinusoidal
muscle stretch, one would expect two things: 1) the motoneuron should be recruited to a relatively high rate because of the boost provided by the simultaneous activation of the plateau and 2) the rate modulation of the motoneuron subsequent to recruitment should not be influenced by the tonically activated plateau currents. That is, the firing rate of a motor unit should increase and decrease linearly with synaptic input, and in particular, the firing rate should be able to decrease to a motoneuron’s minimum firing rate without deactivating the plateau. In fact, based on the cat data, the level of depolarizing synaptic input at which a motoneuron is derecruited would have to be lower than the amount needed to recruit the cell to cancel out the depolarizing contribution to the membrane potential from the plateau potential (Bennett et al. 1998a,b). Therefore the initial rate should be higher than the final rate (asymmetrical), as seen for cat motoneurons with plateaus activated below recruitment threshold. The fact that we see such asymmetrical firing profiles in rat motor units that were phasically activated by symmetrical synaptic inputs (i.e., from sinusoidal muscle stretch) provides further evidence for the existence of plateau potentials in the awake animal. Similar asymmetries in recruitment and derecruitment frequencies have been reported for linear triangular force contractions in the human (DeLuca et al. 1982; Gorassini et al. 1997).

**Self-sustained firing.** Finally, we also have speculated that the plateau potential sustained the firing of the motor units between muscle stretches. As shown for cat motoneurons, (Bennett et al. 1998a,b), muscle stretch could activate the plateau tonically and the firing frequency of the motoneuron then was modulated cyclically by the excitatory drive. Alternatively, this pattern of motor unit firing could have been produced by a tonic, steady synaptic drive to the motoneuron pool that was modulated cyclically by the stretch-mediated afferent input. However, as shown in Fig. 6B, a switch from a tonic to phasic discharge pattern can occur without changes in the modulation depth or mean firing rate of a unit during the period of peak muscle stretch. If the phasic discharge pattern resulted from a removal of background tonic drive, then one would expect the peak and mean discharge rates to decrease as well. This did not occur and therefore we conclude that the maintained discharges of the motor units between muscle stretches may have been produced by a sustained activation of the plateau potential.

In addition, when motor units were only phasically activated by the muscle stretch, they discharged more action potentials during muscle shortening than during muscle lengthening (e.g., hysteresic firing in Fig. 5D). The prolonged discharges were readily observable in the firing profiles of the rat motor units (as compared with cat or human motor units, see Introduction) because of their high discharge rates. For example, if there was 100 ms of prolonged depolarization of the motoneuron, this would have resulted in the firing of 5–10 extra MUAPs for motor units firing at rates of 50–100 Hz. Because there is no known effect of fusimotor drive that acts to preferentially increase the discharge of spindle afferents during muscle shortening and not during lengthening (Hulliger et al. 1977), it is unlikely that the prolonged discharges were the result of asymmetrical synaptic inputs during muscle stretch but rather due to the activation of plateau potentials. During the phasic activation of motor units during locomotion, prolonged unit activation does not occur because strong, rhythmic inhibitory inputs that are thought to be activated at the end of a locomotor burst (Hochman and Schmidt 1998) inactivate the motoneuron (and associated plateaus), thereby preventing any self-sustained firing.

**Lack of bistable firing during locomotion and muscle stretch.**

During quiet sitting, Eken and Kiehn (1989) reported that soleus motor units in the intact rat which fire at very low rates (≈10 Hz) can spontaneously, or in response to brief nerve stimulation, jump to higher, sustained levels of discharge (≈20–25 Hz). These discrete jumps in firing rate were suggested to have been produced by the activation of plateau potentials in the motoneurons. The switch between two stable levels of firing caused the cells to fire in a bistable manner and in this case, the plateau would need to have been recruited above the threshold for firing action potentials (see Bennett et al. 1998a; Kiehn and Eken 1997 for discussion). Such bistable firing was not observed during locomotion or muscle stretch in this study. As mentioned previously, motor units were recruited at rates well above 20 Hz, so it is not surprising that discrete jumps in rate were not seen. When motor units were derecruited, however, their firing rates could decrease gradually to <20 Hz without discrete jumps in rate, i.e., the unit was able to reach its minimum rate of firing without the plateau inactivating (see preceding discussion). In fact, during continuous sinusoidal muscle stretch, firing rates in tonically activated units could decrease to ≈10 Hz during muscle shortening, and subsequent muscle stretches only produced gradual increases in firing (e.g., tonic activity of LG unit in Figs. 3 and 4).

How can one, therefore, reconcile the bistable firing of SOL units in the Eken and Kiehn (1989) study? It may be that rat soleus units only are able to fire tonically at 10 Hz when the descending drive from serotonin (5-HT) fibers is low, as occurs during sleep or quiet rest (Jacobs and Fornal 1993). This may make the plateau potential much harder to activate (i.e., increase the threshold) because plateaus require some level of monoaminergic input to be activated (Hultborn and Kiehn 1992; Kiehn 1991). Under these conditions of low monoaminergic drive, tonic firing below plateau threshold (≈10 Hz) may be possible (i.e., the plateau threshold would be above the Na* threshold for spiking). When either the synaptic or descending 5-HT drive is increased, the plateau would then be activated and the soleus units would jump to a higher rate (≈20–25 Hz) and thus, exhibit a bistable firing pattern. It would be interesting, therefore, to look at the effects of various levels of monoaminergic input on plateau threshold and, in addition, to compare initial recruitment frequencies of motor units in intact animals with low levels of endogenous monoamines (Eken et al. 1990; Kiehn et al. 1996; Westerga and Gramsbergen 1994).

**Activation dependent facilitation of motor unit discharge.**

In four of the nine phasically activated motor units, the number of MUAPs fired gradually increased with each successive muscle stretch, and in some cases the unit eventually would become tonically activated. This increase in motor unit discharge occurred even though the input to the motoneuron...
pool remained fairly constant as measured by the firing rates of other tonically activated units. Thus it appeared that the activation-dependent increase in the stretch responses of the motor units was an intrinsic rather than synaptically mediated event. A similar activity-dependent warm-up effect of plateau potentials first was described in turtle spinal neurons (Russo and Hounsgaard 1994; Svirskis and Hounsgaard 1997) and also for in vivo cat motoneurons (Bennett et al. 1998b). Activation-dependent increases in motor unit excitability have been reported in human motor units in response to either repetitive voluntary contractions or muscle vibrations (Gorassini et al. 1997, 1998; Kiehn and Eken 1997; Romaiguere et al. 1993; Suzuki et al. 1990), and this may be explained by the warm-up phenomenon described here. Interestingly, the time course of this facilitation in humans has been shown to be very similar to that seen for warm-up in plateau potentials in cat motoneurons (Gorassini et al. 1997; Romaiguere et al. 1993).

Functional implications of intrinsic properties on motor unit activation

Motor units in this study often were recruited to a high rate of discharge, regardless of the pattern of synaptic activation, i.e., gradual during muscle stretch or abrupt during walking. We have hypothesized that the abrupt recruitment patterns of the motor units were a result of a simultaneous activation of a plateau potential with motoneuron recruitment. High discharge rates at recruitment, especially >100 Hz (i.e., doublets and triplets), have been shown to optimize the speed and amount of force production in a given motor unit (i.e., catch property) (Burke et al. 1969; Stein and Parmiggiani 1979). Thus the activation of intrinsic conductances in the motoneuron may help to produce rapid and efficient force output of motor units, especially during locomotion and postural responses where rapid timing of movements are important. During the phasic activation of motor units in response to muscle stretch, prolonged motor unit discharge was observed. It was noted that the level of afferent input required to reflexively recruit the motor unit was much higher than the levels at which sustained firing could occur during muscle shortening. If one extrapolates the activation of motor units from stretch reflexes to voluntary activation, then one would expect that the level of voluntary effort required to recruit a motor unit should be higher than the level that is needed to maintain its discharge. This is likely because the added depolarization produced by the plateau potential would reduce the amount of synaptic input and thus “effort” required. We recently have looked at the activation of pairs of motor units in the human during tonic and triangular force contractions (Gorassini et al. 1997, 1998; Kiehn and Eken 1997) and found this to be the case. Finally, the fact that it is difficult for human subjects to produce steady motor unit discharge at frequencies <6–8 Hz (reviewed in Freund 1983) may be explained by the activation of plateau potentials because the added depolarization imparted by them would prevent motor units from firing at these low frequencies. From this and the data presented in this study, it is becoming evident that in the awake state, sustained activation of motor units may be aided by the activation of intrinsic conductances, such as plateau potentials, similar to that seen in reduced animal preparations.

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