Activity of hindlimb muscles
Hensbergen, E.

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Chapter V

Circadian and individual variations in duration of spontaneous activity among ankle muscles of the cat.

E. Hensbergen and D. Kernell
ABSTRACT: This article concerns the spontaneous motor behavior of cat hindlimb muscles and muscle regions using 24-h electromyographic (EMG) recordings. Previously, we found marked differences in average daily "duty time" (i.e., the percentage of total sampling time filled with EMG activity) between different muscles, or muscle portions. We have now analyzed systematic differences in duty time between (i) highly active (midday) and relatively inactive (midnight) periods, and (ii) individual cats. Differences between cats seemed to be associated with differences in motor habits. The midnight reduction in activity was particularly striking for muscles with a high midday activity. Quantitative differences in spontaneous activity (duty time), as compared between active and inactive periods of the day or among individual cats, were associated with marked qualitative alterations in the distribution of activity among the sampled muscles, i.e., these quantitative differences could not be described as a simple up- or downscaling of general motor activity. © 1998 John Wiley & Sons, Inc. Muscle Nerve 21: 345-351, 1998

Key words: cat; muscle; electromyography; daily activity; circadian variation; individual variation

CIRCADIAN AND INDIVIDUAL VARIATIONS IN DURATION OF SPONTANEOUS ACTIVITY AMONG ANKLE MUSCLES OF THE CAT

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In a recent article on the daily durations of electromyographic (EMG) activity in spontaneously moving cats,1 we demonstrated that there are very marked differences between different ankle muscles and muscle regions. Furthermore, some of the evidence suggested that most of these intermuscular differences reflected differences in the postural role of the various muscles. One of the prominent postural tasks of muscles in general (particularly relevant for hindlimb muscles), consists in contributing to behaviors involved in counteracting the force of gravity (e.g., while standing or walking). The use of such behavioral programs is subject to pronounced circadian variations, being much less employed during rest periods (sitting, lying down). For this reason, it was necessary to extend our analysis of the 24-h activity levels of ankle muscles to a study of the associated circadian variation; the intermuscular differences in duration of activity, if mainly dependent on differences in postural role, should be less marked during rest than in more active periods of the day.

The present article also deals with the nature of intersubject variations in activity level among different cats. The same individual, whether cat or man, may show different intensities of motor behavior on different days, and such differences in activity level might be even more striking for comparisons between "lively" and "sluggish" individuals. Such differences in activity level may be considered a purely quantitative phenomenon, scaling the activity up or down to equivalent degrees in different muscles. Alternatively, such differences might mainly be associated with qualitative differences in muscle use, reflecting types of motor behavior requiring different patterns of cocontraction (cf. Ref. 11). In this latter case, mean activity levels of different muscles cannot
easily be “normalized” (scaled) in some simple way for further interindividual comparisons.

**METHODS**

The data to be presented in this article were from the same experiments as those of Hensbergen and Kernell,9 in which methods and procedures are described in detail. Briefly, the measurements were collected from 4 adult female cats (aged, 4–6 years) provided with implanted bipolar electrodes for EMG recording from different regions of various muscles of the hindlimb (data from two left-side and four right-side hindlegs; below, the descriptor “muscle” will generally be used also for muscle regions). The recordings were obtained from tibialis anterior on the anterior (TAa, 2 cats) and posterior (TAp, 4 cats) side, peroneus longus on the anterior (PLa, 3 cats) and posterior (PLp, 2 cats) side, lateral gastrocnemius on the anterior (LGa, 1 cat) and posterior (Lgp, 1 cat) side, the anterior side of soleus (SOL, 4 cats), and the posterior side of extensor digitorum longus (EDL, 4 cats). The PL-electrodes were pairs of fine wires, positioned just under the muscle surface. For other recording sites, pairs of electrodes were provided with an insulating back of silicon rubber and fixed to the muscle fascia (“patch-electrodes”). During 24-h measurement sessions the experimental cats stayed within a recording box of 1 × 3 m (1 m high) and were accompanied by another cat from the same animal housing group. The box was large enough for playing and walking around but not, of course, for more extensive jumping and climbing activities. The recording box had a white translucent top-covering, allowing room light to illuminate the interior (lights out between about 5:30 PM and 7:30 AM). In each 24-h session, EMG recordings from two of the bipolar electrodes were collected by telemetric transmission via small senders carried on the back of the cat. With regard to the electrode combinations used for each recording session, we often paired anterior and posterior sites of the same muscle (LG, PL, TA), or the “slow” soleus was often combined with a “fast” muscle site (EDL, TAp; see Table 1B). Each cat was used for several recording sessions (4–25 times per cat, in total 56 successful sessions; see Fig. 3A).

In order to make the collected amount of data more manageable, the 24-h distribution of spontaneous EMG activity was determined using an intermittent sampling process; 4 min EMG was recorded on tape once every 30 min (i.e., 48 samples per 24 h; see Ref. 9 for methodological considerations). For the off-line analysis, the EMG was first rectified and smoothed to produce an “integrated EMG” (iEMG; smoothing time constant, 20 ms). For the present context, the quantification of the signal was limited to measurements of the total accumulated time containing iEMG activity (total “on-time”) during each 4-min sampling period. A voltage discriminator was used for determining during which moments the iEMG had an amplitude clearly exceeding the (minute) noise of the zero baseline. Typically, this threshold was set low enough to enable the detection of even single-unit repetitive activity. The periods during which the voltage discriminator was “on” were measured with an accuracy of 0.5 ms. In order to assess the reliability and reproducibility of the data analysis, the processing of each tape was executed twice, on different days, so that the threshold of the voltage discriminator also had to be adjusted two times independently. In all accepted cases, these two runs gave very similar results; on average they differed by ≤1.7% from each other with regard to the mean duty time per 24 h. For each sampling period, the “duty time” (%) was the ratio between

<table>
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<th>Muscle pair</th>
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<th>Midday, P</th>
<th>Midnight, P</th>
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<tr>
<td>EDL–SOL</td>
<td>6</td>
<td>&lt;0.05</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>TAa-TAp</td>
<td>6</td>
<td>&gt;0.2</td>
<td>&gt;0.1</td>
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<tr>
<td>TAp-SOL</td>
<td>8</td>
<td>&lt;0.01</td>
<td>&gt;0.7</td>
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<tr>
<td>PLa-PLp</td>
<td>6</td>
<td>&gt;0.7</td>
<td>&lt;0.05</td>
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<tr>
<td>LGp-LGa</td>
<td>6</td>
<td>&gt;0.1</td>
<td>&lt;0.06</td>
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**Table 1. Statistical significance of differences between muscle recording sites in their midday or midnight duration of activity (“duty time”).**
total on-time and total sampling duration. For the analysis of circadian EMG variations, the data from each 24-h measurement session were smoothed by first calculating the mean duty times per consecutive 2-h period (i.e., averages of four consecutive sampling periods). Individual recording sessions started at arbitrary moments of the day between 9:30 AM and 5:30 PM, and the cats were placed in the recording box at least 1 h before the first EMG sample was taken. While EMG activity was clearly associated with the absolute time of the day (Fig. 1), it showed no obvious relationship to the moment at which a particular recording session was actually started. Hence, for the analysis of the circadian variations we felt justified in comparing data from different sessions with the measurements all arranged in the same relation to absolute time of day (i.e., as if all recordings had started at 12 o'clock noon, see Fig. 1).

RESULTS

Circadian Variations in Duty Time. Figure 1 shows the mean circadian variations of activity for different muscle recording sites. These data were pooled from all the cats, and mean duty times per consecutive 24-h period (see Methods) were plotted vs. absolute time of day for five highly active recording sites (SOL, PLA, PLp, LGa, LGp; Fig. 1A,B) and for three less active ones (EDL, TAA, TAP; Fig. 1C). We noted previously\(^9\) that the cats did not at once turn less active as the room lights went out. On average, however, most of the recording sites showed a lower duty time during the dark nightly periods than during daytime with room lights on (Fig. 1; lights on between about 7:30 AM and 5:30 PM). There seemed to be a general lack of activity for several hours around midnight and a distinctly higher activity from, roughly, 6 AM to 6–8 PM (Fig. 1).

The same general type of circadian change in duty time was apparent for most of the muscles. However, as might be expected from the different postural roles of the sampled muscles, the absolute and relative degrees of the change differed markedly among the recording sites. The circadian changes were particularly evident for several of the muscles

![Diagram](image-url)

**FIGURE 1.** (A–C) Circadian distributions of duration of EMG activity (duty times, %) over 24-h recording sessions, as plotted from 12 noon to 10 AM the next day. (A) Soleus (SOL, average of \(n = 29\) recordings), anterior peroneus longus (PLA, \(n = 7\)), posterior peroneus longus (PLp, \(n = 7\)). (B) Soleus (SOL, same data as in A, added for comparison), anterior gastrocnemius lateralis (LGA, \(n = 8\)), posterior gastrocnemius lateralis (LGP, \(n = 7\)). (C) Extensor digitorum longus (EDL, \(n = 12\)), anterior tibialis anterior (TAA, \(n = 12\)), posterior tibialis anterior (TAP, \(n = 16\)). All panels are on the same scale. Suggested presence of circadian variations in EDL and TAA, apparently absent in TAP. Plotted data in A–C show averages from all cats and measurement sessions together. Times on the abscissa give the starting time for each calculated 2-h average. Room lights were on between about 7:30 AM and 5:30 PM.
and recording regions with high values of mean 24-h duty time, and the activity levels of the various individual muscles therefore became much more similar during their low-level periods around midnight than during their high-level periods at daytime. For example, in the pooled data, soleus was the muscle which clearly had the highest average level of 24-h activity. During the night, however, the differences between soleus, peroneus longus, and gastrocnemius largely disappeared (for brief nightly periods PLp even had a numerically larger mean duty time than SOL; Figs. 1A, 2B). Previously we showed that when analyzed over the whole 24-h period, there are very consistent regional differences in duty time within the peroneus longus, lateral gastrocnemius, and tibialis anterior muscles. The data of Figure 1 indicate that these regional activity differences may also show a circadian change (e.g., PLA vs. PLp; LGa vs. LGp; TAA vs. TAP).

For a more quantitative analysis of the levels and distributions of EMG activity during active and inactive diurnal periods, we calculated the means for eight consecutive samples obtained during a highly active period (around noon, midday value, 10 AM to 1:34 PM) and, similarly, for eight samples from a period of relative rest (around midnight, midnight value, 10 PM to 1:34 AM). Mean values for the midday and midnight activity are shown in the bar graphs of Figure 2A and B, in which muscle regions have been ordered according to their average activity during the full 24-h measurement session (24-h activity, see circles connected with interrupted lines). For all muscle recording sites together, the mean on-time durations of midnight activity (Fig. 2B) were about 37% of those for the mean midday activity (Fig. 2A; ratios ranging between 0.15 and 0.71). For most of the individual recording sites (TAA, PLA, LGa, LGp, SOL), the midday activity was in each case significantly higher than the midnight activity (paired t-tests, P < 0.05). However, for three of the recording sites no clear difference of this kind was found (TAp and PLp, P > 0.2; EDL, 0.1 > P > 0.05). When compared per 24-h measurement session, the occurrence of a midnight activity greater than the midday activity was significantly more common for the latter group of recording sites (seen in 10 of 35 cases for TAP, PLp, and EDL) than for the remainder (seen in only 6 of 63 cases for TAA, PLA, LGa, LGp, SOL; chi-square test, P < 0.02).

The average midday and midnight values for duty time were, of course, each based on fewer data points (eight per session) than those for the 24-h activity (48 per session); therefore, the midday and midnight values were inherently more "noisy" than those for the full 24 h. This was probably a major reason why the consistent differences that we noted between the 24-h activity of anterior and posterior muscle portions for peroneus longus, lateral gastrocnemius, and tibialis anterior (see Fig. 4 of Ref. 9) were not as clear for separate midday or midnight values. For the six paired recordings from each one of these three muscles, anterior and posterior recording sites were not significantly different for only midday values; in case of the midnight values, only peroneus longus showed a significant anteroposterior difference (Table 1B).

Because of the differences in circadian activity modulation among the different muscle recording sites, average values for midnight activity were ranked in quite a different manner from those col-

**FIGURE 2.** Mean EMG duty times (%) of the various ankle muscle recording sites for midday and midnight samples. Plotted values are means ± SE for duty times obtained between 10 AM and 1:34 PM (midday, A) and between 10 PM and 1:34 AM (midnight, B). Bars for different muscle recording sites (see Fig. 1 for abbreviations) are arranged according to their ranking order for the 24-h duty times, which are indicated by circles joined with interrupted lines (cf. Fig. 3A of Ref. 9). For statistics, see Table 1.
lected during the day. For the eight muscle recording sites of Figure 2, the midday values were well correlated with those for the full 24 h \((r = 0.964, P < 0.001)\) and, in both cases, there were many significant differences among the various investigated muscle sites \([Table 1; analysis of variance (ANOVA), P < 0.001; cf. Ref. 9]\). On the other hand, the midnight values of Figure 2B showed no significant correlation to either those collected at midday \((P > 0.25)\) or to those for the full 24-h recording session \((P > 0.1)\). During the night, the activity levels of the various muscles were clearly more similar to each other \(\text{cf. Fig. 2A and B}\); the similarity was, however, not complete. Also at night, there were significant differences in activity between different sampling sites \((\text{ANOVA}, P < 0.02)\); pairwise comparisons showed that such differences were mainly a result of the fact that the midnight duty time of PLp was significantly greater than that for several other recording sites \(\text{Table 1; see also Fig. 2B}\).

Previously\(^9\) we showed that individual 4-min samples with long-lasting activity frequently occurred for recording sites which also had a high 24-h duty time \(\text{i.e., as analyzed for the frequency of samples with activity covering 20\% or more of the sampling time, "X20\%".}\) There was, on the other hand, no significant correlation between the 24-h duty time and the frequency of samples totally lacking activity \(\text{"X0\%".}\) In this respect, the midday duty times behaved in the same way as the 24-h values, showing a significant correlation with \(X20\%\) (averages compared for the eight recording sites, \(r = 0.94, P < 0.001\)) but not with \(X0\%\) \((r = -0.23, n = 8, P > 0.5)\). The midnight values for duty time behaved in the opposite manner, showing a significant correlation with \(X0\%\) \((r = -0.76, n = 8, P < 0.05)\) but not with \(X20\%\) \((r = 0.55, n = 8, P > 0.1)\).

**Individual Variations in Duty Time.** The data of Figure 3A show the 24-h mean values of duty time for each one of all the 98 successful measurements of the present study \(\text{separate symbols per cat}\). In Figure 3B, the same data are plotted as averages per cat. The results illustrate two important points: (i) that the ranking of muscles and muscle regions with regard to their mean 24-h duty time was on the whole, similar in different cats; the deviation of ranking order seen in Figure 3B concerns differences within each group of either EDL-TAa or PLa-LGp in only 1 of the 4 cats \(\text{cat M}\); and (ii) that, this being true, at certain recording sites the absolute values of duty time could differ considerably among different cats. The plotted mean data of Figure 3B suggest that individual qualitative variations of muscle use must have been of great importance for differences among the different cats. For cat V the mean 24-h duty times of most recording sites were similar to those of the other animals; however, the soleus of cat V had a mean daily duty time that was almost twice that for other cats, and its range of values did not overlap with those for the other animals. For the other 3 animals, the 24-h duty times for soleus showed a wide overlap with those for peroneus longus and gastrocnemius \(\text{Fig. 3A}\), and the averages were closer together. However, also when excluding data for cat V, the mean 24-h duty time of 10.2 \(\pm 2.1\%\) \((SD, n = 18)\) for SOL was significantly larger than the average of 8.0 \(\pm 2.8\%\) \((n = 23)\) for all the gastrocnemius and peroneus longus measurements combined \(\text{t-test for difference, } P < 0.01\).
Another type of individual variation was evident for comparisons between the activity durations of TAa in cats V and M (significantly lower in the latter animal, t-test, \( P < 0.02 \)). As TAa was studied in only these 2 cats, we do not yet know whether this difference represents an unusually low TAa activity in cat M or an unusually high one in cat V. The seemingly high mean value for PLa of cat M (Fig. 3B) did not differ significantly from the mean value for cat V (\( P > 0.4 \); the low PLa value for cat L came from a single recording session, see Fig. 3A).

The extent to which consistent differences in muscle use occurred between different cats is further illustrated in Figure 4, comparing the circadian variations in mean duty time for muscles of cats V and M, the 2 cats with the largest total number of recording sessions. For only two of the six comparisons (SOL and TAa) was there a fully consistent difference between the cats, throughout the 24-h recording periods. Thus, the total duration of SOL EMG activity of cat V was greater than that of cat M (or cats F or L) not because cat V was somehow an overactive cat, but rather because it apparently had other habits of motor behavior (other preferred patterns of muscle coordination). This conclusion was supported by the observation that cat V often preferred to keep standing during occupations that the other cats usually performed in a sitting or lying position (like eating, grooming, or just looking around). Quiet standing would be associated with, in particular, a maintained activation of soleus.\(^6\) We do not yet know which differences in motor behavior led to the consistent differences in TAa activity between cat V and cat M (Fig. 4B).

**DISCUSSION**

One of the main conclusions of the present analysis is that the differences in total daily activity duration (24-h duty time) that we recently observed between different ankle muscles\(^6\) were mainly a result of their behavior during the more active periods of the day; during resting periods at night, activity was still present at a lower level, but the intermuscular differences became less distinct or disappeared altogether (Figs. 1 and 2). These observations further support the view\(^7\) that the differences in total daily activity duration reflect, to an important extent, differences in the postural role of the various muscles. The results are, of course, not unexpected (see above), because postural hindlimb functions (particularly antigravity functions) would certainly be much less prominent when sitting or lying down during rest periods than when standing and moving around in periods of alert motor behavior. Also in accordance with such expectations, quantitative EMG studies of soleus and plantaris in the rat showed that, in these antigravity muscles, the normally present circadian rhythm of activity disappeared on hindlimb suspension.\(^2\)

While the midday duty time was positively correlated with the extent to which sampling periods were filled with long-lasting, presumably postural, discharges (percentage of samples filled to >20% with activity, X20%), this was not true for midnight activity. Instead, the midnight duty time seemed more related to whether or not movements occurred at all that engaged the target muscle (negative correlation with frequency of sampling periods lacking all activity, X0%).

When considering the absolute values of the present duty times (Figs. 1–4), it should be taken into account that in order to ensure a continuously good reception of the telemetric signals, the recording box had limited dimensions (3 m\(^2\) floor space; see also Ref. 1); it was large enough for moving around and playing but too small for more forceful kinds of jumping, climbing, and running. However, even if
space had been less limited, the short-lasting "phasic" activities of the latter kinds would not be expected to have a large impact on 24-h duty time.

In this first analysis of a large and highly complex material, we have limited ourselves to almost the simplest possible type of quantification; the amount of activity has been measured only with regard to the cumulative "on-times" for whole muscles and muscle regions\(^9\) (Figs. 1–4). This restriction means that we have essentially been comparing muscles with regard to the activity times of their most easily recruited units, i.e. presumably their slow-switch S-units (reviews, Ref. 4, 10). Such measurements are interesting in themselves and essential as a starting point for any further extension of the analysis. For a more complete picture of the total daily amount of muscle activity one would also have had to quantify the intensities of muscle activation over time (for examples of such measurements, see Refs. 1, 2, 5, 13, 14). However, as we wanted to compare different muscles, this involves problems of a considerable complexity (e.g., how to calibrate intensity levels of different muscles such that they may be fairly compared, how to evaluate relative degrees of motoneuron recruitment, etc.).

We felt this has to be further analyzed and measured in future investigations. Pool behavior problems of a similar kind have recently been analyzed in complex theoretical models (e.g., Refs. 6, 7).

From a practical point of view the present results demonstrate that, when investigating relative differences in activity level between different muscles, it does matter during which period of the day or night samples of muscle activity are compared (Fig. 2); muscle use is not simply scaled up or down according to circadian variations in the general level of activity. Furthermore, this absence of a simple "scaling" of behavior and the presence of qualitative behavioral differences is not only true for comparisons between more or less active periods of the same individual (Fig. 2) but also for comparisons among individual cats (Figs. 3 and 4). Our data from 4 cats demonstrate the existence of such qualitative problems but not, of course, how often they would appear in a cat population. Differences in the locomotor and reflex behavior of hindlimb muscles in different cats were also recently studied by Loeb.\(^11\) In his animals (n = 6), some muscles showed a stereotyped locomotor activity but variable cutaneous reflex patterns among individuals (e.g., EDL, TA). Other muscles had a variable locomotor activity but consistent reflexes (e.g., flexor digitorum longus), or variable locomotor as well as reflex activity (e.g., PL).

Although 24-h measurements of mean duty time have been previously published from other laboratories for a few muscles\(^1,3\) and motor units,\(^4\) no previous analysis seems to have been made of the diurnal variations in duty time across muscles or individuals. With regard to the tendency of the present animals to rest relatively more during the night than in daytime (Fig. 1), the results confirm classical observations that domestic cats are not to be regarded as eminently nocturnal creatures.\(^15\) In the study of Bowersox et al.\(^5\) (behavior and electroencephalography), the cats were most active during early morning and early evening hours, whereas sleep patterns were maximally expressed in the afternoon and late evening.

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REFERENCES