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Research report

Age-related changes in sleep-wake rhythm in dog

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Abstract

To investigate a sleep-wake rhythm in aged dogs, a radio-telemetry monitoring was carried out for 24 h. Electrodes and telemetry device were surgically implanted in four aged dogs (16–18 years old) and four young dogs (3–4 years old). Electroencephalogram (EEG), electromyogram (EMG) and electrocardiogram (ECG) were recorded simultaneously as parameters to determine vigilance states and an autonomic nervous function. Wakefulness, slow wave sleep (SWS) and paradoxical sleep (PS) were identified according to the EEG and EMG pattern. We also examined whether absolute powers and the low frequency-to-high frequency ratio (LF/HF) derived from the heart rate variability power spectrum could detect shifts in autonomic balance correlated with aging. The aged dogs showed a marked reduction of PS and a fragmentation of wakefulness in the daytime and a sleep disruption in the night. The pattern of 24 h sleep and waking was dramatically altered in the aged dog. It was characterized by an increase in the total amount of time spent in SWS during the daytime followed by an increasing of time spent in wakefulness during the night. Furthermore, LF/HF ratio showed a very low amplitude of variance throughout the day in the aged dog. These results suggest that the aged dog is a useful model to investigate sleep disorders in human such as daytime drowsiness, difficulties in sleep maintenance. The abnormality in sleep-wake cycle might be reflected by the altered autonomic balance in the aged dogs.

Keywords: Aged dog; Autonomic balance; Diurnal drowsiness; Sleep fragmentation; Telemetry system

1. Introduction

A clear circadian rhythm of sleep time in normal adult dogs has been reported by Takahashi et al. [23] and Wauquier et al. [27]. Tobler and Sigg [24] have established a rest-activity rhythm in the laboratory dogs, based on the long-term motor activity recording. They mentioned the dogs were most active after light onset, and a rest period was found at noon and reduced activity during afternoon hours [24]. Kaitin et al. [11] investigated the sleep-wake rhythm in three narcoleptic dogs. They displayed a marked fragmentation and disruption of the sleep-wake cycle, which was characterized by repeated awakenings and frequent shifts in sleep stages. Horner et al. [9] studied a canine model of obstructive sleep apnea (OSA), and suggested that repeated sleep disruption due to the effects of repetitive apneas and hypoxia may lead to an increased rapid eye movement (REM) sleep drive that manifests itself as a REM sleep rebound during recovery sleep after OSA. These dogs have a possibility of a general dysfunction of sleep-wake rhythm.

Several reports have suggested that the free-running period of locomotor activity rhythms shortens with age in nocturnal rodents [16,19,25,26,31]. However, it has been reported that free-running period lengthens with age in mice [13] and canaries [20], and does not change with age in Syrian hamsters [3]. The period of human circadian rhythms has also been reported to shorten with age [30], and together with the high incidence of early morning awakening in older people [4] has led to the hypothesis that an age-related shortening of circadian period may underlie age-related changes in sleep-wake timing [1]. Early morning awakening and difficulty maintaining sleep have been shown to affect 15–20% of older people [4] and it has been suggested that such sleep

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disturbances are associated with increased mortality, sleeping pill usage, and nursing home placement [1,6,21].

Heart rate variability has been proposed as a semi-quantitative method for assessing activities in the autonomic nervous system [8]. It would be important to investigate the underlying autonomic nervous function of animals with age, because it is thought that these heart rate variations are associated with fluctuations in the tone of the autonomic nervous system [22]. However, the autonomic nervous function in the aged dog has not been clearly evaluated.

To clarify age-related changes in the sleep-wake rhythm in the dog, we applied a radio-telemetry monitoring of electrophysiological parameters for aged dogs, and performed a evaluation semiquantitative.

2. Materials and methods

2.1. Animals

Four mongrel old dogs (16–18 years old, 9.8 ± 0.5 kg) and four shiba young dogs (control; 3–4 years old, 10.5 ± 0.8 kg) were used. Although the aged dogs showed characteristic symptoms of aging, such as inactivity, sleepiness and poor mobility, they did not exhibit any paroxysmal activities, and were not taking any medication that could affect sleep. The dogs were housed in individual cages ($80 \times 100 \times 90$ cm) under controlled light-dark cycle (light on from 8:00 to 20:00) and were fed food and water at 10:00. The use of these animals, as well as the procedures performed, were approved by the Animal Research Committee at Tottori University.

2.2. Surgical procedure

The electroencephalogram (EEG), electromyogram (EMG) and electrocardiogram (ECG) electrodes and telemetric device were implanted chronically. The surgical operation was carried out under sodium pentobarbital anesthesia (Nembutal, Abott Inc., USA, 25 mg/kg, iv.). The telemetric device (Model TL10M3-D70-EEE, Data Sciences, St. Paul, USA) was inserted subcutaneously at the upper edge of scapula through the midline incision and the EEG, EMG and ECG leads (silicone insulated, double helix stainless steel) were fed through a 1 mm hole in the chest musculature just off the midline. In brief, a 8–9 cm incision was made on the dorsal midline of the head, the musculature on the dorsum of the head was ablated from the sagittal crest and retracted laterally exposing the bones of the skull. Two holes in diameter of 1.5 mm were drilled with 1 cm distance on one side of parietal bone. EEG leads were grossly cut leaving 3–4 cm extending from the scalp incision, taking into consideration 3 cm for flexing body movements. A few drops of super glue (GC Dental Chemical Co., Tokyo) were used as the adhesive after each lead was placed on the exposed dura. Antibiotics (Mycillinsol, Meiji Seika Co. Ltd.) was dropped onto the surface of the skull prior to closure. A 3 cm incision was made on the dorsal midline of the neck, the wires for the EMG recording were then inserted into the nuchal muscle and the end of wires were fixed with subcutaneous tissues by ligation. The remaining two leads were ligated with subcutaneous tissues at the positions of Apex-Base lead for ECG recording. Antibiotics (20 000 U/kg) was intramuscularly injected.

2.3. Recording

One week after the surgical operation, radio transmissions of EEG, EMG and ECG data from the animal were detected by a receiver plate (Model RLA2000, Data Sciences, St. Paul, USA). The plate was located on the roof of their home cage and converted the 455 kHz pulses to 5 V square waves. Since the receiver had an 1 m radius of reception, the animal was able to freely move within the cage. Data were captured using both digital recordings via computer (Macintosh Power Book 1400cs, Apple, USA) using a digital data acquisition program (Chart version 3.5.1, AD Instruments, USA) through the consolidation matrix and an analogue polygraph through a digital-to-analogue converter. Bipolar EEG signals were recorded with a sampling rate of 200 Hz throughout the recording period, and were collected on-line for later analysis.

We collected data from one implanted animal at a time for 48 h. On the first day, we started recording at 8:00, and finished at 8:00 on the third day. A continuous polygraph tracing was divided into two parts. The first 24 h recording was used for checking animal conditions, including an influence of human entry into the room, then the later 24 h recording was accepted for further analysis. Information about EEG, EMG and ECG is not affected by the receiver's detection of signal intensity.

2.4. Computer analysis

Polygraph recordings were scored in 15 s epochs for the states of wakefulness (W), slow wave sleep (SWS) and paradoxical sleep (PS), using Mitler and Dement [14] criteria with slight modifications. For each recording, a 24 h sleep plot (hypnogram) was generated, and the total number of episodes of each behavioral state was calculated. The percentages of time spent in each behavioral state were calculated for 20 min epochs and total percentages of time spent in each behavioral state per 24 h were also calculated.

A computer analyzing system (MacLab, AD Instruments, USA) for power spectral analysis of heart rate

variability was followed according to a previous report [10] with slight modifications. Briefly, the computer program first detected R waves and calculated the R–R interval tachogram as the raw ventricular rate variability in sequence order. From this tachogram, data sets of 512 points were resampled. We then applied each set of data to the Hamming window and the fast Fourier transform to obtain the power spectrum of the fluctuation. Squared magnitudes and the products of the computed discrete Fourier transforms were averaged to obtain spectral estimates. The vagal tone monitor was set to evaluate the high-frequency (0.1–0.4 Hz) or the low-frequency (0.03–0.1 Hz) components of heart rate variability. Further, the LF/HF ratio which is considered as an index of the cardiac sympathovagal balance was computed [12].

2.5. Statistical analysis

Results are expressed as mean ± S.E. Data were evaluated by Student's t-test, and a probability level of $P < 0.05$ was taken to be statistically significant.

3. Results

Typical polygraphic recordings of EEG and EMG in a young dog are shown in Fig. 1. Each behavioral state was clearly distinguished and no cataplexy was observed throughout the experimental period. Based on the visual analysis of the polygraph recorder, the time spent in each behavioral state was calculated.

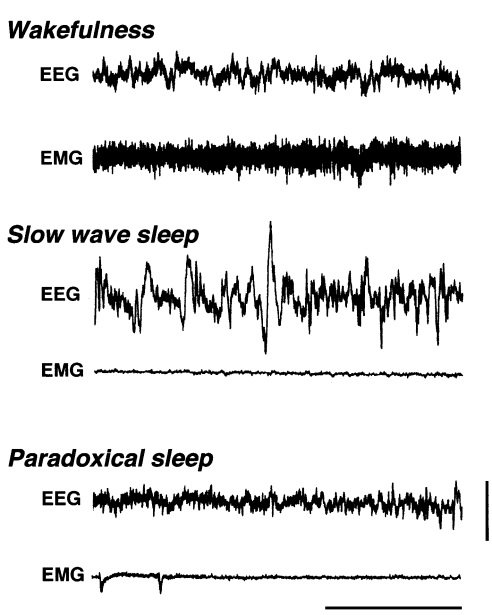

Fig. 1. Polygraphic patterns in each behavioral state in a young dog. In the wakefulness state, EEG showed prominent low amplitude fast activities and EMG showed marked activity. In the slow wave sleep state, EEG showed high amplitude slow activities and EMG showed little activity. In the paradoxical sleep state, EEG showed low amplitude fast activities and EMG showed little activity with transient small discharges. The calibration marks show 5 s and 200 μV.

Examples of 24 h hypnograms for a young and an old dog are shown in Fig. 2. Frequent shifts between wakefulness and SWS were observed in both groups. The hypnogram of the aged dog reveals frequent sleep epochs in the daytime. Whereas the young dogs regularly displayed a smooth transition from wakefulness to SWS and PS, and the waking was maintained for most of the day, except for a nap at around noon. Characteristic changes in the aged dogs were an increase of wakefulness and a decrease of PS in the dark period.

The disturbed quality of the sleep-wake pattern of the aged dogs was reflected in the number of episodes of wakefulness and SWS states as well as in the total amount of these states combined (Table 1). Further, the aged dogs exhibited a substantial decrease in the number of episodes of PS throughout the experimental period.

The time spent in each behavioral state in 24 h was shown in Fig. 3. The plotted graph in the young dog revealed a clear circadian rhythm, which is very similar to those reported by Takahashi et al [23]. Marked difference in the aged dogs was recognized by comparing with that of the young dogs. The peak of sleep time (SWS + PS) in the daytime occurred between 12:00 and 16:00 h in the young dogs, whereas the aged dogs showed fragmented peaks of sleep during the daytime.

These age-related changes in behavior are evident in the total time spent in each state (Fig. 4). In the aged dogs, the amount of SWS significantly ($P < 0.05$) increased in the daytime, and wakefulness had a tendency of increase at night. Total time spent in PS in the aged dogs was significantly reduced to 2.3% ($P < 0.05$) and 2.5% ($P < 0.01$) in the daytime and night, respectively. In contrast, total time spent in wakefulness or PS in the young dogs was very similar to the previous reports [23,27].

Changes in the LF, HF power and LF/HF ratio from the power spectrum analysis of heart rate variability are shown in Fig. 5. LF power was similar in both groups, and was stable throughout the day. Interestingly, the HF power of the aged dogs was augmented in the daytime, and the time course of changes in HF power corresponded to the peaks of sleeping time. In contrast in the young dogs, differences between the HF power in the daytime and in the night were very clear, showing a suppressed HF power in the daytime. Furthermore, the LF/HF ratio, which is an index of the cardiac sympathovagal balance, was suppressed even in the daytime. As a result of suppressed LF/HF ratio, the sympathovagal balance of the aged dogs showed little change throughout the day.

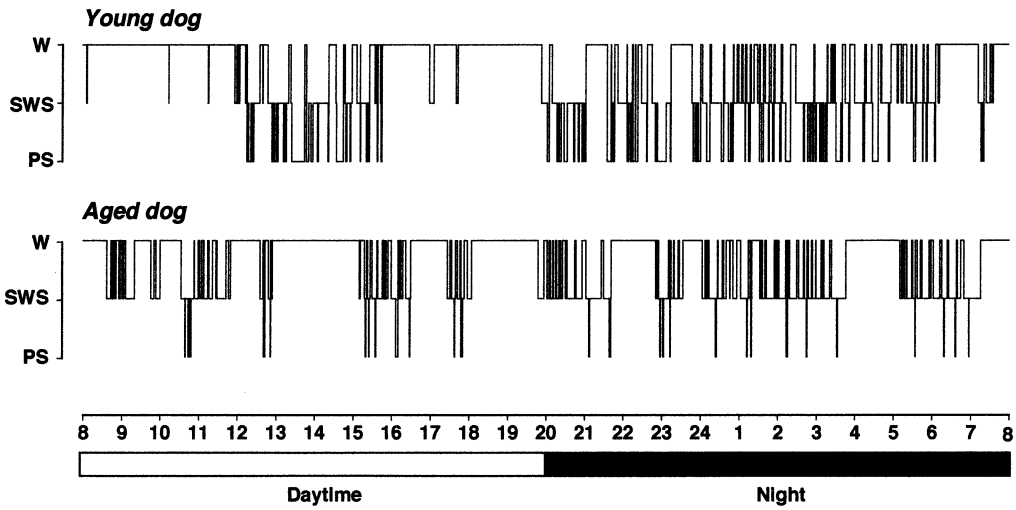

Fig. 2. Typical examples of 24 h sleep plots (hypnograms) for a young and an aged dog. Open and shaded portion of the bar represents daytime and night period, respectively. W; wakefulness, SWS; slow wave sleep, PS; paradoxical sleep.

Table 1
Mean number of episodes for each behavioral state in young and aged dog

	Young dog		Aged dog	
Episode	Daytime	Night	Daytime	Night
Wake	26.2±5.4	42.3±8.6	38.1±7.9	54.8±103
Slow wave sleep	40.5±8.9	60.3±12.7	46.2±9.2	67.4±15.1
Paradoxical sleep	26.3±6.2	53.1±10.2	12.3±3.6	15.9±4.1*
Total	92.±11.8	155.4±32.6	96.4±12.6	136.1±35.3

Value represent means ±S.E.($n=4$). *$P<0.05$ compared with young dog.

4. Discussion

Major alterations of the sleep-wake rhythm in the aged dogs were observed in the time spent in each behavioral state. These included a reduction of PS, an increase in SWS in the daytime, and a decrease in total sleep (SWS and PS) at night. These changes were accompanied sleep epochs in the daytime. Haimov and Lavie [5] suggested that age-related changes in sleep propensity may contribute to the difficulties in initiating sleep and to the early morning awakening in humans. Further, they mentioned that elderly persons complain of daytime drowsiness and difficulties in initiating and maintaining sleep. These patterns of abnormal sleep may be a common phenomenon in human and dogs.

Similar changes in the sleep-wake rhythm were reported in narcoleptic dogs [11]. The narcoleptic dogs displayed marked fragmentation and disruption of the sleep-wake cycle characterized by repeated awakenings and frequent shifts in sleep stages. This previous report [11] demonstrated a severe disturbance of the normal sleep pattern in canine narcoleptics. The nocturnal sleep disturbances seen in canine and human narcoleptics suggest the possibility that a circadian rhythm disturbance is correlated in this disorder [15]. Clinical reports of a disturbed core temperature rhythm for 24 h [17] and a disordered pattern of prolactin and growth hormone secretion [2,7] in narcoleptics further support this possibility. Nishino et al. [18] reported that the cholinergic stimulation in the basal forebrain (BF) triggers cataplexy in canine narcolepsy, and some neurons mediate sleep stage changes after cholinergic stimulation in the BF.

Cholinergic insufficiency in Alzheimer's disease (AD) is associated with reductions in deep non-REM and REM sleep [1]. In addition, the circadian distribution of sleep is affected in AD patients having significant amounts of daytime sleep, and poorly consolidated nocturnal sleep. The aged dogs in the present study did exhibit reductions in PS time and the diurnal distribution of sleep, thus showing a high degree of similarity with AD patients. Further study will be necessary to determine whether the cholinergic insufficiency is associated with the age-related changes of the sleep-wake cycle in the dog.

Some mechanisms have been shown to induce age-related changes in the sleep-wake cycle. Several lines of evidence suggest that the circadian pacemaker oscillates more rapidly and with a lower amplitude in older than in younger animals and human beings. First, the free-running period of the rest-activity rhythm shortens with age in rodents [16,19] and humans [28]. Second, mouse studies have shown an age-related reduction in the amplitude of the rest-activity and sleep-wake cycles [29]. Our findings suggest that the aged dogs have less ability than young dogs to sleep at night, and the daytime sleep compensates for the reduction of night time sleep. In any case, the observation of decreased

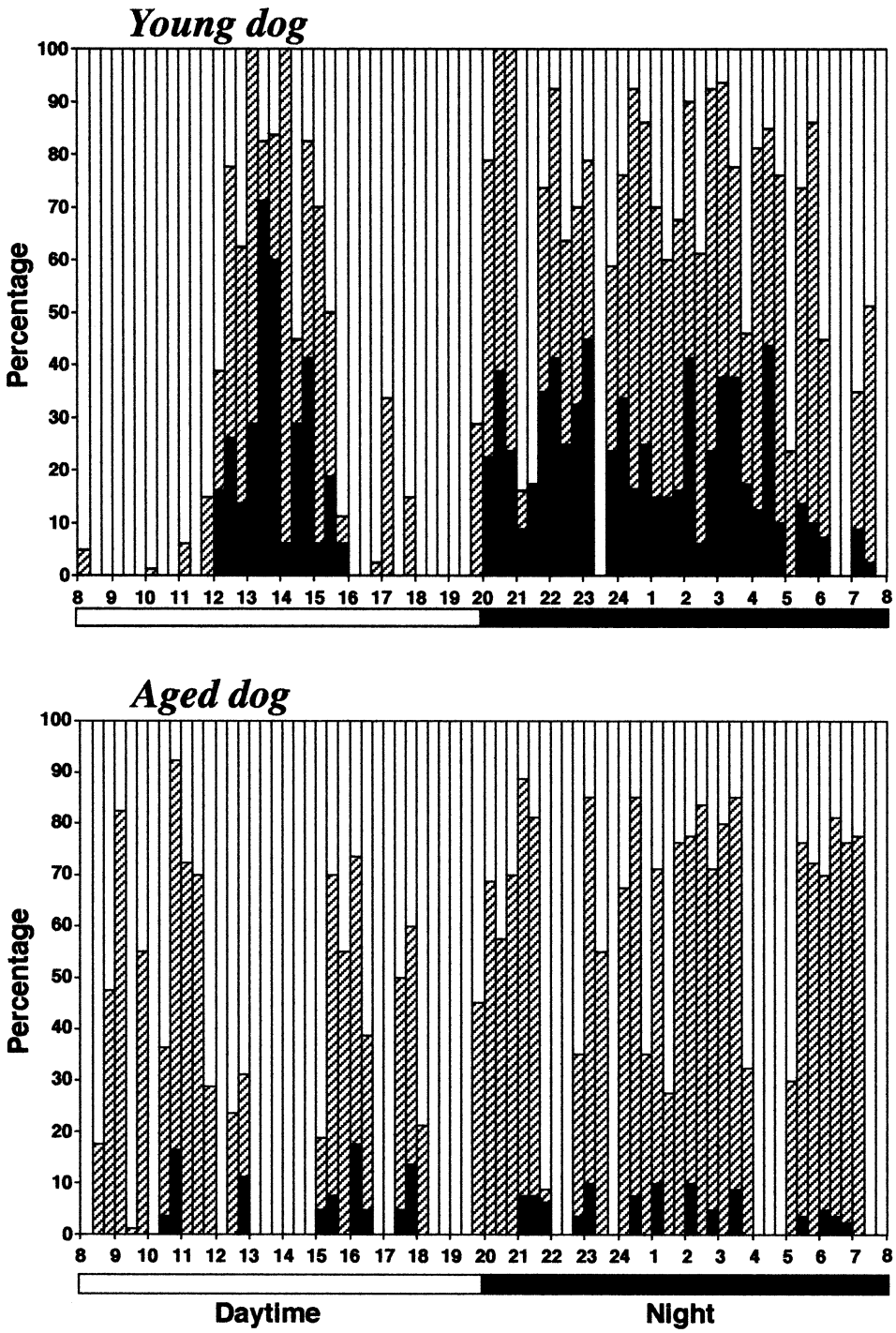

Fig. 3. Typical examples of distribution of the vigilance states waking (open bar), slow wave sleep (shaded bar) and paradoxical sleep (solid bar) in young and aged dogs. The bars under the time scale represent daytime and night periods.

total sleep at night of the healthy aged dogs does not imply that they are at greater risk for excessive daytime sleepiness. The changes in the distribution of sleep and waking in the aged dogs, particular in the daytime, might be explained by a reduction of the amplitude of the oscillation of the circadian pacemaker.

The HF power obtained from heart rate variability is generally thought to reflect primarily parasympathetic nervous function, while the LF power reflects both the sympathetic and parasympathetic nervous functions [10]. Further, the LF/HF ratio is considered as an index of the cardiac sympathovagal balance [12]. In the present study, it is clearly demonstrated that the HF power in the aged dogs was enhanced even in the daytime. This is strikingly different from that in the young dogs. Further, the time course of the enhanced

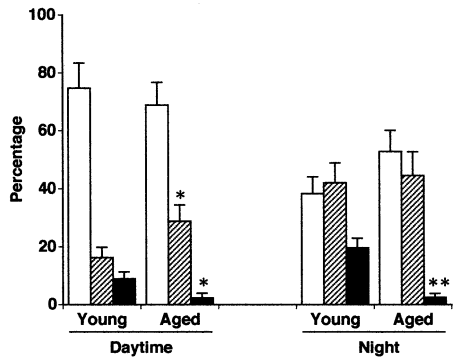

Fig. 4. Changes in percentage of time spent in waking (open bar), slow wave sleep (shaded bar) and paradoxical sleep (solid bar) calculated from polygraphic records in young and aged dogs. Each value represents the mean ± S.E. of 4 dogs. *, ** Significant differences compared with young dog at $P < 0.05$ and $P < 0.01$, respectively.

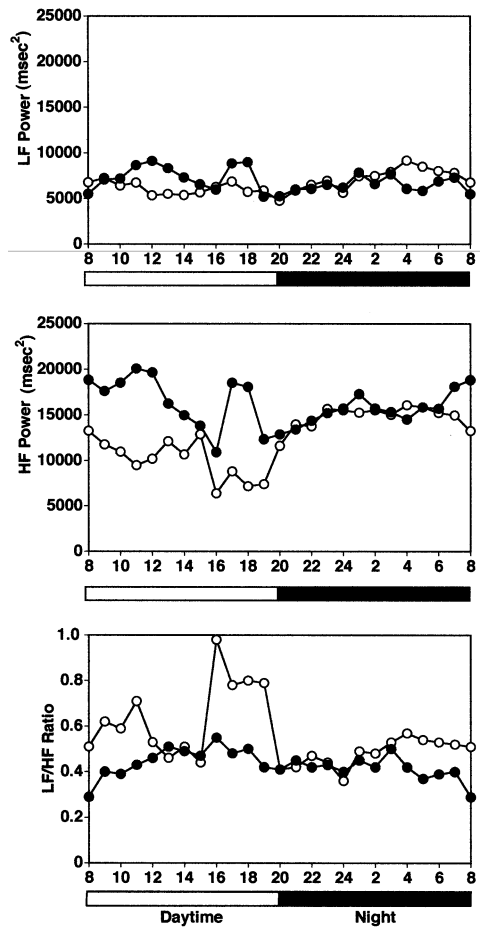

Fig. 5. Changes in values of LF, HF power and LF/HF ratio by power spectral analysis of heart rate variability for 24 h in young (open circle) and aged dogs (solid circle). Dots represent mean values of 4 dogs. The bars under the time scale represent the 12 h light-12 h dark cycle.

HF power was highly correlated with the sleep cycle in the aged dogs. Together with these results, the abnormally augmented parasympathetic nervous tone may reflect the aged-related change in the sleep-wake cycle in the dogs. Further studies, including circadian pacemaker activity and metabolism of neurotransmitters are necessary to clarify the precise mechanisms of the age related changes in the sleep-wake cycle in the aged dogs.

In conclusion, the present study demonstrates the age-related changes in sleep-wake rhythm in the dogs, suggesting that the aged dog is a useful model for an analysis of sleep disorders such as daytime drowsiness, difficulties in initiating and maintaining sleep and reduction of PS in elderly people.

Acknowledgements

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