Neurobiology of Sleep and Circadian Rhythms 1 (2016) 8–18

Contents lists available at ScienceDirect

Neurobiology of Sleep and Circadian Rhythms

journal homepage: www.elsevier.com/locate/nbscr

Research paper

Diurnal changes in core body temperature, day/night locomotor activity patterns, and actigraphy-generated behavioral sleep in aged canines with varying levels of cognitive dysfunction

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A R T I C L E I N F O

Article history:
Received 26 May 2016
Received in revised form
6 July 2016
Accepted 11 July 2016
Available online 13 July 2016

Keywords:
Circadian rhythm
Spatial working memory
Cognition
Dog
Diurnal
Body temperature

A B S T R A C T

Core body temperature (CBT) rhythm, locomotor activity, and actigraphy-sleep were evaluated in geriatric dogs with cognitive dysfunction. Dogs ($n=33$; 9–16 yrs) performed a spatial working memory task and divided into three memory groups: Low, Moderate, and High, with subsequent evaluation of learning and attention. Rectal CBT was recorded 6 times over a 17.5 h period and Actiwatch® activity monitoring system for 5 days while housed indoors with 12 h light/dark schedule. Rhythm of daily activity data was evaluated using the traditional cosinor analysis and generation of non-parametric measures of interdaily stability, intradaily variability, and relative amplitude. CBT differed with time (F (5, 130)=11.36, $p<0.001$), and was the highest at 19:00C. CBT at 19:00 was positively related ($p<0.01$) to memory ($r(31)=0.50$) and 3-domain cognitive performance index (memory, learning, attention; $r(31)=0.39$). Total daytime or night-time activity did not differ between memory groups, but hourly counts at 8:00 were positively related ($p<0.05$) to memory ($r(31)=0.52$), learning ($r(31)=0.36$), and 3-domain cognitive performance index ($r(31)=0.53$). There were no significant differences between age or memory groups for any circadian rhythm measures. Daytime naps were inversely related to memory accuracy ($r(31)=-0.39$; $p<0.05$) and BT at 15:00 ($r(30)=-0.51$; $p<0.01$). Lower peak BT and increased napping may predict some aspects of cognitive performance of working memory, learning, and/or attention processes in these geriatric dogs, but minimal diurnal rhythm disruption of locomotor activity is observed when these cognitive processes decline.

1. Introduction

Many physiological processes oscillate with circadian rhythmicity and are regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus. Circadian regulation can become altered with advanced age and in cognitive disorders or neurodegenerative diseases such as Alzheimer's disease (Harper et al., 2005; van Someren, 2000). Although SCN-mediated circadian timing provides temporal organization to most neurobehavioral, sleep/wake, physiological, and biochemical processes, multiple and divergent synaptic projections from the SCN differentially regulate these circadian rhythms (Fuller et al., 2006). Therefore, dysfunction in the regulation of some circadian processes will not necessarily predict dysfunction of others. Some of the processes that become altered with normal aging in dogs and people include locomotor activity, hormone secretion, thermoregulation, as well as sleep and food intake patterns. Changes in these processes can be a manifestation of many physical and neurological deficits that accumulate over the lifetime.

Dogs, like humans, show age-dependent cognitive decline with accompanying neuropathology, which raises the possibility that dogs may develop a similar syndrome of cognitive dysfunction to that seen in people with cognitive decline and/or dementia. (Head et al., 2008; Milgram et al., 2010). Furthermore, veterinarians can identify a cognitive dysfunction syndrome (CDS) in dogs through behavior-based impairment that becomes progressively more severe with increased age (Bain et al., 2001; Landsberg and Ruehl, 1997; Ruehl et al. 1995; Ruehl and Hart, 1998). Recently, we have demonstrated in aged dogs that several cognitive domains are functionally independent, and an age-related deficit in visuospatial working memory does not predict dysfunction in learning or selective attention processes (Zanghi et al., 2015). Canine cognition, like that of human cognition, subsumes several diverse domains (Milgram et al., 2010; Summers and Saunders, 2012), which

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http://dx.doi.org/10.1016/j.nbscr.2016.07.001
2451-9944/

include, but are not limited to, visuospatial function, learning, working memory and executive function. Yet, it is not clear how deficits in specific cognitive domains are related to dysfunction of various circadian or diurnal rhythms in aged dogs, as it is in humans with neurodegenerative diseases. Therefore, understanding age-related and diurnal changes in behavioral, physiological, and cognitive patterns associated with cognitive dysfunction may offer insight to the development of interventions to partially counteract both canine and human brain aging.

A link between circadian oscillation of core body temperature (CBT) and sleep/wake cycles exists in people, and decreased sleep quality appears to be partially related to age-related changes in thermoregulation (van Someren, 2000). Although circadian oscillation of CBT has been reported in healthy adult dogs (Refinetti and Piccione, 2003), the effect of advanced age and/or cognitive dysfunction on 24-h CBT oscillation has not been reported either independently, or in conjunction with behavioral sleep patterns or locomotor activity data in domestic dogs.

Previous work has established that healthy adult dogs exhibit diurnal locomotor activity/rest patterns (Nishino et al., 1997; Siwak et al., 2003; Tobler and Sigg, 1986; Zanghi et al., 2012) and also show a complex relationship between locomotor activity, age, cognition and housing environment (Siwak et al., 2003). Canine aging has been associated with changes in locomotor activity patterns (Siwak et al., 2002, 2003; Zanghi et al., 2012) and sleep patterns (Takeuchi and Harada, 1986; Zanghi et al., 2013), but not phase shifts of early morning onset or delayed morning onset of locomotor activity (Zanghi et al., 2012). Siwak et al. (2003) observed that young dogs (1–4 yrs old) exhibit higher activity levels than aged dogs (9–14 yrs old), but also reported that cognitively impaired, aged dogs were more active and showed a delayed peak of daytime activity compared to unimpaired, aged dogs. This potential link may parallel age-related activity and cognitive changes observed to be exaggerated in dementias, such as AD (van Someren et al., 1996; Witting et al., 1990), and can be manifested as either hyper- or hypoactivity (Satlin et al., 1991).

In this study, we sought to determine whether cognitive impairment in the dog, using a test of working memory capacity, would be related to dysfunction of diurnal behavioral and physiological rhythms. Therefore, the objective of the study was to evaluate rhythms of locomotor activity, behavioral sleep, and CBT in aged dogs that displayed varying working memory proficiency, with subsequent evaluation of learning and attention performance. Working memory was assessed by performance on a variable delayed non-matching to position (vDNMP) paradigm.

2. Materials and methods

To briefly summarize the experimental design, CBT and locomotor activity were measured in dogs who were also being evaluated for neuropsychological test performance. This cognition data was previously published (Zanghi et al., 2015) and a subset of that neuropsychological test performance data is used here for further analysis of canine cognition and related physiological biomarkers.

2.1. Animals, housing, and feeding regimen

Aged Beagle dogs (*Canis familiaris;* $N=33$; 9–16 yrs) were in good health and adequate body condition, as assessed by a general health evaluation. The veterinary examination included verification of healthy haematology and blood-chemistry parameters. Selection of candidate dogs was also based on their responsiveness on cognitive tests and evidence of impairment during the baseline cognitive testing described below.

Prior to the study, the dogs were housed indoors with exposure to natural light cycles with daily outdoor access. The dogs were housed in groups of two to four per kennel-run (1.5 m wide $\times$ 4.6 m in length) based on compatibility and sex. All dogs were housed in the same kennel-run location with ability to see other dogs in adjacent and opposing runs. Each dog had direct interaction with caretaker staff on daily basis and had access to toys (durable balls and breed-size appropriate chew toys). Two weeks prior to the start of this study, the dogs were switched to a 12-h light-dark cycle with lights on at 7:00 a.m. and off at 7:00 p.m. Dogs were maintained indoors during the study with 30-min of daily group play outside of kennel-run area with caretaker staff and pen-mates. The light-dark cycle was maintained until the completion of the study.

Before the start of the study, dogs were fed individually once daily (Purina ProPlan Adult Maintenance Chicken and Rice formula; Nestlé Purina PetCare, St. Louis, MO) between 8:00 and 10:00 a.m. within their normal housing area. Upon study initiation, dogs were switched to a prototypical complete and balanced dry extruded senior chicken and rice-based formula (Nestlé Purina PetCare, St. Louis, MO) over a 1 week period that contained 7.8% moisture, 28.5% crude protein, 14.3% crude fat, 1.3% crude fiber, and 6.7% ash. The food was formulated to meet or exceed the nutrient requirements outlined by the Association of American Feed Control Officials (AAFCO) guidelines. Food was provided twice daily at 9 a.m. ($\pm$ 30 min) and 6 p.m. ($\pm$ 30 min) with the total daily ration divided equally into two rations and water available *ad libitum*. Total food provided was regularly adjusted to permit the dogs to maintain their body weight over the duration of the study. The study was conducted in accordance with approved Animal Care and Use Committee protocols.

For CBT measurements, dogs were temporarily housed individually in metabolism kennels for 36 h to monitor body temperature, as reported by Refinetti and Piccione (2003) to observe the diurnal variation. Dogs were allowed free access to water and fed on the same schedule while in metabolism kennels. Dogs were allowed exercise with their kennel-run mates for environmental enrichment during the regularly scheduled times over this 36-h period.

2.2. Animals, cognitive test apparatus

The testing apparatus consisted of a wooden box that was approximately 0.609 m $\times$ 1.15 m $\times$ 1.08 m (Milgram et al., 1994) in size and was a modified version of the Wisconsin General Test Apparatus widely used in cognitive assessment of primates. The front contained three height-adjustable gates through which the dog responded. The experimenter was separated from the dog by a plastic partition containing a one-way mirror and a hinged door. The tray was made of Plexiglas and contained either one medial food well and two lateral food wells, or four equally spaced food wells, depending on the task. The food reward was the Pro Plan Adult Wet Dog Food Chicken & Rice Entree (Nestlé Purina Petcare, St. Louis, MO). Approximately, 1 g of the food constituted each reward, resulting in a maximum of 12 g of additional food, depending on the task.

2.3. Cognitive testing schedule

Initiation of a twice-daily feeding regimen was day 1 of the study. Cognitive testing for 60 dogs began 10 days after beginning the twice-daily feeding regimen (days 10–25). All dogs were first trained over 10 days on a spatial memory task using the vDNMP paradigm to assess baseline memory performance (Zanghi et al., 2015). The dogs were then trained over 30 days on a variable object oddity task to assess learning and selective attention.

To briefly summarize the stratification and group size,