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Does daily activity level determine muscle phenotype?

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Summary

The activation level of a muscle is presumed to be a major determinant of many mechanical and phenotypic properties of its muscle fibers. However, the relationship between the daily activation levels of a muscle and these properties has not been well defined, largely because of the lack of accurate and sustained assessments of the spontaneous activity levels of the muscle. Therefore, we determined the daily activity levels of selected rat hindlimb muscles using intramuscular EMG recordings. To allow comparisons across muscles having varying activity levels and/or muscle fiber type compositions, we recorded EMG activity in a predominantly slow plantarflexor (soleus), a predominantly fast plantarflexor (medial gastrocnemius, MG), a predominantly fast ankle dorsiflexor (tibialis anterior, TA) and a predominantly fast knee extensor (vastus lateralis, VL) in six unanesthetized rats for periods of 24 h. EMG activity levels were correlated with the light:dark cycle, with peak activity levels occurring during the dark period. The soleus was the most active and the TA the least active muscle in all rats. Daily EMG durations were highest for soleus (11–15 h), intermediate for MG (5–9 h) and VL (3–14 h) and lowest for TA (2–3 h). Daily mean EMG amplitudes and integrated EMG levels in the soleus were two- to threefold higher than in the MG and VL and seven- to eightfold higher than in the TA. Despite the three- to fourfold difference in activation levels of the MG and VL *vs* the TA, all three predominantly fast muscles have been reported to have a similar, very low percentage of slow fibers. Comparing these relative EMG levels to the published fiber type profiles of these muscles yields a very poor relationship between daily activity level and fiber type composition in the same muscles across several species. Although it is clear that changing the levels of activity can modulate the expression of the myosin phenotype, these results indicate that factors other than activation must play critical roles in determining and maintaining normal phenotypic properties of skeletal muscle fibers.

Supplementary material available online at
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Key words: daily integrated EMG, daily EMG duration, muscle fiber type composition, motor unit recruitment, rat.

Introduction

The role of activation levels in the expression and maintenance of the morphological, physiological and biochemical properties of the skeletal musculature is a topic of continued debate, in part due to the paucity of data on the normal activity levels of different muscles. The majority of previous studies have focused on the role of activation levels in maintaining or modifying muscle mass and phenotype through perturbations of normal activity (Edgerton and Roy, 1996; Grossman et al., 1998; Lewis et al., 1997; Pette and Vrbova, 1999; Pierotti et al., 1991; Roy et al., 1991c; Salmons and Sreter, 1976; Windsich et al., 1998). However, a number of observations demonstrate that the level of activation alone does not determine either normal muscle mass or phenotype (Edgerton et al., 2002; Pette et al., 1992). For example, there is an initial decrease in the daily activity levels in the extensor muscles when the hindlimbs of rats are unloaded, but the activity levels of the major plantarflexors increase towards normal, pre-unloading levels within a few days (Alford et al., 1987). The masses of these muscles, however, continue to decrease, demonstrating that simply activating the muscles is insufficient to maintain muscle mass. When the extensor muscles of unloaded rats are allowed to bear weight and generate forces during short daily periods of normal cage ambulation, treadmill locomotion, grid climbing or centrifugation, the loss in muscle mass is attenuated significantly (Edgerton and Roy, 1996; Roy et al., 1991c). Because neither the normal levels of activity of the muscles nor the levels of activation

imposed by these interventions were determined in any of these studies, the 'dose–responses' for the effects of activation on these muscles are, at best, known only very generally.

The role of activity in defining muscle properties has also been examined using chronic electrical stimulation to influence the enzyme expression and contractile properties of skeletal muscles. These observations have been used to argue that the level of activation largely defines the properties of the muscle (Eken and Gundersen, 1988; Pette and Vrbova, 1992). However, chronic stimulation of a muscle often induces muscle atrophy (Eisenberg et al., 1984; Salmons and Sreter, 1976), and in many cases the changes in phenotype are minimal or at best incomplete, even when the muscles are stimulated for as much as 24 h day^{-1} (Edgerton et al., 1996; Lewis et al., 1997; Pette et al., 2002).

In the cat soleus, a muscle that usually is composed exclusively of slow fibers, even the most active motor units were reported to be active for less than 4 h day^{-1} (Hensbergen and Kernell, 1997). For other muscles containing slow fibers, such as the tibialis anterior (TA) in cats and Rhesus monkeys, even the most recruited motor units appear to be active for less than 1 h day^{-1}, suggesting that prolonged periods of activation are not necessary to sustain even the slow fiber phenotype (Hodgson et al., 2001; Hensbergen and Kernell, 1997; Pierotti et al., 1991).

Given the extensive use of the rat model for studying the mechanisms by which a muscle fiber phenotype is determined as well as muscle mass maintained or even enlarged (Booth and Baldwin, 1996), it seems important to define quantitatively the normal daily activity levels of muscles having different fiber type compositions and functions. While we postulate that only relatively short periods of muscle fiber activation are sufficient to sustain the normal mass and phenotype of slow muscle fibers, the absence of a clear understanding of what the normal activity levels are for flexor and extensor and for slow and fast muscles of rats housed in standard cages precludes a clear conclusion. Thus, the purposes of this study were to determine and compare (1) the daily activity levels of a slow plantarflexor (soleus), a fast plantarflexor (medial gastrocnemius, MG), a fast dorsiflexor (TA), and a fast knee extensor (vastus lateralis, VL) in adult female rats housed in typical sedentary cages; and (2) the relationship between the daily activity level and the reported (Delp and Duan, 1996) fiber type composition (percent slow fibers) of each muscle studied. Preliminary results have been presented in abstract form (Zhong et al., 2003, 2004).

Materials and methods

Adult female Sprague-Dawley rats (N=6; 219±4 g mean body mass at the time of surgery) were used for this study. The studies were approved by the UCLA Chancellor's Animal Research Committee and followed the American Physiological Society Animal Care Guidelines.

EMG implants

The rats were anesthetized with ketamine hydrochloride (100 mg kg^{-1} body mass) and xylazine (8 mg kg^{-1} body mass) administered intraperitoneally (i.p.). Supplemental doses (30% of the initial dose, i.p.) were given as needed. Under aseptic conditions, a skin incision was made along the sagittal suture of the skull. The scalp musculature and underlying connective tissues were reflected laterally and the exposed skull was dried thoroughly. Three screws were anchored firmly to the skull and a 9-pin (gold-plated) amphenol connector was cemented (dental cement) to the skull and screws. Eight multistranded Teflon®-insulated stainless-steel wires (AS 632, Cooner Wire Co, Chatsworth, CA, USA) were led subcutaneously from the connector to the hindlimb (see below). The ninth wire was embedded in the middle back region and served as a common ground. The undersurface of the headplug between the pins and the wires was sealed with epoxy resin to prevent any body fluid seepage into the contact area.

Skin incisions were made in the hindlimb to expose the soleus, MG, TA and VL muscles. Two wires from the headplug were inserted into each of the following: the midbelly of the soleus, and a deep region (i.e. close to the bone) of the midbelly of the TA, MG and VL. These anatomical locations were chosen to ensure a consistent sampling site across rats and to sample a predominantly slow fiber type area in the soleus, and an area having the highest proportion of slow fibers (~30–35% slow fibers) in the deep regions of the MG, TA and VL (Delp and Duan, 1996). The wires were inserted into each muscle region (~2–3 mm apart) by passing them individually through a 23-gauge hypodermic needle. Recording electrodes were made by removing ~0.5 mm of insulation from each wire. Following back-stimulation of the muscle through the headplug to ensure the proper placement of the electrodes, each lead was secured with a suture at its entry and exit from the muscle. This procedure effectively secured the electrodes in the muscle belly. The bared tips of the wires were covered by gently pulling the Teflon® coating over the tips to avoid recording extraneous potentials. All incisions were closed using 4-0 Ethilon® suture. These procedures are used routinely in our laboratories (Roy et al., 1985; Roy et al., 1991b).

The rats were allowed to fully recover from anesthesia in an incubator (27°C) and were given lactated Ringers solution (5 ml, subcutaneously). PolyFlex®, a general antibiotic, was administered (100 mg kg^{-1}, subcutaneously, twice/day) during the first 3 days of recovery. The rats were housed in polycarbonate cages (26 cm × 48 cm × 20 cm) individually and the room was maintained at 26±1°C, 40% humidity and a reversed 12 h:12 h light:dark cycle (dark from 9:00 h to 21:00 h). Rats were supplied with Purina® rat chow and water *ad libitum*.

EMG recordings and analyses

All electromyogram (EMG) recordings were performed using the same cages in which the animals were normally housed and were initiated at least 1 week after the implant surgeries. After this 1 week of recovery, no evidence of

appreciable signal artefacts with the animals at rest or in motion were observed. The housing conditions were considered to be 'normal' for experimental rats, since they are the usual conditions under which rats are housed before characterization of the muscle properties of a 'control' population of animals. A nine-conductor swivel (Alice King Chatham Medical Arts, Inglewood, CA, USA) was mounted on the top of each cage, allowing the animals to move freely during the recordings. Signals were amplified (×1000, custom-built portable amplifiers) upon exiting the swivel, and then recorded digitally at 2 kHz on a desktop computer using custom acquisition software. Visual inspection of the EMG signals indicated stable baseline levels with no fibrillation potentials, which remained stable during passive manipulation of the limb. Raw EMG data were transferred to CD-ROM for subsequent analysis. Recordings began between 08:00 h and 10:00 h and were concluded 24 h later.

The EMG data were analyzed using in-house software developed using LabVIEW (National Instruments, Houston, TX, USA). The methods have been reported in previous publications (Edgerton et al., 2001; Hodgson et al., 2001). Briefly, all EMG data were first reviewed by displaying the recorded data on a computer monitor at selected time resolutions ranging from fractions of a second to several minutes of data on the computer screen. Segments of data containing interference were identified and excluded from further analysis. Typically these were sections containing 60 Hz noise or large amplitude, relatively slow transients across all channels. The remaining data were digitally high-pass filtered at 10 Hz and rectified. Mean EMG values of 40 ms time epochs were calculated from these data, effectively smoothing with a 12.5 Hz low pass filter and decimating the data to 25 samples s^{-1}. Amplitude histograms were constructed from the processed EMG signals from each muscle for each hour of the day. Integrated EMG (IEMG) values were calculated by multiplying each bin count by its corresponding amplitude, and then summing these values over all bins. The duration of EMG activity was calculated by summing all bin counts above a threshold level. The threshold level was determined by generating amplitude histograms of EMG data when no activity was apparent in any muscle and the animal was assumed to be inactive. The threshold level was set at the highest bin required to exclude 95% of the baseline data. Mean burst amplitudes of EMG activity were calculated by dividing the burst integral by the burst duration. Data were corrected to properly represent each hour of activity in those instances where interference excluded some data from the analyses. The daily mean EMG amplitude was calculated by dividing the IEMG by 24 h.

Statistical procedures

Group means and standard deviations (S.D.) are given where appropriate to show trends and variability in the data. Ranges in the data and the number of observations are provided in the tables and figures. In many cases, these ranges did not overlap. The Wilcoxon Signed-Rank test was used to test for significant differences between pairs of muscles from each animal. Significance was set at $P<0.05$. The Wilcoxon test gave P values of 0.028 (N=6) or 0.043 (N=5) for the differences found to be significant. Pearson product correlation coefficients were used to determine the relationship between fiber type composition and activity level.

Results

Examples of raw EMG signals recorded during postural-like and locomotor-like activity are shown in Fig. 1. In each case, the data were selected to illustrate relatively high levels of EMG activity to demonstrate the quality of EMG recordings. The thicker black lines indicate the same data rectified and then smoothed using a moving average calculated over 40 ms time epochs.

Daily activity

A diurnal cycle of EMG activity was apparent: the integrals (Figs 2A, 3A) and durations (Figs 2B, 3C) of EMG activity were significantly higher (approximately double) during the dark (from 09:00 h to 21:00 h) than the light period. During the dark period, EMG burst amplitudes were 97% of the light period values, suggesting that although the muscles were less active during the light period, the amplitudes of the bursts were very similar. The mean amplitude values were combined in Fig. 3B, since the two values were similar.

The soleus was the most active and the TA the least active muscle in terms of daily IEMG (Fig. 3A), daily EMG duration (Fig. 3C) and mean EMG amplitude (Fig. 3B). These values for the MG and VL were intermediate between the soleus and TA. The daily mean EMG amplitude represents the mean amplitude of the EMG for an entire 24 h period and showed an identical pattern (Fig. 3D) to the daily IEMG (Fig. 3A), since it was calculated by dividing IEMG by 24 h. The low values for the MG and VL ($P>0.05$) and the lower value for the TA daily mean EMG amplitude reflect the longer periods when these muscles were not active relative to the soleus. There was no overlap in the ranges for the daily IEMG or daily EMG duration among the soleus, MG and TA, reflecting the significant differences between these muscles, whereas the VL values generally overlapped the ranges for the other muscles (Table 1). The ranges in mean EMG burst amplitudes were similar for the MG, TA and VL, whereas the range of values for the soleus only overlapped with the upper values for the MG. The mean EMG burst amplitude in the soleus was significantly higher than in the other three muscles (Fig. 3B).

The distributions of EMG amplitudes (based on 40 ms bins) during a 24 h period for each muscle in a representative rat are shown in Fig. 4A (thick line). The broad peak extending from the zero amplitude bin (leftmost portion of curve) shows that the soleus was active at low levels for prolonged periods, whereas the other muscles had higher counts at zero amplitude and sharper declines in the amplitude count, indicating longer periods of inactivity in these muscles. Another way to represent the duration of activity at different recruitment levels

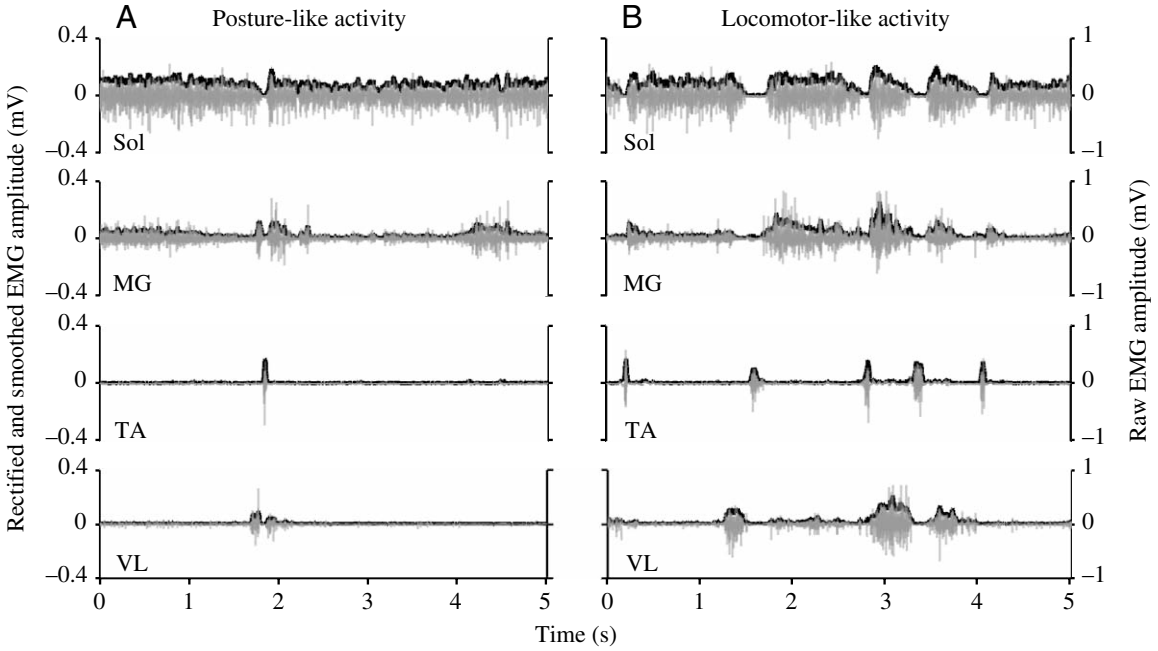

Fig. 1. Examples of EMG data recorded during daily cage activity. Raw EMG (gray lines) and smoothed rectified EMG (black lines) are shown during periods of sustained (posture-like) activity (A) and phasic (locomotor-like) activity (B). Sol, soleus, MG, medial gastrocnemius, TA, tibialis anterior, VL, vastus lateralis.

is to plot the time that the EMG activity exceeded a given amplitude level (Fig. 4B). Mathematically, these curves plot the integrals of all data points to the right of the corresponding EMG amplitudes in Fig. 4A. Note that the *y*-axis is a logarithmic scale in Fig. 4A, but a linear scale in Fig. 4B. These plots indicate that almost all of the activity in all of the muscles was at relatively low levels when compared to their peak EMG amplitudes. For example, the soleus muscle had peak amplitudes of ~0.2 mV in all of the animals, yet the EMG amplitudes remained below 0.05 mV (i.e. 25% of the peak) for ~21 h of the day. This observation indicates that some muscle fibers, even in the relatively highly active soleus muscle, are

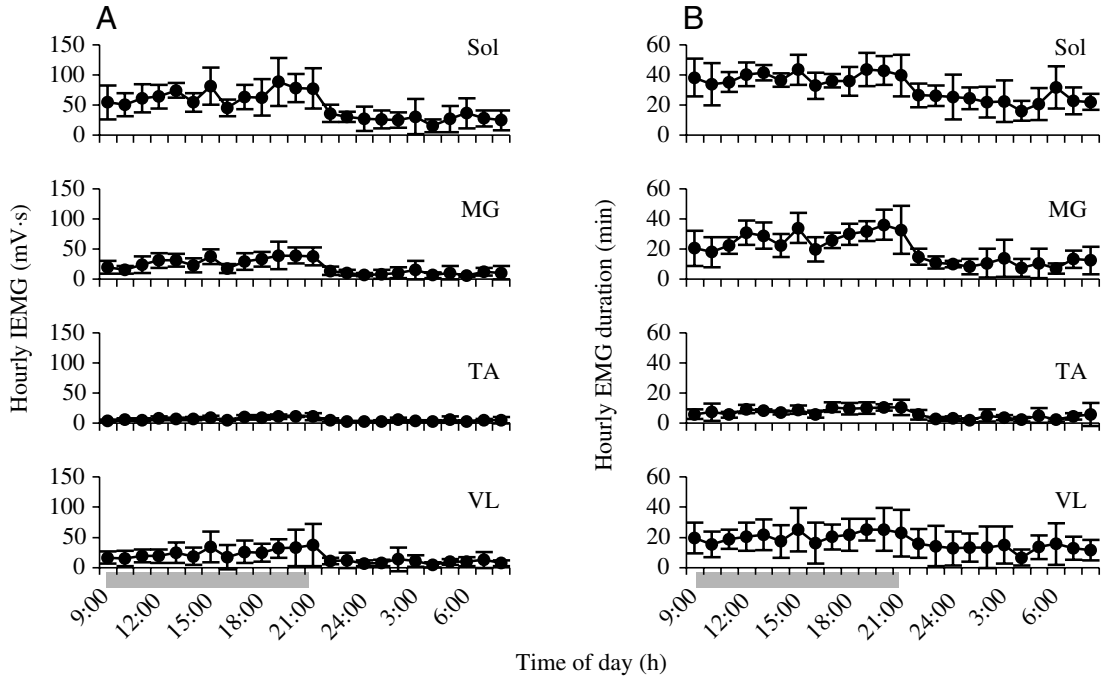

Fig. 2. (A) Mean integrated EMG (IEMG) and (B) mean duration of EMG h^{-1} over a 24 h recording period for six rats. The rats were in darkness between 9:00 h and 21:00 h daily (shaded bars on abscissa). Values are means ± 1 S.D. Abbreviations as in Fig. 1.

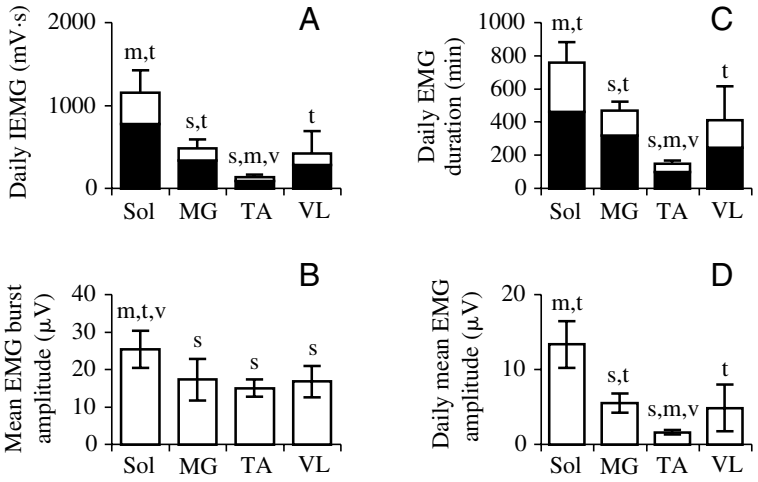

Fig. 3. (A) Mean daily integrated EMG (IEMG), (B) mean EMG burst amplitude, and (C) mean daily duration of EMG over an entire 24 h period for six rats. The dark and light portions of the bars in (A) and (C) represent the dark and light periods. (D) Mean daily EMG amplitude over the entire 24 h period, i.e. including all periods of inactivity. Values are means ± 1 S.D. Abbreviations as in Fig. 1. s, m, t and v, significantly different from soleus, MG, TA and VL, respectively.

activated for relatively short periods of the day (i.e. <3 h). Data from the other muscles indicate much lower levels of activity for a majority of the day.

If we make the conservative assumption that EMG amplitudes greater than 0.1 mV represent full recruitment of the soleus muscle, then we can estimate that the duration of activity above 0.1 mV represents the total time that all soleus motor units were active. The soleus muscles in our sample of animals were active at >0.1 mV for 15 s to 41 min per day. A higher amplitude for full recruitment would indicate even shorter periods of full recruitment. For example, if we assumed full recruitment at 0.15 mV, the total duration for which all fibers were active would drop to between 5 and 32 s day^{-1}. The daily distribution of EMG amplitudes in the other three muscles actually reached higher amplitudes than in the soleus (Fig. 4A). This was confirmed in all of the rats studied. A threshold of 0.15 mv (~50% peak amplitude) for the other three muscles yields durations for full recruitment between 40 s and 17 min day^{-1}. A threshold of 0.225 mV (70% peak amplitude) reduced the durations to 5 s to 3 min day^{-1}.

Discussion

A novel feature of the data presented herein is a detailed description of the distribution of EMG amplitudes in four rat hindlimb muscles throughout a 24 h period. We demonstrate that the observed EMG patterns suggest that populations of both slow and fast motor units remain inactive for all but a few hours, or even minutes, of the day. These observations have critical implications for the projected durations of daily activity required to maintain the mass and phenotype of all fiber types. We have also synthesized the presently available data, much of them from our own laboratory, which relate the 24 h activity patterns with the fiber type composition of several muscles across a range of species.

Variation in activity levels across rat hindlimb muscles

The relative levels of EMG activity of hindlimb muscles in the rats in the present study are consistent with the results of previous EMG studies (Alford et al., 1987; Blewett and Elder, 1993; Fournier et al., 1983) and with the duration of activities reflected in whole body movements (Block and Zucker, 1976; Moore and Bickler, 1976; Mouret and Bobillier, 1971) over prolonged periods of time during normal cage activity. The present study, however, provides the most comprehensive analysis of the daily activity of four rat hindlimb muscles. A majority of the muscle activity occurred during the dark phase of the circadian cycle and the soleus was the most active muscle studied. Interestingly, the mean EMG amplitudes during periods of muscle activity were almost exactly the same during the light and dark periods, suggesting similar levels of recruitment but for much shorter periods of time

Table 1. *Ranges of measures of daily EMG activity in selected hindlimb muscles of rats*

	Daily IEMG (mV·s)	Mean EMG burst amplitude (μV)	Daily EMG duration (h)	Daily mean EMG amplitude (μV)*
Soleus (6)	814–1529	17.7–31.6	10.5–15.6	9.4–17.7
MG (6)	346–644	11.5–26.3	6.8–9.3	4.0–7.5
TA (5)	100–175	12.2–18.3	2.1–3.0	1.2–2.0
VL (6)	185–944	9.2–21.8	2.9–12.0	2.1–10.9

MG, medial gastrocnemius; TA, tibialis anterior; VL, vastus lateralis.
The number of rats studied is shown in parentheses. See Fig. 3 for mean values (± S.D.).
*Mean EMG amplitude for the entire day, including periods of no activity.

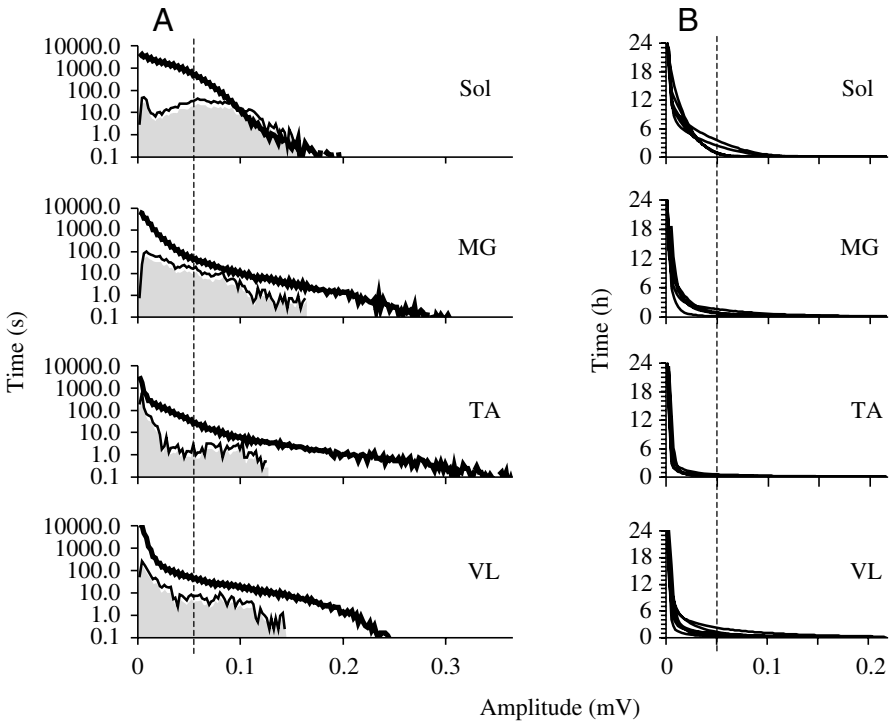

Fig. 4. (A) Distributions of the daily EMG amplitudes for the four muscles from one rat over a 24 h recording period (thick line). Note that the ordinate is a logarithmic scale. The lower maximum amplitude of the soleus was verified from the raw EMG data and was typical for all rats. The thin black line shows the distributions predicted for 20 min and the light gray shaded area 10 min of locomotor-like activity. These predictions were based on the distribution of EMG amplitudes recorded during a bout of phasic EMG activity (see locomotor-like activity in Fig. 1). (B) Graphs of the total time that EMG activity exceeded the amplitude level identified on the abscissa. Results from the six rats are plotted as separate curves to show the consistency across animals. Note that the Sol muscle had higher amplitudes for a greater amount of time and that the TA clearly had lower amplitudes for a lesser amount of time compared to the other muscles studied. The vertical dotted line in both (A) and (B) highlights an EMG amplitude of 0.25 mV, to illustrate the duration of activity at this relatively low amplitude. See text for details.

during the light period. In the present experiments, daily IEMG in soleus across all rats was between 2–4, 1–6 and 7–10 times the corresponding values for the MG, VL and TA, respectively. These results are consistent with previous data from rats, e.g. the soleus total daily IEMG activity has been reported to be between 3–10 and 20 times higher than for the MG and TA, respectively (Alford et al., 1987; Fournier et al., 1983). The daily mean EMG amplitudes for the MG, VL and TA bursts were generally similar, i.e. at ~5% of the peak values recorded, and the soleus daily mean EMG amplitude was about twice the amplitude for the other muscles, i.e. at about 10% of its peak value. Since the duration of activity is closely linked to the overall activity measured as IEMG, this suggests that the duration of time that a motor pool is activated provides a rough estimate of the total activity of that motor pool. Some activity was detected in the soleus for 11–15 h per day, similar to the findings in a report by Blewett and Elder (1993), whereas the TA was active for only 2–3 h per day. The MG was active for 5–9 h and the VL, which was the most variable of the muscles recorded, was active for 3–14 h day^{-1}. Thus, the slow plantarflexor (soleus) was more active than the fast plantarflexor (MG) and fast knee extensor (VL) muscles, which in turn were more active than the fast dorsiflexor (TA) muscle. These activity durations would be expected to represent only the most readily excitable motor units within each motor pool. However, each of these muscles contains a population of slow type motor units, which must be maintained by the short durations of activity observed in these muscles.

We also attempted to evaluate the contribution of locomotion to the daily activity observed in our subjects. Superimposed on the distribution of daily activity in Fig. 4 are distributions of EMG amplitudes predicted from the locomotor-like bursting patterns of activity as shown in Fig. 1. The shape of these distributions is quite characteristic for locomotion. The shaded plot has been scaled to represent the distribution of amplitudes predicted for 10 min and the dark thin line above it for 20 min of locomotor-like activity, i.e. the time of each bin has been divided by the duration of the original data and then multiplied by either 10 or 20 min. Any activity that appears above the curve representing the total daily activity (thick line) exceeds the measured daily activity and, therefore, is an overestimate of the activity occurring within a 24 h period. Note that portions of the curve representing 20 min of locomotor-like activity lie outside the total daily curve for the soleus, indicating that the maximum time that the rats could have spent in locomotor-like activity was between 10 and 20 min day^{-1}. The first peak on the ordinate of the soleus EMG distribution during locomotor-like activity corresponds to the near zero amplitude in this muscle during the swing phase of locomotion and the second peak represents the predominant EMG amplitude during stance (see Fig. 1). The majority of soleus EMG amplitudes in the daily distribution seem to occur at amplitudes slightly below the highest burst amplitudes observed during the stance phase of the locomotor-like activity, suggesting that a majority of the soleus activity occurs during prolonged periods of posture

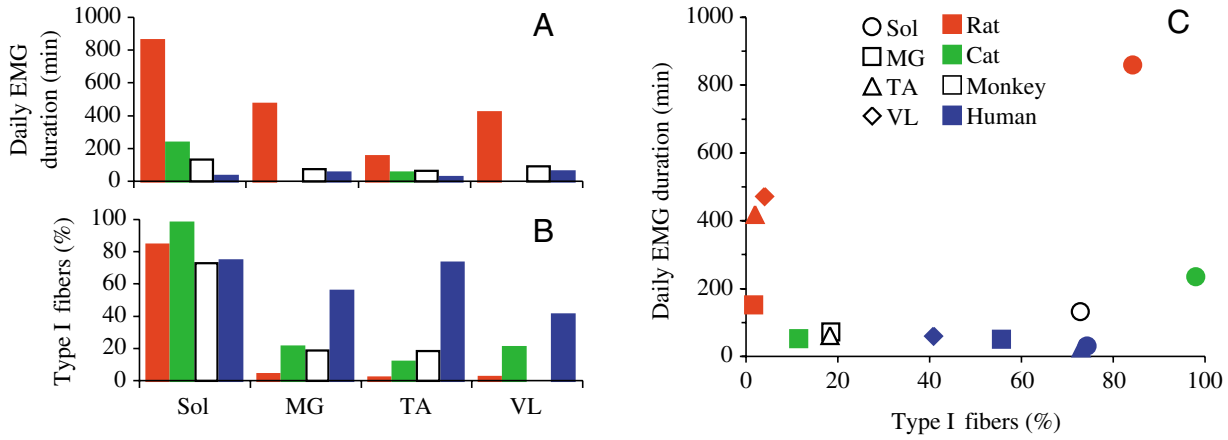

Fig. 5. (A) A survey of normal daily muscle activity from rat, cat, monkey and human muscles and (B) the percentage of type I (slow) muscle fibers. (C) The relationship between muscle fiber type and the duration of daily activity. The species are identified by the color of the bars and symbols and the muscles are denoted by symbol shape. The phenotype data are from the following sources: rats (Ariano et al., 1973; Armstrong and Phelps, 1984; Delp and Duan, 1996), cats (Ariano et al., 1973; Braund et al., 1995; Talmadge et al., 1996), Rhesus monkeys (Grichko et al., 1999; Roy et al., 1991a) and humans (Saltin and Gollnick, 1983). Additional EMG data are taken from the following sources: rats (Alford et al., 1987; Blewett and Elder, 1993), cats (Alaimo et al., 1984; Hensbergen and Kernell, 1997, 1998), Rhesus monkey (Hodgson et al., 2001) and humans (Edgerton et al., 2001; Kern et al., 2001). Abbreviations as in Fig. 1.

(compare the EMG amplitudes during posture *vs* locomotor-like activity in Fig. 1).

Lack of a close relationship between muscle activity level and fiber type composition

Since the activity levels of a muscle can modulate the muscle fiber phenotype over a period of weeks (Al-Amood et al., 1991; Booth and Baldwin, 1996; Eisenberg et al., 1984; Hennig and Lomo, 1987; Lewis et al., 1997; Pette and Vrbova, 1992, 1999; Roy et al., 1991c; Windsich et al., 1998), we examined the degree to which these two variables are coupled. Of the hindlimb muscles for which normal daily EMG activity has been studied (Fig. 5A), the soleus muscle has one of the highest percentages (~70–100%) of slow fibers in all species (Fig. 5B). With the exception of humans, the overall percentage of slow fibers in the other muscles studied is below 20% (Ariano et al., 1973; Armstrong and Phelps, 1984; Braund et al., 1995; Delp and Duan, 1996; Grichko et al., 1999; Roy et al., 1991a). In humans, slow fibers comprise between 40–70% of the fiber population in these same muscles (reviewed in Saltin and Gollnick, 1983).

There is a poor correlation between the percentage of slow fibers in a muscle and the daily duration of activation (r=0.08) (Fig. 5C) and IEMG (r=0.06, data not shown). These weak relationships are apparent for the muscles within a species and across the four species for which data are shown. For example, there is a threefold range in the duration of activity for the rat MG, TA and VL muscles, but the percentages of slow fibers are about the same in each muscle. Also, the percentage of slow fibers ranges from ~0 to 80% for muscles that are active for less than 200 min day^{-1} across species. We noted a strong negative correlation (r=–0.95) between the percent slow phenotype composition and the duration of EMG activity in the human muscles surveyed (Fig. 5C). Similar comparisons from other investigators have revealed mixed results (Kern et al., 2001; Monster et al., 1978). Our conclusion differs from that drawn by Kernell and Hensbergen (Kernell and Hensbergen, 1998) who demonstrated a positive correlation (r=0.76) between duty time, i.e. the ratio between total on-time and total sampling time, and fiber type composition for three predominantly fast muscles in cats, i.e. the TA, extensor digitorum longus and peroneus longus. If these authors had added the data for the soleus muscle to that plot, however, the soleus data point would fall at a much lower value of duration than predicted from the regression line through the other three muscles. Our general conclusion is that although the level of activity can play some role in the modulation of fiber type, it does not play a predominant role.

Hypothetical muscle fiber recruitment model

An ideal examination of the relationship between fiber type and activity levels would be based on actual recruitment patterns of individual motor units relative to the phenotype of that unit, but such direct observations have not been reported. There have been some reports of the activity patterns of single motor units over prolonged periods from muscles of a predominant phenotype (Fishbach and Robbins, 1969; Hennig and Lomo, 1987). In these cases, however, there was a high probability that the recordings were from the most excitable (smaller) motor units.

Our data suggest a wide range of durations of activity even within an individual fiber phenotype. Fig. 6 illustrates this notion using a hypothetical model of the activation of a muscle by relating the recruitment of motor units and muscle fibers to EMG amplitude. The broken red line in Fig. 6 is a plot of the data presented in Fig. 4B and shows the duration of soleus

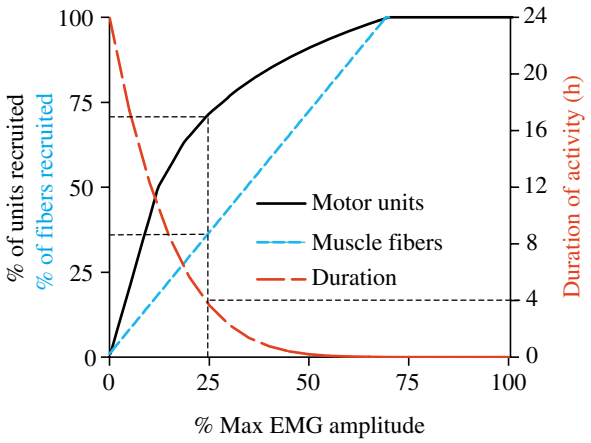

Fig. 6. A diagram illustrating the hypothetical relationship between EMG amplitude, muscle fiber (black) and motor unit (blue) recruitment, and the duration of EMG activity (red) observed in the present study. See text for details.

EMG activity at the EMG amplitude indicated on the abscissa. The black line illustrates the hypothetical recruitment of motor units, measured as a percentage of the total pool for the EMG amplitudes displayed on the abscissa. This example shows a motor pool that is 100% recruited at ~70% of the maximum EMG amplitude of the muscle. The remaining muscle output would be achieved by increasing the discharge frequency of the already recruited motoneurons. The blue line represents the number of muscle fibers recruited at the EMG amplitudes displayed on the abscissa. A linear relationship between the number of muscle fibers recruited and the EMG amplitude is assumed until all muscle fibers are recruited. The vertical dotted line at 25% of the maximum EMG amplitude illustrates our finding that the slow soleus muscle is active at ~25% of its maximum for ~4 h day^{-1} (Fig. 4). Thus, all of the muscle fibers recruited above this EMG amplitude (threshold) would be active for less than 4 h day^{-1}. When the EMG is at 25% maximum, ~35% of the muscle fibers (dotted blue line) and ~70% of the motor units (black line) would be recruited. Although the precise relationships among EMG amplitude, number of motor units and the number of muscle fibers cannot be determined directly, the model serves to illustrate the high probability that some slow muscle fibers in the rat soleus muscle may be recruited for relatively short periods of time each day, suggesting that prolonged periods of activation may not be a requisite for the maintenance of fiber mass or phenotype. The model may be further explored using the down-loadable excel file supplied as supplementary material.

Several assumptions were necessary to construct the model shown in Fig. 6:

(1) The maximum EMG amplitude recorded within the 24 h periods was the maximum EMG level that the muscle was capable of attaining. Underestimating the maximum EMG amplitude would shift the red curve to the left, and thus decrease the duration of activity estimated for any given EMG amplitude.

(2) The muscle is maximally recruited at 70% of maximum EMG amplitude. This value is probably quite low. The effect of higher EMG amplitudes for maximum recruitment would stretch the motor unit and muscle fiber curves to the right, reducing the length of the plateau region. This would result in lower numbers of muscle fibers and motor units being recruited for any given EMG amplitude and thus would further reduce the estimates of active motor unit and muscle fiber numbers for any given EMG amplitude. Lowering the EMG amplitude would have the opposite effect, but would still result in substantial numbers of muscle fibers with relatively short activity durations. For example, a reduction to 50% maximum EMG amplitude for full recruitment would recruit approximately 80% of the motor units and 50% of the muscle fibers at the 25% EMG amplitude level.

(3) The motor units within a muscle are organized such that units recruited early are smaller than those recruited late, and that the units are recruited in a consistent order regardless of the nature of the movement. Although there are a number of examples demonstrating that there are some exceptions to this general principle (Enoka and Fuglevand, 2001), the impact of these exceptions to the size principle (Hennemann, 1965) on the distribution of activity within motor units of a muscle are likely to be small.

This simple model illustrates a key finding of this study: muscle fiber phenotype is maintained even in those fibers that are inactive for substantial periods of time. Assumptions 1 and 2 are conservative assumptions regarding the relationship between EMG and the number of active motor units. Because the estimates chosen for maximum EMG are likely to be lower than actually occurs, our predictions of the duration of activation during normal activity are most likely high.

These observations are inconsistent with the hypothesis that prolonged daily activity is the major determinant of the contractile and biochemical properties of skeletal muscles. In the TA, for example, the low-threshold, presumably slow, muscle fiber population must be maintained by activity that lasts for only 2–3 h day^{-1} whereas some high-threshold, presumably fast, muscle fibers may be maintained by as little as 1–2 min of activity per day. If activity or activity patterns were the sole determinant of muscle properties, these limitations might be expected to apply to all muscle fibers of the same phenotype, regardless of the muscle in which they are found. Based on this logic, conversion to a slow fiber phenotype should be accomplished by less than 3 h of activation per day. Typically, the patterns of daily stimulation used to demonstrate activity-related changes in muscle properties are applied for much longer periods of time, as noted above, and the phenotype conversion is consistently incomplete (Edgerton et al., 1996).

Furthermore, chronic electrical stimulation paradigms often result in fiber atrophy in muscles of control rats (Eisenberg et al., 1984) and are ineffective in maintaining the size of inactivated or unloaded muscles (Al Amood et al., 1991; Canon et al., 1998; Hennig and Lomo, 1987; Leterme and Falempin, 1994). In contrast, short bouts of load-bearing

activity attenuate the loss of muscle mass associated with chronic periods of decreased neuromuscular activity (Roy et al., 1991c; Edgerton and Roy, 1996). For example, extensive attempts to reverse muscle atrophy as a result of spaceflight or hindlimb unloading suggest that skeletal muscles may require specific and finely tuned patterns of activity that integrate several consequences of neural activation, particularly the development of force, to maintain mass and phenotypic properties that would be considered 'normal' (Alkner and Tesch, 2004; Dudley et al., 1999; Edgerton and Roy, 1996; Roy et al., 2002). Therefore, some factors such as muscle loading may be a consequence of activation under specific conditions, but it seems clear that electrical activation alone does not adequately define the conditions that lead to the expression of normal muscle properties.

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