Circadian Force and EMG Activity in Hindlimb Muscles of Rhesus Monkeys

J. A. HODGSON,1,2 S. WICHAYANUPARP,1 M. R. RECKTENWALD,1,2 R. R. ROY,2 G. MCCALL,1 M. K. DAY,1,2 D. WASHBURN,4 J. W. FANTON,5 I. KOZLOVSKYAYA,6 AND V. R. EDGERTON1,3

1Department of Physiological Science, 2Brain Research Institute, and 3Department of Neurobiology, University of California, Los Angeles, California 90095; 4Department of Psychology, Georgia State University, Atlanta, Georgia 30303; 5Oregon Regional Primate Research Center, Beaverton, Oregon 97006; and 6Institute of Biomedical Problems, Moscow 123007, Russia

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Hodgson, J. A., S. Wichayanuparp, M. R. Recktenwald, R. R. Roy, G. McCall, M. K. Day, D. Washburn, J. W. Fanton, I. Kozlovskaia, and V. R. Edgerton. Circadian force and EMG activity in hindlimb muscles of Rhesus monkeys. J Neurophysiol 86: 1430–1444, 2001. Continuous intramuscular electromyograms (EMGs) were recorded from the soleus (Sol), medial gastrocnemius (MG), tibialis anterior (TA), and vastus lateralis (VL) muscles of Rhesus during normal cage activity throughout 24-h periods and also during treadmill locomotion. Daily levels of MG tendon force and EMG activity were obtained from five monkeys with partial datasets from three other animals. Activity levels correlated with the light-dark cycle with peak activities in most muscles occurring between 08:00 and 10:00. The lowest levels of activity generally occurred between 22:00 and 02:00. Daily EMG integrals ranged from 19 mV/s in one TA muscle to 3339 mV/s in one Sol muscle: average values were 1245 (Sol), 90 (MG), 65 (TA), and 209 (VL) mV/s. The average Sol EMG amplitude per 24-h period was 14 μV, compared with 246 μV for a short burst of locomotion. Mean EMG amplitudes for the Sol, MG, TA, and VL during active periods were 102, 18, 20, and 33 μV, respectively. EMG amplitudes that approximated recruitment of all fibers within a muscle occurred for 5–40 s/day in all muscles. The duration of daily activation was greatest in the Sol [151 ± 45 (SE) min] and shortest in the TA (61 ± 19 min). The results show that even a “postural” muscle such as the Sol was active for only ~9% of the day, whereas less active muscles were active for ~4% of the day. MG tendon forces were generally very low, consistent with the MG EMG data but occasionally reached levels close to estimates of the maximum force generating potential of the muscle. The Sol and TA activities were mutually exclusive, except at very low levels, suggesting very little coactivation of these antagonistic muscles. In contrast, the MG activity usually accompanied Sol activity suggesting that the MG was rarely used in the absence of Sol activation. The results clearly demonstrate a wide range of activation levels among muscles of the same animal as well as among different animals during normal cage activity.

INTRODUCTION

A variety of factors influence the regulation of the morphological and biochemical properties of a skeletal muscle. Among these are some combination of the level and frequency of muscle recruitment and/or loading and the duration of the activation periods. The amounts and relative importance of these variables in maintaining muscle properties remain the subject of continued speculation due to the paucity of quantitative information on the patterns of muscle electromyographic (EMG) activity and forces during a normal daily routine. The purpose of this paper is to document daily patterns of muscle activity in normal healthy animals to better define the conditions under which muscles retain their normal properties.

Efforts have been made to estimate long-term EMG activity in some muscles of rats (Alford et al. 1987; Fournier et al. 1983; Hennig and Lümo 1985), cats (Alaïmo et al. 1984; Hensbergen and Kernell 1997, 1998; Pierotti et al. 1991), and humans (Monster et al. 1978). Muscle activity recorded from rats follow circadian cycles that correlate well with their nocturnal behavior (Blewett and Elder 1993), i.e., rats are most active in the dark and sleep during daylight (Block and Zucker 1976; Moore and Bickler 1976; Mouret and Bobillier 1971). EMG activities in both the rat soleus (Sol) and plantaris (Plt) during the dark cycle were double the activities observed during the light cycle (Blewett and Elder 1993). On average, muscles in cats were active three times longer during the daytime than they were at night (Hensbergen and Kernell 1998). However, circadian cycles in felines appear to be weak and easily entrained to the surrounding activity cycles, particularly in domestic cats (Kavanau 1970, 1971; Lancel et al. 1991), suggesting that dark-light cycles may have less influence on felines than other environmental factors such as food availability. Nevertheless, these data suggest that all muscles are subjected to significant differences in their activity levels over prolonged periods of time during any 24-h period.

The Sol muscle is an ankle extensor muscle containing a high proportion of slow-twitch muscle fibers and is one of the most active muscles in all species studied. Daily EMG integrals for the rat Sol were 3 to 10 times higher than for the medial gastrocnemius (MG), a predominantly fast ankle extensor muscle and 20 times higher than the tibialis anterior (TA), a predominantly fast ankle flexor muscle (Alford et al. 1987; Fournier et al. 1983). A “turns” analysis of EMG data indicates that the rat Sol muscle was almost twice as active as the rat Plt muscle during normal cage activity.
muscles without long periods of continuous activation. The Sol species other than the cat that exhibit the ability to sustain slow muscle fibers, suggesting that only minimal activity is needed to maintain the normal morphological and physiological properties of fibers, despite this, there is little evidence of accompanying atrophic infrequently even in the predominantly slow Sol muscle. De- spite this, there is little evidence of accompanying atrophic infrequently even in the predominantly slow Sol muscle. The widely accepted model of motor unit and muscle behavior, such as locomotion. Such data would be extremely helpful in estimating the range of activation patterns that may be recruited during routine cage activity.

Our data suggest that a majority of the activation of the muscles occurs during postural activities and that recruitment of all motor units in a muscle, including the predominantly slow Sol muscle, probably occurs quite infrequently. These observations add to a growing body of evidence indicating that muscle fibers require only brief periods of activity each day to sustain their normal morphological, biochemical, and physiological properties. Further, the range in amount and frequency of activity must vary widely from muscle fiber to muscle fiber, both within and among muscles and among individual animals.

**METHODS**

The present studies were undertaken to obtain a better understanding of the activation patterns in several leg muscles of caged Rhesus monkeys. We were particularly interested in identifying species other than the cat that exhibit the ability to sustain slow muscles without long periods of continuous activation. The Sol muscle of Rhesus monkeys is composed of between 50 and 90% slow, type 1 fibers, whereas ~25% of the MG fibers are type 1 (Bodine-Fowler et al. 1992; Cordonnier et al. 1995; Fitts et al. 1998; Roy et al. 1991). Recent publications report similar fiber type compositions from muscle biopsies of the Rhesus used in this study (Fitts et al. 2000; Roy et al. 1999). Furthermore, quantification of the EMG amplitudes observed during any 24-h period relative to that recorded during treadmill locomotion (Recktenwald et al. 1999) and other high level activation of muscles provided a means for estimating the proportion of the motor unit population that may be recruited during routine cage activity.

Twelve juvenile male Rhesus monkeys (Macaca mulatta) were implanted with intramuscular EMG electrodes in the MG, TA, and VL muscles of the left leg. Additionally, a tendon force transducer (TFT) was placed on the MG tendon. The monkeys were housed individually in standard 4 × 3 × 4 ft (d × w × h) stainless steel cages at the Institute of Biomedical Problems, Moscow (IMBP). The monkeys typically would have to take three steps to move from the back to the front of the cage. Rows of cages were arranged along opposite walls of the colony room. Access to adjacent cages was prevented by a stainless steel sheet mounted to one side of each cage. Windows in the colony rooms provided natural lighting. Sunrise in Moscow on September 1 was ~05:30 and sunset was ~19:30. On January 1, sunrise and sunset were ~08:30 and 17:00. Telemetered recordings of EMG and tendon force during normal daily cage activity were begun by 10.22.023.5 on October 26, 2016 http://jn.physiology.org/ Downloaded from

**CIRCADIAN ACTIVITY IN RHESUS HINDLIMB MUSCLES**

Twelve juvenile male Rhesus monkeys (Macaca mulatta) were implanted with intramuscular EMG electrodes in the MG, TA, and VL muscles of the left leg. Additionally, a tendon force transducer (TFT) was placed on the MG tendon. The monkeys were housed individually in standard 4 × 3 × 4 ft (d × w × h) stainless steel cages at the Institute of Biomedical Problems, Moscow (IMBP). The monkeys typically would have to take three steps to move from the back to the front of the cage. Rows of cages were arranged along opposite walls of the colony room. Access to adjacent cages was prevented by a stainless steel sheet mounted to one side of each cage. Windows in the colony rooms provided natural lighting. Sunrise in Moscow on September 1 was ~05:30 and sunset was ~19:30. On January 1, sunrise and sunset were ~08:30 and 17:00. Telemetered recordings of EMG and tendon force during normal daily cage activity were begun by 10.22.023.5 on October 26, 2016 http://jn.physiology.org/ Downloaded from

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EMG and TFT implantation procedures

EMG electrode and TFT assemblies were purchased from a commercial source (Konigsberg Instruments, Pasadena, CA). The EMG implants consisted of nine Teflon-coated multistrand stainless steel wires (AS 632; Cooner Wire, Chatsworth, CA). The external portion of the implant terminated in a multi-pin connector. Wires exiting the connector were protected by a 20-cm length of 1.5-mm diameter silicon rubber-filled silicon rubber tube. This arrangement protected the external portion of the implant. The TFT was a titanium oval with a removable crosspiece to facilitate tendon insertion. Strain sensors were located in a hermetically sealed cavity within the transducer structure. Wires exiting the buckle were coiled in a 1.5-mm diameter silicon rubber-filled silicon rubber tube. The wires terminated in a small multi-pin connector.

All surgical procedures were performed at the IMBP and were supervised by U.S.-qualified veterinarians. The animals were sedated with a mixture of ketamine HCl (10 mg/kg im) and atropine sulfate (0.04 mg/kg iv). An orotracheal tube was inserted and anesthesia was maintained with isoflurane gas (1.25%) in 100% oxygen. Heart rate, core body temperature, respiratory rate, respiratory pressure, and tidal volume were monitored continuously throughout the surgery. Lactated Ringer solution (10 mL/kg/h) was infused at a continuous rate for the duration of anesthesia.

A 4-cm incision was made at the midline of the back with the upper limit of the incision at the level of the caudal border of the scapula. Skin incisions (2–4 cm) were made in the left leg over the bellies of the VL, the triceps surae, and the TA. The muscles were exposed as clearly as possible, and the distal tendon of the MG was dissected from the Achilles tendon. The sensor cables were passed through small punctures in the skin close to the back incision. This procedure ensured a tight seal between the skin and the implants. The TFT and the EMG wires then were routed subcutaneously from the back to the appropriate locations in the hindlimb using a Teflon rod. Pairs of EMG electrodes were inserted into the midbelly of the Sol, the medial, deep (close to the bone) region of the MG, the midbelly of the TA, and the lateral, deep region of the VL. A loop of the MG tendon was pulled through the buckle and a crosspiece was inserted through the loop in the tendon. Tension in the tendon held the crosspiece in place. Each wire was coiled near the implant site to provide stress relief. A second stress relief loop was arranged in the back region, and the wires were anchored with sutures to the deep fascia. All incision areas were irrigated liberally with 0.1% povidone iodine (Betadine) in saline and closed in layers.

Incision sites were cleansed with the irrigating solution and then a canvas jacket was fitted on the monkey to prevent access to the external cables. Butorphanol HCl (0.5–1.0 mg/kg im) was administered as an analgesic and supplemented as needed. Cephapirin sodium (10 mg/kg im) was administered preoperatively and continued for 3 days after surgery. The monkeys were monitored continuously until they regained their equilibrium and were able to sit upright. Thereafter the animals were placed on prednisone (10 mg/kg) im. Enrofloxacin (5 mg/kg im) was administered if the exit sites became infected.

Data collection

EMG and TFT implants were connected to a radiotelemeter (Konigsberg Instruments, Pasadena, CA; EMG band-pass; 0–750 Hz, force band-pass; 0–20 Hz), which was placed in a pocket on the back of the jacket. The telemeter weighed ~200 g and did not appear to interfere with the behavior of the monkeys or their performance on a treadmill. For the recordings, the Rhesus were placed in cages in the normal colony room, which differed from their normal cages only by the location in the room and by the antennae placed in close proximity to the cage. The antennae were connected to a receiver in an adjacent room where the signals were demodulated, digitized at 2,000 samples/s using commercial software (WINDAQ/200, Datag Instruments, Akron, OH) and stored on high-capacity optical media (Sierra, Pinnacle Micro, Irvine, CA). All activities related to data collection were conducted outside of the normal colony room to avoid any disruption to normal daily activity patterns. The Rhesus experienced the normal daily routine of husbandry and their usual interactions with other members of the colony throughout the recording period. Treadmill data were recorded on separate days. Details of the methodology and results for the treadmill locomotion portions of the study are reported in Recktenwald et al. (1999). Briefly, output from the telemetry receiver was recorded on FM tape (TEAC Model XR-510, TEAC, Montebello, CA) as the Rhesus performed a sequence of ~10 quadrapedal steps at 1.33 m/s. Five steps from this sequence were analyzed for comparison with other data. The criteria for selection were that the Rhesus maintained a constant fore-aft position on the treadmill, did not make contact with the treadmill enclosure, and were not distracted by other events in the recording environment. Steps under these conditions exhibited the least variability in EMG burst duration, cycle period, and mean amplitude.

TFT calibration procedures

A static calibration for each TFT was provided by the manufacturer and was used as a baseline value for force estimation. Since the calibration is subject to individual animal anatomy, a second calibration method (in vivo) adapted from Komri et al. (1987) was used. This method was originally used to calibrate force transducers implanted on the human Achilles tendon. Each Rhesus was anesthetized deeply with a short-acting anesthetic (ketamine HCl, 10 mg/kg im) and placed in a restraint chair (model R001, Primate Products, Redwood City, CA). The implanted leg was secured with the foot held against a hinged plate and positioned so that the pivot point of the plate coincided with the center of rotation of the ankle joint. A calibrated force transducer attached to the plate recorded the external force generated by passive and active torques around the ankle. Anesthesia was maintained at a level that ensured the legs were flaccid during the calibration procedure. Stimulation via the MG EMG electrodes at several intensities elicited contractions that registered at both the TFT and the foot plate force transducer. The foot plate data provided a measure of torque around the ankle. The force developed by the MG muscle was calculated from the ankle torque and the Achilles tendon moment arm, measured from the center of the lateral malleolus to the Achilles tendon. The force then was correlated with the output of the TFT to provide a calibration specific to the individual animal. This in vivo calibration was determined near the times of the recordings, ~4 mo before launch and 1 mo after recovery of the capsule. Figure 1 shows a representative in vivo calibration curve for the TFT. Typically, at lower stimulus intensities, the relationship between the output of the TFT and ankle torque was linear. As stimulus intensity increased the TFT output attenuated relative to ankle torque, even though the static calibrations performed by the manufacturer indicated that the transducer respond lineadly to loads exceeding 10 kg. This response was observed with both twitch and tetanic stimulation and is attributed to stimulus spread to adjacent plantarflexor muscles that would increase ankle torque without affecting the load detected by the TFT on the MG tendon. Calibration constants were calculated from a linear regression of only those data points on the linear portion of the calibration curve.
Other instances of noise, probably generated by the use of high-power electrical equipment, were more difficult to reject automatically. In these cases, the start and end times of the sequences of noisy data were noted and the data excluding the noisy sequences reanalyzed. Figure 2 summarizes the yield of data per 2-h period in the Rhesus where samples were obtained throughout the entire 24-h period. The 96 recording periods, representing 192 h of recorded data, yielded 152 h of useable data (average of 95 min per 120-min recording period). The three shortest periods (7.6, 22.5, and 33.2 min) were from the preflight session of Rhesus 484 between 20:00 and 04:00. All measures of activity that included a time component were corrected to represent a full 2-h period (see Fig. 7A).

EMG and TFT data were available from 8 of the 12 implanted Rhesus. Data from five animals allowed analysis throughout complete 24-h periods (484, 447, 448, 501, and 513). Telemetry transmission failures, possibly due to the animals resting with the back-mounted telemeters against metal cages, accounted for several incomplete datasets. Computer failures and power outages interrupted data acquisition in other instances. Individual monkeys have been identified to facilitate comparisons between the present results and related data published elsewhere. A majority of the data illustrated are from Rhesus 484, one of the two animals selected for the spaceflight. The preflight results from this Rhesus were typical of those observed for all of the monkeys.

Attempts were made to record more than one 24-h session from each Rhesus, but complete datasets from a second recording were obtained for only three animals. A second recording on Rhesus 447 was obtained during normal cage housing, thus duplicating the first recording. A second recording from Rhesus 484 was obtained 14 days after the end of the 14-day spaceflight, and a second recording from Rhesus 501 was obtained 14 days after the ground-based simulation of the spaceflight (see Recktenwald et al. 1999 for further details of the flight and simulation procedures). The long delays between the experimental manipulations in Rhesus 484 and 501 probably allowed substantial recovery of normal activity and, therefore, are of questionable value in interpreting the effects of experimental manipulations on 24-h muscle activity. The results are presented to provide examples of repeatability over multiple days of recording (see Fig. 5). Data from the second recording sessions from 501 and 484 have not been included in any further analyses.

Statistics

Combined data from several Rhesus were presented as means ± SE. Regression lines were calculated for scatterplots of "boxcar" averaged MG EMG versus TFT force (i.e., unsmoothed data were divided into 40-ms segments and averages calculated for each segment, reducing the data to 25 samples/s). The slopes, intercepts, and correlation coefficients were calculated using built-in spreadsheet formulae. Tests

![Figure 1](http://jn.physiology.org/)

**Figure 1.** Representative data from a tendon force transducer (TFT) calibration showing the voltage output of the telemeter force channel plotted against the estimated force on the Achilles tendon based on the force recorded by the external transducer and the lever arms of the foot pedal and calcaneus. The departure from a linear relationship at the higher forces is probably due to stimulus spread to other ankle extensor muscles, thus increasing Achilles tendon force, which would not be detected by the transducer on the medial gastrocnemius tendon. The data points used to calculate the calibration constant for the TFT (0–0.76 kg). The peak force recorded from this Rhesus (501) during a 24-h period was 8.9 kg.

![Figure 2](http://jn.physiology.org/)

**Figure 2.** Histogram showing the total duration of samples of useable electromyographic (EMG) data collected per 2-h period in the 4 Rhesus having complete datasets. Average sample duration was 96 ± 3 min per 2-h period.
for significant differences between linear regressions were not attempted since correlations between successive data points of time series data artificially inflate significance.

**RESULTS**

Figure 3 shows examples of muscle activity recorded from *Rhesus 484* and illustrates the correspondence between the raw EMG signal (A) and the rectified, averaged data (B) from which integrals were calculated. These data represent periods of relatively high activity in the cage and during treadmill locomotion. Averaged MG EMGs are plotted against MG tendon force in Fig. 3C. Regression lines are shown to aid in the comparison between the different activities and demonstrate the considerable variability in the relationship between EMG and force. EMG amplitude distributions for 2-h intervals throughout the day consistently showed maximum counts at

![Image](image-url)
the baseline level, indicating that the muscles were at rest for a majority of the time, even during the most active periods of the day (Fig. 4).

Circadian patterns of activity

A circadian pattern was evident from the 24-h recordings taken from each of the Rhesus: muscle activity in a majority of cases, and particularly in the more active muscles, was highest during the day and lowest during the night (Figs. 5 and 6). The integrated EMG activity for each of the four muscles studied during 2-h periods throughout a day is shown in Fig. 5. The similarities in the results for the two recording sessions for each animal, particularly for the more active Sol and VL muscles, show considerable consistency in the pattern and level of activation over 24 h. Minor fluctuations in the activation of the minimally active TA and MG muscles could account for the instances where the circadian pattern is not clear. Diurnal cycles were evident for each muscle when the data from the eight subjects were combined (Fig. 6). None of the data used in Fig. 6 were associated with any experimental perturbations (i.e., spaceflight or simulation). Although the

![Diagrams showing circadian activity patterns](https://www.jn.org/article-figures/1435f4.png)
amount of integrated EMG activity differed among animals and muscles, most of the animals exhibited the greatest muscle activity between 08:00 and 10:00. The amount of activity generally decreased as the day progressed. The minimum amount of activity was most commonly recorded between 22:00 and 02:00. The diurnal patterns were also evident in the durations of activity throughout the day (Fig. 7).

**General levels of muscle activity over 24-h periods**

The total daily EMG activity was calculated by summing the integrals for each 2-h period in the six complete datasets obtained from five Rhesus (Fig. 8A). The Sol exhibited the greatest amount of total activity during each 24-h recording period with a mean level of 1,245 mV/s (range of 448 to 3339 for 484). The lowest amounts of total EMG activity were recorded from the MG and TA muscles with mean daily activities of 90 (range of 35 for 448 to 171 for 447) and 65 (range of 19 for 448 to 141 for 484) mV/s/day, respectively. The mean VL activity was 209 mV/s/day (range from 24 for 448 to 366 for 484). On average, the Sol was 14, 20, and 6 times more active than the MG, TA, and VL, respectively. These relative activities were consistent across all animals. The greatest amount of Sol activity was recorded from *Rhesus 484* from 08:00–10:00 (Figs. 4 and 5). During this time interval, Sol activity was 803 mV/s, whereas MG, TA, and VL activities were 41, 32, and 81 mV/s, respectively. All recordings showed the TA to be the least active muscle. The greatest amount of TA activity was 85 mV/s and occurred from 08:00 to 10:00 in *Rhesus 357*. However, this value was only 11% of the highest Sol activity recorded during any 2-h interval in the same Rhesus.

EMG activation of the four muscles studied in the five Rhesus with complete datasets also was expressed as durations of activation (Fig. 8B), as mean EMG amplitudes (Fig. 8C) and relative to activation levels recorded during treadmill locomotion (Fig. 8E). A majority of the data counts (84–97%) in the EMG amplitude histograms resided in the amplitude bins representing inactivity (Figs. 4 and 9). The duration of daily activity for individual muscles was between 42 and 248 min for the Sol, 48 and 100 min for the MG, 18 and 87 min for the TA, and 23 and 144 min for the VL (Fig. 8B). The EMG amplitudes averaged over the entire day were typically in the low microvolt range, reflecting the long periods of electrical silence (Fig. 8C). The Sol 24-h average EMG amplitude was 14.4 μV and ranged between 0.7 and 17% of the corresponding EMG amplitude recorded during treadmill locomotion (Fig. 8E). The MG, TA, and VL 24-h mean EMG amplitudes were 1.0, 0.7, and 2.4 μV, respectively, ranging between 0.7 and 2, 0.8 and 5, and 0.6 and 7% of their respective mean EMG amplitudes during treadmill locomotion (Fig. 8E). Mean EMG amplitudes per step were calculated over a sequence of five steps when the animals were walking quadrupedally and consistently on a treadmill at 1.33 m/s. The average EMG during locomotion includes the data during both stance and swing. The duration of locomotor activity that would be required to generate the same amount of activity as the 24-h integrals was calculated (Fig. 8E). On average, an animal would need to walk at a rate of 1.33 m/s for 174 min to generate the same amount of activity during the 24-h period.
m/s for 93 min (range from 10 to 238 min) for the Sol muscle
to generate an integrated EMG level equal to 24 h of normal
daily activity. The corresponding estimates for the MG, TA
and VL muscles were 15 (range from 5 to 27), 32 (range from
10 to 71), and 60 (range from 9 to 105) min, respectively. The
mean EMG levels during periods of activity (Fig. 8
F) were calculated by dividing the daily integral (Fig. 8A)
by the duration of activity (Fig. 8B). The Sol muscle was activated at
the highest levels with a mean of 103 mV (range from 32 to
224). Mean amplitudes for the MG, TA and VL were 18 (range
from 7 to 30), 20 (range from 5 to 41), and 33 (range from 17
to 50) mV, respectively.

**Locomotor activity**

Comparisons of the distributions of EMG activity over 24 h
with the distributions from a short sequence of five steps during
treadmill locomotion suggest that only a small fraction of the
daily activity was comparable to that obtained during treadmill
locomotion. Figure 9 shows the distribution of EMG amplitudes for a 24-h session and for a session of treadmill locomotion
recorded on a different day for Rhesus 484. The distributions derived from the locomotion data have been scaled in
time to represent locomotion lasting for 15, 30, and 45 min.
The distribution of EMG activity during locomotion must be completely enclosed by the 24-h distribution (bold lines in Fig.
9) from the same muscle for that activity to have occurred
within the 24-h period. Even the distributions representing 30
min of locomotion fail to meet this requirement in three of the
four muscles (MG, Sol, and TA) and in the MG, a small
portion of the 15-min distribution falls outside the 24-h distri-
bution. These data therefore suggest that, based on amplitude
distribution, locomotor-like activity accounted for <30 min of
the 210 min of activity recorded over the course of a day in
Rhesus 484. Rhesus 484 was the most active animal and similar
estimates from the other animals suggest that some Rhesus
engaged in locomotor-like activity for as little as 10 s of the
day.

**High-intensity activity**

All of the distributions of 24-h activity showed the presence
of high-amplitude EMG activity, although the incidence was
very low (e.g., see Fig. 9). There were brief periods in all
muscles when the EMG amplitudes exceeded the amplitudes
observed in the Sol muscle during locomotion. This suggests
that all of the muscles studied in all subjects may have been
briefly activated at levels sufficient to recruit all of the motor
units of the muscle during the 24-h recording period. Rhesus
484 showed the highest incidence of signals >1 mV. This

![FIG. 6. Circadian pattern of integrated mean EMG activity for four hind-
limb muscles of the Rhesus. Each point represents the mean 2-h EMG integral
of 5–8 animals prior to any experimental manipulation. The time of recording
is shown on the x axis, beginning at 06:00. Note the different scale on the
ordinate for each muscle. Bars, standard errors. Abbreviations are as in Fig. 3.](http://jn.physiology.org/)

![FIG. 7. Histograms showing the distribution of durations of activity recorded throughout a 24-h period. A: data from Rhesus 484 showing the actual
duration of activity observed in each 2-h period (○) and the estimated total
duration of activity after correcting for the loss of data during each recording
period. Note the different scale on the ordinate for each muscle. B: averaged
data from all subjects (n = 8). Bars, standard errors. Abbreviations are as in
Fig. 3.](http://jn.physiology.org/)
activity was close to the highest 40-ms average EMG amplitudes observed during locomotion in Fig. 9 and was probably close to maximum activation. In this animal, the total time per day that the mean EMG exceeded 1 mV was 33, 21, 37, and 20 s in the Sol, MG, TA, and VL, respectively. In other animals, EMG levels >1 mV occurred for as little as 5 s/day in all the muscles. Figure 3 shows an example of high-level activity in *Rhesus* 484. The MG force reached 4 kg, i.e., about four times the forces recorded during treadmill locomotion. In this example, the mean Sol EMG reached 1.5 mV and exceeded 1 mV for a total of 2.7 s during the 4-s period illustrated. Mean MG EMG exceeded 1 mV for 220 ms and VL EMG exceeded 1 mV for 1.9 s.

**Coordination between muscles**

The incidence of co-contractions between pairs of muscles was investigated by plotting JPDs of EMG amplitudes. Figure 10 summarizes the distribution of co-contractions between the Sol and TA and between the Sol and MG over 24-h periods in three animals. The Sol:TA plots indicate a generally antagonistic relationship with one muscle usually inactive when the other is active. Comparisons with locomotion data suggest that the 24-h data had a higher incidence of Sol and TA co-contractions than a majority of the locomotion data (see Figs. 12 and 13 in Recktenwald et al. 1999). Coincident activity in the Sol and TA always occurred at relatively low amplitudes and probably represents a transition as activity in one muscle decayed while activity in the other muscle increased.

The Sol:MG JPDs from 24-h recordings appear quite similar

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**Fig. 8.** A summary of the daily activity for 6 complete datasets from 5 monkeys (447, 448, 484, 501, and 513) housed in colony cages. A: the daily integral is the integral of EMG for the entire day. B: the duration of activity is the total time that EMG activity was detected in the muscle. C: the daily mean EMG is the daily integral divided by 24 h (86,400 s). D: mean EMG per step is the mean EMG amplitude calculated over the entire step. It was calculated from 5 steps of treadmill locomotion at 1.33 m/s. E: daily activity (% locomotion) is the percentage of the day that the Rhesus would have to spend walking at 1.33 m/s to generate the daily EMG integral equivalent for each muscle, assuming that they participated in no other activity. The duration of the locomotion in min is shown above the bar for each muscle. F: the mean EMG of activity is the total daily activity divided by the duration of activity (i.e., A/B). Bars, standard errors. Abbreviations are as in Fig. 3.

**Fig. 9.** The distribution of EMG amplitudes recorded throughout a 24-h period and during stepping at 1.33 m/s on a motorized treadmill for *Rhesus* 484. The amplitudes are averages for every 40-ms epoch of data. The thick lines show the distributions of EMG amplitudes over 24 h expressed as the (log) total duration of activity in each amplitude bin. Locomotor data were derived from a sequence of 5 steps at 1.33 m/s. The shaded areas are the distributions of EMG amplitudes predicted for a 30-min sequence of locomotion at 1.33 m/s. Thin lines above and below the shaded area show the distributions for 45 and 15 min of locomotion, respectively. Note that the log scale on the ordinate distorts the appearance of the threefold change in times for the distributions of locomotor EMG activity. Abbreviations are as in Fig. 3.
to those observed during locomotion (see Figs. 10 and 11 in Recktenwald et al. 1999) with a predominance of probabilities along the Sol axis, indicating that the Sol is generally more active than the MG. There were, however, some instances when the Sol amplitudes were lower than the MG amplitudes, suggesting that Sol and MG may be activated independently under some conditions. Data from *Rhesus 513* suggest a possible alternative explanation. The JPD from this animal has a modest “depression” at a Sol amplitude of ~1.5 mV and an MG amplitude of ~0.4 mV, indicating that the paired amplitudes defined by this region are less common than those observed in nearby regions. Previous studies of Sol:gastrocnemius EMG relationships during locomotion report that the Sol activity slightly leads the gastrocnemius activity to produce a loop-like relationship in the scatterplots (de Guzman et al. 1991; Prilutsky et al. 1994). Fluctuations in the amplitudes of both muscles generally “filled in” the middle of the loops when multiple steps were plotted. JPDs, therefore have not detected a consistent lower probability of data in the center of the distribution. The 24-h data from *Rhesus 513* seem to be an exception, suggesting a remarkable consistency of phasing and coordination between the Sol and MG in this animal. These data strongly suggest that the observations of MG activity in the absence of Sol activity may be explained by a slight phase difference between the activities in the two muscles rather than an independent activation of the MG in the absence of Sol activation. Less consistent relationships between the amplitudes of the MG and Sol would obscure the “hole” in the center of the JPD and result in a more uniform distribution, such as that observed for *Rhesus 484*. Figure 11 shows the sequence of hourly JPDs from *Rhesus 513* illustrating the general predominance of Sol over MG activity and the consistent loop-like structure surrounding an area containing no data, particularly between 07:00 and 15:00.

**Forces recorded over 24 h**

It was not possible to analyze the MG tendon forces in the same way as the EMG data due to considerable baseline fluctuations over the course of the recordings. Passive forces due to changes in limb position, a modest baseline drift, and possible direct effects on the force transducer such as occurred when the Rhesus rested its foot with the Achilles tendon on a bar of the cage all contributed to uncertainty about the precise interpretation of force data recorded in the relatively uncontrolled cage housing conditions where animals were permitted to choose their own activities. However, active forces were estimated by viewing the force and EMG data on a computer screen, identifying baseline conditions of low, steady force in the absence of EMG activity and observing fluctuations above the baseline coincident with MG EMG activity. In general, the patterns of force development were similar to the patterns of EMG activity, i.e., a majority of data indicated only baseline levels of force, although force amplitudes were not accurately predictable from EMG amplitudes. In some instances, high forces were accompanied by relatively modest levels of EMG activity, whereas in other cases, high EMG activities were accompanied by modest force levels. These observations were expected given the spectrum of behavioral opportunities available to the Rhesus, including eccentric and concentric contractions. Figure 3 provides some examples of forces and EMG amplitudes under different recording conditions. Slopes of the regressions through the mean EMG/force data differ by 40%, and there appears to be a shift in the distribution of data points among the slow locomotor-like data points in the cage, the high-intensity cage activity and treadmill locomotion. The example of high-intensity activity also shows a 100% change in the EMG amplitude between the two force peaks of similar amplitude, presumably due to differences in the combination of lengths, forces and velocities of the muscle encountered at different times during the activity.
Figure 12 provides a second example of the variable relationship between EMG and muscle force output. The MG EMG signal was rectified and smoothed with a low-pass filter setting of 10 Hz and superimposed on the force record. The plot of EMG versus tendon force (Fig. 12B) shows considerable scatter in the relationship although there is a clear correspondence between fluctuations in the EMG and slightly delayed fluctuations in the force signal (superimposed thin line in Fig. 12A, bottom plot). This electromechanical delay must account for some of the variability in the EMG/force relationship. The relationship between these two parameters was only slightly altered if the EMG signal was shifted by 50 ms although fluctuations in EMG and force were mostly coincident (superimposed thick line in Fig. 12A, bottom plot). The regression line through the delayed EMG/force data had a similar slope to the regression using the nondelayed EMG, but had a slightly higher correlation coefficient, i.e., 0.90 versus 0.83 (Fig. 12, B and C).

The maximum forces recorded during the 24-h periods ranged from 4.2 kg in 484 (Fig. 4) to 8.9 kg in 501 (Fig. 12) with a mean of 6.0 ± 0.5 kg. The average mass of four MG muscles removed from two age-matched Rhesus (body weights, 4.4 and 4.8 kg) was 10.5 ± 0.4 g, suggesting a maximum isometric tetanic tension of ~6.5 kg, based on estimates published by Roy et al. (1991). Thus Rhesus may be expected to develop muscle forces reaching close to the maximum isometric tetanic tension during their daily activities. However, our observations of only brief periods of MG activity, both in terms of force and EMG, suggest that these force levels occur for only a few seconds of each day.
Interpretation of differences between levels of EMG activity

The EMG data collected during treadmill locomotion provided a level of confidence that differences among individual Rhesus were due to differences in behavior rather than to variability in our recording conditions. Mean EMG amplitudes per step for the Sol during locomotion were quite consistent, lying between 200 and 300 mV for all subjects. The greatest relative variability between individual mean EMG amplitudes per step for locomotion was in the TA and ranged from 33 to 61 mV. Figures 6 and 7 in Recktenwald et al. (1999) further illustrate the similarities in EMG burst amplitudes during treadmill locomotion among five of the Rhesus used in these experiments. The lower mean values in this paper reflect the difference between the mean amplitude of the burst calculated in Recktenwald et al. (1999) and the mean amplitude of the entire step, including swing, calculated in this paper.

A comparison of the results from Rhesus 501 and Rhesus 484 further illustrates the consistency of EMG recordings across subjects. These two Rhesus had the greatest difference in total daily EMG activity levels. The mean Sol EMGs for these two Rhesus during locomotion were 200 and 233 μV, respectively, whereas the daily integral for the Sol muscle was more than 30 times greater for Rhesus 484 than for Rhesus 501. The lower levels of cage activity in Rhesus 501 compared with Rhesus 484 are illustrated in Fig. 5 (○ represent normal cage activity). Sol EMG activity in 501 was detected for a total of 42 min during the 24-h period illustrated in Fig. 5, which is about one-sixth of the duration of the EMG activity detected in 484. The mean EMG amplitude of 501 during the 42 min of activation was 50 μV, about one-fifth of the 224 μV mean EMG amplitude in 484. Thus both the amplitude and duration of the EMG activity contributed to the 30-fold difference in the Sol activation between these two animals. Figures 3 and 12 show that periods of intense muscle activity resulted in similar EMG amplitudes in monkeys 484 and 501, even when comparing different muscles. These data strongly support the validity of comparing the absolute EMG levels among subjects and muscles.

DISCUSSION

The major findings regarding the activity of Rhesus leg muscles are: a circadian cycle of activity with the highest activity in the morning and lowest activity at night; all muscles were inactive for ≈20 h or more per day; the Sol was 14, 20, and 6 times more active than the MG, TA, and VL, respectively; the average levels of integrated EMG activity per day were equivalent to walking for ≈90 min/day, although the distributions of EMG amplitudes suggested that the actual daily duration of locomotor activity ranged between 10 s and 30 min; all muscles exhibited brief periods (5 s to 1 min/day) of intense activity that would be sufficient to recruit all motor units in the muscles; coactivation of the TA and Sol muscles was only observed at low amplitudes, suggesting that strong coactivation of antagonist muscles is uncommon; the MG muscle was usually coactivated with the Sol, suggesting that there are few occasions when the MG (containing a high proportion of fast muscle fibers) is preferentially recruited over the Sol (containing predominantly slow muscle fibers); and the general pattern of MG tendon forces followed the pattern of MG EMG activation, although there was substantial variability in the force/EMG relationship.
Circadian activity patterns

The circadian cycle of activity exhibited by all of the animals tested demonstrates that Rhesus are most active during the daytime, particularly in the morning, and least active at night. This pattern of activity was seen in all of the Rhesus regardless of test conditions, implying that the levels of muscle activation are, in large part, determined by the time of day. The circadian cycle of EMG activity for Rhesus is consistent with daily EMG activity patterns found in cats (Hensbergen and Kernell 1997, 1998) and with behavioral observations of Rhesus (Weed et al. 1997).

Amounts of muscle activity

The short duration of EMG activity per day, the low mean EMG amplitudes, and the small amount of locomotor activity that is equivalent to the daily activity suggest that the muscles of Rhesus are at rest for the majority of the day. Weed et al. (1997) documented daily Rhesus behavior from videotapes and from counts of gross body movements detected by an ultrasonic motion detector. They used two groups of animals from different sources and detected an average of two movements per minute in one group and six movements per minute in the other group. Video records indicated that the animals were inactive for 30–40% of the day and only 8–12% of the activity, i.e., ~35–70 min/day, was within their classification of “gross locomotor” activity. Our estimates of leg muscle activity using intramuscular EMG recordings suggest similar brief durations of activity throughout any 24-h period. The results from cats were similar to monkeys with low durations of activity, although peak activity in cats occurred later in the day (Hensbergen and Kernell 1998). The data from cats were based on relatively short-duration sampling, i.e., ~4 min every half-hour. Total daily “on time” for the Sol in the cat was estimated as ~14% of the day, i.e., 3 h and 20 min. Corresponding times for the cat TA and LG were 43 min (~3% of the day) and 1 h and 48 min (~8% of the day), respectively. In the monkey, the Sol was active for an average of 2 h and 11 min (~9% of the day), and TA and MG were active for 61 min (~4% of the day) and 1 h and 12 min (~5% of the day), respectively. A graph of the distribution of duty, or on time, for each hour in the cat Sol indicates a maximum on time of 30%, i.e., ~20 min (Hensbergen and Kernell 1998), corresponding to the cumulative activity of ~40 min in the peak 2-h period between 08:00 and 10:00 for the Rhesus. Muscle activity in both species declined to almost zero for periods lasting 1–2 h during the night.

These data add to a considerable body of literature showing that the Sol muscle, composed of a high proportion of slow muscle fibers, is more active than other hindlimb muscles which contain higher proportions of fast fibers. It is clear, however, that in the domestic cat and the Rhesus, the Sol is not continuously active. On the contrary, it appears to be inactive for a major portion of the day. Clearly long periods of activation are not necessary to maintain the muscle mass nor the physiological and biochemical properties of cat or Rhesus hindlimb skeletal muscles. It is not clear whether this is true for the rat where much longer periods of activation have been observed (Blewett and Elder 1993; Hennig and Lømo 1985).

Comparison of 24-h and locomotor activity on a treadmill

Relating 24-h EMG data to locomotor activity data from the same animal provides a common baseline for comparison among animals and a basis to interpret the overall levels of activity. It is clear that during the normal course of daily activity, the four muscles studied were inactive for prolonged periods. Even during more active periods, EMG amplitudes were usually lower during cage activity than during treadmill locomotion, although in some instances the two values were similar (Fig. 3). In the cat, the Sol is near maximally activated during locomotion and shows relatively high levels of activation even during posture (Gregor et al. 1988; Walmsley et al. 1978). Thus it seems reasonable that during a 24-h period of normal cage activity, the Sol generally would be recruited at levels closer to those observed during locomotor activity than the other leg muscles studied. During some periods of daily activity, the TA, MG, and VL muscles were recruited at higher levels than during treadmill locomotion (Figs. 3 and 9). A rough estimate of the duration of locomotor-like activities was made by comparing the distribution of amplitudes recorded during locomotion with those obtained over a 24-h period. The data from Rhesus 484 (Fig. 9) suggest that locomotor-like activity occupied <30 min of the total daily activity, i.e., less than one-eighth of the total duration of activity recorded from the Sol of 484 (248 min). This animal was the most active Rhesus of the group, and similar estimates for other Rhesus indicate that the time spent performing locomotor-like activity in a cage probably amounted to much less than 1 h of the day and in two cases (501 and 447) seemed to be negligible. For comparison, the duration of treadmill locomotion at a moderate speed (1.33 m/s) required to produce the same Sol EMG integral as an entire day of cage activity ranged from ~10 min to almost 4 h (average duration of 93 min for 5 Rhesus). The MG showed the least amount of activity throughout the day and would require <30 min (average of 15 min) of locomotion to generate the equivalent of a normal day of activity. In general, during the normal course of daily activity, the four muscles studied were inactive for prolonged periods of time.

Coordination and independent activation of muscles

The patterns of activities of the Sol and TA suggest that these two muscles consistently function as antagonists. There were no circumstances observed when high levels of Sol and TA activity occurred simultaneously. These data suggest that co-contractions are uncommon between these antagonists. In contrast, we observed a very high level of synergy between the synergistic Sol and MG muscle. The significantly higher levels of Sol activity, relative to MG, observed in all subjects over a 24-h period confirm that the Sol is the muscle of choice for most actions requiring calf muscle activity. Furthermore, the data indicate that the MG is probably recruited when additional effort is required above that which can be supplied by the Sol. While there are many reports of alternative recruitment schemes, such as task-related recruitment (Hensbergen and Kernell 1992; Howell et al. 1995) and cycling of motor units, and even muscles, during a single task (Tamaki et al. 1998; Westgaard and deLuca 1999), it appears that these alternative schemes are not commonly utilized in a majority of the routine daily cage activity in the Rhesus.
Despite a general pattern of coordination between the Sol and MG, the present data demonstrate considerable variability in the relationship between Sol and MG activation, suggesting that the two muscles have some degree of independent control. In certain circumstances, it appears that the activation of the two muscles may be linked by a relatively consistent phase delay combined with control inputs which modulate relative activity between the two motoneuron pools. One potential source of modulation may be the different sensory-mechanical environments influencing the Sol and MG outputs. For example, knee flexion reduces the activation of the MG relative to the Sol in voluntary plantarflexor movements (Tamaki et al. 1997).

Distribution of activity across motor units

The present data indicate that minimal neuromuscular activity is sufficient to maintain normal properties in muscles having a mixed population of fiber types (MG, TA, and VL). This also appears to be true for the predominantly slow Sol. Even the most active motor units in the Sol were active for <5 h of the day. Furthermore, the data indicate that all muscles studied were activated submaximally for a majority of their on-time. The highest levels of activation occurred for only a few seconds per day, suggesting that some motor units (muscle fibers) experience only very brief and infrequent periods of activity. Single motor unit activation patterns from the rat Sol and EDL muscles support the notion that some motor units are active for only brief periods of time within a 24-h period (Hennig and Lömo 1985). For example, the total daily duration of discharges of EDL motor units assumed to be fast, fatigable was only between 0.5 and 3 min/day, although Sol motor units were active for 5–8 h/day.

Optimum activities for the maintenance of normal muscle properties

The present results leave little doubt that normal morphological, biochemical, and physiological properties of both slow and fast muscle fibers in Rhesus prevail even when the muscles are inactive for a majority of each day. This is probably particularly true for the muscle fibers comprising the higher threshold motor units and suggests that, with the appropriate choice of exercise, relatively short-duration activities can maintain normal functioning muscles. The predominant use of the muscles tested was at submaximal activation levels, most probably associated with postural activity. Only a small portion of the total daily muscular effort was expended in locomotor-like activity. Housing conditions may have played a significant role in the choice of activities undertaken, but morphological, biochemical, and physiological characterization of the muscles from biopsies of these Rhesus and from Rhesus in previous investigations (Fitts et al. 1998, 2000; Grichko et al. 1999; Roy et al. 1991, 1999) indicate that our recordings were under conditions that maintained normal muscle properties. Locomotion involves cyclical stretching and shortening of muscles with cyclical loading, whereas postural activities may be accomplished with mostly isometric loading of the muscles. Interestingly, isometric contractions have been shown to be more effective than shortening and/or lengthening contractions in reversing the effects of muscle decreased use, both in terms of changes in MHC phenotype (Diffee et al. 1993) and muscle mass (D’Aunno et al. 1992; Edgerton et al. 1996; Zhong et al. 1996–1998).

There is a growing body of experimental evidence to support the view that the morphological, biochemical, and physiological properties of both slow and fast muscle fibers can be maintained by relatively brief periods of muscle loading lasting only a few minutes each day (Booth and Kirby 1992; Caiozzo et al. 1996; Stauber et al. 1994; Wong and Booth 1988). There are several examples where exercising for an hour, or less, per day reduces the atrophy induced by hindlimb unloading (reviewed in Booth and Kirby 1992; Edgerton and Roy 1996; Edgerton et al. 1996). In one report, 60 min of standing per day, divided into four sessions of 15-min duration evenly spaced throughout the day, completely prevented atrophy during 7 days of hindlimb unloading (D’Aunno et al. 1992). However, similar durations of activity using treadmill locomotion, grid climbing, or higher levels of postural loading in a centrifuge (1.2 G) failed to completely prevent muscle atrophy in rats subjected to 1–4 wk of hindlimb unloading (D’Aunno et al. 1992; Graham et al. 1989a,b; Hauschka et al. 1988; Herbert et al. 1988). All of these results suggest that the mechanisms responsible for the maintenance of muscle mass are finely tuned to the specific patterns of loading and/or activation that are encountered during normal behavior. The present data provide the first comprehensive examination of the relative levels of activation of four motor pools and of muscle force during routine, spontaneous motor behaviors. Similar data from a wider range of motor pools of free ranging animals could provide considerably more insight into the neural control strategies that might be operating on a routine basis.

In conclusion, the total daily duration of activation of selected hindlimb muscles in Rhesus is relatively short, generally <4 h for the Sol, between 2 and 4 h for the VL, and <2 h for the MG and TA. The distribution of EMG amplitudes recorded during the active periods suggests that a majority of the motor units in each muscle were recruited much less frequently. This is supported by the low forces recorded from the MG tendon. Very infrequent bouts of high level activity (<1 min/day) were observed. These levels of activity may have been sufficient to recruit all of the motor units within a muscle of the Rhesus. Together, these findings indicate that quite minimal levels and/or durations of activation and/or loading are required to maintain the normal structural, biochemical, and functional properties of skeletal muscles of Rhesus monkeys.

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