

## **Development of a non-invasive polysomnography technique for dogs (*Canis familiaris*)**

Anna Kis<sup>a,b\*</sup>, Sára Szakadát<sup>a,c,d\*</sup>, Enikő Kovács<sup>a</sup>, Márta Gácsi<sup>e</sup>, Péter Simor<sup>c</sup>, Ferenc Gombos<sup>f</sup>, József Topál<sup>b</sup>, Ádám Miklósi<sup>a,e</sup>, Róbert Bódizs<sup>d,f</sup>

<sup>a</sup> Department of Ethology Eötvös University, H-1117 Pázmány P. stny. 1/c Budapest, Hungary

<sup>b</sup> Research Centre for Natural Sciences, Hungarian Academy of Sciences, H-1132 Victor Hugo u. 18-22. Budapest, Hungary

<sup>c</sup> Department of Cognitive Science, Budapest University of Technology and Economics, H-1111 Egy József u. 1. Budapest, Hungary

<sup>d</sup> Institute of Behavioural Sciences, Semmelweis University, H-1089 Nagyvárad tér 4. Budapest, Hungary

<sup>e</sup> MTA-ELTE Comparative Ethology Research Group, H-1117 Pázmány P. stny. 1/c Budapest, Hungary

<sup>f</sup> Department of General Psychology, Pázmány Péter Catholic University, H-2087 Egyetem u. 1. Piliscsaba, Hungary

\* These two authors have contributed equally to this work.

Correspondence should be addressed to AK (email: [vargane.kis.anna@ttk.mta.hu](mailto:vargane.kis.anna@ttk.mta.hu), tel.: +36 1 3812179, fax: +36 1 3812180, postal address: H-1117 Pázmány P. stny. 1/c Budapest, Hungary) and SS (email: [szurtrikk@gmail.com](mailto:szurtrikk@gmail.com))

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## **Abstract**

Recently dogs (*Canis familiaris*) have been demonstrated to be a promising model species for studying human behavior as they have adapted to the human niche and developed human-like socio-cognitive skills. Research on dog behavior, however, has so far almost exclusively focused on awake functioning.

Here we present a self-developed non-invasive easily replicable canine polysomnography method. N=22 adult pet dogs (with their owners present) and N=12 adult humans participated in Study I. From these subjects N=7 dogs returned on two more occasions for Study II.

In Study I. we give a descriptive analysis of the sleep electroencephalogram of the dog and compare it to human data. In order to validate our canine polysomnography method in Study II. we compare the sleep macrostructure and the EEG spectrum of dogs after a behaviorally active versus passive day.

In Study I. we found that dogs' sleep EEG resembled that of human subjects and was generally in accordance with previous literature using invasive technology. In Study II. we show that similarly to previous results on humans daytime load of novel experiences affects the macrostructural and spectral aspects of subsequent sleep.

Our results validate the family dog as a model species for studying the effects of pre-sleep activities on the EEG pattern under natural conditions and thus broaden the perspectives of the rapidly growing fields of canine cognition and sleep research.

**Keywords:** Dog; *Canis familiaris*; Polysomnography; Day-time experience

## **Highlights**

We present a non-invasive, easily replicable, canine polysomnography method

The method is validated by direct comparison of sleep EEG spectrum to human data

Dogs' sleep EEG spectrum is related to their age

Macrostructural and spectral aspects of dogs' sleep are affected by daytime activity

## **1. Introduction**

Although the intertwined nature of sleep and awake states is widely accepted, there is still no unifying and quantitative theory of sleep, and its universal role in information processing across species is also debated (Siegel 2009). The investigation of mammalian sleep within a comparative framework and a unified methodology would thus be indispensable. The dog (*Canis familiaris*) has been proposed to be a promising model species for studying the evolution of human cognition by the means of a comparative method (Hare et al. 2002; Miklósi and Topál 2013) because it has adapted to the human niche and developed human-analogue socio-cognitive skills. However research on dog behavior has so far focused almost exclusively on awake functioning. Recent advances in the field have attempted to complement behavioral data with recording dog electroencephalogram in order to study awake brain activity, but these techniques were either minimally invasive (used needle electrodes: Howell et al. 2011, 2012) or required extensive prior training (1.5 years: Kujala et al. 2013; Törnqvist et al. 2013).

The non-invasive canine polysomnography method we describe here can be easily applied to naive pet dogs and thus enables the study of the dog as a new natural model of sleep research. As the method has been developed following the recording technique used in human studies it also allows for a more direct comparison to human data, as opposed to rodent experiments.

## **2. Ethic statement**

No special permission for use of pet dogs in such non-invasive studies is required in Hungary. The relevant committee that allows conducting research without special permissions regarding animals is: University Institutional Animal Care and Use Committee. (UIACUC, Eötvös Loránd University, Hungary). All owners volunteered to participate in the study. The person shown in the Fig. 1a gave written consent to the publication of the photo. The human study protocol was approved by the Ethical Committee of the Semmelweis University. Subjects volunteered to participate without monetary compensation and provided informed consent before the onset of the experiment.

## **3. STUDY I.**

### **3.1. Methods**

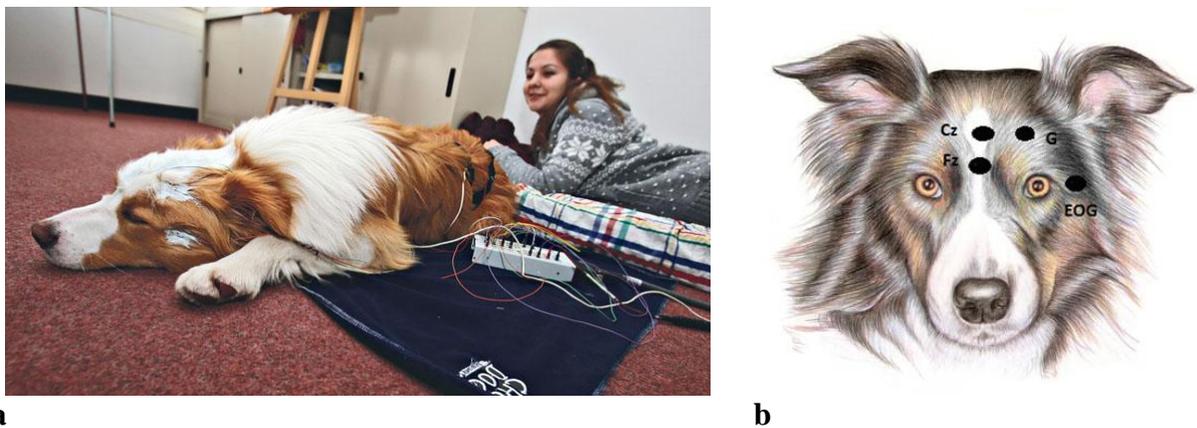
Our subjects were N=22 privately owned adult (1-8 years old) pet dogs (9 males, 13 females) from 8 different breeds (5 Border Collies, 4 Golden Retrievers, 1 Belgian Shepherd, 1 Border Terrier, 1 Labrador Retriever, 1 Miniature Schnauzer, 1 Puli) and 8 mongrels with highly

variable head shape and size (7-32 kg of weight). There were no specific requirements for participation except that dogs had to be older than 1 year. As a reference group, 12 young adult (23-34 years old, mean age:  $26.92 \pm 3.00$ ) human subjects were also included in the study (5 men, 7 women).

All subjects participated in a 3-hour-long sleeping occasion (an afternoon napping). The timing of the recording could vary depending on the preferences of the participating dog owners and human subjects, but was restricted to the period between 12 pm and 6 pm as (apart from night time) dogs, similarly to humans, show the highest propensity to sleep during the afternoon (Takahashi et al. 1972). The sleep laboratory was equipped as an ordinary room in the Department of Ethology, ELTE, Budapest. There was a mattress on the floor with a blanket next to it. Owners could decide whether they preferred their dog to sleep on the mattress with them or on the floor next to them. Windows in the room were covered with curtains to provide constant light conditions. In case of the canine subjects after a 5-10 minutes exploration and familiarization period the owner took place on the mattress and assisted the two experimenters throughout the process of fixing surface attached electrodes onto the dog. The dog was rewarded with food during electrode placement if the owner deemed it necessary; social reinforcement (praise, petting) was used in all cases. While the dog was resting or sleeping the owner watched a movie with an earphone and was asked to stay quiet and still in the mattress (**Fig. 1a**). Similarly to the canine subjects, after a 5-10 minutes familiarization period human participants were asked to take place on the mattress for the process of fixing surface attached electrodes on them.

Sleep was monitored by polysomnography (PSG) that allows the parallel recording of several physiological variables – such as neural oscillations (EEG), eye movements (EOG), muscle tone and movements (EMG), heart rhythm (ECG) and respiration patterns – during sleep. The canine PSG technique was developed following the methodology of human PSG studies. Prior to the acceptance of the final design we conducted pilot studies on 3 dogs (7-9 years old; 1 Belgian Shepherd, 1 Sheltie, 1 mongrel; all males) to find the most efficient setup and placement of the electrodes. When all of our a priori articulated criteria (production of clear and interpretable signal; impedances below 15 k $\Omega$ ; both NREM and REM phase during the recording interval) were met, our PSG design (**Fig. 1b**) was accepted and applied identically for both dogs and humans. Surface attached scalp electrodes were placed over the anteroposterior midline of the skull (Fz, Cz) – similarly to the study of (Howell et al. 2011) – and on the zygomatic arch (*os zygomaticum*) next to the left eye (O) for electrooculography (EOG). The ground electrode (G) was placed on the left *musculus temporalis*, the Cz

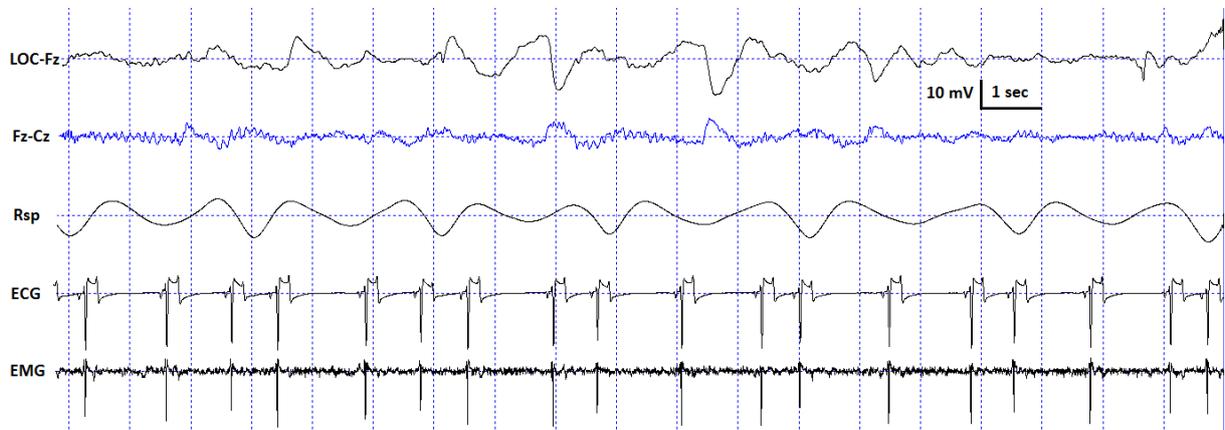
electrode served as reference. All EEG and EOG electrodes were placed on a bone for both dogs and humans so artifacts resulting from muscle movements were minimal. Electrodes were placed bilaterally on the *musculus iliocostalis dorsi* for electromyography (EMG) and over the second rib for electrocardiography (ECG). Respiratory movements were also monitored by a respiratory belt attached to the chest. Gold-coated Ag/AgCl electrodes fixed with EC2 Grass Electrode Cream (Grass Technologies, USA) were used for the recordings. Impedances for the EEG electrodes were kept below 15 k $\Omega$  for dogs and below 10 k $\Omega$  for humans. Signals were collected, prefiltered, amplified and digitized at a sampling rate of 249 Hz/channel by using the 30 channel Flat Style SLEEP La Mont Headbox with implemented second order filters at 0.5 Hz (high pass) and 70 Hz (low pass) as well as the HBX32-SLP 32 channel preamplifier (La Mont Medical Inc., USA).



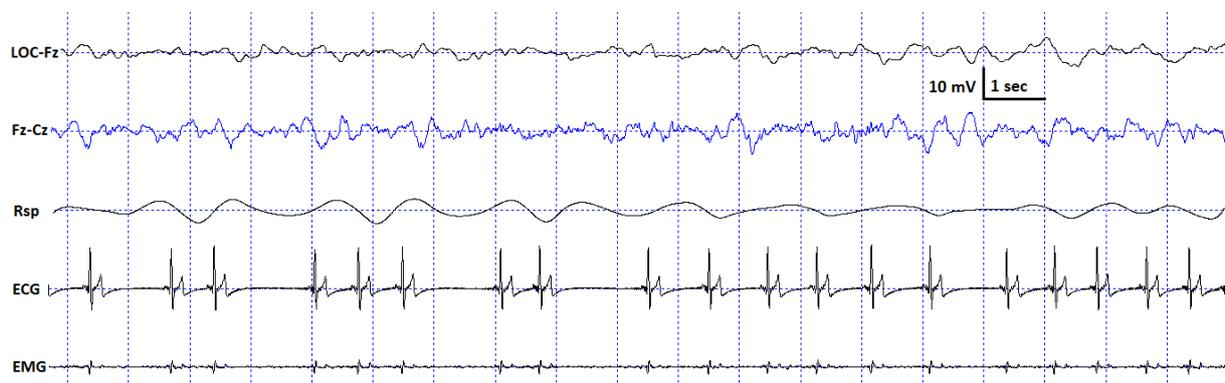
**a**  
**Figure 1.** Photograph of the recording setup (a) and schematic drawing of the electrode placement (b). The photograph was taken by BLIKK (permission obtained from Marcell Murányi).

Sleep recordings of both dog and human subjects were visually scored by two experienced sleep researchers (AK, SS) according to standard criteria (Rechtschaffen and Kales 1968; Wauquier et al. 1979). In case of both dogs and humans the *wakefulness stage* was defined as the occurrence of fast activity in the EEG (Fz-Cz derivation), high amplitude and frequency eye movements in the EOG (LOC-Cz derivation), elevated muscle tone and frequent movements (EMG channel). For dogs *drowsiness* was defined as fast EEG activity in the EEG channel (Fz-Cz derivation) accompanied by decreased amplitude and frequency eye movements in the EOG (LOC-Fz derivation), lowered but observable muscle tone (EMG channel) and fairly regular respiration (Rsp channel) (**Fig. 2**). For humans *Stage 1 sleep* was defined as the absence of alpha (8-12 Hz) waves and sleep spindles (see SWS for comparison) with the possible occurrence of vertexes (narrow – brief – and focal waves with an amplitude of 50-150  $\mu$ V) in the EEG (Fz-Cz derivation), no or low amplitude eye movements in the

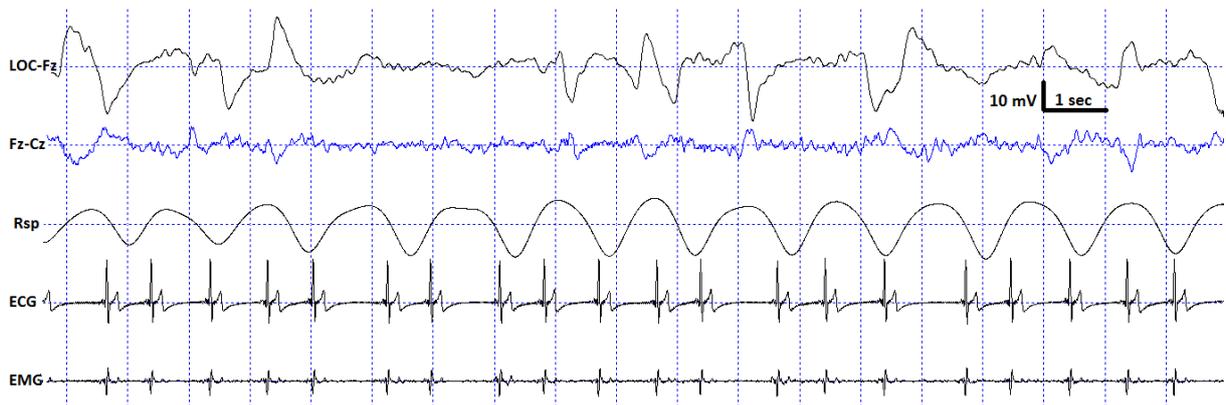
EOG (LOC-Fz derivation), relatively regular respiration (Rsp channel) and decreased muscle tone (EMG channel). *Slow wave sleep* (SWS) for both dogs and humans was defined as the occurrence of  $\geq 15 \mu\text{V}$  delta (1-4 Hz) activity and/or sleep spindles (waves with 12-16 Hz frequency and  $\geq 0.5$  sec duration) in the EEG (Fz-Cz derivation), no or low amplitude eye movements in the EOG (LOC-Fz derivation), relatively regular respiration (Rsp channel) and decreased muscle tone (EMG channel) (**Fig. 3**). *REM sleep* was defined for both dogs and humans as the occurrence of rapid eye movements in the EOG (LOC-Fz derivation) – also seen as artefacts in the EEG (Fz-Cz derivation) –, fast EEG activity (Fz-Cz derivation), muscular atonia (EMG channel), irregular respiration (Rsp channel) and heart beat (ECG) (**Fig. 4**).



**Figure 2.** Characteristic polysomnographic view of dogs' drowsiness. The EEG channel (Fz-Cz derivation) is characterized by fast activity accompanied by decreased amplitude and frequency eye movements in the EOG (LOC-Fz derivation), lowered but observable muscle tone (EMG channel) as well as fairly regular respiration (Rsp channel) and heart beat (ECG)



**Figure 3.** Characteristic polysomnographic view of dogs' slow wave sleep. The EEG (Fz-Cz derivation) was characterized by the occurrence of  $\geq 15 \mu\text{V}$  delta (1-4 Hz) activity and/or sleep spindles (waves with 12-16 Hz frequency and  $\geq 0.5$  sec duration), accompanied by no or low amplitude eye movements in the EOG (LOC-Fz derivation), relatively regular respiration (Rsp channel) and heart beat (ECG) as well as decreased muscle tone (EMG channel).



**Figure 4.** Characteristic polysomnographic view of dogs' REM sleep. The EOG (LOC-Fz derivation) was characterized by the occurrence of rapid eye movements – also seen as artefacts in the EEG (Fz-Cz derivation) –, fast EEG activity (Fz-Cz derivation), muscular atonia (EMG channel), irregular respiration (Rsp channel) and heart beat (ECG).

A program developed by our laboratory (Fercio, © Ferenc Gombos 2012) was used to export the following macrostructural variables: sleep latency (time elapsed until the first non-drowsiness sleep, min), sleep efficiency (ratio of time spent asleep to the total length of the recording, %), drowsiness / S1 duration (for dogs / humans respectively, min), SWS duration (min), REM duration (min) and sleep cycle duration (min). A descriptive analysis was conducted on these macrostructural variables (mean±SD, minimum and maximum values). Artifact rejection was carried out manually on 4 s epochs before further automatic analyses. Average power spectral densities (1 Hz to 30 Hz) were calculated by a mixed-radix Fast Fourier Transformation (FFT) algorithm applied to the 50% overlapping, Hanning-tapered 4 sec windows of the EEG signal of the Fz-Cz derivation using the DADiSP program (DSP Development Corp. USA). Relative spectral power values were obtained for each frequency bin (width: 0.25 Hz) by dividing the absolute power of the given frequency bin with the total spectral power (the sum of the absolute power of the whole range of analysis between 1–30 Hz). The relative power values reflect the relative contribution of a given frequency range to the total spectrum. The relative spectra for the different vigilance states are provided in **Fig. 5**. Comparisons of the sleep stages (wake+drowsiness/S1, SWS, REM sleep) in each frequency bin were performed using analysis of variance (ANOVA). In order to address the issue of multiple comparisons we used the procedure of descriptive data analysis delineating the so called Rürger's areas (Abt 1987). Rürger's areas are defined as sets of conventionally significant ( $p < 0.05$ ) results which are accepted or rejected as significant as a whole, instead of individual results of statistical tests. Taking the results of the statistical tests as a matrix we defined Rürger's areas along the dimension of frequency bins. Starting from the lower frequencies a Rürger's area is the range of all the neighboring, consecutive frequency bins

which contain a significant result surrounded by bins containing non-significant results. After defining these areas of significance, the number of significant results within the area was calculated, and it was investigated whether at least half of these results were significant at least at 1/2 of the conventional  $p=0.05$  significance level (that is, whether they were below 0.025) and at least one third of them were significant at least at 1/3 of the conventional  $p=0.05$  significance level (that is, whether they were below 0.0167). If both of these conditions were fulfilled, the area as a whole was considered significant. With this method, a single significant statistical test with  $p<0.0167$  theoretically counts as a significant Rüger's area, however, we would not have considered single-bin results as an area (although there were none).

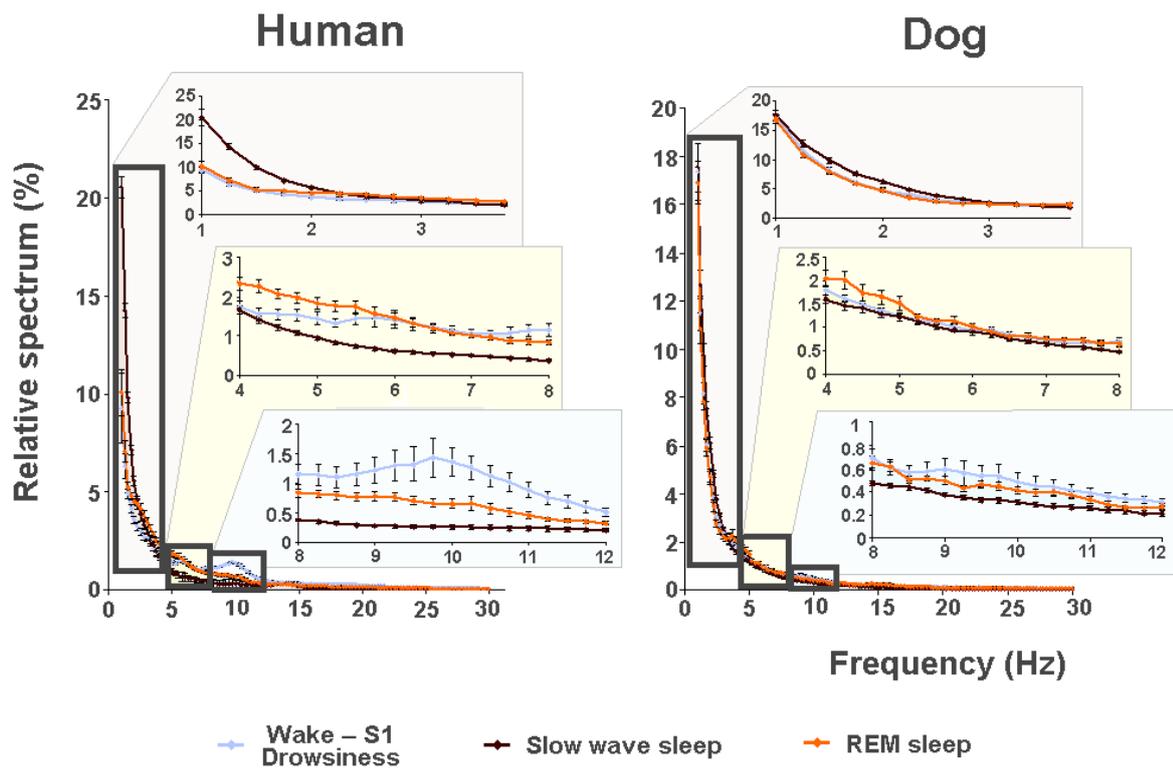
### 3.2. Results

In line with previous studies using invasive methodology (Takahashi et al. 1972) dogs showed polyphasic sleep with frequent shifts between different vigilance states including the wakeful state. Furthermore a high inter-individual variation could be observed in case of all variables, for instance sleep efficiency (the ratio of time spent asleep to the total length of the recording) ranged from 7.7 to 81.4 % in case of dogs ( $42.7\pm 23.3$ ) and from 0.9 to 92.9% in humans ( $70.4\pm 32.2$ ).

	Sleep efficiency (%)		Sleep latency (min)		Drows. (min) dog	S1 (min) hum	SWS (min)		REM (min)		Sleep cycle (min)	
	dog	hum	dog	hum			dog	hum	dog	hum	dog	hum
<b>mean</b>	42.7	70.4	28.3	16.9	37.6	10.1	21.6	98.3	16.5	20.1	48.7	106.0
<b>SD</b>	23.3	32.2	29.6	22.9	20.1	7.8	21.5	50.0	20.5	11.8	20.4	47.4
<b>min</b>	7.7	0.9	4.3	6.0	3.0	1.6	0.0	0.0	0.0	0.0	25.6	49.4
<b>max</b>	88.9	92.9	136.0	88.0	91.0	23.0	89.3	138.0	60.3	37.6	102.0	167.3

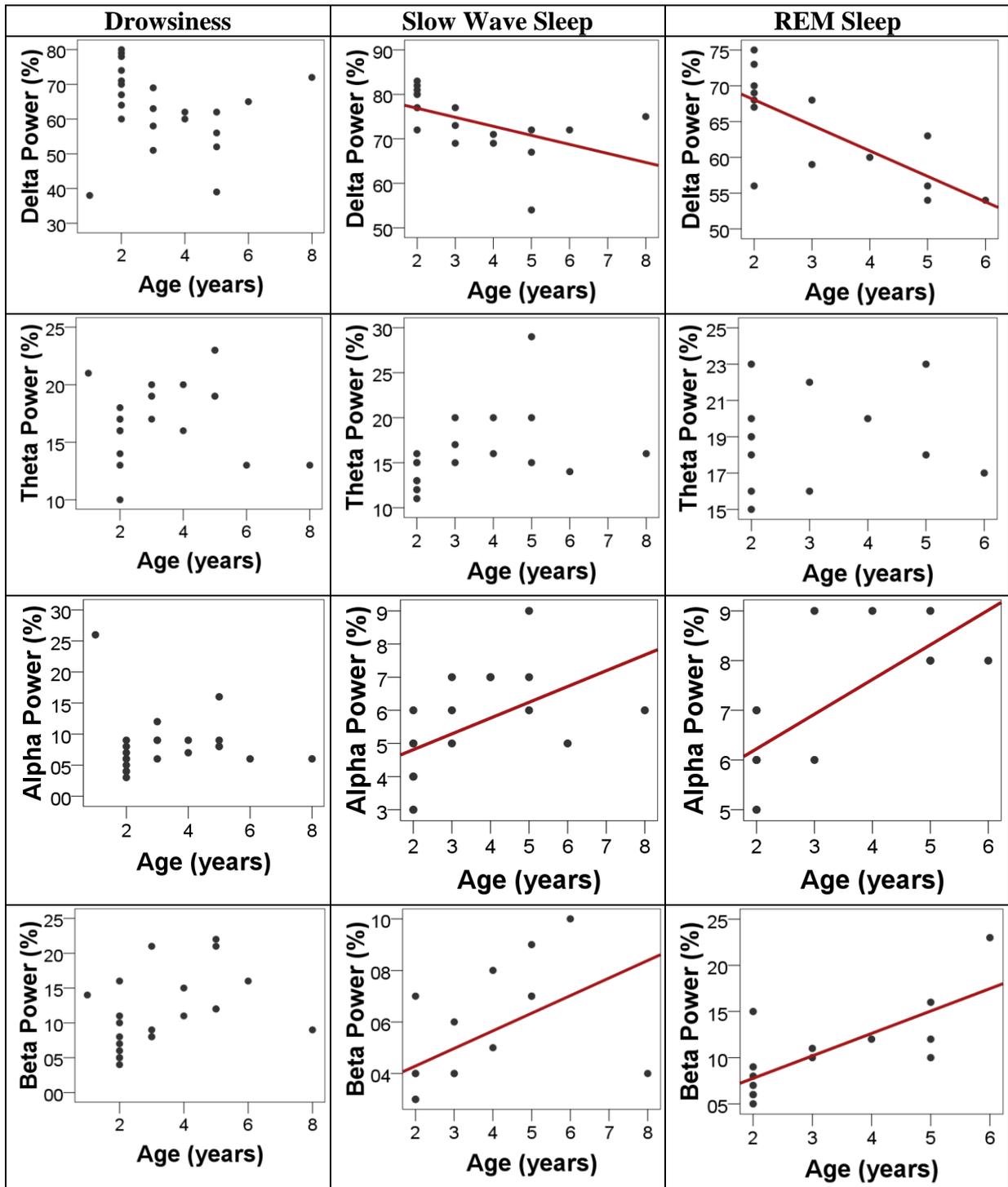
**Table 1.** Descriptive results of sleep macrostructure for dog and human subjects. Sleep efficiency is the ratio of time spent asleep (total drowsiness / S1, SWS and REM sleep time) to the length of the recording (in this case 3 hours). Sleep latency was defined as the minutes elapsed until the first non-drowsiness sleep. Descriptive data of sleep cycle duration is based on the recordings of 15 canine subjects and 10 human subjects as 7 dogs and 2 humans had no SWS and/or REM sleep during the recording.

Dogs' sleep EEG spectrum resembled that of human subjects and was generally in accordance with previous literature using invasive technology (Wauquier et al. 1979). Namely, drowsiness in dogs and calm awake + S1 in humans was characterized by an increased activity in the high frequency (alpha, beta) range, while in both dogs and humans SWS was characterized by an increased activity in the low frequency (delta) range and REM sleep was characterized by an increased activity in the theta range compared to the other sleep stages (**Fig. 5**).



**Figure 5.** Relative power spectra of drowsiness, slow wave sleep and REM sleep in human (on the left) and dog (on the right) sleep in the frequency range of 1-30 Hz. Comparisons of the sleep stages show that in dogs drowsiness is characterized by increased high frequency (alpha: 8.75-12.25 Hz and beta: 12.75-30 Hz) activity, SWS sleep is characterized by increased low frequency (delta, 1.5-3 Hz) activity and REM sleep is characterized by increased theta (4.25-4.5 & 7-8 Hz) activity; similarly in humans calm awake + S1 is characterized by increased high frequency (alpha: 8.75-12.75 Hz and beta: 15-30 Hz) activity, SWS sleep is characterized by increased low frequency (delta: 1-2.75 Hz) activity and REM sleep is characterized by increased theta (3.5-4.5 Hz) activity.

Furthermore dogs' age was related to the spectral features of the sleep EEG, paralleling previous findings on humans (Carrier et al. 2001) (Pearson correlations), with older dogs showing a decrease in relative delta power during SWS ( $r = -0.515$ ,  $p=0.029$ ) and REM sleep ( $r = -0.732$ ,  $p=0.003$ ) and an increase in relative alpha (SWS:  $r = 0.530$ ,  $p=0.024$ , REM:  $r = 0.716$ ,  $p=0.004$ ) and beta (SWS:  $r = 0.540$ ,  $p=0.021$ , REM:  $r = 0.743$ ,  $p=0.002$ ) power (**Fig. 6**).



**Figure 6.** Relatedness of dogs' age and spectral features of the EEG in the different sleep stages. Older dogs show a decrease in relative delta power during SWS ( $r = -0.515$ ,  $p=0.029$ ) and REM sleep ( $r = -0.732$ ,  $p=0.003$ ), but not during Drowsiness ( $r = -0.161$ ,  $p=0.474$ ) and an increase in relative alpha (SWS:  $r = 0.530$ ,  $p=0.024$ , REM:  $r = 0.716$ ,  $p=0.004$ , but not Drowsiness:  $r = -0.092$ ,  $p=0.068$ ) and beta (SWS:  $r = 0.540$ ,  $p=0.021$ , REM:  $r = 0.743$ ,  $p=0.002$ , but not Drowsiness:  $r = 0.381$ ,  $p=0.080$ ) power; no correlations were found in the theta range (Drowsiness:  $r = 0.070$ ,  $p=0.758$ , SWS:  $r = 0.402$ ,  $p=0.098$ , REM:  $r = 0.276$ ,  $p=0.340$ ). Regression lines are displayed for significant correlations only. Note that some of the subjects had no SWS and/or REM sleep thus the sample sizes and age ranges vary among sleep stages.

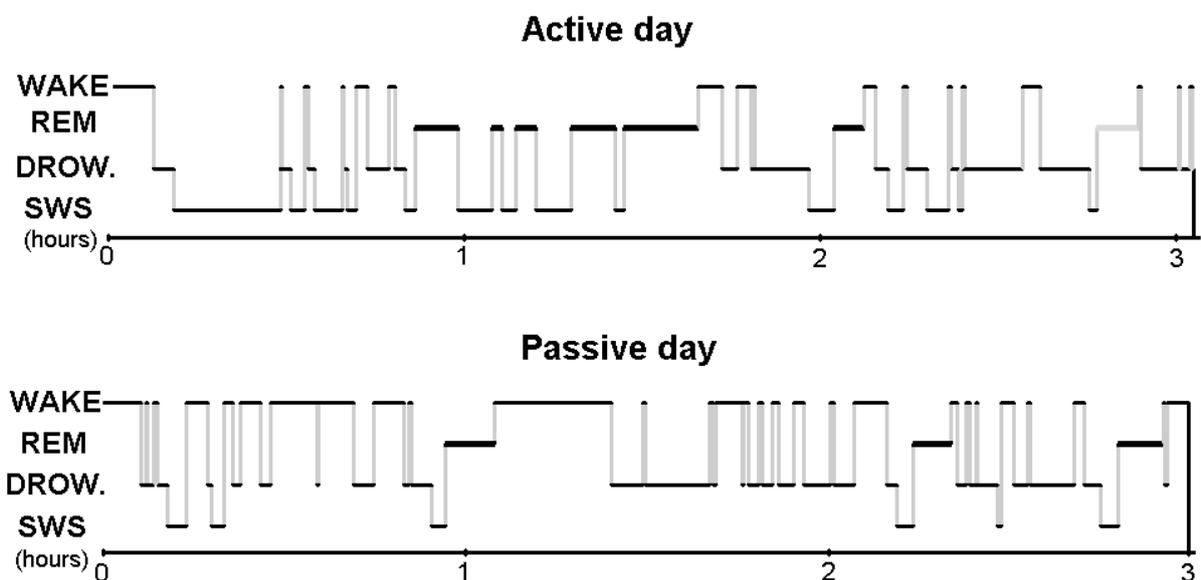
## **4. STUDY II.**

### **4.1. Method**

To validate our canine polysomnography method described above we investigated whether increased load of novel experiences and mild sleep deprivation during the day have effects on subsequent sleep as predicted by the synaptic homeostasis hypothesis (Tononi and Cirelli 2006) and other theories supporting the information processing role of sleep (Horne and Minard 1985; Diekelmann and Born 2010) put forward in the human literature.

From the dog subjects that participated in the above study N=7 (age: 2-4 years; 2 males, 5 females; 2 Border Collies, 1 Golden Retriever and 4 mongrels; 15-34 kg of weight) returned on two more occasions (an active day and a passive day condition, in a counterbalanced order) in the afternoon between 1 pm and 6 pm. As these were the 2<sup>nd</sup> and 3<sup>rd</sup> sleep recordings for all subjects, they were already familiarized with the laboratory setting and the environment, thus the risk of an order effect (also known from the human literature (Agnew et al. 1966) as the first-night effect) affecting our results was minimal. In the active day subjects were requested to engage in 6–8 hours of sleepless activity including locomotion and social interactions such as going for a walk/excursion, attending a dog training school, etc. In the passive day subjects were requested to spend a usual day at home with the owner involving the least social interaction possible. The active day included for all subjects 4 to 6 hours walking (in the city: N=3 or in the forest: N=4, out of these N=1 subject was walking with a group of other dogs) and approximately half an hour transportation to the department (by car: N=1, with public transport: N=6), during which the owners ensured that the dogs did not fall asleep. Additional (1-2 hour long) activities included training (N=2) and playing with the owner (with a ball: N=2 or with a Frisbee: N=1). During the passive day all dogs stayed at home (N=6) or at the owner's workplace (N=1) for at least 6 hours prior to the experiment and all owners reported that the dogs spent some time asleep. During transportation to the department (by car: N=1, with public transport: N=6) dog were also allowed to sleep. The maximum difference between the starting time of the two nappings for the same individual was 1 hour and the active and the passive conditions had to be either both during the week or both at the weekend. Although there are certain methodological shortcomings of this experimental design manifested in many uncontrolled variables of the behaviorally active day (such as the type of new experiences, amount and intensity of exercise or social interactions) our intention was to sleep deprive our subjects and present them with a set of novel experiences and interactions that would yield robust changes in the quality of subsequent sleep.

After hypnogram scoring (for an illustrative example see **Fig. 7**) the effects of two factors ('active vs. passive day' and '2<sup>nd</sup> or 3<sup>rd</sup> sleeping occasion') were tested on the macrostructural data as dependent variables with Generalized Estimating Equation in a within subject design. The relative power spectrum of the EEG signal of the Fz-Cz derivation was calculated separately for the three vigilance states in the frequency ranges of delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-30 Hz) known from human and rat sleep studies (Colrain 2011) and also previously used in laboratory dogs (Wauquier et al. 1979) To compare the spectral features of the sleep EEG after a behaviorally active vs. passive day paired sampled t tests (with Benjamini false discovery rate correction: Benjamini et al. 2001) were used. All statistical tests were carried out with SPSS18 (IBM USA).

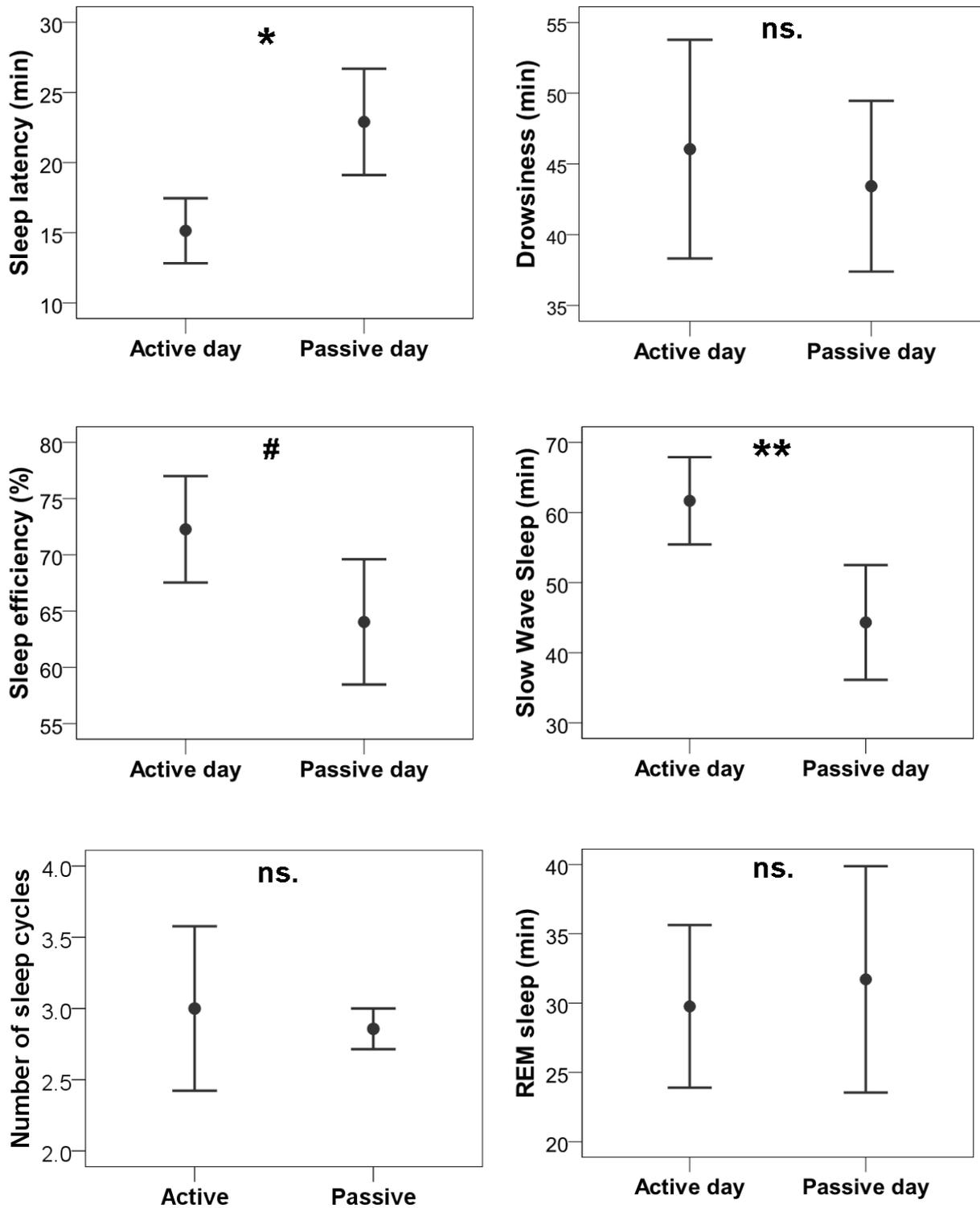


**Figure 7.** Sleep EEG differences after an active and a passive day were reflected in sleep macrostructure, as shown by a sample hypnogram from one of the subjects

## 4.2. Results

In accordance with previous studies on the effect of experience (Horne and Minard 1985) and sleep deprivation (Borbély et al. 1981) in humans a Generalized Estimating Equation revealed that the macrostructure of dogs' sleep differed between the active and the passive days in several aspects (**Fig. 8**) while no order effects (2<sup>nd</sup> or 3<sup>rd</sup> occasion, all  $p > 0.1$ ) or interaction among the two factors (all  $p > 0.1$ ) could be observed. Sleep latency was shortened after an active day ( $\chi^2_{(1)} = 4.665$ ,  $p = 0.031$ ). Sleep efficiency (%) was only marginally affected by condition type (active/passive day,  $\chi^2_{(1)} = 2.998$ ,  $p = 0.083$ ). This was probably due to the fact that drowsiness duration ( $\chi^2_{(1)} = 0.409$ ,  $p = 0.523$ ) and REM duration ( $\chi^2_{(1)} = 0.041$ ,  $p = 0.840$ ) did

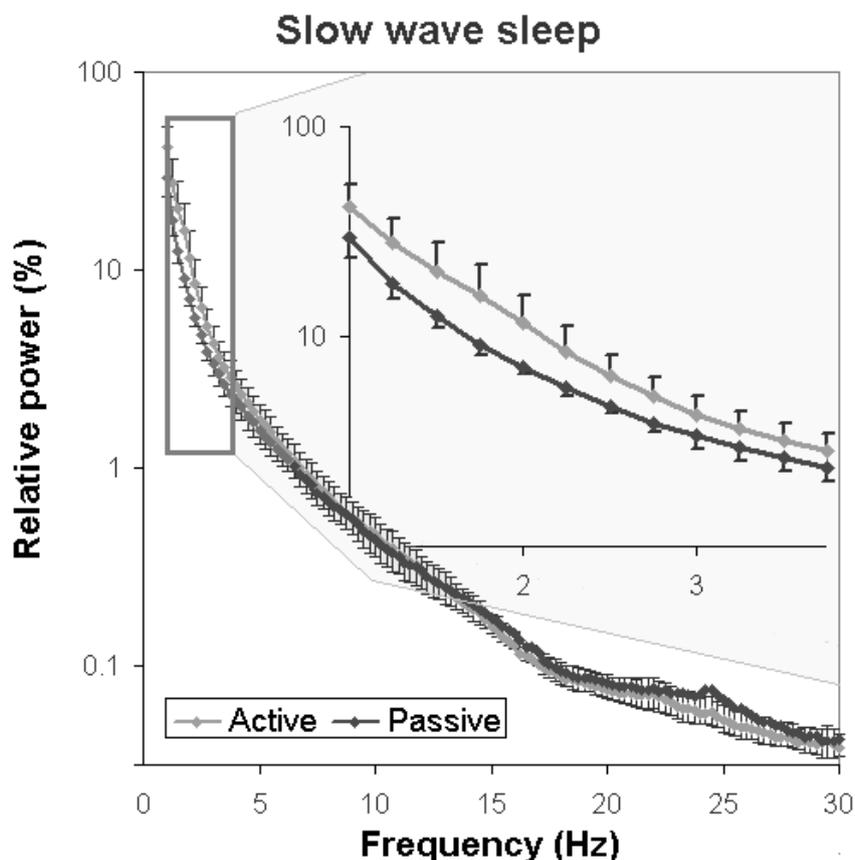
not differ among active and passive days. However the amount of slow wave sleep was higher following an active day ( $\chi^2_{(1)}= 6.829$ ,  $p=0.009$ ). The number of sleep cycles was also not affected by a preceding active/passive day ( $\chi^2_{(1)}=0.085$ ,  $p=0.770$ ).



**Figure 8.** Differences in sleep macrostructure between the active and the passive day. \*\*:  $p<0.01$ , \*:  $p<0.05$ , #:  $p<0.1$ ; ns.:  $p>0.1$ . *Sleep latency* was shortened after an active day ( $\chi^2_{(1)}=4.665$ ,  $p=0.031$ ; no

order effect (2<sup>nd</sup> or 3<sup>rd</sup> occasion,  $\chi^2_{(1)}=0.303$ ,  $p=0.582$ ) or interaction among the two factors ( $\chi^2_{(1)}=0.000$ ,  $p=0.990$ ) could be observed). *Sleep efficiency* (%) was only marginally affected by a preceding active/passive day ( $\chi^2_{(1)}=2.998$ ,  $p=0.083$  and no order effect (2<sup>nd</sup> or 3<sup>rd</sup> occasion,  $\chi^2_{(1)}=0.003$ ,  $p=0.953$ ) or interaction among the two factors ( $\chi^2_{(1)}=0.427$ ,  $p=0.514$ ) could be observed). This was probably due to the fact that *drowsiness duration* ( $\chi^2_{(1)}=0.409$ ,  $p=0.523$ ) and *REM duration* ( $\chi^2_{(1)}=0.041$ ,  $p=0.840$ ) did not differ among active and passive day. (Also no order effect (2<sup>nd</sup> or 3<sup>rd</sup> occasion, drowsiness:  $\chi^2_{(1)}=0.245$ ,  $p=0.625$ ; REM:  $\chi^2_{(1)}=0.627$ ,  $p=0.429$ ) or interaction among the two factors (drowsiness:  $\chi^2_{(1)}=1.255$ ,  $p=0.263$ ; REM:  $\chi^2_{(1)}=1.141$ ,  $p=0.285$ ) could be observed.) However – just like in a similar human study (Horne and Minard 1985) the *amount of slow wave sleep* was higher following an active day ( $\chi^2_{(1)}= 6.829$ ,  $p=0.009$ ; no order effect (2<sup>nd</sup> or 3<sup>rd</sup> occasion,  $\chi^2_{(1)}=0.489$ ,  $p=0.484$ ) or interaction among the two factors ( $\chi^2_{(1)}=1.502$ ,  $p=0.220$ ) could be observed). The *number of sleep cycles* was also not affected by a preceding active/passive day ( $\chi^2_{(1)}= 0.085$ ,  $p=0.770$ ; no order effect (2<sup>nd</sup> or 3<sup>rd</sup> occasion,  $\chi^2_{(1)}=0.085$ ,  $p=0.771$ ) or interaction among the two factors ( $\chi^2_{(1)}=0.464$ ,  $p=0.496$ ) could be observed).

In line with previous human studies (Borbély et al. 1981) the spectral features of dogs' sleep differed between the behaviorally active and passive days: after correction for multiple comparisons (Benjamini false discovery rate adjustment (Benjamini et al. 2001)) we found that following the active day alpha (8-12 Hz) activity decreased ( $t_{(6)}=2.760$ ,  $p=0.033$ ) during drowsiness, while during SWS delta (1-4 Hz) activity increased ( $t_{(6)}=3.173$ ,  $p=0.019$ ; **Fig. 9**) and alpha (8-12 Hz;  $t_{(6)}=2.866$ ,  $p=0.029$ ) and beta (12-30 Hz;  $t_{(6)}=2.847$ ,  $p=0.008$ ) activity decreased; there were no significant differences during REM sleep (**Table 2**).



**Figure 9.** Sleep EEG differences after an active and a passive day were reflected in the relative EEG spectrum especially in the delta (1-4 Hz) frequency range during slow wave sleep

Sleep stage	EEG frequency	Result	$t_{(6)}$	p	False Discovery Rate
Drowsiness	Delta (1-4 Hz)	Passive = Active	3.439	0.014	0.012
	Theta (4-8 Hz)	Passive = Active	2.138	0.076	0.050
	<b>Alpha (8-12 Hz)</b>	<b>Passive &gt; Active</b>	2.760	0.033	0.050
	Beta (12-30 Hz)	Passive = Active	2.781	0.032	0.022
SWS	<b>Delta (1-4 Hz)</b>	<b>Passive &lt; Active</b>	3.173	0.019	0.022
	Theta (4-8 Hz)	Passive = Active	2.205	0.070	0.050
	<b>Alpha (8-12 Hz)</b>	<b>Passive &gt; Active</b>	2.866	0.029	0.050
	<b>Beta (12-30 Hz)</b>	<b>Passive &gt; Active</b>	3.847	0.008	0.012
REM	Delta (1-4 Hz)	Passive = Active	2.498	0.047	0.012
	Theta (4-8 Hz)	Passive = Active	1.901	0.106	0.022
	Alpha (8-12 Hz)	Passive = Active	1.584	0.164	0.050
	Beta (12-30 Hz)	Passive = Active	0.694	0.514	0.050

**Table 2.** Differences in the spectral features (relative EEG power) of sleep between the active and the passive day. Differences that remain significant after False Discovery Rate correction are marked with bold.

## 5. Discussion

Our newly developed canine polysomnography technique yielded comparable results to both data from human (Horne and Minard 1985) and mammalian (Takahashi et al. 1972) sleep studies. This methodological development represents a significant advance in the fields of both sleep and canine cognition research providing an easily applicable and non-invasive method to study neural oscillations of the dog. Our design allowed us to compare the macrostructure and EEG spectrum of sleep in different conditions and revealed experience- and sleep deprivation-dependent changes in dogs' sleep. Furthermore the high inter-individual variation we observed opens the way for investigating the correlates of different information processing mechanisms and other underlying variations (e.g. age of the subjects, as shown in the present study). In summary, our results validate the family dog as a model species for studying the effects of pre-sleep activities on EEG pattern under natural conditions and thus broaden the perspectives of the rapidly growing field of canine cognition research.

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