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Effect of age and feeding schedule on diurnal rest/activity rhythms in dogs

Brian M. Zanghi[a], Wendell Kerr[a], Christina de Rivera[b], Joseph A. Araujo[b], Norton W. Milgram[b]

[a]Nestlé Basic Research Center, St Louis, Missouri; and
[b]CanCog Technologies, Toronto, Ontario, Canada.

Abstract Dogs exhibit biorhythms like diurnal sleep/wake and rest/activity behavior patterns with circadian rhythmicity and, similar to other mammalian species, show age-dependent changes in activity rhythms. This study sought to further characterize night/day locomotor activity patterns in adult dogs of various ages, and to examine the effect of feeding frequency (once vs. twice). Three groups (early adults [EA], 1.5-4.5 years; late adults [LA], 7-9 years; senior, 11-14 years) of beagles (N = 48) wore the Actiwatch activity monitoring system for 3 days while housed indoors with 12-hour light/dark schedule. Activity recording devices were used to collect actigraphy data when dogs were on a once- or twice-daily feeding regimen. All dogs demonstrated a circadian activity pattern with high levels of daytime activity and low levels of nighttime activity. The main effect of age differed for both daytime ($P < 0.0001$) and nighttime ($P = 0.002$) activity counts. Daytime activity in senior dogs was 17% and 42% lower compared with LA and EA dogs, respectively. With nighttime activity, both LA and seniors had significantly lower counts compared with the EA. Compared to once-daily feeding, twice-daily feeding significantly increased night activity, night/day activity ratio, and activity counts 60 minutes before the light phase across all 3 age-groups. This study provides a comprehensive characterization of age-related changes in locomotor behavior patterns in dogs and uniquely demonstrates that frequency of feeding can influence nighttime activity levels, which is partially associated with level of prelight onset of activity.
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Introduction

Normal aging is accompanied by declining locomotor activity, altered circadian rhythms, as well as altered sleep and food intake patterns, which can be a manifestation of many physical and neurological deficits that accumulate over a lifetime. The hypothalamic/hypocretin system is partially responsible for regulating wakefulness, locomotor activity, and feeding in animals (Baumann and Bassetti, 2005), and recent research in rodents indicates that there is loss of hypocretin neurons with age (Brownell and Conti, 2010; Kessler et al., 2011). In dogs, aging has been associated with changes in sleep patterns (Takeuchi and Harada, 2002). Age-dependent decline in activity levels has also been observed under laboratory conditions (Siwak et al., 2002, 2003) as well as by pet owners (Houpt and Beaver, 1981; Neilson et al., 2001; Bain et al., 2001).

Address for reprint requests and correspondence: Brian M. Zanghi, PhD, Nestle Research Center, One Checkerboard Square, St Louis, MO 63164; Tel: 001-716-532-5186; Fax: 001-314-982-5857.
E-mail: Brian.Zanghi@rdmo.nestle.com

Yet, it is not clear how different feeding regimens influence circadian locomotor activity patterns in dogs, and whether age influences activity patterns relative to varying feeding regimens.

Previous work has established that healthy adult dogs exhibit diurnal locomotor activity/rest patterns (Tobler and Sigg, 1986; Nishino et al., 1997; Siwak et al., 2003) and also show a complex relationship between locomotor activity, age, cognition, and housing environment (Siwak et al., 2003). Siwak et al. (2003) observed that young dogs (1-4 years old) exhibit higher activity levels than aged dogs (9-14 years old). In addition, evidence of a link between cognitive decline and activity was observed, in which cognitively impaired, aged dogs were more active and showed a delayed peak of daytime activity than unimpaired, aged dogs. This potential link may parallel age-related activity and cognitive changes observed to be exaggerated in dementias, such as Alzheimer's disease (Witting et al., 1990; van Someren et al., 1996), and can be manifested as either hyper- or hypoactivity (Satlin et al., 1991).

Characterizing locomotor activity patterns in aged dogs is important for practical as well as theoretical reasons. In humans, age-related changes in sleep have been linked to disruptions in mechanisms underlying circadian rhythm generation (reviewed by Weinert, 2000; Hofman, 2000; Espiritu, 2008), which has also been linked to cognitive dysfunction. Therefore, this study was conducted to confirm previous observations that day/night activity is influenced by age, while simultaneously evaluating effect of daily feeding frequency on various measures of day/night activity and circadian locomotor rhythms across 3 distinct age-groups of adult dogs.

Methods

Animals, housing, and feeding regimen

Adult beagle dogs (N = 48, all spayed or neutered) were divided equally into 3 age-groups: early adult (EA, 1.5-4.5 years; N = 16), late adult (LA, 7-9 years; N = 16), and senior (11-14 years; N = 16). The dogs were housed indoors in groups of 2-4 per pen (1.5 m wide × 4.5 m long), randomly grouped by age, and based on compatibility and sex. All animals were housed in the same kennel location, with ability to see other dogs in adjacent and opposing runs. All dogs were observed daily, given access to toys, and had daily interaction with staff. Pens were cleaned between 9 AM and 11 AM.

Before the study, the dogs were fed once daily and had exposure to natural light cycles. Two weeks before the start of this study, they were switched to a 12-hour light/dark cycle by covering the windows and were only exposed to kennel lighting from 7 AM to 7 PM. This was maintained until the completion of the study. Water was available ad libitum.

During the 3-week baseline period, dogs were fed Purina Pro Plan Adult Maintenance Chicken and Rice formula (Nestlé Purina Petcare, St. Louis, MO) once daily between 8 AM and 9 AM. Immediately after the baseline activity recording period, all dogs were switched to a twice-daily feeding at 8 AM and 6 PM. by dividing the once-daily food ration equally into 2 rations for the remainder of the study. Total food provided was regularly adjusted to permit the dogs to maintain their body weight at baseline levels. The study was conducted in accordance with approved Animal Care and Use Committee protocols.

Activity recording and data analysis

Twenty-four-hour activity rhythms were assessed for 3 consecutive days using an omnidirectional accelerometer (Mini-Mitter Actiwatch-16 activity monitoring system, Respironics Co., Inc., Bend, OR). The Actiwatch is an omnidirectional accelerometer (Ancoli-Israel et al., 2003), and was placed inside a specially designed case and attached to a collar around the dog's neck, permitting normal activity, rest, exercise, and feeding (Siwak et al., 2003). Once-daily feeding activity recordings occurred in September. Twice-daily feeding activity rhythms were subsequently monitored 35 days later (in November; referred to as twice-P1), and again following an additional 70 days (in January; referred to as twice-P2).

The monitors recorded activity counts on a 30-second epoch setting, and activity data were downloaded to a personal computer immediately after the completion of the data recording period for later analysis. Total daily, light phase (day), and dark phase (night) activity counts were generated by the Actiware software provided with the Actiwatch recording system, along with dark/light phase activity counts ratio. Morning onset activity counts were calculated manually and represent the sum of activity counts recorded within 1 hour before the start of the light phase at 7 AM.

Circadian rhythm analysis

A traditional cosinor analysis was performed, as described by Nelson et al. (1979), to calculate the circadian rhythm variables: acrophase and circadian quotient (mesor/amplitude ratio). Before performing the cosinor analysis, raw activity data recorded every 30 seconds during the first and all subsequent 60-minute intervals were transformed to represent raw activity counts based on 60-minute increments. Total activity counts per 60-minute increment were transformed using natural logarithms of the counts + 1.

The traditional cosine model used for circadian rhythm was:

$$\text{Activity} = M + A \times \cos(\omega \times \text{time} + t)$$

where M (mesor) = value about which oscillation occurs; A (amplitude) = half the difference between the highest

and lowest values; ω (angular frequency) = degrees/unit time, with 360° representing a complete cycle (or 2π radians representing a complete cycle); t (acrophase) = timing of high point in radians.

The data were analyzed from day 1 at 7 AM through day 4 at 7 AM to represent 3 recording periods, each of 24 hours duration. Therefore, 7 AM was hour 0 for the calculation of the corresponding clock time of acrophase, as the cosinor model generates acrophase in radians.

R^2 values from fitting the 1 cycle per day cosine model were generated using the raw counts, the natural logarithms of the counts + 1, and the square roots of the counts. In general, the R^2 values using the natural logarithms of the counts + 1 transformation gave the best fit to the data, but only slightly better than the raw counts (data not shown). The natural log-transformed data were used for the statistical analysis. R^2 values for the natural logarithms transformation ranged from 0.20 to 0.55 for all dogs.

Statistical analysis

A repeated-measures analysis of variance (ANOVA) was used to examine the effect of age and feeding frequency on behavioral activity over a 3-day block. All statistical analyses were performed using PROC MIXED in SAS (SAS 9.1.2., SAS Institute Inc., Cary, NC), with age-group, day, and feeding frequency as fixed effects; dog nested within age-group as a random effect; and day as the repeated measure. The dependent variables—total daytime activity counts, total nighttime activity counts, night/day activity counts ratio, morning activity onset counts, and traditional cosinor statistics (mesor, amplitude, acrophase)—were generated from the accelerometer-based recorded activity counts. These locomotor activity statistics were analyzed for main effects of age, feeding frequency (3 periods), day (3 consecutive days), and the 2-way and 3-way interactions of the main effects. A significance level of α = 0.05 for the ANOVA was used to determine statistical differences. Post hoc analyses were performed using a protected ($P < 0.05$) Fisher least significant difference test to separate means of dependent variables that differed with age or feeding frequency.

Results

Activity counts were recorded over 3 consecutive 24-hour periods to characterize age-related changes in locomotor activity rhythms while on a once- or twice-daily feeding schedule. Figure 1 illustrates 3-day rest/activity profiles for a representative dog recorded during each period. A repeated-measures ANOVA revealed that no age × feeding frequency or 3-way interactions were observed for all locomotor activity statistics analyzed, but there were several significant main effects of age or feeding frequency. The main effects of age or feeding frequency are described separately further in the text.
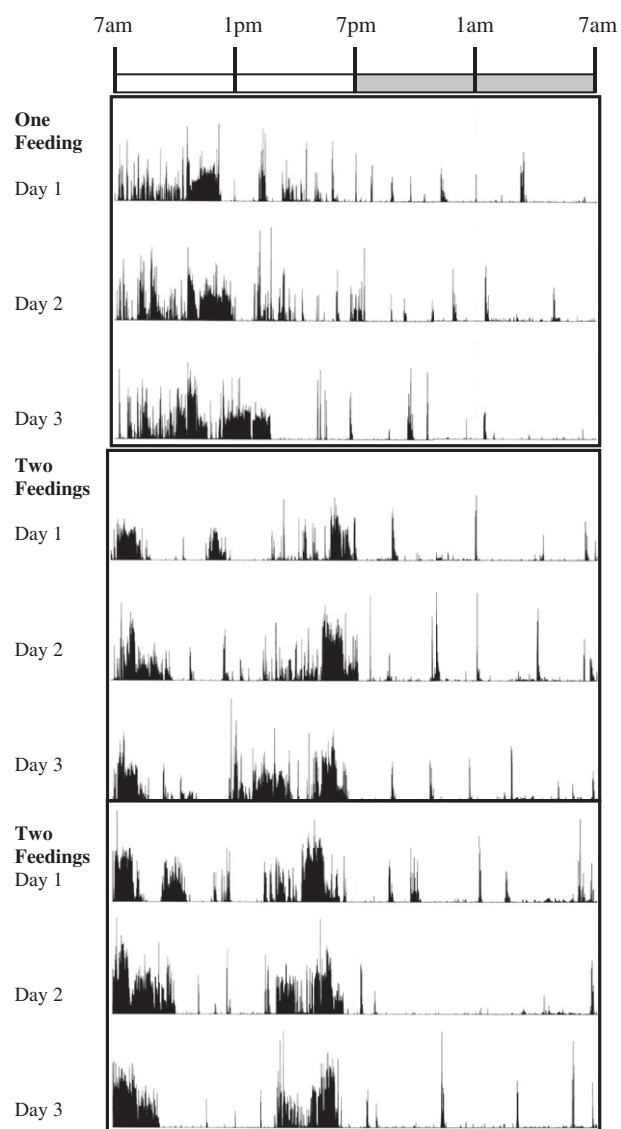

Figure 1   Representative 24-hour rest/activity profiles of 1 dog from each activity recording period over 3 consecutive days from 7 AM to 7 AM, with exposure to a 12-hour light/dark cycle. Activity counts recorded on 30-second epochs. All y-axes are 0 to 2,000 counts. Once-daily feeding (1 feeding) was recorded in September, and twice-daily feeding (2 feedings) was recorded in November and February. Dogs were fed once daily between 8 AM and 9 AM, whereas twice-daily feeding occurred at 8 AM and 6 PM.

Day/night activity and age

To examine the effect of age, a repeated-measures ANOVA compared activity counts between 3 age-groups of dogs, and reported means are calculated means across all feeding frequency periods. Both daytime (light phase; ANOVA $P <0.0001$) and nighttime (dark phase; ANOVA $P = 0.002$) activity counts decreased with age (Figure 2). Specifically, least squares means analysis indicated that total daytime activity counts in senior dogs were reduced ($P < 0.05$), on average, by 42% compared with EA dogs
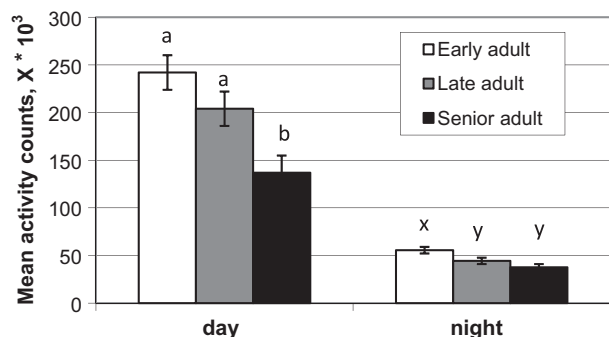
Figure 2 Mean (±SEM) total day and night activity counts by age-group. Letters a/b indicate differences ($P < 0.05$) between age-groups for total day activity counts, whereas x or y indicate differences ($P < 0.05$) between age-groups for total night activity counts.

Figure 4 Mean (±SEM) total activity counts within each period recorded during 60 minutes before lights on at 7 AM. Letters a, b, or c indicate differences ($P < 0.05$) between periods. Once represents dogs fed once daily, twice-P1 and twice-P2 represent dogs fed twice daily.

and by 17% compared with LA dogs. Total nighttime activity counts in both senior and LA dogs were significantly lower ($P < 0.05$) by 32% and 20%, respectively, compared with EA dogs. Nighttime activity counts were not different between senior and LA dogs. In addition, night/day count ratios were calculated, and the ANOVA for the main effect of age did not differ. The mean ratio for EA and LA dogs was 0.25 ± 0.02 SE and for senior dogs was 0.27 ± 0.02 SE.

Day/night activity and feeding frequency

To examine the effect of daily feeding frequency, a repeated-measures ANOVA compared activity counts recorded 3 times when dogs of all ages were fed either once daily or twice daily, with twice-daily feeding recorded on 2 separate occasions and differentially distinguished as twice-P1 (recorded in November) and twice-P2 (recorded in January). The main effect of feeding frequency for nighttime (12-hour dark phase) activity counts differed significantly (ANOVA: $P < 0.0001$), but not for daytime activity counts (Figure 3). Least squares means analysis indicated that total nighttime activity counts were higher ($P < 0.05$) when dogs were fed twice daily by 44% or 62% for
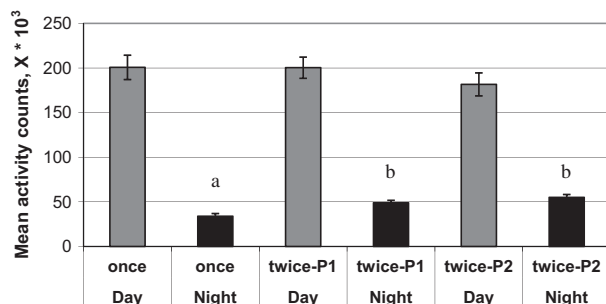
twice-P1 and twice-P2, respectively, compared with once-a-day feeding. In addition, the ANOVA for night/day activity count ratio was highly significant ($P < 0.0001$) for the feeding frequency effect. The least squares means ratio was different ($P < 0.05$) between all 3 activity recording periods, which increased from 0.19 ± 0.02 SE for once-a-day feeding to 0.27 ± 0.01 SE for twice-P1 feeding and 0.31 ± 0.02 SE for twice-P2 feeding repeated 70 days later.

Locomotor A/M activity onset counts

A repeated-measures ANOVA was used to analyze activity onset counts for age and daily feeding frequency effects (Figure 4). The ANOVA for activity onset counts differed with feeding frequency ($P < 0.0001$), but did not differ with age or age × feeding frequency interaction. When dogs were transitioned from a once-daily to twice-daily feeding schedule, activity onset counts increased 213%. As the twice-daily feeding regimen was maintained for another 70 days, activity onset was observed to further increase by 62%, which was a 400% increase over the initial baseline activity recording when dogs were on a once-daily feeding. It is notable that the increase in activity onset between the initial twice-feeding data (twice-P1) and second twice feeding (twice-P2) was largely driven by EA and LA dogs, as counts were, on average, 41% and 49% greater, respectively, whereas seniors increased less than 11%.

Rhythmicity of locomotor activity recordings

Observational assessment of the actigraph plots clearly indicate that feeding frequency had a considerable influence on the rhythmicity of locomotor activity. Therefore, the activity count data was evaluated by using the traditional cosinor analysis to calculate the acrophase and the circadian quotient (amplitude/mesor) to assess the locomotor circadian rhythmicity. Estimates of mesor (M),

Figure 3 Mean (±SEM) total day and night activity counts by period. Letters a/b indicate differences ($P < 0.05$) between periods for total night activity counts. Total day activity counts were not different between age-groups.
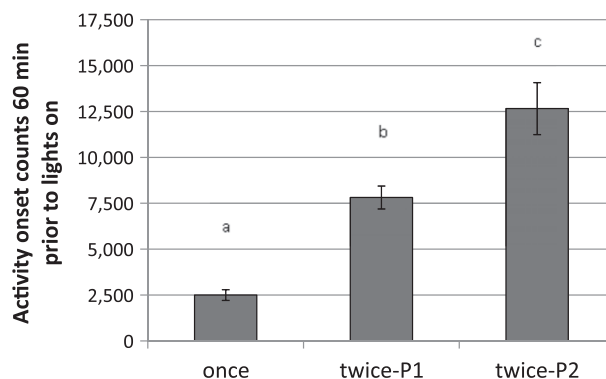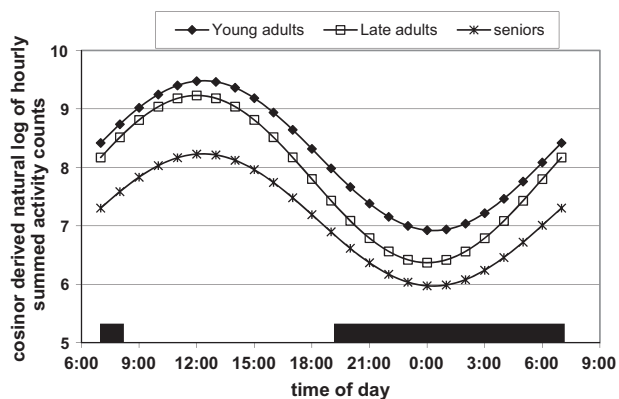
Figure 5 Plot of traditional cosinor model over 24 hours for each age-group. The cosine curve is calculated based on the model variables listed in Table 2.

Figure 6 Plot of traditional cosinor model over 24 hours for different feeding frequency periods. The cosine curve is calculated based on the model variables listed in Table 2.

amplitude (A), acrophase, and circadian quotient (A/M) were generated based on 1 cycle per day. The cosine curves are plotted over a 24-hour period for each age-group (Figure 5) and feeding frequency regimen (Figure 6). These curves show that all groups have clear diurnal rhythmicity and also clear differences in amplitude.

A repeated-measures ANOVA was used to analyze cosinor statistics for age and daily feeding frequency effects. No age × feeding frequency interaction was observed for any cosinor model variable. The M (ANOVA $P = 0.01$) and A (ANOVA $P = 0.02$) significantly differed with the main effect of age. Least square means analysis indicated that young dogs have a significantly greater ($P < 0.05$) M compared with both middle-aged and senior dogs, whereas A of senior dogs was only significantly lower ($P < 0.05$) compared with middle-aged dogs. Acrophase did not differ (ANOVA $P = 0.25$) with age, which indicates that time of peak and trough activity occurred at the same time of day for all dogs (Table 1, Figure 5). The A differed (ANOVA $P = 0.002$) with the main effect of feeding frequency, as least squares means analysis indicated that the switch from once- to twice-a-day feeding regimen resulted in a significant reduction in A ($P < 0.05$, Table 2). Both M and acrophase also differed (ANOVA $P < 0.003$) with feeding frequency, but interestingly, differed significantly between twice-P1 and twice-P2 when all dogs were similarly fed twice daily, in which a decline in M and an earlier acrophase was observed during twice-P2.

Discussion

The overall objective of this study was to examine the effects of age and feeding pattern on activity rhythms in dogs. The analysis was based on both total activity counts and use of the traditional cosinor analysis using canine locomotor activity data to assess circadian rhythmicity, which is more typically used in human subjects for the same pu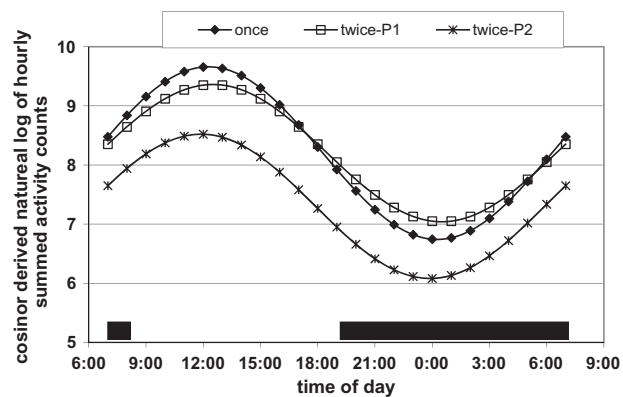rpose. This study confirms that daytime activity declines with canine aging, but uniquely characterizes activity rhythms across multiple age-groupings, and shows that both day and night activities are influenced by age. We also described the novel observation that feeding frequency influences activity patterns across all ages of dogs examined here. In addition, this study uniquely models canine activity data across both age and feeding frequency to characterize circadian rhythmicity statistics for the first time in dogs.

Age-related changes in animals such as dogs are not only relevant within veterinary research, but also provide an opportunity to understand similarities and distinct differences between canine aging and human aging. Understanding behavioral changes in dogs provides value not only as a model of human cognitive and behavioral aging, but also because there is an increasing importance placed by pet owners on health and wellness of their aging pets, particularly because there is an increased life-expectancy of pets (Stratton-Phelps, 1999; Kraft, 1998).

The activity count data confirmed that dogs exhibit strong diurnal activity/rest rhythms, which is indicated by increased daytime over nighttime activity across all age-groups of adult dogs. These observations are in agreement with previous canine research in which day/night locomotor activity (Tobler and Sigg, 1986; Nishino et al., 1997; Siwak et al., 2003) and electroencephalogram-detected sleep/wake status (Lucas et al., 1977; Gordon and Lavie, 1984; Tobler and Sigg, 1986; Takeuchi and Harada, 2002) were measured. In the present study, all animals lived in an indoor environment and had their activity recorded while in their home pen with exposure to a 12-hour light/dark cycle. Previous research has indicated that the housing environment also impacts day/night locomotor activity patterns and is important in revealing age-related changes in dogs (Siwak et al., 2002).

Total daytime activity of young and middle-aged adult dogs ranged from 3 to 8 times greater than nighttime activity levels, and was approximately 65%-89% of total daily activity. This is consistent with previous observations

Table 1   Mean (±SEM) of traditional cosinor model parameters of locomotor activity for adult dogs of different age-groups[a]

Variable	Age-groups			ANOVA P value
	Young	Middle	Senior	
Mesor	8.2 ± 0.1[b],*	7.8 ± 0.1[†]	7.7 ± 0.1[†]	0.01
Amplitude	1.28 ± 0.08*,†	1.43 ± 0.08*	1.13 ± 0.08[†]	0.02
Acrophase, radians[c]	−1.40 ± 0.04	−1.31 ± 0.04	−1.39 ± 0.04	0.25
Acrophase, time[d]	11:20 AM	11:00 AM	11:18 AM	
Circadian quotient, A/M	0.31	0.37	0.32	

A/M, amplitude/mesor.
[a]Data expressed as natural log.
[b]Different symbols (*,†) indicate least squares means differed using protected Fisher least significant difference test ($P < 0.05$).
[c]Negative radian values reflect negative degrees, 0° to −360° (Nelson et al., 1979).
[d]Acrophase as time of day was calculated by first converting radians to degrees, which was used to calculate time of day within a 24-hour cycle.

of locomotor activity in dogs measured over a 24-hour period, in which 60%-75% of total activity occurred during the 12-hour daytime period (Tobler and Sigg, 1986; Nishino et al., 1997).

Characterization of locomotor activity and age effects

Locomotor activity declined as a function of age, and this study uniquely demonstrated that age-related changes occurred differently with daytime compared with nighttime activity. Decreased activity in aged dogs could reflect numerous factors, including loss of vision, arthritis, and excess weight. To partially control for these factors, the dogs used in the current study were not medicated for osteoarthritis, were within an ideal body condition to exclude issues with excess body weight, and did not have notable loss of vision from cataract formation. Therefore, alteration in locomotor activity with age was interpreted to be manifestations of changes in circadian rhythms or other age-related influences.

Although both mean daytime and nighttime activity levels decreased with age, daytime activity in senior dogs >10 years of age was significantly reduced compared with young and middle-aged dogs. By contrast, a significant

age-related reduction in nighttime activity was observed in dogs at an earlier age, as dogs aged >6 years began to show declining locomotor activity, but this was most striking when dogs were fed once daily and variability was notably greater when dogs were fed twice daily.

Siwak et al. (2003) and Zanghi et al. (2008) similarly observed a decrease in total daytime activity between young (1-4 years old) and senior (9-14 years old) dogs. It is notable that Siwak et al. (2003) did not observe an age-related change with nighttime activity, but a significant age-related decline was observed by our laboratory in an initial age-characterization study (Zanghi et al., 2008). Age-related decreases in activity have been reported previously in many other animals, such as nonhuman primates (Emborg et al., 1998; Aujard et al., 2007; Terrien et al., 2009), rats (Dorce and Palmero-Neto, 1994; Goodrick, 1971), mice (Elias et al., 1975; Elias and Redgate, 1975; Goodrick, 1975; Rosenthal and Morley, 1989), ringdoves (Garau et al., 2006), and zebrafish (Zhdanova et al., 2008). However, declining locomotor activity related to advanced age is exhibited differently for various diurnal vertebrate species. Specifically, ringdove (Garau et al., 2006), zebrafish (Zhdanova et al., 2008), and rhesus monkey (Emborg et al., 1998) as models of aging and circadian locomotor rhythms have demonstrated that older animals

Table 2   Mean (±SEM) of traditional cosinor model parameters of locomotor activity for adult dogs on different meal feeding frequency[a]

Variable	Feeding frequency			ANOVA P value
	Once daily	Twice daily-P1	Twice daily-P2	
Mesor	8.2 ± 0.1[b],*	8.2 ± 0.1*	7.3 ± 0.1[†]	0.001
Amplitude	1.46 ± 0.06*	1.16 ± 0.06[†]	1.22 ± 0.06[†]	0.002
Acrophase, radians[c]	−1.38 ± 0.07*	−1.44 ± 0.07*	−1.28 ± 0.03[†]	0.003
Acrophase, time[d]	11:16 AM	11:30 AM	10:53 AM	
Circadian quotient, A/M	0.36	0.28	0.33	

[a]Data expressed as natural log.
[b]Different symbols (*,†) indicate least squares means differed using protected Fisher least significant difference test ($P < 0.05$).
[c]Negative radian values reflect negative degrees, 0° to −360° (Nelson et al., 1979).
[d]Acrophase as time of day was calculated by first converting radians to degrees, which was used to calculate time of day within a 24-hour cycle.

exhibit reduced daytime activity, but little change in nighttime activity. In contrast, recent research with other nonhuman primates, including lemurs (Aujard et al., 2007; Terrien et al., 2009), has shown age-dependent decreases in nighttime activity. Conflicting evidence exists in other research with ringdoves (Paredes et al., 2006), in which daytime activity remained unchanged with aged birds, but increased nighttime activity was observed.

Canine research with home-living pets demonstrates that locomotor activity data can be effectively collected with pet-owner participation in clinically coordinated trials and is significantly influenced by owner lifestyle and schedule. However, it remains to be observed whether age-related changes in locomotor activity and/or night/day activity patterns can be observed with home-living pets.

Once- versus twice-daily feeding on locomotor activity

The present study also examined the effect of feeding frequency. In most animal studies, feeding frequency is held constant and not considered as a variable of interest, particularly when feeding is not associated with the main study objectives. From a facility or resource management perspective, this is obviously rooted in practical management of animal resources, animal facilities, and personnel resources. Similarly, home-living pets are largely fed once daily and possibly with ad libitum access as well. However, at least among dog owners, more than 50% feed twice daily, and for those who feed once daily, feeding time is nearly equally divided into those who feed in the morning or at night. Therefore, understanding how feeding frequency impacts an animal's behavioral patterns is of value.

The results clearly demonstrated that feeding schedule impacts locomotor rhythm. In the present study, twice-daily feeding increased nighttime locomotor activity by approximately 50% compared with once-daily feeding, but did not cause a change in daytime locomotor activity. Because the afternoon feeding occurred at 6 PM, which was 1 hour before the start of the 12-hour dark cycle at 7 PM, the afternoon activity counts associated with the PM feeding did not carry over into the dark phase and, therefore, did not artificially elevate nighttime activity counts. A limitation of the study is that we did not control for the transition of feeding frequency. Consequently, it leaves the question unanswered as to the length of time it would take for the effect to be reversed after switching from a twice-daily feeding to once-daily feeding, particularly because the oldest dogs appear to adjust more slowly.

Morning locomotor activity onset

Siwak et al. (2003) noted that puppies and young dogs showed an average activity onset at 20-30 minutes after lights on, whereas senior dogs became active nearly 2 hours after lights on. These data were generated with dogs that were fed once daily. Although we do not report the estimated time of activity onset with this data set, our unpublished analysis of these data confirms that older dogs have a later activity onset time than young dogs. However, the absolute time of activity onset in our study compared with that reported by Siwak et al. (2003) differs significantly by several hours. Our unpublished data also indicate that transition from a once- to twice-daily feeding regimen resulted in an earlier activity onset time by 20-30 minutes (Zanghi, unpublished work). Here, we simply report total activity counts within the first hour before the start of the light phase for all periods. We observed that morning activity levels increased 2-3 times from 6 AM to 7 AM when fed twice daily, which occurred with all dogs. Interestingly, it appears that young and middle-aged dogs continue to have an increase in morning activity levels when tested again 35 days later (ranging from 4 to 10 times greater), whereas senior dogs did not. Although we did not manually analyze the activity data before the afternoon meal, it would be interesting to see if a similar level of premeal anticipatory activity occurs. A final notable bias that could be contributing to the earlier morning activity onset is that morning feeding was slightly earlier with twice-daily feeding frequency, thereby possibly contributing to a slight phase shift toward waking earlier. In addition, because all dogs were transitioned from once-daily feeding to twice-daily feeding, a slight bias exists for time effect, as once-daily was recorded in September (vs. November).

The relationship between activity onset and time of feeding may be linked to the hypothalamic/hypocretin system, which is partially responsible for regulating wakefulness, locomotor activity, and feeding (Baumann and Bassetti, 2005). In situations where food availability occurs once a day and scheduled at a fixed time, animals have been observed to adapt to the feeding regimen and demonstrate increased locomotor activity a few hours before feeding (Mistlberger, 1994; Stephan, 2002). Recent evidence in rodent models indicates that loss of hypocretin neurons is related to advancing age (Brownell and Conti, 2010; Kessler et al., 2011). Therefore, it is possible that age-related changes in activity onset in dogs may be related to some aspect of age-related loss of hypothalamic/hypocretin system. Moreover, blood levels of hypocretin follow a diurnal pattern, with levels rising during the day and falling during the night (Zeitzer et al., 2003). This pattern is consistent with a role in promoting food anticipatory activity. Although it was believed that a food-entrainable oscillator was independent of the suprachiasmatic nucleus, Mieda et al. (2004) demonstrated that hypocretin neuron–ablated mice had a severe deficit in displaying the normal food anticipatory increase in wakefulness and locomotor activity when fed once daily. Future work in the dog should also examine hypocretin secretion patterns to see whether the results obtained from rodents are translatable to other species.

Circadian rhythm

Finally, locomotor circadian rhythm variables using the traditional cosinor analysis were generated to uniquely assess the magnitude of the circadian oscillation of day versus night activity and alignment of the rhythm with the light/dark cycle in dogs. The traditional cosinor analysis is a widely used method within the field of human sleep research (reviewed by Ancoli-Israel et al., 2003), as well as in assessing neurological diseases associated with circadian impairment like Alzheimer's disease (Satlin et al., 1991; Ancoli-Israel et al., 1997, 2003). Other means of assessing the circadian rhythm of day/night activity can include the night/day activity count ratio, which also has been previously used to assess the circadian rhythm of Alzheimer's patients (Satlin et al., 1991; Mishima et al., 1998).

Although senior dogs had a decrease in the relative amplitude and mesor of the cosine curve compared with young dogs, synchronization of circadian locomotor patterns with the light/dark cycle appear to remain intact in aged dogs, as indicated by the absence of age differences in acrophase (time of peak activity). A similar observation was documented in aged versus young ring doves when characterizing locomotor activity rhythms (Garau et al., 2006). Because acrophase was not sensitive to detect a phase delay in senior dogs, this aspect of "normal" canine aging clearly differs from the regularly observed phase advance in sleeping patterns in aged people (Hofman, 2000, Wolkove et al., 2007). The minimal circadian impairment is also reinforced with our evidence that aged dogs retain a fairly robust night/day activity ratio and that the circadian quotient did not differ between any age-groups, which indicates that a relatively strong circadian rhythm is maintained in these older dogs.

Conclusions

This study extends previous work on canine activity rhythms by providing quantitative estimates of activity rhythm parameters, and by demonstrating that rhythms vary as a function of feeding schedule. In addition, we provide further clarification of the impact of age on activity rhythms, confirming the existence of age-dependent changes. Specific evidence of changing activity patterns in aging canines living in a kennel environment is demonstrated and continues to advance the understanding of canine aging. Because locomotor activity data are regularly used to estimate sleep/wake status in people, future analyses with these canine activity data will be used to generate actigraphy-estimated sleep variables in dogs. This will also provide additional advancement in our understanding of sleep/wake patterns in young and aged dogs. Circadian alterations are more prevalent in people with various neurodegenerative diseases (Satlin et al., 1991; Ancoli-Israel et al., 1997, 2003); therefore, it remains to be determined whether cognitively impaired canines also experience exacerbated locomotor circadian rhythmicity with severely advanced or delayed activity rhythms or irregular sleeping patterns.

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