Spontaneous Electromyographic Activity in Adult Rat Soleus Muscle

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Eken, Torsten. Spontaneous electromyographic activity in adult rat soleus muscle. J. Neurophysiol. 80: 365–376, 1998. Single-motor-unit and gross electromyograms (EMG) were recorded from the soleus muscle in six unrestrained rats. The median firing frequencies of nine motor units were in the 16–25 Hz range, in agreement with previous studies. One additional motor unit had a median firing frequency of 47 Hz. This unit and one of the lower-frequency units regularly fired doublets. Motor-unit firing frequency was well correlated to whole-muscle EMG during locomotion. Integrated rectified gross EMG revealed periods of continuous modulation, phasic high-amplitude events, and tonic low-amplitude segments. The tonic segments typically were caused by a small number of motor units firing at stable high frequencies (20–30 Hz) for extended periods of time without detectable activity in other units. This long-lasting firing in single motor units typically was initiated by transient mass activity, which recruited many units. However, only one or a few units continued firing at a stable high frequency. The tonic firing terminated spontaneously or in conjunction with an episode of mass activity. Different units were active in different tonic segments. Thus there was an apparent dissociation between activity in different single motor units and consequently between single-motor-unit activity and whole-muscle EMG. It is proposed that the maintained tonic motor-unit activity is caused by intrinsic motoneuron properties in the form of depolarizing plateau potentials.

INTRODUCTION

Regulation of muscle force is attributed to the orderly recruitment and derecruitment of motor units within a task group and to frequency regulation within the individual active motor unit. The current view on motor-unit recruitment and firing characteristics is largely based on studies of rather stereotypical repetitive contractions, i.e., reflexes, locomotion, or repetitive voluntary movements (Grimby 1984; Hoffer et al. 1981; see review by Calancie and Bawa 1990). Such studies in unfunctional muscles have shown that individual motoneurons are reliably recruited when the target muscle electromyogram (EMG) approaches a reproducible level, and once recruited, their firing frequencies closely follow the whole-muscle EMG signal (Hoffer et al. 1987). Thus in stereotypical contractions, active motoneurons appear to differ only by their intrinsic thresholds and by the relative strength of the common inputs they receive.

Motoneuron firing characteristics during spontaneous motor behavior in unrestrained animals had not been investigated until Hennig and Lomo (1985) addressed the question in a single-motor-unit EMG study of the slow soleus and the fast extensor digitorum longus (EDL) muscles in the rat. Their work provided detailed statistical information about firing properties in one kind of soleus unit and two types of units in the EDL, demonstrating a close relation between motoneuron firing properties and muscle fiber contractile properties. However, the study did not include much information about the characteristics of individual activity episodes and did not relate the activity to animal behavior. Some aspects of these questions have been dealt with by Eken and Kiehn (1989). The aim of the present investigation was to study in more detail the behavior of individual soleus units in unrestrained rats and to relate the activity of those units to the behavior of other motor units in the soleus muscle and to animal motor behavior. A brief report has been published elsewhere (Eken and Lomo 1993).

METHODS

Single-motor-unit and gross-EMG electrodes were implanted in adult male Møll-Wistar rat soleus muscles (n = 56). The technical difficulties in obtaining stable recordings from single motor units during spontaneous behavior are considerable. Interference is increased due to a large number of active units, and the shape of a motor-unit potential is not constant during high-amplitude movements. This is conceivably mainly due to the relative movement of muscle fibers in relation to recording electrodes, but changes in muscle fiber conduction velocity also may contribute (Trontelj 1993). Thus long-lasting stable discrimination of single motor units was only obtained in a small fraction of the experiments, and results from six rats weighing between 220 and 460 g (median 290 g) are presented. However, gross-EMG and single-motor-unit activity during periods of weaker muscle activation were studied in some detail in every implanted animal, and even though they were excluded from further analysis, all animals supported the general picture that is presented in this paper.

Single-motor-unit electrodes (Hennig and Lomo 1985) were made from 3-cm, 25-μm-diam teflon-insulated platinum/iridium wires (7750, A-M Systems). Each wire was soldered to a 30-cm multilament stainless steel wire (AS 632, Cooner Wire). The soldering points were insulated with silicone elastomer (Silastic Medical-Grade Elastomer, MDX4-4210, Dow Corning) and three fine wires were twisted tightly together, and the assembly was strengthened by a 0.5-cm silicone tube with 0.9 mm OD (Silastic Medical-Grade Tubing, 602-135, Dow Corning) at the level of the soldering points. The electrode bundle was cut transversely, and all electrodes were tested for leaks by submerging them partly into a drop of saline and attempting to pass a 5 μA current into the fluid. Gross-EMG electrodes were made from two individual 6-mm, 50-μm diam platinum/iridium wires (7760, A-M Systems) that each were soldered to a 30-cm multilament stainless steel wire. The terminal 1.5 mm of insulation was removed from each electrode, and the distal 2-mm segment was bent back 180° to make a hook. The proximal end of each single-motor-unit and gross-EMG electrode was soldered to a socket contact (E363/0, Plastics One).

All procedures were approved by the local ethics committee. The soleus muscle was exposed under deep general anesthesia (Equithesin, initial dose 0.4–0.5 ml per 100 g body wt ip), and the single-motor-unit electrode assembly was pushed gently into the muscle in a distal-to-proximal direction through a small slit in the fascia, ensuring that the imaginary line between the centers of
the recording surfaces was oriented perpendicular to the direction of the muscle fibers (Andresen and Rosenfalck 1978). The gross-EMG electrodes were introduced separately in the proximal and distal part of the muscle, and a ground electrode consisting of a 30-cm multilament stainless steel wire with ~5 mm insulation removed was placed in the lower leg at some distance from the soleus muscle. The wires were secured with 6-0 sutures and taken subcutaneously to an electrode mount (MS363, Plastics One), which subsequently was fixed to the head of the animal by stainless steel bone screws (No. 40-77-8, FHC) and dental cement (Simplex Rapid, Austenal Dental). A wire loop under the dorsal skin of the animal prevented pull on the electrodes during movements. In one experiment, diaphragm EMG was obtained from two multilament stainless steel wires with a bare 1-mm segment that were sutured to the abdominal surface of the left hemidiaphragm. Electrode positions were verified in all experiments after the animal was killed (Equithesin overdose).

After waking up from anesthesia, the rats showed normal behavior, exploring the environment, grooming, feeding, and sleeping regularly. Recordings were made from the first day after the operation. All recordings were performed in subdued light. The rat was placed on the sawdust-covered bottom of a metal cage (47 × 33 × 34 cm) with one glass wall and a mirror on the opposite wall, and a 45-cm six-lead spring-covered lightweight flexible cable [363-OPEN 45CM 6VHB (C), Plastics One] was connected to the head mount in the lower end and to a custom-built swivel in the upper end. Recordings were made differentially, and single-unit EMG was recorded from the pair of single-motor-unit electrodes in the assembly that gave best spike discrimination. The signals from the gross-EMG and single-motor-unit electrodes were fed into differential A. C. amplifiers and preampillifiers (Medelec MS6 oscilloscope mainframe with 2 sets of AA6 Mk III and PA 63), band-pass filtered at 80 Hz to 32 kHz, and taken to the recording equipment. In addition, the signal from the single-motor-unit electrodes was high-pass filtered (custom-built 2nd-order Bessel filter), and the signal from the gross-EMG electrodes was fed into a custom-built rectifier and integrator with a time constant of 200 ms. This relatively high value was necessary to avoid a sawtooth appearance of the output signal when only one or two units were firing. The two derived signals were displayed on a dual-beam storage oscilloscope (Tektronix 5113) equipped with a custom-built autoerase circuitry, which was activated when the beams reached the rightmost limit of the screen.

The recording equipment was modified from Eken and Kiehn (1989). A video camera with a macro lens kit (WVF-F10E CCD with WV-KT100E, Panasonic) filmed the oscilloscope screen, and another camera with a telephoto zoom lens and gen-lock adapter (WVP-F10E CCD with WV-KT200E) filmed the animal through the glass wall in the cage. The two video signals were mixed (Panasonic WJ-S1E) to ascertain time locking, and the composite video signal was fed into a U-matic video cassette recorder (Sony VQ-9600P). The unprocessed signals from the single-motor-unit and gross-EMG electrodes were recorded on the two sound tracks (bandwidth 50 Hz to 15 kHz). Individual video frames could be printed on a video copy processor (Mitsubishi P65E) for documentation.

During analysis, the unprocessed signal from the single-motor-unit electrodes was played back from the video recorder, high-pass filtered at the frequency that gave optimal discrimination, usually 2 or 5 kHz, and fed into a spike discriminator (Slope/Height Window Discriminator, Frederick Haer). The output spike detection pulses were displayed on a storage oscilloscope underneath the high-pass filtered single-unit EMG signal to ascertain that every spike in a train was detected. At the same time, the detection pulses triggered an oscilloscope with signal delay and storage circuitry (MS6 with SDS 6, Medelec), which displayed all the unprocessed motor-unit potentials superimposed at high sweep speeds, making it possible to verify visually that the discriminated potentials were of similar shape and hence probably belonged to the same motor unit. In addition, a single unprocessed spike from the start of the recording was displayed on a digital storage oscilloscope (Tektronix 5116 with 5D10 waveform digitizer). Incoming spikes were displayed superimposed on this “template” spike for a selected time period, providing visual verification that the current spikes had a similar shape. Spike-triggered averages of the gross-EMG signal obtained at different times was used as additional evidence of stable single-motor-unit recording conditions and confirmed that gross-EMG recordings were also stable throughout the recording period (cf. Fig. 3). With the current technique, a gross-EMG electrode might pick up some low-amplitude activity from neighboring muscles, whereas a single-motor-unit electrode did not do so. This was demonstrated by EMG recordings in four rats after the soleus nerve was cut selectively. No muscle-fiber activity was seen until fibrillatory activity appeared. The nerve transection was performed under general anesthesia as described earlier, and the animals showed no distress after the operation.

The unprocessed EMG signals, the single-motor-unit spike detection pulses, and the integrated rectified gross-EMG signal also were fed into a computer (Macintosh IIx, Apple Computer) equipped with an analog/digital input/output card and a DMA card (NB-MIO-16H-9 and NB-DMA-8-G, National Instruments). Applications for data acquisition and analysis were custom built by the author and consisted of “virtual instruments” developed with LabVIEW 2 (National Instruments), with extensions written in a high-level language (THINK Pascal, Symantec) and assembly language (THINK C, Symantec).

Analyses of single motor units during spontaneous locomotion were particularly difficult due to interference between simultaneously active units. Thus an improved detection and verification algorithm had to be applied. The signals from the single-motor-unit and gross-EMG electrodes were digitized in parallel at 42 kHz. The signal from the single-motor-unit electrodes was enhanced digitally off line according to an algorithm devised by Edin et al. (1988), and the enhanced signal was processed by a digital spike detector. Each automatically detected spike was accepted or rejected after visual inspection of its unenhanced waveform. The signal from the gross-EMG electrodes was root-mean-square (RMS) processed to obtain enhanced time resolution compared with the integrated rectified gross EMG used elsewhere in this study. RMS values from the gross-EMG signal were computed for 40-ms segments every 5 ms. The resulting RMS-EMG curve was computer fitted to the instantaneous frequency curve according to the equation $F(t) = k \times E(t)$, where $F(t)$ represents instantaneous frequency of motoneuron discharge, $r$ represents the set of occurrence times of spikes, and $E(t)$ represents the values of the RMS-EMG profile at such times (Hoffer et al. 1987). Frequencies outside the 5–80 Hz range (5–200 Hz in the fast unit), i.e., doublets and intervals between individual activity episodes, were excluded before curve fitting.

RESULTS

General appearance of gross-EMG activity

The soleus muscle was active in both locomotor and postural tasks. Analysis of integrated rectified gross EMG revealed periods of continuous modulation as well as phasic high-amplitude events and tonic low-amplitude segments in all animals (Fig. 1). Continuous modulation at rather high amplitudes typically was seen during exploratory behavior when the rat was moving around in the cage. Tonic segments appeared at several different levels, typically when the animal was standing still or lying down, and also while it ap-
FIG. 1. Integrated rectified gross electromyogram (EMG) during spontaneous behavior. A–C were obtained in early afternoon; D was recorded shortly after midnight. Note periods of continuous modulation, phasic high-amplitude events, and tonic low-amplitude segments. High amount of continuous-modulation periods in D was typical for night recordings. Labels below plots indicate figures where sections are shown in greater detail. Recordings were obtained from rats 298 (A; 3 days after electrode implantation), 504 (B; 2 days after implantation), and 290 (C and D; 8 days after implantation). Dashed horizontal lines represent signal ground level and vertical bars represent 200 μV in all plots of integrated rectified gross EMG.

peared to be sleeping. They often were initiated and terminated by phasic high-amplitude events caused by gross movements of the limb. However, changes in tonic activity also could be elicited by barely detectable movements, often without any visible change in limb position. A startle response, e.g., provoked by a sudden sharp metallic sound, also could initiate long-lasting tonic activity.

Tonic segments

The amplitude of a rectified and integrated gross-EMG signal is determined by several factors. The number of motor units firing, their firing frequency, their size, and the shape of their motor-unit potential as picked up by the electrode are important. With the present electrode arrangement, the position of units relative to the recording electrodes would be important for determining sizes and shapes of motor-unit potentials. Due to interference between the large number of units that was picked up during mass activity, the single-unit firing activity underlying phasic high-amplitude events could not be analyzed through the gross-EMG electrodes. However, at low activity levels it was often possible to follow one or a few units through the gross-EMG electrode. Thus some tonic segments were analyzed further to determine the underlying single-unit activity.

Figure 2 (top) shows an expanded portion of Fig. 1C. It represents 10 min of integrated rectified gross-EMG activity with four labeled tonic segments (1–4), all initiated and the first three also terminated by phasic events as the rat made minute transient postural adjustments. The rat was lying down throughout the recording, and there was no overt change in posture between the segments. Figure 2 (middle) depicts the raw EMG signals underlying the individual labeled segments. On the basis of previous studies (e.g., Hoffer et al. 1987), one would expect that lower amplitude of the integrated rectified gross-EMG signal would be caused by lower firing frequencies in participating units and derecruitment of previously active units, provided that the activity was not shifted between different task groups. However, each tonic segment was caused by two or three motor units firing at similar and rather stable frequencies for the full duration of the segment, and different constellations of units were active in different segments. There was no fixed order of recruitment and derecruitment. This is illustrated in Fig. 2 (bottom), which shows further expanded portions that distinguish at least eight different units. Each unit was given a unique label (a–h) only if it could be clearly differentiated from the others. Thus the unit firing concurrently with units b and f in segment 3 is not labeled as it may be the same as c in the first segment. This approach probably leads to an underestimate of the number of participating units. Similar cycling of tonic activity between motor units was observed in the two other muscles with long-lasting tonic segments and stable single-motor-unit recording conditions that were
FIG. 2. Cycling of tonic activity between motor units. Ten-minute period from the recording in Fig. 1C. Top: integrated rectified gross-EMG activity with 4 individually labeled tonic segments (1–4) separated by phasic events. Middle: unprocessed gross-EMG signal underlying the tonic segments; labels correspond to those in top. Tonic firing of several motor units at relatively high frequencies is evident. Bottom: parts of the signal further expanded; this part also reveals that different groups of motor units were active in different tonic segments. For labeling of individual units, see text.

analyzed. Thus in the present material, there was no simple relation between gross-EMG activity level and the participation of individual units in tonic segments. Furthermore, all the active units tended to fire stably in the 20- to 30-Hz range regardless of the gross-EMG level.

For the conclusions drawn from Fig. 2 to be valid, it is essential to ascertain that the recording conditions were stable. Although there were no overt changes in posture, the electrodes might conceivably have moved slightly during the phasic events, so that a single unit could have been recorded with different waveforms at different occasions. Conversely, apparently similar waveforms could have originated from different units due to the relatively large pickup volume of the gross-EMG electrodes. Thus unequivocal identification of the units in Fig. 2 would have to be based on their appearance as seen through the single-motor-unit electrodes. For each unit, an attempt was made to obtain spike-triggered averages from both the single-motor-unit and gross-EMG electrodes (Fig. 3A). Four of the eight units were available for this kind of analysis. The remaining four units could not be reliably discriminated due to interference with other simultaneously active units. In segment 1, averages of units a and b could be obtained. Segment 2 provided averages of units d and e, and in segment 3 unit b could be averaged. Superposition of the averages of unit b confirmed that its waveform did not change appreciably in spite of two phasic high-amplitude events during the 3-min period between the recordings. Unit d also could be averaged twice, as it was active in a tonic segment 2 min after the end of Fig. 2. Again, superposition of the two averages, which were obtained 6.5 min apart, revealed only insignificant changes in appearance in spite of three intervening phasic high-amplitude events. Thus the two units provide evidence of stable recording conditions for 8 min starting at segment 1. Units a and e were only seen once during the 10-min period in Fig. 2 and could not be discriminated during the remaining 12 min of the recording session. Figure 3A shows that they are clearly different from b and d, and the stable recording conditions evident from analysis of the latter two rules out the possibility that they could have been lost because of electrode movements. Further evidence of stable conditions was obtained from the unit shown in Fig. 3B, which was discriminated 7 min before and 4 min after the activity period shown in Fig. 2. The only difference that could be seen was a slight shift of the last components of the waveform with respect to the first ones. The different elements of the waveform are likely to have arisen from muscle fibers with end-plates in different distances from the recording electrode. Changes in muscle length, and thus in conduction distance, would change the difference between arrival times of action potentials originating in different muscle fibers. In addition, changes in fiber length may lead to differential changes in
muscle-fiber conduction velocities (Trontelj 1993). There were several periods of gross movements during the 21 min between the averages in Fig. 3B. In contrast, no overt change in posture was evident between the segments in Fig. 2. Thus it was confirmed that recording conditions in Fig. 2 were stable in spite of the phasic events, justifying the earlier conclusions regarding the behavior of participating units.

Recordings through single-motor-unit electrodes were necessary to uncover further details in the firing pattern of individual units. In this work, thorough documentation of firing as a function of time and motor behavior was emphasized rather than the collection of a large number of spikes from each unit. As can be seen from Table 1, the firing frequencies of nine units corresponded to those reported by Hennig and Lømo (1985), who made 24-h recordings from six soleus units. The single unit that fell outside this range will be discussed separately.

The initiation of a typical tonic gross-EMG segment, recorded in parallel from single-motor-unit and gross-EMG electrodes, is shown in Fig. 4. The rat was lying down with eyes closed during the recording. Initially, a single unit firing tonically at ~20 Hz could be seen through the gross-EMG electrode (B, middle and bottom). A brief phasic event occurred as the animal made a minor postural adjustment, and a second unit, picked up by both the gross-EMG and single-motor-unit electrodes, started firing at ~10–15 Hz (single unit in A; B now shows the activity of both units, which have similar amplitude). Another phasic event occurred, and the firing frequency of the second unit was shifted to ~20 Hz. Individual units in the gross-EMG recording were lost during the phasic events, but the first unit can be seen to undergo only slight changes in firing frequency in spite of major changes in the second unit (cf. instantaneous frequency plot in C). In many cases a unit could be recruited directly to the 20- to 30-Hz range, or undergo a slow acceleration (cf. Eken and Kiehn 1989). This and similar recordings confirmed the earlier notion that there was no simple relation between the participation of a unit in an individual tonic segment and the activity of other units and that active units tended to fire at a preferred frequency in the 20- to 30-Hz range.

In all animals, ~1-Hz oscillations in motor-unit firing frequency were seen during tonic firing. This is illustrated in Fig. 4; note that the oscillations in the two participating units were in phase. Thus in spite of the apparent dissociation during tonic activity, the units still responded to common inputs. Examination of the video tapes suggested that the 1-Hz oscillations were related to respiration. This was confirmed by the experiment illustrated in Fig. 5, which shows a simultaneous recording from the soleus (top) and the diaphragm (bottom). The frequency oscillations in the soleus motor units were phase locked to the diaphragm EMG and could be either in phase or in counter phase (not shown). This activity might participate in counteracting the postural effects of a shifting center of gravity caused by movements of the diaphragm. The present observations are in good agreement with the earlier findings of Hennig and Lømo (1985) and Hennig and Hennig (1987)

![Image](http://jn.physiology.org/)

**TABLE 1. Firing-frequency characteristics of 10 soleus motor units**

<table>
<thead>
<tr>
<th>Unit ID</th>
<th>No. of Intervals</th>
<th>Firing Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.1</td>
<td>9,263</td>
<td>18 (14–22)</td>
</tr>
<tr>
<td>07.2</td>
<td>15,339</td>
<td>20 (19–22)</td>
</tr>
<tr>
<td>290.3</td>
<td>160,833</td>
<td>25 (22–31)</td>
</tr>
<tr>
<td>298.1</td>
<td>70,192</td>
<td>22 (20–25)</td>
</tr>
<tr>
<td>504.2</td>
<td>2,249</td>
<td>16 (11–21)</td>
</tr>
<tr>
<td>504.3</td>
<td>27,953</td>
<td>22 (20–24)</td>
</tr>
<tr>
<td>504.4</td>
<td>21,029</td>
<td>22 (18–25)</td>
</tr>
<tr>
<td>661.1</td>
<td>4,867</td>
<td>21 (16–25)</td>
</tr>
<tr>
<td>661.3</td>
<td>10,203</td>
<td>24 (22–26)</td>
</tr>
<tr>
<td>641.1</td>
<td>2,441</td>
<td>47 (34–61)</td>
</tr>
</tbody>
</table>

Number of intervals (total 324,369) and firing-frequency characteristics (median and quartiles) in each of the included units. The unit ID consists of rat number and chronological unit number in the individual rat, separated by a point.

**Fig. 3.** Spike-triggered averages of motor-unit potentials. One hundred-sweep averages as seen through the single-motor-unit electrodes (top) and the gross-EMG electrodes (bottom); sampling frequency 20 kHz, identical gain settings. A: motor units shown in Fig. 2, with corresponding labeling. b: averages from segments 1 (left) and 3 (middle). Rightmost panel: averages superimposed. Shape and amplitude of the waveforms showed only small changes, verifying that the electrodes did not move significantly in relation to the muscle fibers they recorded from. d: averages from segment 2 and from a segment 2 min after the end of Fig. 2 (cf. Fig. 1). a and e: averages from segments 1 and 2, respectively; the units are clearly different from b and d. B: motor-unit averages obtained 7 min before the beginning and 4 min after the end of the time period shown in Fig. 2. Unit could not be discerned in the intervening time. Offset traces in the rightmost panel show the signals shifted 0.45 ms to obtain better superposition of the last part of the potentials.
FIG. 4. Recruitment of one motor unit to tonic firing while another fires at a stable frequency. A: single-motor-unit potentials recorded through the single-motor-unit electrodes (bottom) with instantaneous frequency values (● unit 504.3; same as in Fig. 9). Frequency fluctuations in the 1-Hz range are emphasized by a curve that represents smoothing of the instantaneous frequency plot with a 250-ms symmetrical time window. B: simultaneous recording through the gross-EMG electrodes. Middle: unenhanced signal, expanded portions of which are depicted in the bottom trace. Top: integrated rectified signal. The gross-EMG electrodes initially picked up activity in a single unit that fired at a stable frequency ~20 Hz throughout. A phasic event occurred, after which the unit recorded by the single-motor-unit electrodes in A, firing at ~10–15 Hz, could be discerned as well. The two units had similar amplitudes and still could be followed after the 2nd phasic event, both firing at ~20 Hz. C: instantaneous frequency plot of the initially active unit in B. Times of occurrence of spikes were obtained in parallel from the signals in A and B. For each spike in A, 1 spike in B was deleted if it occurred within ±1 ms of the reference spike. Outliers in C with frequencies at approximately half value of the neighboring points correspond to instances where only 1 potential was discerned, and removed, due to superposition of spikes from the 2 units. Phasic events were deleted due to interference.
agreement with previous studies that have demonstrated respiratory influence on hindlimb extensor motoneurons (e.g., Meyer-Lohmann 1974). Recently, Sasaki et al. (1991) have shown that the majority of caudal medullary expiratory neurons in the cat extend their axons to the sacral or lower lumbar segments and distribute collaterals in L₅–L₇.

Tonic firing in single units at stable frequencies around 20–30 Hz typically lasted for up to several minutes (cf. Hennig and Lømo 1985) and most often was terminated by a phasic event. However, it also could terminate by a sudden drop in frequency that appeared not to be related to leg movements (Fig. 6).

**Phasic events**

**Postural tasks.** Phasic events initiating or separating tonic segments usually were caused by activity in several motor units, some of which continued firing in the tonic segment. The accompanying movements were often barely detectable. Figure 7 shows an example of a unit (504.2) firing transiently at the start of a tonic segment (A). The unit approached 50 Hz and fell off rapidly to ∼20 Hz, from where it decelerated at a slower rate. The gross-EMG signal is shown in B. Initially, a low-amplitude unit firing at ∼23 Hz could be seen through the gross-EMG electrode. The phasic event recruited several other units; the largest one could be clearly discerned and continued tonic firing at ∼18 Hz. The similarity with the recording in Fig. 4 is obvious.

The unit in Fig. 7A usually fired brief trains of up to 6 s duration, but it was also capable of firing longer tonic trains. Firing episodes of up to ∼70 s were observed during a 25-min recording session. It was also, together with the putative fast unit (see further), capable of firing high-frequency doublets. This property has not previously been documented in rat soleus motor units. Altogether 29 doublets were found (Fig. 8). They were remarkably similar in interspike interval (median 2.75 ms; quartile interval 2.65–2.76 ms), always occurred at the start of brief firing episodes, and often as stand-alone firing events. They were usually accompanied by small postural adjustments or only barely visible movements.

**Locomotor tasks.** Locomotor behavior of rat motor units has not previously been studied, and the literature on spontaneous animal locomotion on uneven surfaces is scarce. In the present material, 87 step cycles from five units comprising 2,637 intervals were analyzed in detail. Figure 9 shows the firing pattern of a soleus motor unit during spontaneous locomotion on an uneven surface (same unit as in Fig. 4). Instantaneous firing frequencies are superimposed on a curve representing RMS-processed gross EMG (cf. METHODS). Figure 9 bears a striking resemblance to cat data published by Hoffer et al. (1987). Thus as in the cat, the firing frequency of an individual unit seems to be closely related to the gross-EMG level, indicating a tight coupling between the members of the motoneuron pool participating in the locomotor event. This is in contrast to the apparent dissociation during tonic firing (cf. Figs. 2 and 4).

**Fast soleus unit**

One soleus motor unit differed markedly from the rest of the present material (Table 1, unit 641.1) and from the units seen in previous studies (Hennig 1987; Hennig and Lømo 1985). It was recruited at relatively high gross-EMG levels during postural activity, and its high firing frequency and the broad scatter within individual trains were almost identical to those of a motor unit recorded from the EDL muscle (unpublished observations). The unit was followed for 10 min, and all recorded trains were shorter than eight seconds. Figure 10 shows the unit firing two bursts without any high-amplitude phasic transients in gross-EMG activity. The behavior of the unit during locomotor activity (33 step cycles, 1,025 motor-unit potentials) was also different from that of the other soleus units (Fig. 11). In contrast to them (Fig. 9), it typically started firing well into the stance phase, as the contralateral foot was lifted off the ground and moved forward. However, its firing frequency still followed the gross-EMG envelope closely.
FIG. 7. Transient activation of a single unit at the start of a tonic segment. A: single-motor-unit potentials (unit 504.2) with instantaneous frequency plot. B: simultaneous gross-EMG activity with integrated rectified EMG; note continued activity of high-amplitude unit (cf. Fig. 4). Recording was made 2 days after electrode implantation.

The first interspike interval in firing episodes was often remarkably short, down to 5.7 ms (Fig. 10). Furthermore, the unit regularly showed high-frequency doublets within trains (Fig. 11). The median interspike interval of 108 manually measured doublets was 2.78 ms (quartile interval 2.57–3.13 ms), remarkably similar to that of the other unit firing doublets. This is in good agreement with the position of the delayed depolarization relative to the original motoneuron spike (Granit et al. 1963). The immediately following interspike interval was significantly longer than other nondoublet intervals in trains (median 30.0 and 18.5 ms respectively; \( P < 0.0001 \), Mann-Whitney \( U \)). This phenomenon is probably due to summation of after hyperpolarizations and confirms that both spikes originate in the same motoneuron even though the shape of the second spike may be slightly distorted (cf. Fig. 8B). Prolonged postdoublet intervals previously have been seen in cats (Hoffer et al. 1987; Zajac and Young 1980) and in humans (Bawa and Calancie 1983), and they are also common in intracellular recordings of doublets caused by delayed depolarization (Calvin and Schwindt 1972).

**DISCUSSION**

The current study presents spontaneous motor activity in freely moving rats as opposed to most previous work, which has focused on more stereotypical motor behavior. This approach has resulted in observations that would not have been made if stereotypical repetitive movements were studied. The material is in agreement with previous investigations of rat soleus motor-unit activity (Hennig and Lømo 1985). However, in the present investigation, the methodological emphasis has been changed so that firing frequency in individual units as a function of time was studied as well as correlation of motoneuron activity with whole-muscle activity and motor behavior.

**Classification of single units**

According to studies of twitch contraction times (Close 1967), the soleus muscle consists of \( \sim 30 \) motor units, \( \sim 90\% \) of which are slow and \( \sim 10\% \) have intermediate contractile properties. In male Wistar rats of approximately the same age and from the same supplier as in the present study, Gundersen et al. (1988) found on the basis of histochemical staining of myofibrillar actomyosin ATPase that 98.6 ± 1.3\% (mean ± SD) of soleus fibers were Type I, 0.3 ± 0.8\% were Type IIA, and 1.1 ± 1.3\% were Type IIC. These values differ to some extent from those reported elsewhere in the literature (e.g., Ariano et al. 1973). The differences may be due to a number of factors, e.g., strain, age, and method of typing.

Eight of 10 units in the present study corresponded to previously described rat soleus units (Hennig and Lømo 1985). As suggested by Hennig and Lømo (1985) on the basis of their common occurrence, they are probably slow Type I units. The assumption is supported by their relatively low median firing frequency and long-lasting tonic firing. These properties are known to induce and maintain slow contractile properties in muscle fibers (Eerbeek et al. 1984; Eken and Gundersen 1988; Westgaard and Lømo 1988; see review by Pette and Vrbova 1992). Slow cat motor units produce maximum tension-time area per pulse, and thus should be most energy efficient when trains with interpulse intervals 1.31 times twitch contraction time (\( T_c \)) are applied (Burke et al. 1976). According to Close (1967), \( T_c \) of slow
FIG. 8. Fixed-interval initial doublets in a slow motor unit. A: interval histogram for all 29 doublets obtained during a 25-min recording session (unit 504.2; same as in Fig. 7). Intervals were measured manually as the distance between motor-unit potential peaks. B: analog signals showing the first 6 doublets. Second and 5th doublet were stand-alone firing episodes. The rat was lying down, and the firing events were only followed by small postural adjustments. Note the distorted appearance of the 2nd potential in the doublets.

FIG. 9. Locomotor activity in a slow motor unit. A: single-motor-unit instantaneous frequency values ($\bullet$; unit 504.3; same as in Fig. 4) and root-mean-square (RMS)-processed gross-EMG activity (line). B: analog signals; single-motor-unit potentials (top) and gross-EMG activity (bottom). Firing frequency of the slow motor unit was well correlated to the gross-EMG activity. The rat was outside reach of the video camera; see Fig. 11 for demarcation of swing phase during locomotion.

FIG. 10. Postural activity in a fast motor unit. A: single-motor-unit instantaneous frequency values ($\bullet$) and RMS-processed gross-EMG activity (line; scaling factor obtained from Fig. 11). B: analog signals; single-motor-unit potentials (top) and gross-EMG activity (bottom). The unit (641.1; same as in Fig. 11) fired 2 high-frequency bursts; note large scatter of instantaneous frequency values and short initial interspike interval.
corded from motoneurons when the gross-EMG records originally had been obtained.

The fast unit (Fig. 11) behaved differently from the others during locomotion. In addition to its higher firing frequency and broader scatter in frequencies, it was often active only during the rather late part of the stance phase when the contralateral leg was in the swing phase. However, once recruited, its firing frequency still followed the gross-EMG envelope closely. Thus it might still belong to the same task group as the concurrently active slow units. There was no evident increase in gross EMG on recruitment of the fast unit. This observation would be expected if all the slow units, which constitute a vast majority of the motoneuron pool, were recruited earlier in the stance phase and already were firing near their maximal rate.

Apparent dissociation between tonically active motor units

There was a considerable degree of firing-frequency dissociation between individual slow soleus units, and thus between single units and gross EMG, during tonic firing. This is in sharp contrast to the tight coupling between the individual unit and the rest of the motoneuron pool during locomotion. The gross electrode commonly detected a small number of single units, often one or two, that were tonically active at rather high, stable frequencies for several minutes without any detectable activity in other units (Fig. 2). At a later instance, the previously active units were silent while others fired in the same frequency range. Furthermore, a silent unit could be recruited to fire at a rather high frequency with hardly any changes in the firing frequency of already active units (Fig. 4). We have shown previously that there may even be a slight drop in the firing frequency of a unit firing at ~30 Hz when a second unit jumps abruptly from ~10 Hz to the frequency range of the first unit (Fig. 6 in Eken and Kiehn 1989).

Individual members of a motoneuron pool are expected to show a repeatable recruitment pattern (see review by De Luca and Erwin 1994). Motor units are believed to be recruited in order of increasing size when muscle activity is increased, a phenomenon often referred to as the “size principle” (Henneman et al. 1965). In addition to their higher recruitment thresholds, motoneurons of larger motor units innervate more and larger muscle cells and thus have larger muscle action-potential spikes and larger force-generating capability. Because the slow units in the soleus muscle are remarkably homogeneous both in terms of contractile properties and fiber type (Close 1967; Gundersen et al. 1988; Kugelberg 1976), one would expect them to follow each other closely in recruitment and firing-frequency modulation. Whereas this was obvious during phasic firing, the classical concepts for motoneuron pool excitation do not seem to be able to explain the firing-frequency dissociation during tonic activity. In the latter case, individual motor units appeared to be offset by 10–20 Hz from other members of the motoneuron pool, often through abrupt steplike changes in firing frequency (Fig. 4).

One might assume the apparent dissociation between participating motoneurons to be caused by varying constellations of active units due to activity in different task groups. However, the motoneuron pool would have to be divided

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**Figure 11.** Locomotor activity in a fast motor unit. A: single-motor-unit instantaneous frequency values (●, note broken axis) and RMS-processed gross-EMG activity (line). Horizontal bars indicate swing phase, identified from the video recording. B: analog signals; single-motor-unit potentials (top) and gross-EMG activity (bottom). The unit (641.1) was recruited during the latter part of the stance phase, after which its firing frequency closely followed the gross-EMG activity profile. Recording was obtained 7 days after electrode implantation.
into a large number of task groups with minimal overlap in excitatory drive to obtain the observed lack of hierarchy in the recruitment to tonic firing at relatively high frequencies. This seems unlikely, as there is a limited number of tasks in which the soleus can participate. Alternatively, one might suggest the existence of specialized high-precision spinal circuitry underlying this kind of firing, but the complexity of the circuitry would have to be considerable to achieve the high degree of firing-frequency dissociation during tonic firing.

An alternative proposition is that the mechanism behind the apparent dissociation between motor units during tonic firing resides in individual neurons. This proposition is based on the discovery of depolarizing plateau potentials in cat and turtle spinal α-motoneurons (Conway et al. 1988; Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988). These long-lasting nifedipine-sensitive depolarizing potentials are dependent on monoamines, particularly serotonin and noradrenaline, and they can be activated and deactivated by synaptic excitation and inhibition respectively (see review by Kiehn 1991). Activation of such potentials not only increases the firing frequency of an active unit but allows single motoneurons to fire at a fixed frequency for prolonged time periods without any activity in neighboring neurons. The proposition is supported by previous experiments in unrestrained rats, where we have demonstrated that delivery of synaptic excitation through electrical stimulation of Ia afferents induced jumps from tonic low-frequency firing to sustained firing at rather high frequencies, similar to Fig. 4 (Eken and Kiehn 1989). Conversely, synaptic inhibition mediated through cutaneous afferents shifted the tonic firing from high to low frequencies. The monoaminergic influence on motoneurons is believed to be exerted by descending fibers from the brain stem, in particular from the nucleus raphe pallidus and nucleus raphe obscurus, which project directly to α-motoneurons (Holstege and Kuypers 1987). Monoaminergic brainstem neurons are known to possess pacemaker properties (Aghajanian and VanderMaalen 1982) and to be activated preferentially in association with motor output, especially during changes in muscle tone and during responses mediated by central pattern generators (Jacobs and Fornal 1993). We have shown that the long-lasting activity in single units disappears after selective depletion of descending monoaminergic fibers (Kiehn et al. 1996) and that this characteristic feature of adult motor behavior appears only after the third postnatal week (Eken et al. 1990), when the descending monoaminergic projections mature (Bregman 1987).

The spontaneous firing behavior of individual soleus motor units is demonstrated in the present material seems consistent with a system that has the following essential components:

1) There is a gradient of recruitable among motoneurons, i.e., the “size principle.” However, the soleus motoneuron pool is highly homogeneous, leading to rather similar recruitment thresholds and firing-frequency profiles (Fig. 9).

2) A depolarizing offset of membrane potential is introduced when a plateau potential is activated, e.g., due to excitatory synaptic input (Fig. 4A; same unit as in Fig. 9). This makes individual motoneurons fire tonically for extended time periods, apparently dissociated from the rest of the pool (Figs. 4A and 2). However, they are still subject to common drive (cf. respiratory oscillations in Fig. 4). Inhibitory synaptic input to the motoneuron will inactivate the plateau potential and lead to cessation of tonic firing (phasic events in Fig. 2). Decay of the plateau potential due to decreased monoaminergic influence probably also would lead to rather abrupt termination of tonic activity (cf. Fig. 6).

3) The susceptibility of individual motoneurons to recruitment of the plateau potential varies over time. Thus, a seemingly random factor, e.g., membrane noise, determines which of the similarly sized units is recruited to long-lasting tonic firing for a given common excitatory input to the pool.

Seemingly random recruitment of motor units to long-lasting tonic firing as described in the present work appears not to have been reported in the literature before. This may be due to several factors. Most single-unit EMG studies have been performed during rather stereotypical repetitive contractions, conditions that are probably not ideal for observing the phenomenon. Studies focusing on long-lasting low-amplitude activity have primarily used noninvasive gross-EMG techniques, which are generally inadequate for picking up and following several single motor units selectively for a sufficient duration of time. In addition to methodological problems, plateau potential activation may not be under “conscious” control (Kiehn 1991), so that the phenomenon may not have been brought into action in a particular experimental setting. Furthermore, motoneuron firing behavior due to activation of plateau potentials might not have similar appearance in different species, e.g., due to different thresholds for activation relative to the threshold for repetitive firing of the motoneuron (see, e.g., Kiehn and Eken 1997). However, Lang and Vallbo (1967) have described long-lasting tonic activity in human soleus muscle after transient synaptic excitation delivered through electrical stimulation of the tibial nerve. Recently, we have reported that transient vibration of human soleus and tibialis anterior muscle tendon can induce previously silent motor units to jump to a high frequency and continue to fire for a sustained time period (Kiehn and Eken 1997). Similar behavior could be seen when silent units were recruited voluntarily. These results resemble our findings in single motor units studied in intact rats (Eken and Kiehn 1989) and may provide a starting point for further investigation.

Random recruitment of individual units to long-lasting tonic firing may have several functional implications, both for the animal as a whole and for the individual motor units. According to the 24-h recordings performed by Hennig and Lomn (1985), the median firing frequencies of their six soleus units (18–21 Hz) corresponded to ~80% of maximal tetanic muscle tension. Individual units could be active for more than one-third of the total time (range 22–35%), the longest trains recorded in each unit lasting for 300–548 s. The present material is in agreement with these findings. This activity is conceivably important for muscle tone, and thus for keeping the animal’s posture, both during wakefulness and sleep. One also might suggest that the heat produced during long-lasting firing could be important for maintenance of body temperature. The soleus muscle is located deep in the leg and has a rich blood supply, thus making it well suited for conserving heat and conveying it to the rest
of the body. Maintained firing activity is probably also essential for keeping the soleus muscle fibers slow and fatigue resistant (Eerbeek et al. 1984; Eken and Gundersen 1988; Westgaard and Lømo 1988). Brief muscle contractions during resting or sleep may actually serve to cyclic tonic activity between motor units (cf. Figs. 1, 2, 4, and 7). This will enable all units to receive a comparable high amount of endurance training at the same high activity levels and could be responsible for the remarkably homogeneous muscle-fiber properties in the muscle. Thus descending monoaminergic innervation of motoneurons may have implications ranging from gain setting for classical synaptic transmission (for review, see Holstege and Kuyper 1987) and control of muscle tone and posture (Eken and Kiehn 1989) to regulation of motor-unit contraction speed and endurance.

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