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Repeated afternoon sleep recordings indicate first-night-effect-like adaptation process in family dogs

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Summary

The importance of dogs (*Canis familiaris*) in sleep research is primarily based on their comparability with humans. In spite of numerous differences, dogs' comparable sleep pattern, as well as several phenotypic similarities on both the behavioural and neural levels, make this species a most feasible model in many respects. Our aim was to investigate whether the so-called first-night effect, which in humans manifests as a marked macrostructure difference between the first and second sleep occasions, can be observed in family dogs. We used a non-invasive polysomnographic method to monitor and compare the characteristics of dogs' ($N = 24$) 3-hr-long afternoon naps on three occasions at the same location. We analysed how sleep macrostructure variables differed between the first, second and third occasions, considering also the effects of potential confounding variables such as the dogs' age and sleeping habits. Our findings indicate that first-night effect is present in dogs' sleep architecture, although its specifics somewhat deviate from the pattern observed in humans. Sleep macrostructure differences were mostly found between occasions 1 and 3; dogs slept more, had less wake after the first drowsiness episode, and reached drowsiness sleep earlier on occasion 3. Dogs, which had been reported to sleep rarely not at home, had an earlier non-rapid eye movement sleep, a shorter rapid eye movement sleep latency, and spent more time in rapid eye movement sleep on occasion 3, compared with occasion 1. Extending prior dog sleep data, these results help increase the validity of further sleep electroencephalography investigations in dogs.

KEYWORDS

dog model, neuroethology, non-invasive electroencephalography

1 | INTRODUCTION

The dog (*Canis familiaris*) has been proved to be an interesting and valid animal model of human socio-cognitive skills not just at the

behavioural level (Miklósi & Topál, 2013), but also in the area of neurocognitive research, including sleep-related cognition (Bunford, Andics, Kis, Miklósi, & Gácsi, 2017). One prominent line of canine neuroscience literature focuses on awake functioning, mainly using

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functional magnetic resonance imaging (Andics et al., 2016; Berns, Brooks, & Spivak, 2012) and electroencephalography (EEG)-based (ERP) methods (Howell, Conduit, Toukhsati, & Bennett, 2011; Kujala et al., 2013). A somewhat independent line of research investigates the characteristics of the dogs' sleep, mainly building on the fact that the general architecture of human sleep is better approximated by dog sleep, and not by the most commonly used laboratory animals (Toth & Bhargava, 2013). Strong interrelatedness has been discovered between dogs' sleep and awake functioning, including memory consolidation (Iotchev et al., 2017; Kis, Szakadát, et al., 2017) and emotion processing (Kis, Gergely, et al., 2017). Early studies on dogs' sleep focused on neurological conditions (e.g. epilepsy), and used invasive methods on laboratory dogs (Shimazono et al., 1960; Wauquier, Verheyen, Broeck, & Janssen, 1979). Recently, a non-invasive polysomnography (PSG) method has been developed to investigate the sleep architecture of family dogs (Kis, Szakadát, Kovács, et al., 2014), which has been successfully used to monitor natural sleep in dogs as a function of pre-sleep experiences and/or individual differences (Bunford et al., 2018; Kis, Gergely, et al., 2017; Kis, Szakadát, et al., 2017).

However, the basic characteristics of dogs' natural sleep patterns (without pre-sleep treatment) have only been tangentially investigated (Kis, Szakadát, Kovács, et al., 2014), whereas a descriptive analysis of sleep macrostructure was provided only for a single recording session. To gain more insight into the basic mechanisms of natural sleep in family dogs, we conducted three consecutive sleep recordings without affecting the dogs with any pre-sleep activity or handling. Our primary goal was to assess the so-called first-night effect (FNE), a phenomenon well known in human sleep research, which manifests in marked macrostructure differences between the first and second sleep occasions measured by PSG. The major factors that contribute to FNE are unfamiliar surroundings (e.g. sleep laboratory), discomfort and limited mobility caused by electrodes, and psychological pressure of being under observation (Le Bon et al., 2001).

It is a common practice in human sleep studies to consider the first sleeping occasion as an adaptation session and thus discard it from further analysis without direct comparison to the following occasions. Studies that have assessed the differences between the sleep macrostructure of the first versus second night spent in the laboratory have consistently found that FNE is associated with an increased state of alertness, which results in alterations in the sleep pattern: increased number of awakenings and more time spent awake after sleep onset (WASO) leading to less time spent sleeping, increased wakefulness, less rapid eye movement (REM), longer REM latency and increased slow-wave sleep (SWS) latency (Agnew, Webb, & Williams, 1966; Le Bon et al., 2001). Although the phenomenon is called the FNE, human studies indicate that, for certain parameters, more than 1 night is needed to achieve stability, for example REM-related variables (Le Bon et al., 2001; Schmidt & Kaelbling, 1971).

All previous non-invasive dog PSG measurements were either carried out with the exclusion of the first sleep occasion and counterbalancing pre-sleep treatments between the second and third occasions (Bunford et al., 2018; Kis, Gergely, et al., 2017; Kis, Szakadát,

et al., 2017; Kis, Szakadát, Kovács, et al., 2014), or exclusively focused on one-time recordings (Iotchev et al., 2019). This was based on the assumption that, similarly to humans (Agnew et al., 1966; Le Bon et al., 2001), there must be specific differences in dogs' sleep patterns and brain activity between the consecutive sleep occasions. Additionally, some of these studies (Kis, Gergely, et al., 2017) reported a lack of order effect between the second and third sleep occasions, indicating that at that stage, adaptation effects could be of smaller magnitude compared with the effect of pre-treatments used. In light of our scarce knowledge on the number of occasions needed for dogs to adapt to sleep with electrodes on their heads and bodies in a new environment, a more thorough analysis of FNE has become essential.

Age is an important factor that both influences sleep-wake rhythm (Takeuchi & Harada, 2002) and the EEG spectrum of dogs (Kis, Szakadát, Kovács, et al., 2014; Takeuchi & Harada, 2002) and humans (Carrier, Land, Buysse, Kupfer, & Monk, 2001). Thus, in order to gain pure insight into the effect of repeated laboratory testing (PSG recordings) on dogs' sleep structure, variables such as age need to be included as potential confounding factors in the analyses. Moreover, the sleep laboratory is a novel and potentially perceived as a stressful environment, which might have a determinant role in sleep quality (Lima, Rattenborg, Lesku, & Amlaner, 2005; Voss, 2004). As dogs tend to vary with regard to their sleeping habits (frequency of sleeping away from home), it can be assumed that differences will emerge between dogs that rarely versus those that often sleep away from home, making it imperative to control for such environmental factors.

Similarly to humans, dogs' sleeping pattern is sensitive to the timing of sleep: at night-time, dogs tend to sleep more, spend more time in non-rapid eye movement (NREM) and REM and less time in drowsiness, and wake after first drowsiness (WASO 1, for more details on WASO 1 in dogs, see Section 2.5) compared with daytime (Bunford et al., 2018). However, during the day, dogs are also prone to sleep, especially during the afternoon period (Tobler & Sigg, 1986). Thus, for practical reasons (e.g. in order to reach an adequate sample size), all dog studies so far have been based on afternoon sleep recordings. Though we are not aware of any human studies documenting FNE in the context of the afternoon nap, it is plausible to assume that an adaptation effect is also present in repeated afternoon sleep sessions. Further differences compared with human studies arise from the fact that due to practical reasons (availability of dog owners volunteering for the study), dog PSG measurements are not recorded on consecutive days, but with gaps of several days/weeks/months between occasions. The effect of this procedural confound has not yet been addressed but, based on general habituation-dishabituation theory, the time between measurements might interfere with the general adaptation (FNE) effect.

Taken together, prior research into human sleep indicates significant and relevant differences in sleep structure between the first two (and potentially more) sleep occasions. We suggest that in order to run valid comparative EEG studies, it is an important

precondition to examine the process of dogs' adaptation to the PSG test situation. We assumed that human-like FNE could be observed between the first/second and possibly the second/third measurement in dogs, resulting in relatively similar changes in their sleep structure to that of humans, including more intermittent wake time (WASO), decreased sleep efficiency (percentage of time spent sleeping during the 3-hr-long measurement), less REM sleep, longer REM and sleep latencies during the first occasion. We also examined the effects of age and sleep habits on the relevant sleep parameters.

2 | METHODS

2.1 | Subjects

We measured 30 family dogs who attended the canine EEG lab with their owners. Six dogs were excluded either due to the lack of sleep (four dogs) or recording artefacts caused by high muscle tone (two dogs). The 24 dogs whose data were used for the analysis were 7 months–9 years old; nine males and 15 females (14 out of the 15 female dogs had been neutered, and the remaining one female dog was not in heat during any of the recording occasions); from nine different breeds and 10 mutts.

Owners were recruited from the Family Dog Project (Eötvös Loránd University, Department of Ethology) database. All experimental protocols were approved by the Scientific Ethics Committee for Animal Experimentation (Állatkísérleti Tudományos Etikai Tanács) of Budapest, Hungary (number of ethical permission: PE/EA/853-2/2016). The location of the measurements was a fully equipped laboratory for canine EEG measurements at the Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology.

2.2 | Procedure

Participation in the sleep EEG research did not require prior training. All subjects were measured on three occasions at the same location. All the recordings were conducted during the afternoon, with a start time between 12:00 hours and 17:00 hours. For each individual dog, the starting time of the three different nap opportunities was kept within a ± 2 hr interval between recording occasions. Recordings were conducted within 11.03.2017 and 22.12.2018 interval. For one dog all three recordings were conducted within the same season (during autumn); while for others they were spread out from spring to summer $N = 3$; from spring to autumn (first two recordings in spring, third recording in autumn) $N = 8$; from autumn to winter $N = 10$; from summer to autumn $N = 2$. Between occasions 1 and 2, 1–4 weeks passed, while between occasions 2 and 3 for a subgroup ($N = 10$) 3–4 weeks passed, and for the other subgroup ($N = 14$) 5 weeks–6 months

passed. Applying this set-up allowed us to investigate the effect of time elapsed between the recordings.

All measurements were carried out after a relatively active day (i.e. a physically and mentally loaded day due to advanced training, excursion). Though the activity level could be slightly different between dogs, an individual's activity level was the same before all sleeping occasions. Before the measurement, the experimenter explained the process to the owner while the dog could explore the room (5–10 min). Then the owner settled on the mattress with the dog and held the dog's head gently while the experimenter was placing the surface electrodes. During electrode placement, dogs were rewarded using social (e.g. petting, praise) and/or food reward. After the electrode placement and the check of the PSG signals, owners were asked to mute their cell phones and engage in a quiet activity such as reading, watching a movie on a laptop with earphones or sleeping during the measurement. The experimenter left the room and monitored the measurement on a laptop in the adjacent room. In case of the rare event of the malfunction of an electrode, the experimenter replaced or changed the electrode. The canine sleep laboratory is a room with no window, thus an inbuilt heating and air-conditioning system was set to keep the temperature at the same level (about 22°C), and a reading lamp was provided for those owners who wished to read (which was then on for all recordings).

2.3 | PSG placement and monitoring

Sleep was monitored by PSG, which allowed the parallel recording of EEG, electrooculogram (EOG), electrocardiogram (ECG), respiration (PNG) and electromyography (EMG). In this research project, we followed the previously validated PSG method on dogs (Kis, Szakadát, Kovács, et al., 2014) with the single addition of another electrode on the right zygomatic arch. With this new set-up instead of two, four EEG channels and an eye movement channel had been recorded. The two electrodes placed on the right and left zygomatic arch next to the eyes (F8, F7) and the scalp electrodes over the anteroposterior midline of the skull (Fz, Cz) were referred to the G2, a reference electrode that was in the posterior midline of the skull (occiput; external occipital protuberance). The ground electrode (G1) was attached to the left musculus temporalis. ECG electrodes were placed bilaterally over the second rib. See Figure 1 for photo of a dog with electrode placement; Figure 2 for detailed drawing of a dog with the names and exact placement of the electrodes; and Figure 3 for examples of PSG data from the four different sleep stages.

For the recordings, gold-coated Ag/AgCl electrodes were used, secured by Signa Spray Electrode Solution (Parker) and EC2 Grass Electrode Cream (Grass Technologies). The impedance values of the EEG electrodes were kept under 20 k Ω during the recordings. The signals were collected, pre-filtered, amplified and digitized at a sampling rate of 1,024 Hz per channel, using the 25-channel SAM



FIGURE 1 Photo of a dog with electrode placement before the measurement. Electroencephalogram (EEG) and electrooculogram (EOG) electrodes were placed on the scalp, electrocardiogram (ECG) electrodes were placed bilaterally over the second rib, and electromyogram (EMG) electrodes were attached on the musculus iliocostalis dorsi. Respiration was recorded by a respiratory band. Note: during the measurement the owner's hand was not on the dog

25R EEG System (Micromed), and the System Plus Evolution software with second-order filters at 0.016 Hz (high pass) and 70 Hz (low pass).

2.4 | Questionnaire

We sent out an online questionnaire to all participating owners about the sleeping habits of the dogs. This was done based on the assumption that dogs, which sleep frequently outside their home environment, might find it less stressful to sleep in a laboratory. In the questionnaire owners had to rate their dogs on a 0–3 (never; rarely; often; very often) scale on the following statements: How often does the dog sleep (a) in a *novel environment* in the presence of the owner (who is engaged in other activities; work, meeting) during the *afternoon*; (b) not at home but in a *familiar environment* in the presence of the owner (who is engaged in other activities; work, meeting) during the *afternoon*; (c) in a *novel environment* in the presence of the

owner *at night*; (d) not at home but in a *familiar environment* in the presence of the owner *at night*?

Most owners reported that their dogs never or rarely slept not at home in a familiar (79%) or new environment (100%) during the night. Moreover, most owners (75%) reported that their dogs never or rarely slept in the afternoon in a new environment. Therefore, only the second question yielded reasonable variability in responses, therefore we included it as a factor in our analysis, lumping never and rarely responses ($N = 0 + 10$; mean age = 4.8 ± 2.2) and often and very often responses ($N = 10 + 4$; mean age = 4.9 ± 2.9) so that in the end we had two categories for “sleep habits”: rarely sleeping away from home (RSAH); and often sleeping away from home (OSAH).

2.5 | Data analysis

Sleep recordings were visually scored in accordance with standard criteria (Berry et al., 2015), adapted for dogs (Kis, Szakadát, Kovács, et al., 2014). A self-developed program (by Ferenc Gombos; Fercio's EEG Plus, 2009–2019) was used to analyse and export data. The recordings were manually scored, and the program provided data for exporting macrostructural variables. This manual coding reliably identifies the stages of wake, drowsiness, NREM and REM in dogs (Kis, Szakadát, Kovács, et al., 2014). A notable difference in the canine sleep stage scoring, compared with human studies, arises from the fact that in dogs there is a stage called drowsiness that bears characteristics of both human Stage 1 NREM sleep and quiet awake. Drowsiness is characteristic of insectivore and carnivore mammals (including dogs), as in these taxa the transition from wakefulness to sleep is not as clear as in humans (Zepelin et al., 2005). Due to this difference, we used two approaches/measures to determine WASO, sleep latency and REM latency. In case of WASO and REM latency, WASO 1 and REM latency 1 were measured from the first drowsiness episode, while WASO 2 and REM latency 2 were measured from the first NREM episode. In case of sleep latency, sleep latency 1 was measured until the first drowsiness

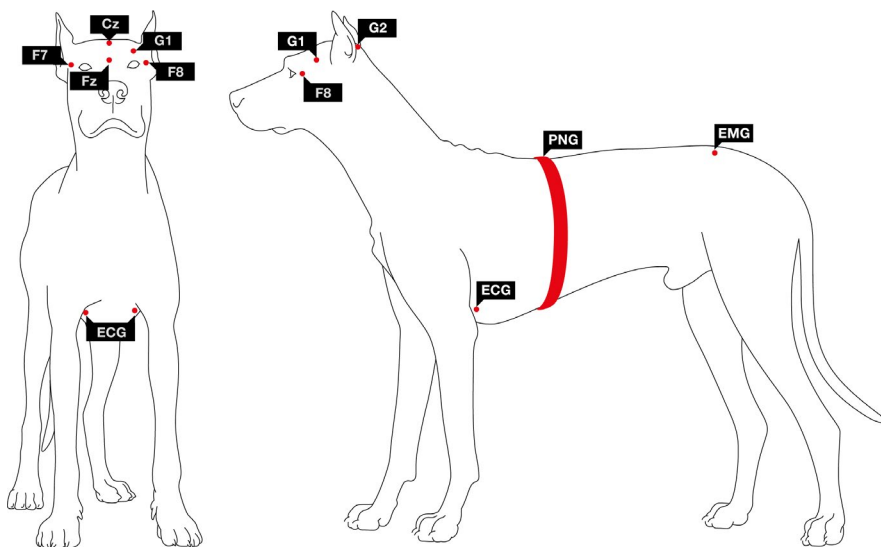


FIGURE 2 Placement of the electrodes and the respiratory belt (Fz-Cz: frontal and central midline; F7-F8: right and left electrodes placed on the zygomatic arch; G2: reference electrode; G1: ground electrode; ECG: electrocardiographic electrodes; EMG: electromyography electrodes; PNG: respiratory belt – respiratory inductance plethysmography)

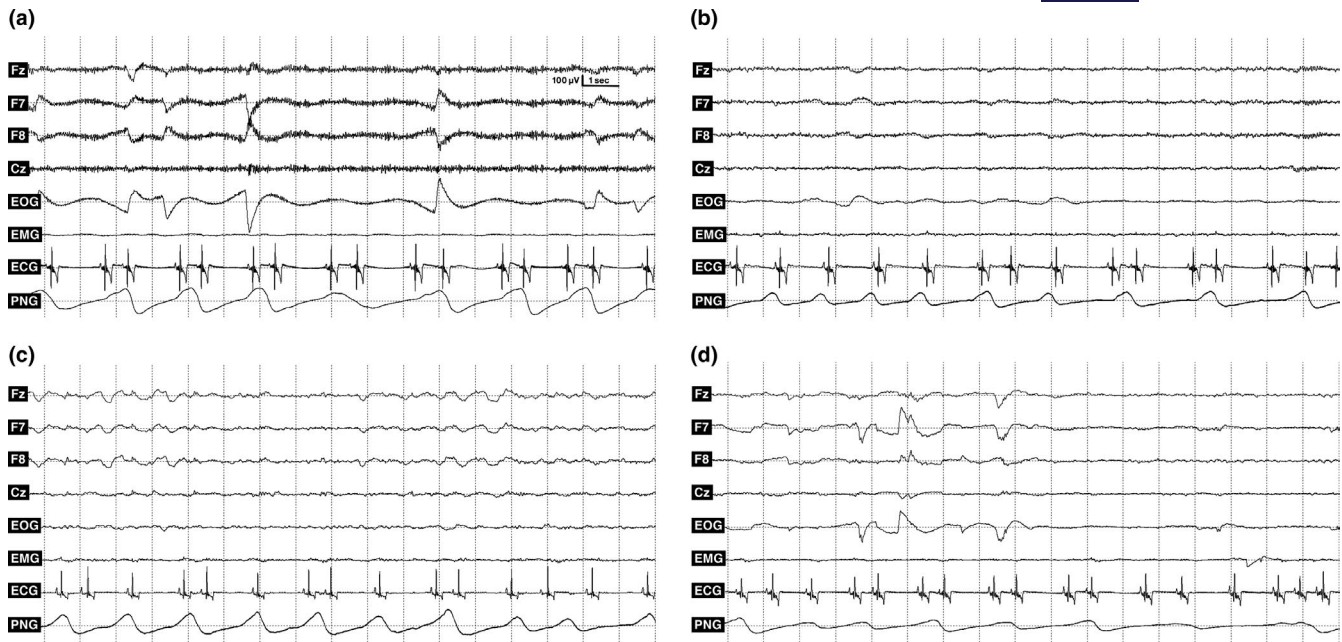


FIGURE 3 Representative polysomnographic (PSG) traces from the sleep stages of (a) wake, (b) drowsiness, (c) non-rapid eye movement (NREM), (d) rapid eye movement (REM)

episode, while sleep latency 2 was measured until the first NREM episode. It also needs to be noted that in dog sleep research the different stages of NREM are not distinguished, but handled uniformly as SWS or NREM sleep (Kis, Szakadát, Kovács, et al., 2014).

2.6 | Analytic plan

For all statistical models the 10 dependent macrostructural variables of interest were: sleep efficiency (the percentage of time spent asleep: drowsiness + NREM + REM during the 3-hr-long measurement), the duration of time spent awake after the first epoch scored as drowsiness (WASO 1) and NREM (WASO 2) sleep, the latency to first drowsiness (sleep latency 1) and NREM (sleep latency 2) sleep, the proportion of time spent in drowsiness, NREM and REM sleep, the latency to REM sleep after drowsiness (REM latency 1) and NREM (REM latency 2) sleep.

To measure the effect of the time elapsed between occasions, we ran separate generalized linear mixed models, where the subject ID was included as a random factor, the macrostructural variables were entered as targets, and time intervals between occasions were entered as fixed factors.

To measure FNE, the difference between sleep macrostructure (for the above-described 10 variables of interest) on sleep occasions 1, 2 and 3 was analysed with generalized linear mixed models with backward elimination. Statistical analyses were performed using IBM's SPSS 25.0 software. The subject ID was included as a random factor. The macrostructural variables were entered as targets, whereas occasion, dogs' sleep habits, age (in years), and the interaction of occasion and sleep habits were entered as fixed factors.

In case of sleep and REM latencies the statistical analyses were conducted in R 3.6.1 (R Core Team, 2014). These variables

were analysed using Mixed Effects Cox Models (R package 'coxme'; Therneau, 2015), with occurrence of REM sleep as terminal event. The subject ID was included as a random factor. In all initial models, the effect of occasion, sleep habits, age (in years), and interaction of occasion and sleep habits were included as fixed factors.

3 | RESULTS

Elapsed time between occasions had no effect on the sleep variables (all $p > .05$), thus we did not include it in further analyses.

3.1 | Sleep efficiency

Occasion had an effect on sleep efficiency ($F_{2,65} = 19.874$, $p < .001$), which was greatest on occasion 3. Pairwise post hoc analysis revealed a difference between occasions 1 and 3 ($p < .001$) and occasions 2 and 3 ($p = .019$), but no difference between occasions 1 and 2 ($p = .111$; Figure 4a). Dogs' sleep habits had an occasion-specific effect on sleep efficiency ($F_{2,65} = 3.655$, $p = .031$): OSAH dogs slept more during occasion 1 compared with RSAH dogs ($p = .016$; Figure 6). Moreover, age had a main effect on sleep efficiency: older dogs slept more, compared to younger dogs ($F_{(2,65)} = 8.382$, $p = .005$).

3.2 | WASO 1 and WASO 2

Occasion affected WASO 1 ($F_{2,67} = 5.344$, $p = .007$). More specifically, dogs spent less time awake after the first drowsiness episode

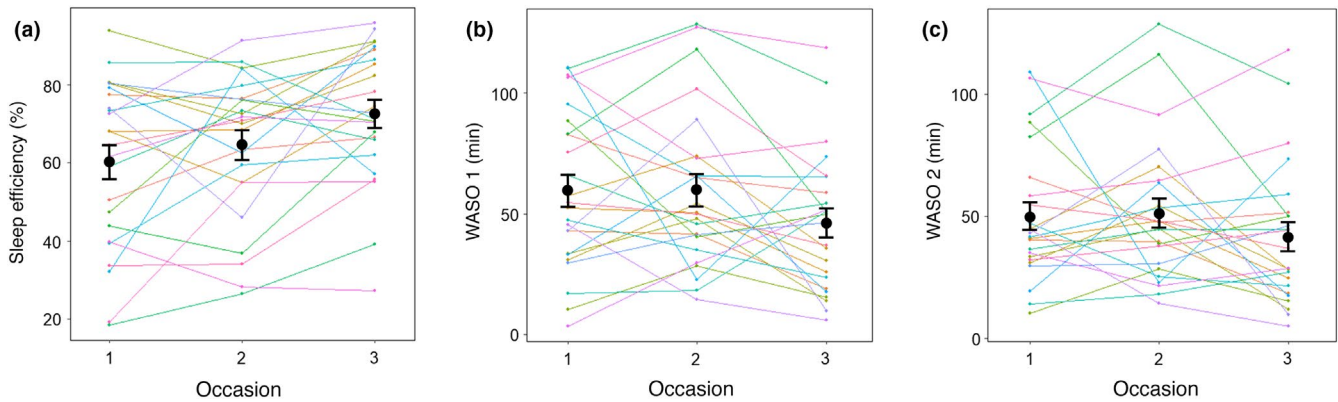


FIGURE 4 The effect of occasion on (a) sleep efficiency, (b) wake after first drowsiness and (c) wake after first non-rapid eye movement (NREM) episode. Note: sleep efficiency, wake after first drowsiness and NREM sleep in dogs individually are indicated with coloured lines; mean values (\pm SE) are indicated with black

on occasion 3, compared with occasion 1 ($p = .013$) and occasion 2 ($p = .05$), while occasion 2 did not differ from occasion 1 ($p = .989$; Figure 4b). Age affected WASO 1: older dogs spent less time awake after their first drowsiness episode, compared to younger dogs ($F_{2,67} = 5.658, p = .020$). Moreover, dogs' sleep habits had a tendency effect ($F_{2,67} = 3.725, p = .058$): OSAH dogs spent less time awake, compared to RSAH dogs.

Occasion showed a trend on WASO 2 ($F_{2,68} = 2.943, p = .059$; Figure 4c). In addition, age had a main effect on WASO 2 ($F_{1,68} = 5.580, p = .021$): older dogs spent less time awake after their first NREM episode, compared with younger dogs. Dogs' sleep habits showed no effect ($F_{1,67} = 2.580, p = .113$).

3.3 | Sleep latency 1

The Cox model proved to be significant in case of sleep latency 1, and occasion had a main effect ($\chi^2_3 = 25.65; p < .001$; AIC = 19.65). Dogs reached drowsiness sleep later on occasion

1, compared with occasions 2 ($\exp(\beta) = 0.379, z = -2.82, p = .001$) and 3 ($\exp(\beta) = 0.243, z = -3.89, p < .001$), but no difference was found between occasions 2 and 3 ($\exp(\beta) = 0.384, z = -1.34, p = .375$; Figure 5a). Moreover, age and sleep habits had no effect (all $p > .05$).

3.4 | Sleep latency 2

The Cox model proved to be significant in case of sleep latency 2, including an interaction of occasion and sleep habits ($\chi^2_6 = 23.46; p < .001$; AIC = 11.46). RSAH dogs reached NREM sleep later on occasion 1, compared with occasions 2 ($\exp(\beta) = 0.251, z = -2.56, p = .028$) and 3 ($\exp(\beta) = 0.135, z = -3.53, p = .001$), while occasion 2 did not differ from occasion 3 ($\exp(\beta) = 0.54, z = -1.24, p = .433$). Moreover, RSAH dogs reached NREM sleep later on occasion 1, compared with OSAH dogs ($\exp(\beta) = 0.245, z = -2.019, p = .043$; Figure 5b). Age showed no effect ($\exp(\beta) = 1.087, z = 0.81, p = .42$).

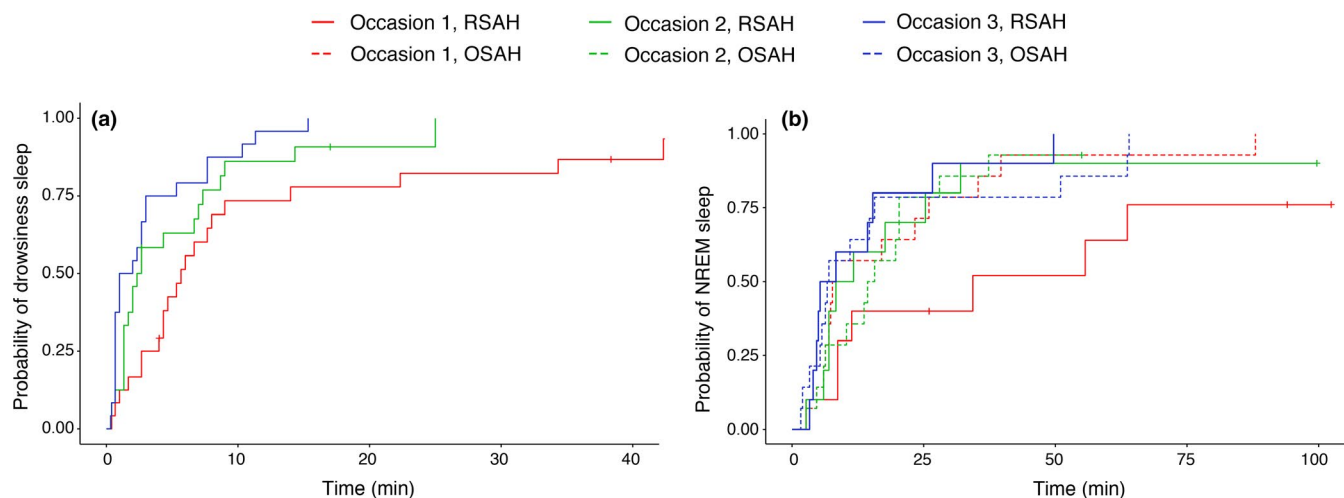
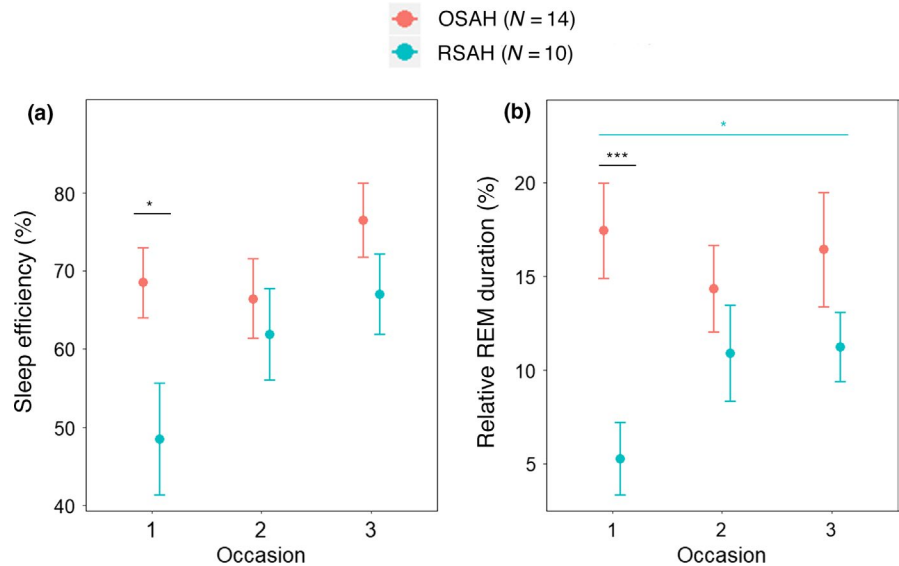


FIGURE 5 The effect of occasion on sleep latency 1 (a); and occasion-specific effects of dogs' sleep habits on sleep latency 2 (b)

FIGURE 6 Occasion-specific effects of dogs' sleep habits on (a) sleep efficiency (mean \pm SE) and (b) relative rapid eye movement (REM) duration (mean \pm SE). The effect of occasion on rarely sleeping away from home (RSAH) dogs (mean \pm SE) (b). * $p < .05$; *** $p < .001$



3.5 | Relative drowsiness duration

Occasion, age and dogs' sleep habits did not influence the relative duration of drowsiness (all $p > .05$).

3.6 | Relative NREM duration

Occasion, age and dogs' sleep habits did not influence the relative duration of NREM sleep (all $p > .05$).

3.7 | Relative REM duration

Occasion did not influence the relative REM duration ($F_{2,66} = 1.005$, $p = .372$), but the dogs' sleep habits had a significant main effect ($F_{2,66} = 8.070$, $p = .006$): OSAH dogs spent more time in REM sleep compared with the RSAH dogs. A significant interaction of occasion and sleep habits on relative REM duration was also found

($F_{2,66} = 3.265$, $p = .044$); on occasion 1, OSAH dogs spent more time in REM sleep compared with RSAH dogs ($p < .001$; Figure 6). Age had no effect ($F_{2,65} = 0.321$, $p = .573$).

As OSAH dogs did not seem to show significant FNE, we ran an additional model to assess the effect of occasion in relative REM duration on RSAH dogs. We found a main effect of occasion ($F_{2,27} = 13.116$, $p < .001$), that is, RSAH dogs had less relative REM duration on occasion 1, compared with occasion 3 ($p < .001$), but no difference between occasions 1 and 2 ($p = .19$), and between occasions 1 and 3 ($p = .92$; Figure 6b, blue line).

3.8 | REM latency 1

The Cox model proved to be significant in case of REM latency 1, including an interaction of occasion and sleep habits ($\chi^2_6 = 23.8$; $p = .005$; AIC = 11.8). In the subgroup of RSAH dogs we revealed longer REM latency on occasion 1 compared with occasions 2 ($\exp(\beta) = 0.236$, $z = -2.19$, $p = .03$) and 3 ($\exp(\beta) = 0.239$, $z = -2.34$,

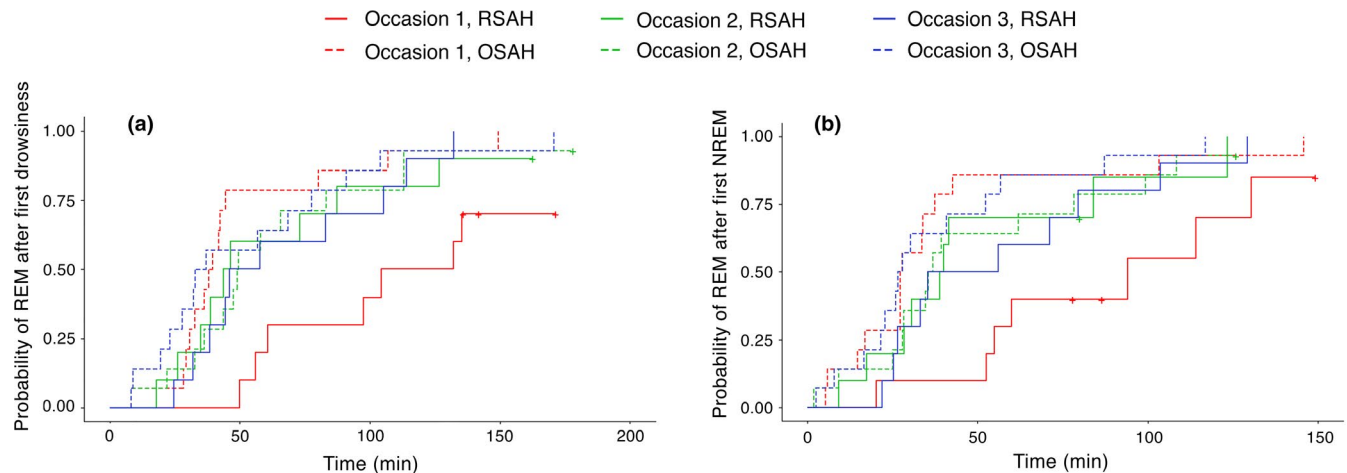


FIGURE 7 Occasion-specific effects of dogs' sleep habits on rapid eye movement (REM) latency 1 (a) and 2 (b)

$p = .02$), but no difference between occasions 2 and 3 ($\exp(\beta) = 0.013$, $z = 0.027$, $p = .99$). Moreover, RSAH dogs reached REM sleep later on occasion 1, compared with OSAH dogs ($\exp(\beta) = 0.123$, $z = -3.24$, $p = .001$; Figure 7a). Age had no effect ($\exp(\beta) = 1.049$, $z = -0.53$, $p = .59$).

3.9 | REM latency 2

The Cox model proved to be significant in case of REM latency 2, including an interaction of occasion and sleep habits ($\chi^2_6 = 20.29$; $p = .002$; AIC = 8.29). In the subgroup of RSAH dogs we neither revealed difference between occasions 1 and 2 ($\exp(\beta) = 0.315$, $z = -2.043$, $p = .10$), nor between occasions 2 and 3 ($\exp(\beta) = 0.951$, $z = -0.100$, $p = .99$), but dogs tended to have longer REM latency on occasion 1, compared with occasion 3, although the effect did not reach significance ($\exp(\beta) = 0.300$, $z = -1.93$, $p = .054$). Moreover, RSAH dogs reached REM sleep later on occasion 1, compared with OSAH dogs ($\exp(\beta) = 0.180$, $z = -2.807$, $p = .005$; Figure 7b). Age had no effect ($\exp(\beta) = 1.017$, $z = -0.20$, $p = .84$).

4 | DISCUSSION

A complex pattern of differences was revealed between sleep occasions when conducting repeated afternoon sleep recordings in family dogs. These adaptation effects on sleep macrostructure present both similarities to and differences from the FNE phenomena described in humans when conducting sleep recordings on consecutive nights. Dogs experience (sleep habits) and age also seem to affect the sleep architecture, which parallels human findings.

Sleep occasion had an effect on dogs' sleep macrostructure; however, contrary to humans (Agnew et al., 1966; Le Bon et al., 2001), in dogs most significant differences were found not between the first two occasions, but between occasions 1 and 3. One possible explanation for the mostly "second-night" instead of the "first-night" effect might be the variation in dogs' sleeping habits (frequency of sleeping away from home). These findings are in line with human studies suggesting that a novel and potentially dangerous environment has a determinant role in sleep quality, for example reducing the time spent in REM sleep (Lima et al., 2005). With NREM-REM cycles, the low and high arousal thresholds alternate, and REM sleep with high arousal threshold results in low behavioural awareness and high vulnerability to dangerous surroundings (Lima et al., 2005; Voss, 2004). In human studies, a reverse version of the FNE has also been reported; in contrast to healthy subjects, in whom the novel environment results in lower quality of sleep (Agnew et al., 1966), in patients with insomnia the awareness of being watched had a promoting effect on sleep quality, presumably because the environment was interpreted as more secure (Hauri & Olmstead, 1989). Previous studies on monkeys and laboratory rats also found that sleep architecture (e.g. sleep efficiency, number of arousals and proportion of time spent in REM) was sensitive to the perceived security of the

environment (e.g. laboratory rats were exposed to a laboratory cat under various conditions; Bert, Balzamo, Chase, & Pegram, 1975; Broughton, 1973). Our results regarding REM-related variables, sleep latency and sleep efficiency suggest that dogs that rarely sleep away from home are more sensitive to the new test situation (sleeping in a new environment with electrodes) compared with dogs that often sleep away from home.

However, this does not explain the lack of a linear habituation process over the sleep occasions at the individual level. Dogs are known to show individual-level variation that outnumbers humans' by several magnitudes both in morphological and behavioural features (McGreevy et al., 2013), as well as physiological parameters (Bálint et al., 2019). Such individual variations might mask a linear habituation process, but as we did find differences between occasions (1 and 3), it is unlikely that the noise caused by individual variability would produce such a pattern.

Prior human FNE studies that showed convincing data on FNE were conducted on 43 (Agnew et al., 1966), 26 (Le Bon et al., 2001) and 12 (Lorenzo & Barbanoj, 2002) subjects on consecutive nights. Our data were obtained from a sample of 24 dogs, which – in the light of the remarkable individual differences – might not be large enough. Moreover, due to practical reasons, our measurements were conducted neither on three consecutive days nor during the night. Although we cannot exclude that the relatively great time intervals between the measurements interfered with our results, the present data do not provide statistical evidence for the effect of the elapsed time between occasions. However, as our set-up followed that used in dog PSG studies, the minimum time elapsed between recordings was 1 week, thus our findings regarding this circumstance might not be extendable to a situation where recordings are performed on consecutive nights. Lorenzo and Barbanoj (2002) collected data on 12 nights, with a minimum of 1 month between three periods, and one period consisted of 4 consecutive nights. They found that the FNE was only present in the first night of the first period (called the "very first night"). This study is in line with earlier data obtained in a research implying that FNE might last for more than 1 night, specifically, REM-related parameters are more sensitive and their stability process might extend up to 4 nights (Le Bon et al., 2001). Interestingly, we found FNE in REM latency, but marked differences were also present between occasions 1 and 3. These findings strengthen the assumption that REM-related parameters need more sleep occasions to stabilize.

Previous studies have already documented that several sleep parameters including EEG spectrum (Kis, Szakadát, Simor, et al., 2014) and sleep spindles (Iotchev et al., 2019) co-vary with age. Here we found that older dogs slept more and spent less time awake after the first drowsiness and NREM sleep. The same macrostructural variables were also suggested in humans to be a marker of age-related sleep changes (Carrier, Monk, Buysse, & Kupfer, 1997), but the relationship is opposite to the one we found in dogs. Human studies have also documented an age-specific effect on FNE, for example it has been reported that children and elderly need 3 instead of 2 nights to adapt (Schmidt & Kaelbling, 1971). No indication of an interaction between age and occasion was found here in dogs.

In sum, our findings indicate that in case of dogs' afternoon sleep recordings, the effect of adaptation during the first occasion is considerably smaller than what we expected based on the human literature. However, the human FNE literature is based on data from consecutive night-time recordings, we could not find any study investigating adaptation effects for afternoon recordings in humans. Most macrostructural differences in our study were detected between sleep occasions 1 and 3, which raises issues for future dog PSG research. Alternatively, the adaptation sleep might not be necessary (considering the few significant effects between occasions 1 and 2). It is, however, imperative to control for dogs' sleep habits. Future research – evaluating the effects of, for example attachment, personality and sensitivity – needs to confirm that a simplified measurement procedure without adaptation and/or the inclusion of experienced dogs is indeed feasible.

It has been suggested that the way FNE manifests in humans at the individual level could be used as a diagnostic criterion in certain sleep disorders and psychiatric conditions (e.g. a more pronounced FNE can be observed in patients with idiopathic nightmares; Kis, Szakadát, Simor, et al., 2014), while a less pronounced FNE is characteristic of patients with depression (Toussaint, Luthringer, Staner, Muzet, & MacHer, 2000). Considering that the family dog is increasingly recognized as a model for human neuropsychiatric conditions, including obsessive-compulsive disorder (Ledford, 2016), autism (Topál, Román, & Turcsán, 2019), and sleep disorders, like narcolepsy (Ripley, Fujiki, Okura, Mignot, & Nishino, 2001), sleep-disordered breathing (Hinchliffe, Liu, & Ladlow, 2019), our findings might open up new directions for the investigations of the links between environmental factors and brain mechanisms underlying cognitive (dys)functions, which could help better understand complex dog and even human phenotypes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, V. R., A. K., P. S., R. B. and M. G.; methodology, V. R., A. K., P. S., R. B. and M. G.; software, F. G.; validation, V. R., A. K.

and M. G.; formal analysis, V. R. and A. K.; investigation, V. R.; data curation V. R.; writing – original draft, V. R.; writing – review and editing, all authors; visualization, V. R.; supervision, M. G.; funding acquisition, M. G.

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