Long-term 24-hour rest-activity pattern of sheep in stalls and in the field

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Summary. Motor activity of sheep was continuously recorded for 2–3 weeks with an ambulatory monitoring device. Recordings were obtained from free-ranging animals in the field and from animals maintained under various controlled conditions in stalls. The sheep were diurnal under all conditions. While the daily amount of activity and the frequency of rest episodes showed only small differences between the conditions, the rest-activity pattern showed prominent differences. The pattern differed particularly between the field and the stalls. In the field, activity started to increase one hour after dawn, reaching a first maximum towards noon; a second, higher peak in the evening was followed by a rapid decline after dusk. In the stalls the onset and offset of activity was more abrupt; activity peaks coincided with feeding and human activity; the onset of rest with lights off. Activity was lowest and rest most prominent in those stalls where the animals were most isolated from human influence.

Key words. Sheep; rest-activity rhythm; field; stall.

The effect of the light-dark (LD) cycle on the rest-activity rhythm has been an important area of research on circadian rhythms[1]. It has been shown that behavioral rhythms in sheep can be entrained to an LD-cycle of 12:12 h, although entrainment was only weak[2,3]. Sheep have particularly attracted interest in the investigation of the role of the photoperiod in breeding[4,5]. However, most investigations of sheep have been undertaken in more-or-less controlled, artificial environments with systematically varying LD-cycles[6–10], and studies of the behavior of sheep under natural conditions are scarce. Particularly, continuous long-term observations are lacking. Squires[11] investigated the activity patterns of a small flock of Merino sheep for 20 days. Activity was determined automatically under semi-confined, natural conditions, by charting the movement of animals past two specific sites, one near the water and self-feeder, the other at an intermediate site. The onset of locomotion in these sheep correlated with sunrise, whereas cessation of activity showed no relation to sunset. A bimodal activity pattern corresponding to two grazing periods, one during the early morning and the other in the late afternoon, were found. The interval between mid-morning and mid-afternoon corresponded to the period of minimum activity[11]. In some studies, continuous 24-h EEG recordings were obtained to determine the vigilance states of sheep. Drowsiness prevailed during 20.1% and sleep during 16% of the 24-h period in sheep kept in metabolic cages[12]. Similar data were obtained in sheep recorded for two months in a constant environment in stalls; however, total sleep time depended on their diet[13]. In neither of these studies was the 24-h distribution of sleep and wakefulness determined.

It is an open question to what extent results obtained in the laboratory reflect natural behavior. Owing to the lack of technical equipment, the rest-activity behavior of animals in the laboratory has only rarely been compared with their behavior in the field. For example, marked effects of stall conditions and feeding on sleeping and waking behavior have been reported for cows and horses[14]. The distribution of sleep episodes in free and foraging cattle was biphasic, with maxima at dawn and dusk, subsequent to the two major grazing periods[15]. The same animals, recorded in a stable, exhibited a monophasic, nocturnal sleep pattern. The nocturnal sleep episodes in the cows tended to begin after a period of rumination. When the cows were isolated in a sound-proof chamber with little external influence and given food ad libitum, the sleep episodes were equally distributed throughout the 24-h period[14].

The development of efficient, miniaturized activity recorders[16] provides the opportunity to record the rest-activity pattern of animals in their natural environment over long periods. A small recording device attached to the collar was applied to monitor the motor activity of dogs for several weeks in the laboratory[17]. The method was useful to assess compensatory mechanisms after sleep deprivation[17] and to determine differences in the amount of activity and rest in isolation and in several stall conditions[18]. In both studies the number of rest episodes (defined as episodes with activity counts $\leqq 5$) was the most sensitive variable of the treatments; therefore, this parameter was also determined in the present study.

Our aim was to determine the feasibility of applying the actometer for long-term recordings in the field and to compare the behavior of free-ranging ewes with their behavior in stalls. In addition, the data were compared with those obtained in rams of a different strain, recorded under more controlled conditions in pens.

Methods

Recording conditions. Motor activity was continuously recorded in ewes (Swiss alpine sheep; age: 9–19 months) for 18–19 days in three different conditions, and compared with data recorded for 13 days in rams (Ile de France; age 2 years), kept in a pen. Recordings were obtained with an ambulatory device worn on the collar. The ewes were selected from a large flock and were recorded in the following three conditions: 1) *stall*, (S) in autumn (19 d; n = 9) when they were kept in groups in a pen (5 × 4 m) (LD 11:13, L: 7–18 h, with daylight penetrating through the windows); 2) *stall-field* (SF); this condition followed immediately upon the stall condition; the same individuals (19 d; n = 9) were allowed to spend 7.75–9 h in an adjacent pasture (between 8.30 and 17.30 h; in both conditions food was supplied at 7.30 h, and a second, smaller food supply was given approx. at 18 h); 3) *field* (F) in the summer of the subsequent year (18 d) when the sheep were allowed to roam for several weeks in the mountains (2300 m above sea level; n = 6–10; four of these sheep were the same individuals that were previously recorded; all individuals belonged to the same flock). The rams were recorded in Toulouse in the spring (13 d; n = 12 rams) in two groups of six each maintained in a sound-attenuated *pen* (P) under constant stall conditions (LD 12:12 h, L: 7–19 h; white fluorescent light). Feeding was scheduled at approximately 10.00 h.

The activity monitor. The activity monitor integrated and stored activity values between 0 and 253 for fixed time intervals. The time interval of 3.75 min was used in all studies except in four sheep in condition F, where the recordings were based on 7.5-min episodes. The storage capacity allowed continuous recording for 21 days. After the period in each condition the monitors were removed from the animals and the data were read into a PDP-11/34 computer. Computations were based on mean activity values for two consecutive 3.75-min episodes. In addition, the number of 7.5-min episodes with activity counts < 5 and < 10 (resting episodes) were computed.

To obtain an estimate of the type of behavior that contributed to the actometer data, two sheep were recorded for 24 h with an infrared camera on a time-lapse video recorder while they were wearing the actometer. The tapes were played back and rest-activity behavior was visually scored and compared with the output of the actometers. Large activity counts were caused by gross motor activity, when the sheep engaged in locomotion and feeding activity, and by jaw movements related to ruminating when the animals were recumbent in a sphinx-like position. The lowest activity values occurred when the sheep were in a recumbent position with the head resting on the ground. In this state frequent twitches of the ears and limbs were observed. Activity during these episodes often exceeded zero and usually reached values between 0–10. This behavior rarely occurred for more than a few minutes. Regardless of whether rest episodes were defined by activity counts < 5 or < 10 similar differences between the conditions were obtained. We decided to restrict our analysis to rest episodes defined by activity counts ≦ 5.

Data analysis. The overall duration of the activity and rest periods was determined to compute the activity-rest (i.e. alpha-rho) ratio for each animal in each condition. The mean 24-h activity plot computed over all days of a condition (fig. 1) served to determine visually the beginning and end of the activity period. These were defined separately for each individual as the time of day when activity rose above or fell below the computed nighttime mean activity value, respectively.

The variability of activity onset and the end of activity within a condition was estimated on the basis of the daily numerical outputs and by computation of the variance.

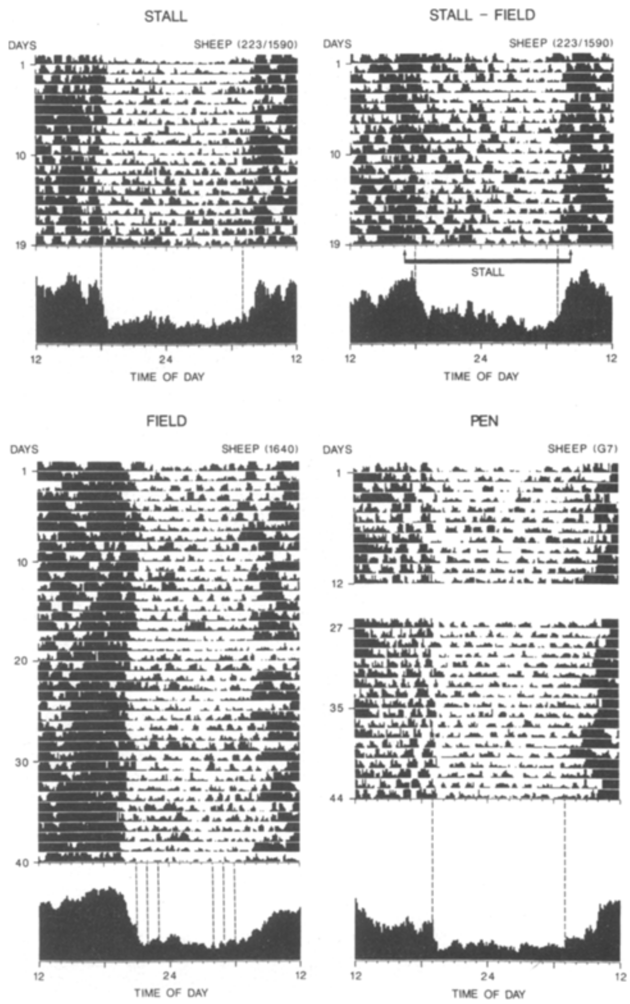

Figure 1. Long-term records of motor activity of three ewes and one ram in the 4 conditions. The plots represent activity records of successive 7.5-min intervals plotted at 11 levels with upper thresholds at 10, 30, 50, 70, 90, 110, 130, 150, 170, 190, and 230 counts. Mean activity (arbitrary units) over all days of each consecutive 7.5-min episode is shown at the bottom of each record. The time of day is indicated at the bottom. The dark period (defined by the artificial light provided in the conditions stall, stall-field and pen) is indicated by the interrupted vertical lines. In the condition 'field' sunset, dusk and beginning of astronomic darkness, and sunrise, dawn and end of 'astronomic darkness' are marked. In the condition 'stall-field', the animals were in the field during the daytime hours. The hours in the stall are delimited by the arrows.

Activity onset was defined as the first consolidated interval of activity lasting at least 30 min and exceeding a threshold of 90 counts per 7.5-min episode. The end of the activity period was defined by the first consolidated rest interval of at least 30 min duration with activity counts below 90 per 7.5-min episode.
Overall effects of the conditions were analyzed by subjecting the activity and rest values separately to an analysis of variance (ANOVA). Whenever significant effects were obtained the Mann-Whitney test or the Rank Sum test was applied to evaluate the differences between the conditions, and a paired t-test to determine differences between the light and the dark period within a condition.

Results

Figure 1 illustrates the rest-activity pattern of individual sheep in the four conditions. The animals were typically diurnal. During the light period large activity bouts were rarely interrupted by rest, whereas during the dark period, rest episodes predominated. The number of rest episodes was in general small (table 1). Figure 2 illustrates the polyphasic rest-activity pattern which was most evident in the dark period. The mean 24-h rest-activity pattern is illustrated in figure 3 for all four conditions. The effect of condition (S, SF, F and P) on the 24-h mean values, and on the values of the light and dark period (table 1) was tested with one-way ANOVA separately for the variables activity and rest (activity: 24-h: $p < 0.004$; dark period: $p < 0.04$; light period: $p < 0.0001$; number of rest episodes: 24-h: $p < 0.0001$; dark period: $p < 0.0001$; light period: $p < 0.0001$). The comparison of the data for the four conditions showed several similar features. Activity was always significantly more prominent in the light period (table 1), whereas rest episodes predominated in the dark period. Usually, activity exhibited a bimodal pattern, with a first activity bout either at the beginning of the light period or towards noon; a second bout occurred in the late afternoon (fig. 3). Bimodality was least evident in the condition P, where the 'morning' peak occurred between 10 and 14 h, and the evening peak was relatively short and small. In the field (SF and F) a drop of activity was sometimes observed between 12 and 15 h.
The amount of rest and activity was affected by the condition in which the animals lived (table 1). The lowest 24-h activity values and the largest number of rest episodes were observed in condition P. In the conditions F and P, daytime activity was significantly lower compared to the other two conditions. The reduced activity was also reflected in the higher frequency of daytime rest episodes. Activity in the dark period was highest in condition SF.
Differences between the conditions were also seen in the distribution of activity within the dark period, when activity values in the first and second halves of the night were compared ($p < 0.0001$; one-way ANOVA for the

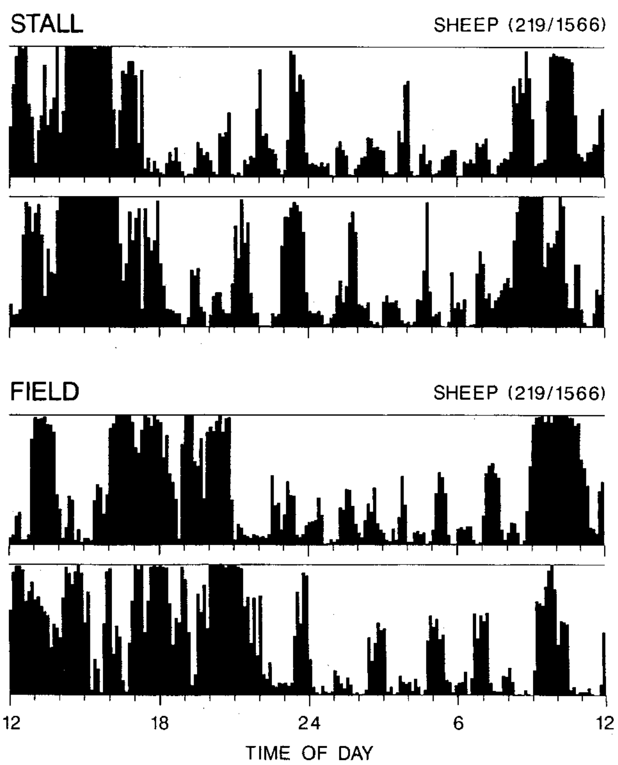

Figure 2. Four 24-h records of one sheep. The bars represent the activity value in one 7.5-min episode which could vary between 0 and 253 counts (for details see methods section).

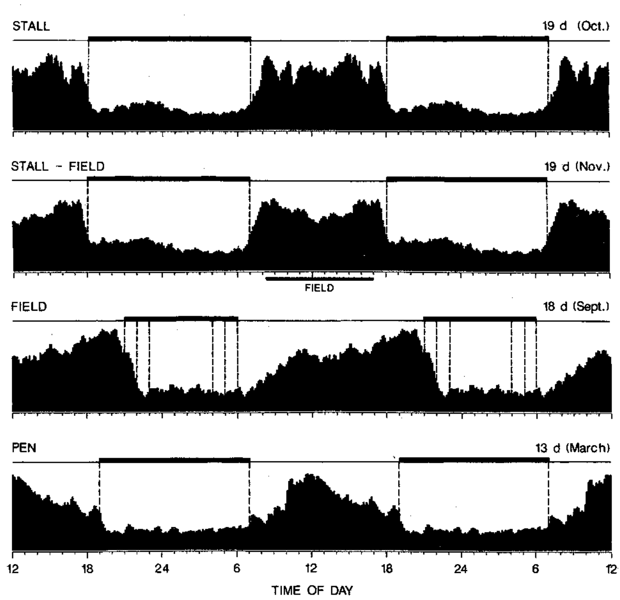

Figure 3. Activity of the sheep for the entire recording period (13–19 days; arbitrary units) separately for the four conditions. Means were computed first for each animal (see fig. 1) and then over all animals. For better visualization of the rest-activity pattern two days are plotted on each line. The dark period is delimited by the black bars. Vertical interrupted lines delimit the light-dark period as in fig. 1.

Table 1. Motor activity and occurrence of rest under four conditions (stall, stall-field, field and pen) of 6–12 sheep recorded continuously for 13–19 days. Mean values ± SEM

	Condition	n	Total (24 h)	L	D
Light-dark schedule	Stall			07.00–18.00	18.00–07.00
	Stall-field			07.00–18.00	18.00–07.00
	Field			06.00–21.00	21.00–06.00
	Pen			07.00–19.00	19.00–07.00
Mean activity count per 7.5-min episode	Stall	9	110.3 ± 1.0	168.2 ± 1.3	61.3 ± 1.6
	Stall-field	9	117.7 ± 1.2	167.1 ± 1.3	76.0[a] ± 1.6
	Field	6	119.7 ± 1.8	156.1[bc] ± 1.7	59.0 ± 2.6
	Pen	12	97.1[def] ± 1.4	138.3[def] ± 1.5	56.0[e] ± 1.7
Number of rest episodes per hour	Stall	9	0.35 ± 0.02	0.06 ± 0.01	0.61 ± 0.03
	Stall-field	9	0.44 ± 0.02	0.10 ± 0.01	0.73 ± 0.04
	Field	6	0.35 ± 0.03	0.20[c] ± 0.02	0.59 ± 0.05
	Pen	12	0.98[def] ± 0.04	0.37[de] ± 0.03	1.59[def] ± 0.07

Comparisons between conditions. [a]S vs SF; [b]SF vs F; [c]S vs F; [d]S vs P; [e]SF vs P; [f]F vs P; $p<0.05$; Mann-Whitney test; or Rank Sum test. Comparisons between L (light) and D (dark, defined by the artificial light, or in the field by the end of dusk and beginning of dawn). *$p<0.05$, paired t-test.

Table 2. Motor activity, frequency of rest episodes and variance of activity and rest onset under four conditions (stall, stall-field, field and pen) of 6–12 sheep recorded continuously for 13–19 days. Mean values ± SE

	Condition	n		
Alpha-rho ratio	Stall	9	0.94**	± 0.03
	Stall-field	9	0.93**	± 0.03
	Field	10	1.83**[bc]	± 0.12
	Pen	12	0.99[f]	± 0.06
Activity (mean counts per 7.5-min episode), ratio of first and second half of night	Stall	9	1.39**	± 0.11
	Stall-field	9	1.43**	± 0.09
	Field	10	1.14[b]	± 0.07
	Pen	12	1.07[de]	± 0.06
Rest episodes (number per h), ratio of first and second half of night	Stall	9	0.51*	± 0.08
	Stall-field	9	0.68*[a]	± 0.06
	Field	10	0.56*	± 0.10
	Pen	12	1.11[def]	± 0.05
Variance of activity onset (h^2)	Stall	9	0.19	± 0.09
	Stall-field	9	0.19	± 0.06
	Field	10	1.64[bc]	± 0.76
	Pen	12	0.97[def]	± 0.55
Variance of rest onset (h^2)	Stall	9	0.26	± 0.15
	Stall-field	9	0.78[a]	± 0.49
	Field	10	0.27[b]	± 0.17
	Pen	12	0.35[de]	± 0.16

Comparisons between conditions: [a]S vs SF; [b]SF vs F; [c]S vs. F; [d]S vs. P; [e]SF vs P; [f]F vs P; $p<0.05$; Mann-Whitney test; or Rank Sum test. Comparisons between first and second half of night: **$p<0.001$, *$p<0.05$, paired t-test.

variable mean motor activity per night half; table 2). A significant activity decrease was observed in the second part of the night in the conditions S and SF, whereas in the conditions F and P the night halves did not differ (fig. 3 and table 2). The number of rest episodes increased significantly in the second half of the night in the conditions S, SF and F, and remained on the same level in both night halves of condition P.

Activity onset and the end of activity were not invariably determined by the LD-schedule (fig. 1). Therefore, the duration of the activity period (alpha) and rest period (rho) were determined for each condition independently of the light exposure. The overall ANOVA for the variables alpha and rho revealed significant differences ($p < 0.0001$). Alpha was significantly shorter than rho in the conditions S and SF, and longer in condition F (table 2). In condition P the durations of alpha and rho did not differ.

Both the end of daytime activity and, particularly, the beginning of activity were relatively abrupt in conditions S and SF, whereas in condition F, activity exhibited a gradual increase until noon (fig. 3). In condition P a first small activity peak occurred approximately at light onset, and a second larger peak was seen at 10 h when the rams were fed. To determine whether the gradual activity changes in conditions F and P were induced by a large intra-individual day-to-day variability, the variance of activity and rest onset were computed. The variability in activity onset was markedly larger in condition F than in the other conditions and lowest in the conditions S and SF, whereas the variability of rest onset was largest in condition SF (table 2).

Discussion

The ambulatory activity recording device proved to be a useful tool for obtaining continuous data on the rest-activity behavior of animals both freely ranging and in stalls. It was not surprising that the sheep were most active during the daytime, since results from former behavioral[2,3,10,11,19–21] and sleep-wake studies[12,13] have shown that sheep are clearly diurnal. Large amounts of activity also took place in the dark, which is consistent with the occurrence of eating, drinking and walking activity observed in rams in the 12-h dark period[2,3]. However, the alpha-rho ratio, which corresponded to the LD-schedule only in condition P, where the animals were most isolated, indicates that not only the photoperiod determined the duration of activity and rest. The largest deviation of the alpha-rho value from the LD-schedule was found in the condition F, where the animals were in the most natural conditions. During the long photoperiod in September (condition F) a prolonged activity period was observed, and activity onset showed the largest variance of all conditions. Activity onset was not determined by light intensity, since dawn occurred at 5.00 h and sunrise at 6.00 h, and the sheep began their daily activity at about 7.30 h. It seems unlikely that the low environmental temperature in the morning hours was responsible for the delayed and gradual activity increase until noon, since also in Merino sheep, the onset of grazing was delayed by over an hour after dawn in early summer, compared to a fast onset of activity which correlated with dawn in the autumn [11]. The sudden onset of activity in the other conditions, particularly in the stalls, was probably induced by human activities and feeding. Thus, in conditions S and SF (which differed only in that the sheep were in the field for some daytime hours in condition SF; the same individuals contributed to the data of both conditions), activity onset was sudden with the least day-to-day variance. The presence of people after 8.00 h and the replenishment of food induced a synchronized activation of the sheep. In the field, the first activity peak occurred many hours later, indicating that the sheep started feeding later than in the stall. Perhaps after the shorter nights in the field the sheep were less hungry than after the longer nights in the stall and pen (7, 12 and 13 h respectively). In condition P, where the sheep were most isolated from exogenous influences and feeding occurred only once per day at about 10 h, the onset of light induced only a small activation, and the larger activity peak correlated with feeding.

The time course of activity within the light period differed between the conditions. Several studies have reported a biphasic occurrence of activity [2, 11, 12]. When food was provided ad libitum, feeding occurred around the clock, but the two most marked peaks were in the morning and in the evening [22]. The clearest biphasic activity pattern in our sheep was present in condition SF. An activity bout occurred in the morning due to the main feeding period, and in the evening, when the ewes returned to the stall from the field. This group (SF) exhibited the most marked drop of activity in the afternoon, although a comparable decrease of activity was observed also in condition F. This behavior is consistent with the reports that in many animal species napping occurs in the early afternoon hours (Tobler [23] for a review).

The sheep that remained in the stall all day (condition S) and those that were allowed into the field during the day (condition SF) exhibited the largest overall activity during the light period. The human activities and the transfer back and forth from the stall to the field must have induced the activation. When external stimuli were lacking (condition P), the evening activity peak was very small, whereas in the natural environment (condition F) the evening activity peak was more marked than the morning peak.

Rest onset was determined by lights off in the controlled environments. In the field, cessation of activity occurred well after sunset, as was the case for the Merino sheep in summer and autumn [11]. The variance of rest onset differed between the conditions S and F, although the identical individuals were recorded in the same stalls, and the recordings were obtained in consecutive weeks in October and November (table 2). The daytime exposure to the field, and the return to the stall approximately 1 h before lights off, induced an activation that sometimes subsided only after several hours. This interpretation is supported by the larger amount of nighttime activity in condition SF compared to condition S.

The presence of humans seems to have influenced the behavior of the sheep considerably. The greater daytime activity in the conditions S and SF was paralleled by the rare occurrence of rest episodes, indicating that the human presence not only activated the sheep, but also prevented rest. This is consistent with the findings in the rams, which were most isolated from human influence and which exhibited the smallest activity values and the largest number of rest episodes. Similarly, sheep spent more time lying down than standing when they were in isolation [22], and exhibited 10% more sleep at weekends than on weekdays [24], indicating a sensitivity to the presence of humans. The overall amount of rest in our study was in general small. The rare episodes with activity values < 5 during the dark period probably coincided with REM sleep episodes. The scoring of the video-tapes allowed the behavioral identification of REM sleep episodes: The sheep lay either on its stomach or outstretched on its side, and the head invariably rested on the ground. The episodes were further characterized by twitches of the extremities and the ears, and by irregular breathing. Activity values reached the lowest levels during these episodes. On the basis of electrographic recordings it has been found that REM sleep episodes in sheep occur only at night and last approx. 2–6 min [9, 22, 25, 26]. It could be expected that sheep in the field would be more active or exhibit less rest due to the lack of security. It is amazing that in condition F, with the largest freedom, the ewes exhibited the same amount of 24-h activity and rest as in the stalls. The large differences in the rest-activity behavior observed between condition P and all other conditions can be attributed to several factors. The rams belonged to a different strain, to a different gender, and were recorded in spring. Rams may be generally less active than ewes. This could explain why also in the dark period, when the animals were undisturbed in all conditions, the rams still exhibited the largest number of rest episodes. Also, it cannot be excluded that seasonal effects contributed to the differences in the rest-activity behavior observed in our study. To our knowledge activity in sheep has not been quantified as a function of the time of year.

In conclusion, the rest-activity pattern of the sheep in stalls was largely determined by the light-dark schedule, by the daily feeding and by human activities, whereas the conditions in the field and isolation in a pen allowed the manifestation of more natural behavior. The differences between the conditions were least evident in the overall 24-h amounts of rest and activity, but were clear in the distribution of rest and activity within the light and the dark periods.

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