frontiers | Research Topics

Electroencephalography and other neuroelectrophysiologic studies in post-MRI generation veterinary medicine

Edited by
Daisuke Hasegawa, Fiona May Keir James and Marcin Adam Wrzosek

Published in
Frontiers in Veterinary Science

ISSN 1664-8714
ISBN 978-2-8325-4808-0
DOI 10.3389/978-2-8325-4808-0

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Electroencephalography and other neuroelectrophysiologic studies in post-MRI generation veterinary medicine

Topic editors

Daisuke Hasegawa — Nippon Veterinary and Life Science University, Japan

Fiona May Keir James — University of Guelph, Canada

Marcin Adam Wrzosek — Wroclaw University of Environmental and Life Sciences, Poland

Citation

Hasegawa, D., James, F. M. K., Wrzosek, M. A., eds. (2024). *Electroencephalography and other neuroelectrophysiologic studies in post-MRI generation veterinary medicine*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4808-0

Table of contents

frontiers | Frontiers in Veterinary Science

TYPE Editorial
PUBLISHED 09 April 2024
DOI 10.3389/fvets.2024.1396967

OPEN ACCESS

EDITED AND REVIEWED BY
Andrea Tipold,
University of Veterinary Medicine
Hannover, Germany

*CORRESPONDENCE
Fiona M. K. James
✉ jamesf@uoguelph.ca

RECEIVED 06 March 2024
ACCEPTED 07 March 2024
PUBLISHED 09 April 2024

CITATION
James FMK, Wrzosek M and Hasegawa D (2024) Editorial: Electroencephalography and other neuroelectrophysiologic studies in post-MRI generation veterinary medicine. *Front. Vet. Sci.* 11:1396967. doi: 10.3389/fvets.2024.1396967

Editorial: Electroencephalography and other neuroelectrophysiologic studies in post-MRI generation veterinary medicine

Fiona M. K. James[1]*, Marcin Wrzosek[2,3,4] and Daisuke Hasegawa[5]

[1]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wrocław University of Environmental and Life Sciences, ul., Wrocław, Poland, [3]NeuroTeam Specialist Veterinary Clinic, Wrocław, Poland, [4]Vet Imaging Veterinary Computed Tomography, Kraków, Poland, [5]Department of Veterinary Medicine, Nippon Veterinary and Life Science University, Musashino, Japan

KEYWORDS

electroencephalography—diagnosis, electroencephalography—methods, electrical impedance myography (EIM), somatosensory evoked potentials (SEPs), veterinary

Editorial on the Research Topic
Electroencephalography and other neuroelectrophysiologic studies in post-MRI generation veterinary medicine

Neuroelectrophysiologic studies have long been integral for functional evaluations of both the central and peripheral nervous systems. In the era preceding the widespread use of magnetic resonance imaging (MRI) and computed tomography (CT) scans, these tests played a crucial role. However, the advent of advanced imaging, particularly MRI, shifted the paradigm in veterinary medicine, seemingly eclipsing the importance of neuroelectrodiagnostics. The assumption was that imaging could provide a comprehensive understanding of both structure and function.

This Research Topic explores the evolving landscape of neuroelectrophysiologic studies in the post-MRI era. Despite the popularity of advanced imaging techniques, veterinarians are re-evaluating the scope and significance of neuroelectrodiagnostics. It is increasingly evident that diagnostic imaging, while proficient in detecting structural abnormalities, falls short in documenting crucial functional aspects of the nervous system.

Several facets of electroencephalography (EEG) are explored in this Research Topic. The first theme comprises advancements in EEG techniques. Luca et al. presented a comprehensive survey of EEG usage and techniques in veterinary neurology. Findings revealed underutilization and barriers such as equipment availability, highlighting the need for standardization and harmonization of EEG techniques in canine neurology. In response, to propose routine clinical short-term video-electroencephalography (vEEG) recordings, Lyon et al. demonstrated its feasibility in unsedated dogs and cats. This standardized, unanesthetized procedure proved clinically informative, positioning EEG as a vital first-line neurological functional exploration test. Folkard et al. ventured into homes, conducting in-home vEEG and actigraphy recordings. Their approach, integrating vEEG and actigraphy, not only assesses behavior but also detects epileptic seizures. This innovative study laid the groundwork for holistic insights into canine behavior in natural settings.

A second theme involved the use of EEG, to understand for example pharmaceutical effects or age- or sleep-related features. Mizuno et al. explored ketamine in cats with temporal lobe epilepsy. Beyond sedation, ketamine emerged as an inducer of epileptiform discharges during EEG recordings. This study prompts a reconsideration of sedation alternatives, offering a nuanced perspective on the potential activating effects of pharmacologic choices on neuroelectrophysiologic studies. Simultaneously, Pellegrino and Gómez Álvarez employed xylazine sedation to unravel EEG features of the developing canine brain. Their meticulous analysis provides a refined understanding of EEG characteristics, emphasizing age-specific interpretation in veterinary neuroelectrophysiology. Examining the senescent brain, Mondino et al. scrutinized sleep-wakefulness cycles and EEG features in senior dogs. Their polysomnographic study unveiled age-related changes correlated with cognitive performance, offering potential markers for canine cognitive dysfunction syndrome.

Williams et al. extended the scope of EEG to the marine world, examining EEG in stranded California sea lions. Abnormalities in EEG recordings shed light on domoic acid toxicosis, emphasizing the importance of understanding EEG patterns in marine mammals. Knipe et al. took a visual perspective, focusing on periodic discharges in veterinary EEG. Their review explores these enigmatic patterns, challenging our understanding and paving the way for further research into the interpretation of these patterns in clinical practice.

Extending neurophysiology to muscle health and neurosurgery was the final theme in this post-MRI compilation. Verga et al. delves into the realm of canine skeletal muscle health, presenting normative values and repeatability for electrical impedance myography. Their study provides a convenient and non-invasive tool for assessing muscle condition, offering insights into age-related changes and gender differences. Okuno et al. address a critical aspect of neurosurgery, evaluating the impact on spinal cord integrity during thoracolumbar intervertebral disk herniation surgery in dogs. Utilizing somatosensory-evoked potentials, they provide a framework for maintaining electrophysiological safety during surgical interventions.

This Research Topic of articles not only establishes the current state of knowledge in post-MRI veterinary neuroelectrophysiology but also propels the field forward with innovative methodologies and insights. These contributions establish a foundation for the future, where neuroelectrophysiology intertwines seamlessly with veterinary clinical practice. Given the rapid development of diagnostic technologies for neurological diseases in animals, both advanced imaging diagnostics (CT and MRI) and all electrodiagnostic techniques, their fusion may be predicted, with consequential development of hybrid diagnostic solutions in veterinary medicine.

Author contributions

FJ: Conceptualization, Writing – original draft, Writing – review & editing. MW: Conceptualization, Writing – review & editing. DH: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

MW was employed by NeuroTeam Specialist Veterinary Clinic and Vet Imaging Veterinary Computed Tomography.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 15 September 2022
DOI 10.3389/fvets.2022.976972

OPEN ACCESS

EDITED BY
Daisuke Hasegawa,
Nippon Veterinary and Life Science University, Japan

REVIEWED BY
D. Colette Williams,
University of California, Davis, United States
Hidetaka Nishida,
Osaka Prefecture University, Japan

*CORRESPONDENCE
Kensuke Orito
oritok@azabu-u.ac.jp

SPECIALTY SECTION
This article was submitted to Veterinary Neurology and Neurosurgery, a section of the journal Frontiers in Veterinary Science

RECEIVED 24 June 2022
ACCEPTED 29 August 2022
PUBLISHED 15 September 2022

CITATION
Okuno S, Katahira H and Orito K (2022) Somatosensory evoked potentials of the tibial nerve during the surgical decompression of thoracolumbar intervertebral disk herniation in dogs. *Front. Vet. Sci.* 9:976972. doi: 10.3389/fvets.2022.976972

Somatosensory evoked potentials of the tibial nerve during the surgical decompression of thoracolumbar intervertebral disk herniation in dogs

Seiichi Okuno[1,2], Hirotaka Katahira[3] and Kensuke Orito[1]*

[1]Laboratory of Physiology II, School of Veterinary Medicine, Azabu University, Sagamihara, Japan, [2]Veterinary Clinic of Neurology, Isesaki, Japan, [3]Laboratory of Environmental Biology, School of Life and Environmental Science, Azabu University, Sagamihara, Japan

This study aimed to identify the impact on spinal cord integrity and determine the electrophysiological safety level during surgery for thoracolumbar intervertebral disk herniation in dogs. A total of 52 dogs diagnosed with thoracolumbar intervertebral disk herniation were enrolled. The tibial nerve somatosensory evoked potential elicited on the scalp by stimulation of the tibial nerve was recorded before and during hemilaminectomy. Both the amplitude and latency of the somatosensory evoked potential were periodically registered, and the percentage changes from the pre-operative control values (amplitude rate and latency rate) were calculated. When the multifidus muscles were retracted after removal from the spinous processes and vertebrae, the somatosensory evoked potential amplitude rate decreased in all dogs, while the latency rate increased in 33 dogs examined. The amplitude rate remained unchanged during the halting procedure, loosening retraction, and hemilaminectomy. After removing the disc material from the spinal canal, the amplitude rate was increased. The somatosensory evoked potential latency increased when the multifidus muscles were retracted and shortened after multifidus muscles closure in four cases. The outcome of all cases showed improvement in clinical signs 7 days after operation. Spinal cord conduction is impaired by retraction of the multifidus muscles and improved by removal of disk materials. Maintaining intraoperative SEP amplitudes above 50% of control may help avoid additional spinal cord injury during surgery. Since we have no case that worsened after the surgery, however, further studies are necessary to confirm this proposal.

KEYWORDS

somatosensory evoked potential, IVDH, hemilaminectomy, dog, intraoperative monitoring

Introduction

Thoracolumbar intervertebral disc herniation (IVDH) is a common disorder in dogs that mainly affects the chondrodystrophic breeds. Backache and neurological disorders in the pelvic limbs are clinical signs of thoracolumbar disk disease, and urinary dysfunction and loss of pain sensation of the pelvic limbs are observed in cases with more severe lesions. Decompression surgery, such as hemilaminectomy, is performed on dogs with neurological deficits and evidence of severe spinal cord compression, with most commonly reported intraoperative complication being insufficient removal of disk materials (1). However, the effects of surgical procedures on spinal cord integrity have not yet been verified and no method has been established to assess spinal cord function during surgery or determine its safety level in veterinary medicine.

Somatosensory evoked potentials (SEPs) are brain and spinal cord responses induced by stimulation of peripheral sensory nerves in the limbs or cranial nerves with sensory function. SEP reflects transmission of the afferent volley from the peripheral sensory nerves to the primary somatosensory cortex, through the myelinated dorsal columns and the medial lemniscal pathways. Cortical recording of SEP is considered to be a field potential arising from the cerebral cortex (2). In humans, SEP has been utilized for evaluation of the spinal cord somatosensory conductive function during spinal surgeries (3–5), and intraoperative SEP monitoring was reported to be useful for reduction of post-operative paraplegia by more than 50–60% (4, 6). In humans, intraoperative SEP amplitude of 40% or less caused post-operative neurological deficit (3), and the absence of SEP during spinal surgery led to new complication or neurological loss of function (4, 6). Therefore, a 50% decrease in SEP amplitude during spinal surgeries is reportedly the alarm threshold for spinal cord damage in humans (4, 7).

In dogs, SEP is recorded when electrical stimulation is applied to the peripheral sensory nerve or cranial nerve with sensory function (8–10). Studies have reported that SEP amplitude in dogs is decreased by spinal cord compression and blocked blood supply to the spinal cord (11–13), suggesting that intraoperative SEP examination is a useful tool for monitoring spinal cord integrity during spinal surgery in veterinary medicine. Notably, we previously reported the usefulness of SEP monitoring during cervical operation in dogs (14). However, to the best of our knowledge, there are no reports of intraoperative SEP monitoring being utilized during a thoracolumbar spinal operation in veterinary medicine.

In this study, we measured the amplitude and latency rates of SEP and evaluated spinal cord integrity during each surgical procedure of hemilaminectomy. Based on the results of this study and previous knowledge of human medicine, we propose that maintaining intraoperative SEP amplitudes >50% of control may help avoid additional spinal cord injury during surgery.

Materials and methods

Animals

This study was conducted in a prospective manner. All dogs enrolled in this study were handled in compliance with the Azabu University Animal Experiment Guidelines (April 2000) (17114-2, 191205-5). Dogs were eligible for enrollment after obtaining informed consent from their owners. All surgery was performed under isoflurane anesthesia.

Dogs with thoracolumbar IVDH of clinical grade 2, 3, and 4 that were determined by conventional neurological examination and MRI, and stable SEP was obtained in the pre-operative examination were included in this study. All the cases were diagnosed as Hansen Type 1 intervertebral disk extrusion. Fifty-two dogs (27 males and 25 females) were enrolled between May 2017 and March 2020. The cases showed clinical grade 5 were not enrolled in this study because cortical SEP could not be elicited as reported previously (15). The cases included 41 miniature Dachshunds, 2 Pomeranians, Papillons, Mixes each, and 1 Chihuahua, Beagle, Boston Terrier, Shiba, and Welsh Corgi Pembroke each. Mean ± SD (range) age and body weight were 7.3 ± 2.9 (2–12) years and 6.1 ± 2.0 (3.1–12.6) kg, respectively. The location of disc herniation differed among the dogs as follows: T12–13 (n = 10), T13–L1 (n = 16), L1–2 (n = 19), and L2–3 (n = 7). The cases were classified according to the clinical thoracolumbar IVDH grade, which was assigned based on the severity of clinical signs according to a previously reported classification system as follows (16): grade 2 = ataxia, conscious proprioception deficit, and paraparesis (n = 9); grade 3 = paraplegia (n = 40); and grade 4 = paraplegia with urinary retention and overflow (n = 3).

Experimental procedures

All dogs were premedicated with atropine sulfate [0.04 mg/kg, subcutaneous (SC); Tanabe Mitsubishi Pharma Corp, Osaka, Japan], midazolam [0.1 mg/kg, intravenous (IV); Dormicum, Maruishi Pharmaceutical Co Ltd, Osaka, Japan], and butorphanol (0.2 mg/kg, IV; Vetorphale, Meiji Seika Pharma Co Ltd, Tokyo, Japan). Anesthesia was induced by alfaxalone (2.0–3.0 mg/kg, IV; Alfaxan. Meiji Seika Pharma Co Ltd, Tokyo, Japan) until the desired effect was obtained and maintained throughout the operation with isoflurane (Isoflu, Zoetis Japan Inc, Tokyo, Japan). Rectal temperature was monitored and body temperature was maintained at between 37.0°C and 38.0°C with heating pads.

Surface disk electrodes (Disk Electrode for evoked EEG NE-132B. Nihon Koden Corp, Tokyo, Japan) were used as recording, reference and ground electrodes to check an impedance below 5 K ohms between skin and electrode. The recording electrode was placed at the juncture of the coronal and sagittal sutures, which were considered to be adjacent to the somatosensory cortex area, and the reference and ground electrode was placed on the spinous process of the axis and dorsal surface of caudal portion of neck, respectively, following the method used by Uzuka et al. (9, 14) (Figure 1). The electrical stimuli lasted 0.2 ms, and rectangular waves at a rate of 3 Hz were applied to the tibial nerve on the same side as the operation site. Stainless needles (TERMO needle NN-2525R. TERMO Corp, Tokyo, Japan) were used for the stimulating electrodes, and these were inserted percutaneously and placed immediately proximal to the tarsal joint on the tibial nerve. The cathode was placed proximal ~1 cm from the anode. The stimulating intensity was adjusted with less than 3.6 mA to produce visible small twitch of 5th digit. A total of 200 responses were averaged with high- and low-pass filters at 0.5 and 3,000 Hz, respectively. The recording condition of SEP was 2 μV/division of amplitude and 5 ms/division of sweep speed.

Somatosensory evoked potential examination was performed as previously described (14). SEP examinations began after induction of anesthesia in the prone position. A stable SEP elicited before the operation served as a control SEP. The SEP amplitude was determined as the peak-to-peak amplitude of the initial positive and subsequent negative waves (Figure 2). The latency from the stimulation artifact (SA) to the peak of the initial positive waves was recorded simultaneously (Figure 2). A system for evoked potentials (Neuropack MEB-9404, Nihon Kohden Corp, Tokyo, Japan) was used to provide electrical stimulations as well as measure the amplitude and latency.

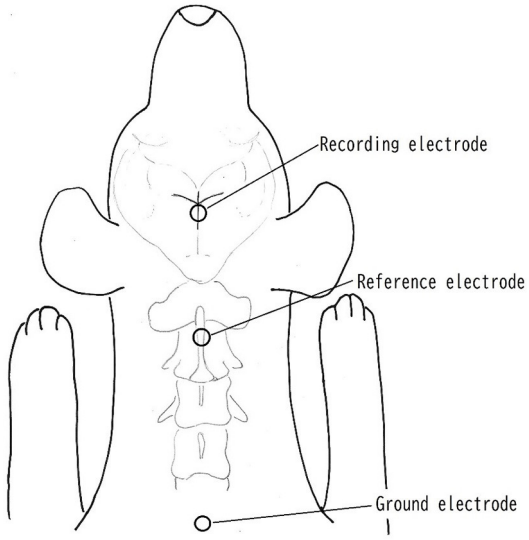


FIGURE 1
Placement of electrodes. The recording electrode was placed at the juncture of the coronal and sagittal sutures, which were considered to be adjacent to the somatosensory cortex area. The reference electrode was placed on the spinous process of the axis. The ground electrode was placed on the surface of caudal portion of neck.

The surgical procedure was performed as follows. The skin and superficial tissues were incised lateral to the midline. The dorsal fascia was incised near each spinous process. The multifidus muscles were peeled off from the spinous and articular processes using an elevator. The multifidus muscles were retracted laterally using Gelpi retractors. The articular processes and vertebral laminae were removed using rongeurs. Disk materials compressing the spinal cord were removed using forceps or curettes.

Somatosensory evoked potential recordings were performed once for each surgical procedure during surgery, starting at multifidus muscles retraction (Retract). When a decrease in amplitude was recognized, surgical manipulations were halted and the retraction of the multifidus muscles was loosened. SEP was then recorded once (Halt). After this recording of SEP (about 2 min), the operation was continued and SEP were recorded at articular processes and laminae resection (Hemilami), intervertebral disk material removal (Remove), and muscle closing (Close). SEP was also recorded at the end of the surgery (End). Representative SEPs and measured amplitude and latency of control and Retract are shown in Figure 2. And the result of SEP recording is shown in Figure 3.

The SEP amplitude is negatively correlated with body size in dogs (10). Low body temperature and low blood pressure cause slowing of conduction velocity and reduced amplitude of SEP, respectively (4, 6). Therefore, the amplitude rate and latency rate were used in this study to evaluate the changes in SEP during surgical procedures of various bleeds.

Amplitude and latency rate were calculated as follows:

$$\text{Amplitude rate (\%)} = \frac{\text{Recorded SEP amplitude}}{\text{Control SEP amplitude}} \times 100$$

$$\text{Latency rate (\%)} = \frac{\text{Recorded SEP latency}}{\text{Control SEP latency}} \times 100$$

Statistical analyses

Data are expressed as mean ± SD (range). Amplitude and latency rates were compared between surgical procedures as a fixed effect by fitting a linear mixed model (LMM), considering individual differences as a random effect (17). The significance of each constructed model was evaluated by a likelihood ratio

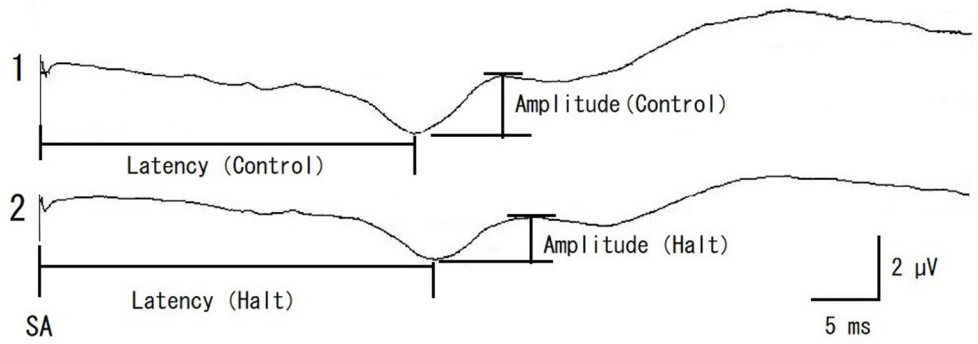


FIGURE 2
Representative recording of control SEP (pre-operative control, 1) and multifidus muscles retracting (Halt, 2) in a case of 11 years old male miniature dachshund enrolled in this study. The SEP amplitude was determined as the peak-to-peak amplitude of initial positive and next negative waves. The SEP latency was determined from the stimulation artifact (SA) to the peak of initial positive wave. The amplitude rate and latency rate were calculated as follows; The amplitude rate = the amplitude (Halt)/the amplitude (pre-operative control) × 100, The latency rate = the latency (Halt)/the latency (pre-operative control) × 100, respectively.

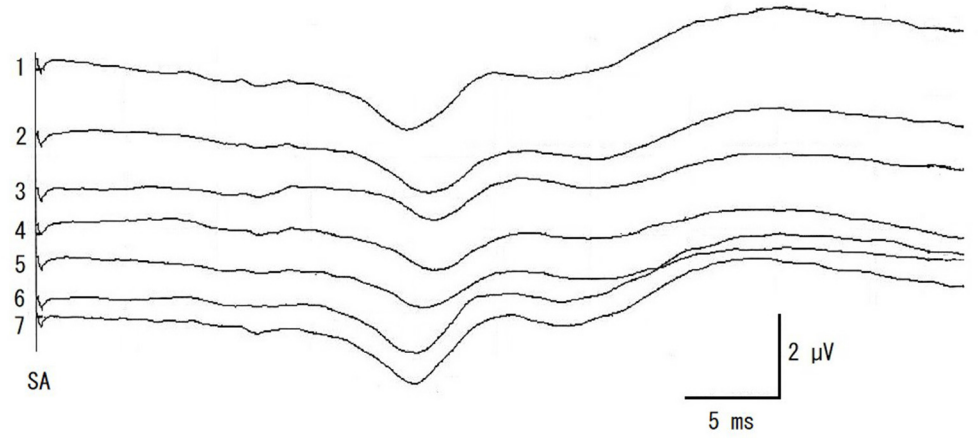


FIGURE 3
The recordings of intraoperative SEP monitoring in a case of Figure 2. 1: pre-operative control, 2: retracting multifidus muscles, 3: halting surgical procedure and multifidus muscles loosened, 4: hemilaminectomy, 5: removing the disk materials from the spinal canal, 6: closing multifidus muscles, 7: end of the surgery. SA, electrical stimulation artifact.

test based on an approximate chi-square distribution against the null model deviance. When the model significance was confirmed, the estimated coefficients for the mean rate at each surgical procedure were compared using Tukey's *post-hoc* honestly significant difference tests. These analyses were performed using the *lme4* and *multcomp* packages in the R 4.0.3 (18). Statistical significance was set at $p < 0.05$.

Clinical outcome evaluation

Clinical outcome evaluation of all cases was performed 7 days after operation using the conventional neurological examinations same as pre-operative examination. An improvement was determined by the following changes in clinical signs: disappearance of neurological deficit in cases of grade 2, and walking without any assistance in cases of grade 3 and 4.

Results

Stable SEPs were recorded throughout the surgery and examined for all cases in this study. Isoflurane concentration was stable and any additional agents was not used throughout operation in all cases. There were no cases in which clinical signs deteriorated after surgery. All of the cases of grade 2 showed no

neurological deficit and all patients of grade 3 and 4 were able to walk without any assistance within 7 days after surgery.

When the multifidus muscles were removed from the vertebrae and retracted, the SEP amplitude decreased in all cases, and its latency increased in 33 dogs examined (Figure 2). The deviances in both constructed models for the amplitude and latency rates differed significantly from those in the null models ($\chi^2 = 191.45$, df = 5, $p < 0.001$ in amplitude; $\chi^2 = 30.01$, df = 5, $p < 0.001$ in latency). The estimated intercept (i.e., the relative difference in the process of the retracting multifidus muscles) in the amplitude rate was significantly lower (69% with the mean value, $t = 36.99$, $p < 0.001$) than in the pre-operative control (Table 1), while in the latency rate was significantly higher (104% with the mean value, $t = 186.91$, $p < 0.001$) than in the control (Table 2). The standard deviations of the intercept variability caused by individual differences were 10.05 in the amplitude rate and 3.62 in the latency rate.

Although amplitude rate in End was still lower than pre-operative control in 34 cases, the *post-hoc* test comparing the pairs of the estimated means, supported the tendency of increasing amplitude rates during the surgical procedure (Table 1). In one case the amplitude rate decreased in Retract, increased in Halt, and then decreased again in Hemilami. It was, however, still higher than that in Retract. Therefore, surgical manipulations were not halted again. Although the mean amplitude rates in Halt and Hemilami tended to be higher than those in Retract, significant differences were not observed (4.49%, $z = 2.60$, $p = 0.097$ and 4.67%, $z = 2.70$, $p = 0.075$, respectively). The mean amplitude rates in Remove, Close, and End were higher (8.63%, 19.21%, and 22.98%, respectively; $p < 0.001$) than those in Retract. The mean amplitude rates in Halt, Hemilami, and Remove also differed from those of Close and End (10.58–22.98% lower, $p < 0.001$ in each comparison). For latency rates, the estimated mean values increased in Retraction, Halt, Hemilami, and Remove. Latency rates decreased from these Halt and Hemilami steps to Close and End (1.03–1.47%, $p < 0.022$ in each comparison) and from Remove to End (1.12%, $p = 0.01$) (Table 2).

Discussion

The effects of surgical procedures of hemilaminectomy on spinal cord integrity were verified using intraoperative SEP monitoring. The multifidus muscles retraction affects spinal cord conductivity. It is suggested that SEP could be the method to assess spinal cord function during surgery in veterinary medicine.

Somatosensory evoked potentials correspond to spinal cord and cortical sensory responses evoked following electrical stimulation of a peripheral sensory nerve or cranial nerves with sensory function; therefore, SEP reflects the function of sensory pathways in the spinal cord and brainstem for cranial nerve studies. Cortical recording of SEP are affected by various factors such as anesthetic agents, nerve stimulate rate, body size (length of pathway), physiological condition and spinal cord integrity of patient (2, 4, 10). Although cortical evoked potentials are attenuated by inhalation anesthetics, isoflurane has little effect on SEP even when bolus doses are administered to obtain a high anesthetic effect during surgery (4, 6). The effect of premedication and induction agent of anesthesia on SEP parameters was unclear. Stimulation frequency affects SEP amplitude. For example, higher amplitude SEP could be obtained by lower frequency stimulation. Because it takes longer time to record, the lower frequency is not feasible for recording of SEP during surgery. Stimulus intensity was determined by small twitch of 5th digit that was induced by slight activation of motor nerves. Activation threshold of the sensory nerve is much lower than that of the motor nerve. Thus, almost all sensory nerve could be activated when the motor nerve was activated slightly (19).

The SEP amplitude evoked by stimulation of the sciatic nerve decreased when compression was applied to the spinal cord, and recovered after release of the applied compression (11). In an experimental study of canine spinal cord ischemia, SEP elicited by stimulation of the sciatic nerve disappeared within a mean ischemic time of 12.4 min. When reperfusion was instituted immediately following the abolition of SEP, SEP recovered and there were no neurologic sequelae. However, when occlusion was maintained for an additional 15 min following abolition of SEP, spastic paraplegia with corresponding histological changes indicative of spinal cord infarction developed (11). Thus, SEP amplitude may be decreased by compression or ischemia of the spinal cord. The release of the compression and the ischemia are important to restore SEP amplitude. In more than 50% of patients, reduction of SEP amplitude and prolongation of its latency after retracting multifidus muscles were not recovered to the pre-operative control values even at the end of surgical procedure. However, all of the patients could walk without any assistance within 7 days after surgery, suggesting that neurological function has been recovered day by day.

In this study, the SEP amplitude rate decreased and the SEP latency rate increased both when the multifidus muscles were removed from the vertebrae and retracted. Therefore, this may indicate that these procedures induce compression of the spinal cord and/or a decrease in blood supply to the spinal cord under IVDH. The SEP amplitude rate continued to decrease and its latency rate continued to increase even after halting surgical manipulation and loosening the muscle retraction and hemilaminectomy, suggesting that perfusion of the spinal cord may not recover sufficiently during these procedures. Removal of the disk material from the spinal canal may release compression from the spinal cord and blood flow recovery, as the SEP amplitude recovers after this procedure. Further studies, however, are necessary to prove this hypothesis

10.3389/fvets.2022.976972

TABLE 1 Number of dogs in each range of amplitude rate of six surgical procedures.

Amplitude rate (% of pre-operative control values)	Retract[a]	Halt[a,b]	Hemilami[a,b]	Remove[b]	Close[c]	End[c]
≥140	0	0	0	0	0	0
130–140	0	0	0	1	1	1
120–130	0	0	0	0	0	2
110–120	0	0	0	1	3	4
100–110	0	1	1	1	8	11
90–100	1	2	1	2	8	8
80–90	6	7	8	12	14	10
70–80	16	20	21	18	13	12
60–70	17	17	17	16	4	3
50–60	12	5	4	1	1	1
<50	0	0	0	0	0	0
Mean ± SD (% of pre-operative control values)	69 ± 11	73 ± 11	73 ± 10	77 ± 14	88 ± 16	92 ± 17
Range (% of pre-operative control values)	52–93	54–100	54–100	58–132	58–132	58–132

Retract, retracting multifidus muscles; Halt, halting surgical procedure and multifidus muscles loosened; Hemilami, hemilaminectomy; Remove, removing the disk materials from the spinal canal; Close, closing multifidus muscles; End, end of the surgery. Procedures with different letters indicate significant difference ($p < 0.05$).

TABLE 2 Number of dogs in each range of latency rate of six surgical procedures.

Latency rate (% of pre-operative control values)	Retract[a,b,c]	Halt[a]	Hemilami[a]	Remove[a,b]	Close[b,c]	End[c]
≥120	0	0	0	0	0	0
110–120	2	3	3	2	1	1
100–110	50	49	49	49	47	45
90–100	0	0	0	1	4	6
<90	0	0	0	0	0	0
Mean ± SD (% of pre-operative control values)	104 ± 4	104 ± 4	104 ± 4	104 ± 4	103 ± 4	103 ± 4
Range (% of pre-operative control values)	100–119	100–119	100–119	98–117	93–114	93–114

Retract, retracting multifidus muscles; Halt, halting surgical procedure and multifidus muscles loosened; Hemilami, hemilaminectomy; Remove, removing the disk materials from the spinal canal; Close, closing multifidus muscles; End, end of the surgery. Procedures with different letters indicate significant difference ($p < 0.05$).

The results of this study indicate that to remove the disk materials that compress the spinal cord may be required to improve spinal cord function. However, surgical manipulation to remove disk materials from the spinal canal poses the risk of compression of the spinal cord. Therefore, it is important to monitor spinal cord function to avoid additional spinal cord compression during surgery.

In this study, the minimal SEP amplitude rate during the surgery was 52% of the control, and the clinical signs of all dogs were improved within 7 days after the surgery. A previous study reported that the safety limit of SEP amplitude was 50% of the control (13). A 50% of the control in SEP amplitude is generally considered alarming in humans (4). In this study, there were no cases SEP amplitude being less than 50% of control. And no cases worsened clinical signs. Therefore, alarm level of neurological deficit of spinal cord cannot be determined. We propose here that maintaining intraoperative SEP amplitudes above 50% of control may help avoid additional spinal cord injury during surgery. Further studies are necessary to confirm this proposal.

There are some limitations in this study. Although intraoperative monitoring of SEP has the potential to increase the safety of spine and spinal cord surgery, SEP can assess only sensory pathway from peripheral sensory nerve to the sensory cortex. Dysfunction of the motor pathway from motor cortex to the muscles cannot be evaluated by SEP. Dysfunction of the motor pathway can be evaluated by motor evoked potential method, utilizing a transcranial magnetic stimulation (20, 21). Because SEP could not be obtained in dogs with grade 5, neurological function cannot be evaluated in such patients.

Conclusions

Intraoperative SEP is useful for monitoring spinal cord integrity during surgical procedure of spine or spinal cord. The retraction of the multifidus muscles under the condition of compressive disk materials would especially cause a decrease in

blood flow and/or compression of the spinal cord. Therefore, additional compression of the spinal cord should be avoided during surgery. Maintaining an SEP amplitude rate above 50% of control is important to avoid excessive spinal cord injury during surgery.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Azabu University Animal Experimentation Committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

SO wrote the majority of the manuscript, performed the surgeries, and SEP examinations. HK wrote statistical analysis parts and performed the statistical analysis. SO and KO designed the study. All authors took part in editing the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

We thank Y. Ohkubo, the Institute of Statistical Mathematics, Tokyo, Japan, for evaluating our data handling.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Sharp N, Wheeler S. Thoracolumbar disk disease. In: Sharp N, Wheeler S, editors. *Small Animal Spinal Disorders: Diagnosis and Surgery*. 2nd ed. St Louis, MO: Mosby Elsevier (2005). p. 121–35. doi: 10.1016/B978-0-7234-3209-8.50012-1

2. Holliday TA. Electrodiagnostic examination. Somatosensory evoked potentials and electromyography. *Vet Clin North Am Small Anim Pract.* (1992) 22:833–57. doi: 10.1016/S0195-5616(92)50079-1

3. Fisher RS, Raudzens P, Nunemacher M. Efficacy of intraoperative neurophysiological monitoring. *J Clin Neurophysiol.* (1995) 12:97–109.

4. Nuwer MR. Spinal cord monitoring. *Muscle Nerve.* (1999) 22:1620–30.

5. Pelosi L, Lamb J, Grevitt M, Mehdian SMH, Webb JK, Blumhardt LD. Combined monitoring of motor and somatosensory evoked potentials in orthopedic spinal surgery. *Clin Neurophysiol.* (2002) 113:1082–91. doi: 10.1016/S1388-2457(02)00027-5

6. Kimura J. Intraoperative monitoring. In: Kimura J, editor. *Electrodiagnosis in Diseases of Nerve and Muscle*. 4th ed. New York, NY: Oxford (2013). p. 573–95. doi: 10.1093/med/9780199738687.003.0021

7. Zentner J, May J. Intraoperative monitoring with somatosensory evoked potentials in neurosurgical operations on the spinal cord. *Cent Nerv Syst Trauma.* (1987) 4:197–207. doi: 10.1089/cns.1987.4.197

8. Vanderzant CW, Schott RJ, Natale JE, Pondo CA, D'Alecy LG. Somatosensory evoked potentials of the dog: recording techniques and normal values. *J Neurosci Methods.* (1989) 27:253–63. doi: 10.1016/0165-0270(89)90087-3

9. Uzuka Y, Saitoh M, Hiramatsu I, Nagata T. Studies of the factors affecting the recording of somatosensory evoked potentials induced by tibial nerve stimulation in dogs. *J Vet Med Sci.* (1995) 57:871–6. doi: 10.1292/jvms.57.871

10. Poncelet L, Michaux C, Balligand M. Influence of body size on tibial nerve somatosensory evoked potentials in dogs. *Am J Vet Res.* (1993) 54:178–82.

11. Dinner DS, Lüders H, Lesser P, Morris HH. Invasive methods of somatosensory evoked potential monitoring. *J Clin Neurophysiol.* (1986) 3:113–30. doi: 10.1097/00004691-198604000-00002

12. Janusz MT, Qayumi AK, Jamieson WRE, Fairholm DJ. Experimental use of somatosensory evoked potential for intraoperative identification of spinal cord blood supply. *J Invest Surg.* (1997) 10:195–203. doi: 10.3109/08941939709032158

13. Shen N, Wang S. Monitoring spinal-cord injury intraoperatively and attempting prognosis by cortical somatosensory evoked potentials: experimental study. *J Reconst Microsurg.* (1998) 14:61–6. doi: 10.1055/s-2007-1006903

14. Okuno S, Nakamura A, Kobayashi T, Orito K. Effectiveness of intraoperative somatosensory evoked potential monitoring during cervical spinal operations on animals with spinal cord dysfunction. *J Vet Med Sci.* (2005) 67:719–22. doi: 10.1292/jvms.67.719

15. Poncelet L, Michaux C, Balligand M. Somatosensory potentials in dogs with naturally acquired thoracolumbar spinal cord disease. *Am J Vet Res.* (1993) 54:1935–41.

16. Sharp N, Wheeler S. Patient examination. In: Sharp N, Wheeler S, editors. *Small Animal Spinal Disorders: Diagnosis and Surgery*. 2nd ed. St Louis, MO: Mosby Elsevier (2005). p. 19–33. doi: 10.1016/B978-0-7234-3209-8.50006-6

17. Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed effects modeling for nested data. In: Zuur AF, Ieno EN, Walker N, et al., editors. *Mixed Effects Models and Extensions in Ecology With R*. New York, NY: Springer (2009). p. 101–42. doi: 10.1007/978-0-387-87458-6_5

18. R Core Team. *A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2020).

19. Kimura J. Somatosensory evoked potential. In: Kimura J, editor. *Electrodiagnosis in Diseases of Nerve and Muscle*. 4th ed. New York, NY: Oxford (2013). p. 477–524. doi: 10.1093/med/9780199738687.003.0019

20. Poma R, Parent JM, Holmberg DL, Partlow GD, Monteith G, Sylvestre AM. Correlation between severity of clinical signs and motor evoked potentials after transcranial magnetic stimulation in large-breed dogs with cervical spinal cord disease. *J Am Vet Med Assoc.* (2002) 221:60–4. doi: 10.2460/javma.2002.221.60

21. Martin-Vaquero P, de Costa RC. Transcranial magnetic motor evoked potentials in Great Danes with and without clinical signs of cervical spondylomyelopathy: association with neurological findings and magnetic resonance imaging. *Vet J.* (2014) 201:327–32. doi: 10.1016/j.tvjl.2014.05.035

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 21 December 2022
DOI 10.3389/fvets.2022.1025528

OPEN ACCESS

EDITED BY
Andrea Tipold,
University of Veterinary Medicine Hannover, Germany

REVIEWED BY
D. Colette Williams,
University of California, Davis, United States
Andrea Fischer,
Ludwig Maximilian University of Munich, Germany

*CORRESPONDENCE
Seward B. Rutkove
srutkove@bidmc.harvard.edu

SPECIALTY SECTION
This article was submitted to Veterinary Neurology and Neurosurgery, a section of the journal Frontiers in Veterinary Science

RECEIVED 23 August 2022
ACCEPTED 21 November 2022
PUBLISHED 21 December 2022

CITATION
Verga SA, Pandeya SR, Kowal JB, Cochran RJ, Lim S, Sabol JC, Coates JR and Rutkove SB (2022) Electrical impedance myography in healthy dogs: Normative values, repeatability, and the impact of age. *Front. Vet. Sci.* 9:1025528. doi: 10.3389/fvets.2022.1025528

Electrical impedance myography in healthy dogs: Normative values, repeatability, and the impact of age

Sarah A. Verga[1], Sarbesh R. Pandeya[1], Joseph B. Kowal[2], Randall J. Cochran[2], Stefanie Lim[2], Julianna C. Sabol[2], Joan R. Coates[2] and Seward B. Rutkove[1]*

[1]Department of Neurology, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA, United States, [2]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, United States

Convenient tools to assess canine skeletal muscle health would be useful for a variety of applications, including standard veterinary assessments of dog fitness, as well as studies of muscle deterioration due to age or disease. One technology that can be applied conveniently to awake dogs with minimal restraint is electrical impedance myography (EIM). In EIM, a weak electrical current is applied *via* surface electrodes to a muscle of interest and consequent impedance characteristics of the muscle are obtained, providing insight into muscle condition and composition. In this study, we assessed a total of 73 dogs (42 males and 31 females), of varied neutering status and breed, ages 0.6 to 13.5 years. We identified age-dependent reference values for the 100 kHz phase value in three pelvic limb muscles, caudal sartorius, cranial tibial, and gastrocnemius. While phase values were generally higher in males than females, the difference did not reach significance. In general, values declined on average with age at about 0.5 degrees/year, but with the decline being most substantial in the oldest dogs. Limited reproducibility assessment of the technique suggested good repeatability with variation in values between measurements being under 5%. These results show that EIM has the potential for the assessment of canine muscle health and may find value in aging muscle research.

KEYWORDS

muscle, neuromuscular, canine, phase, atrophy

1. Introduction

Loss of muscle mass can occur during many catabolic physiological and pathophysiological processes, including physical inactivity and starvation or anorexia, associated with systemic disease. Muscle loss associated with disease is called cachexia, whereas muscle loss associated with aging in the absence of disease is termed sarcopenia (1). Sarcopenia reflects loss of myofibers due to apoptosis and decreases in myofiber size.

Overall muscle circumference is often maintained during early sarcopenia, as lost myofibers are replaced with adipose and fibrous tissue (1). Biochemical assessment of aging canine muscle has confirmed that the upregulation of autophagy occurs in aged dogs leading to myofiber loss (2). Muscle loss is common in animals with chronic diseases (e.g., chronic kidney disease, congestive heart failure, and cancer) or an acute injury or illness and during aging. Because older animals are more likely to develop chronic diseases, sarcopenia and cachexia can occur concurrently.

Visual scoring systems have been used as subjective measures to evaluate body and muscle condition in dogs. While many veterinarians use visual body condition scores (BCS) in which dogs' body condition ranges from 1 (very thin) to 5 (ideal) and to 9 (very overweight) to determine whether dogs are at a correct weight, these systems have significant limitations (3). An important limitation is the fact that visual scores do not adequately differentiate between increased body size due to fat vs. muscle. Animals can have significant muscle loss if overweight (BCS >5). Conversely, animals can have a low BCS (<4) but have minimal muscle loss. Muscle condition score (MCS) is 4-point scale graded as normal, or mild, moderate or severe loss (4). MCS had substantial repeatability and moderate reproducibility for assessment of muscle mass in dogs (5). Thus, body condition score, which is an assessment of fat, and MCS, which is an assessment of muscle, are not directly related.

Clinically relevant methods for quantifying muscle loss are needed. Ultrasonography and computed tomography (CT) of muscle has been used for assessment of epaxial muscles in healthy dogs. An ultrasonographic method for assessment of epaxial muscles has been validated for use on healthy dogs (6). Mean epaxial muscle area measured by ultrasonography was significantly lower in older dogs, compared with younger dogs, whereas epaxial muscle area measured by CT was only significantly lower in older dogs after normalization for vertebral height (7). In addition to loss of skeletal muscle mass, low muscle CT attenuation values suggested that older dogs also had greater muscle fat content than did younger dogs (8).

Electrical impedance myography (EIM) is a tool that has the potential to evaluate individual muscles non-invasively and painlessly, making it possible to use in awake dogs (9, 10). It relies on the application of a very weak, high frequency electrical current to a region of tissue and measurement of the resulting voltages, from which various electrical properties (the impedance) can be inferred. The general concept of EIM is that skeletal muscle can be modeled as a network of resistors and capacitors, with the intracellular and extracellular matrices of muscle tissue acting as resistors and the lipid bilayers that constitute the muscle membranes acting as capacitors. Thus, any global atrophy that reduces the cross-sectional area of muscle tissue would also be expected to increase the resistance (R); loss of both free extracellular and intracellular water will also contribute to increased resistance. As a muscle atrophies, the local capacitance of the muscle increases (more membranes per unit volume of muscle). The capacitance is inversely proportional to reactance (X), and myofiber atrophy, therefore, would be expected to decrease X. Phase angle (or simply phase) (θ) combines R and X into a single value by the equation $\theta = \arctan(X/R)$ as is the most commonly used impedance parameters in EIM studies (11, 12). The advantage of the phase angle is that, to some extent, it corrects for simple volumetric alterations in the tissue, since simply decreasing the size of the muscle will have the effect of increasing X and R to a similar extent. The impedance (Z) itself, is calculated *via* the Pythagorean theorem ($Z = \text{sqrt}(R^2 + X^2)$); however, the impedance itself is rarely used in most applications since R is considerably larger than X and thus impedance tends simply to reflect R. It is a useful outcome parameter since it normalizes to some extent variations in muscle size and electrode geometry. We specifically provide the data on phase (and not resistance or reactance) because phase represents a more inherent muscle property than resistance or reactance values which are far more dependent on electrode size, inter-electrode distances, and muscle size and shape. The integrity of individual cell membranes, myofiber size, and the presence of fat and connective tissue has a significant effect on the tissue's impedance; consequently, a muscle's impedance can be used to measure the tissue's alteration in disease. Indeed, impedance has been shown to change in a variety of neuromuscular conditions and can be used as an overall marker of muscle health (11, 12).

Comparative aging research in companion animals that share the same environment, diet, and exposure to pollutants, etc., as humans may make the canine a more realistic "real-world" model than laboratory rodents. The objective of this study was to assess normal EIM values in selected pelvic limb muscles of healthy dogs, determine its association with age, including preliminarily developing a range of age associated reference values, and assess sex differences and reproducibility.

2. Materials and methods

2.1. Study population

Healthy dogs were recruited by two different mechanisms. One group was recruited at the University of Missouri Veterinary Health Center. These dogs were included in the study if they had no history of neurologic or orthopedic disease, a normal complete blood count and serum biochemistry, and a normal neurologic examination. In addition, boxers were recruited at the American Boxer National Specialty. These dogs were included if they had normal orthopedic and neurologic examinations. All studies were completed with Institutional Animal Care and Use Committee approval and informed consent from the owners. Only dogs with a BCS of 4, 5 and 6 (on the standard 1-9 scale) were included in the data analysis

to reduce the potential impact of excessive body fat on the measurements. Both groups of dogs were studied at single session for practical reasons, as it is very difficult to get these pet dogs back for repeated visits over a short period of time.

2.2. Electrical impedance myography acquisition

A handheld EIM device (mView, Myolex, Inc, Boston, MA) was utilized to obtain impedance measurements from the dogs' hind limb muscles using a small electrode array originally designed for human infants (13). This electrode has three electrode sets embedded within it (Figure 1), two providing current flow along the myofibers and one across myofibers. Based on standard impedance theory (12), the farther apart the electrodes, the great depth of muscle penetration. Thus, only the set that was spaced farthest apart was utilized in this analysis (the outer electrodes for longitudinal measurements, see Figure 1). Five pelvic limb muscles were initially chosen for study based on their easy palpability, superficial location, and size. These included, gastrocnemius, cranial tibial, gracilis, caudal sartorious, and biceps femoris; however, given limitations on testing, including inconsistent values and more limited data sets in gracilis and biceps femoris, results on only three are reported here: cranial tibial, caudal sartorious and gastrocnemius. During data collection, the dogs were awake and gently restrained in lateral recumbency. Clippers were used to remove the hair over the targeted muscle, and saline was used to moisten the skin allowing for effective electrical current conduction from the EIM probe. The probe was placed at the thickest part of the targeted muscle. The skin was re-moistened with a saline-soaked sponge in between each measurement. Three trials of repeated recordings were performed to test the measurements' repeatability. The left side was studied uniformly and the right side less consistently across all dogs. Thus, in this analysis, only left side muscle data is provided.

2.3. EIM data processing

We extracted the 100 kHz values from the multifrequency data set. The third measurement served as the final data set analyzed and the first, second and third were used in the intrarater reproducibility assessments. The main target EIM parameter i.e., phase value was our primary measure for data inclusion and exclusion criteria as any phase value that were negative or $>30^{\circ}$ were excluded as spurious. Similarly, any values that were two standard deviations away from the mean for that muscle were also excluded. Overall, 15 out of 219 (i.e., 6.89%) EIM muscle measurements were filtered and removed. Single muscle data is analyzed as well as the average of 100 kHz data from all three muscles in the limb.

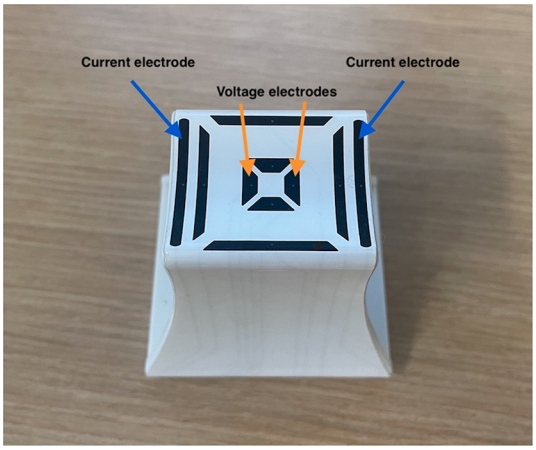


FIGURE 1
Electrode array used to obtain data. The outer electrodes (blue arrows) emit the electrical current and the inner electrodes (orange arrows) measure the resulting voltages.

2.4. Statistical analysis

Analysis was conducted in R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Given its relative independence from simple volumetric effects, we chose the phase value as the primary outcome measure. For this analysis, we used second-degree polynomial regression to observe the quadratic trend of phase value through the fitted regression line along with its lower prediction limit, based on the 95% confidence interval; this regression provided an age-specific lower limit of normal values for each muscle specific muscle. In addition, after assessing for normality with the Kolmogorov-Smirnov test and QQ plots, we used the Wilcoxon rank sum test to conduct comparison tests between the two sexes (male vs. females). To assess the reliability of the EIM measures, we also performed the coefficient of variation (CoV) assessments on the first, second and third repeated measurements from each muscle and their average.

3. Results

3.1. Study population

A total of seventy-three dogs were included in the study, with forty-two males and thirty-one females, all with body conditioning scores between 4 and 6. Of the 42 males, 42 gastrocnemius, 42 caudal sartorius and 20 cranial tibial muscle measurements were obtained. Of the 31 females, 31 gastrocnemius, 30 caudal sartorius and 17 cranial tibial muscle measurements were obtained. Not all muscles were able to be measured due to various circumstances including time

TABLE 1 Mean and standard deviation for age, weight, and BCS for both neutered and intact dogs.

	Females			Males			Total
	Intact females ($N = 20$)	Neutered females ($N = 11$)	Total ($N = 31$)	Intact males ($N = 31$)	Neutered males ($N = 11$)	Total ($N = 42$)	Total ($N = 73$)
Age (years) mean (±SD, range)	2.8 (±2.08, 0.63–7.72)	8.2 (±4.45, 1.05–13.6)	4.3 (±3.58, 0.62–13.5)	3.2 (±3.20, 0.76–11.2)	7.3 (±3.95, 2.0–13.0)	3.9 (±3.56, 0.76–13.0)	4.32 (±3.5, 1–13)
Weight (kg) mean (±SD, range)	26.1 (±3.6, 20.4–31.87)	26.8 (±10.9, 7.7–54.1)	26.8 (±3.2, 7.7–54.1)	31.0 (±3.7, 24.1-41.4)	20.3 (±10.6, 6.8–33.6)	30.3 (±4.2, 6.8–41.4)	28.7 (±1.6, 6.8–54.1)
BCS mean (±SD, range)	4.7 (±0.57, 4–6)	5.2 (±0.75, 4–6)	5.0 5.0 (±0.52, 4–6)	4.8 (±0.45, 4–6)	5.5 (±0.52, 5–6)	4.9 4.9 (±0.4, 4–6)	4.9 (±0.4, 4–6)

restraints, dog owner consent, fur shaving limitations for the show dogs, and other variables. There were different breeds of dogs included in the analysis. These were: Australian Cattle Dog ($n = 1$), Australian Shepherd ($n = 1$), Beagle ($n = 1$), Boxer ($n = 59$), Chinese Crested-Beagle mix ($n = 1$), German Shepherd Dog ($n = 2$), Great Dane ($n = 1$), Greyhound ($n = 1$), Husky mix ($n = 1$), Jack Russel Terrier ($n = 1$), Labrador Retriever mix ($n = 1$), mixed-breed ($n = 1$), Pit Bull mix ($n = 1$), Rat Terrier ($n = 1$) and Treeing Walker Coonhound ($n = 1$). All the Boxers were show dogs and their data was collected on site at the American Boxer National Specialty. However, the remaining dogs were pet volunteers and underwent data collection at the University of Missouri Veterinary Health Center during routine clinical visits. As described in detail in Table 1, mean age was 4.3 years, mean weight of 28.7 kgs, and mean BCS of 4.9. Table 1 further summarizes the number of neutered dogs along with means of age, weight and BCS; neutered dogs tended to be younger than intact animals.

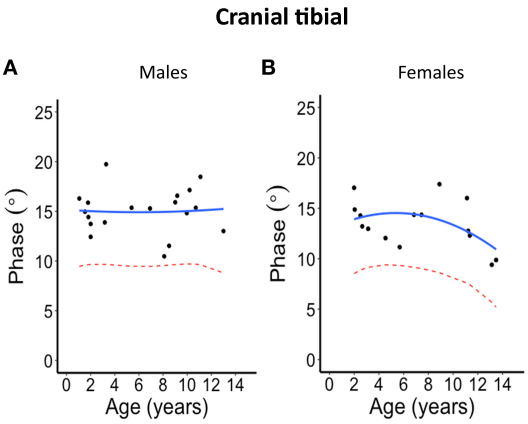


FIGURE 2
Phase angle values at 100 kHz made on cranial tibial muscle for **(A)** males and **(B)** females. The blue solid line represents the normal fit generated from the second-degree polynomial regression through the log-transformed data while the red dotted lines represent the parallel curve that is the lower limit of normal based on the 95% prediction limit of the quadrative curve.

3.2. Evaluation of EIM phase with age in male and female dogs: Establishing lower limits of normal

The second-degree polynomial regression result shows the normal linear or parabolic pattern, and its lower limit fit as given in Figures 2–5 for each muscle and their average for different sexes, as well the lower 95% confidence limit, that can be defined as the lower limit of normal. Despite the relative small sample size, it was evident from all these graphs that phase tends to decrease with age. This is further summarized in Table 2 and provided with age-specific lower limits of normal.

3.3. Relationship of all EIM parameters to sex and age: A multivariate analysis

In the previous analysis, we generated a simple table (Table 2) by which one can identify normal vs. abnormal values for EIM separately by sex for the major EIM parameter of phase. Here we take another view at the data set by incorporating age and sex values into a multivariate regression. Table 3 summarizes these results for all three impedance parameters. As can be seen, of all three impedance parameters, resistance appears to be most age-sensitive, increasing significantly with age for all individual muscles and on average across all the muscles. Phase showed reductions in gastrocnemius and on average across all

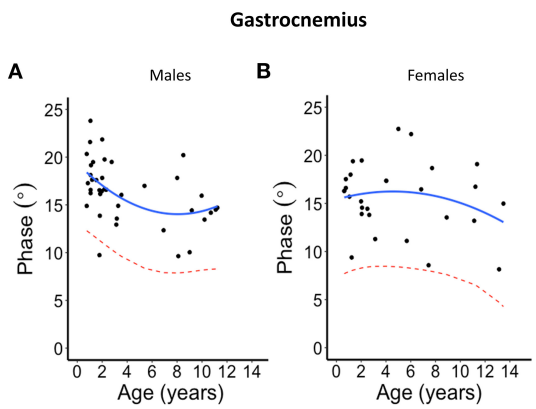


FIGURE 3
Phase angle values at 100 kHz made on gastrocnemius muscle for **(A)** males and **(B)** females. The blue solid line represents the normal fit generated from the second-degree polynomial regression through the log-transformed data while the red dotted lines represent the parallel curve that is the lower limit of normal based on the 95% prediction limit of the quadrative curve.

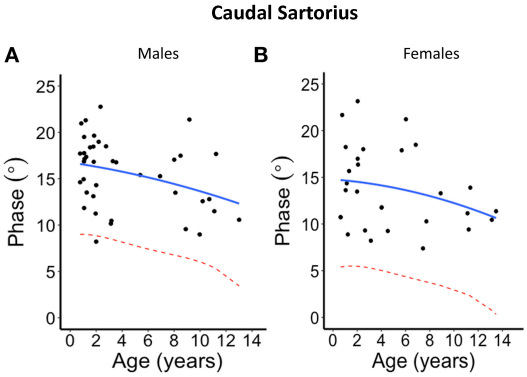


FIGURE 4
Phase angle values at 100 kHz made on caudal sartorius muscle for **(A)** males and **(B)** females. The blue solid line represents the normal fit generated from the second-degree polynomial regression through the log-transformed data while the red dotted lines represent the parallel curve that is the lower limit of normal based on the 95% prediction limit of the quadrative curve.

three muscles with age but not in the other muscles separately. Reactance showed no significant relationship with age. Although samples are relatively small, male and female animals did not show significant differences in this analysis.

3.4. Within-session, single evaluator repeatability of EIM measurements (phase, resistance, and reactance)

To test the repeatability each of the EIM parameters, we used the measurements in the first, second and third trials in rapid succession, as described above, obtained by a single evaluator. Based on the assessment of the three muscle groups *via* coefficient of variation and mean percent difference, it was evident that EIM displayed good repeatability. A detailed summary of these values is given in Table 4.

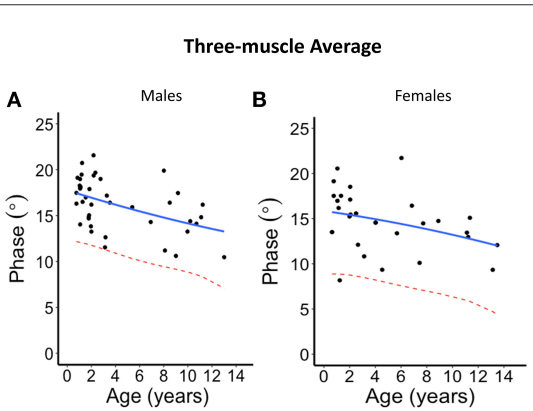


FIGURE 5
Phase angle values at 100 kHz made on average of the three muscles for **(A)** males and **(B)** females. The blue solid line represents the normal fit generated from the second-degree polynomial regression through the log-transformed data while the red dotted lines represent the parallel curve that is the lower limit of normal based on the 95% prediction limit of the quadrative curve.

4. Discussion

In this study, we were able to demonstrate a trend toward decreasing phase values with age in most pelvic limb muscles studied in both male and female dogs of varying breed. This reduction in values mirrors that observed in other animals, including mice (14) and humans (15, 16), where the technique has also been applied. Preliminary studies also show a similar trend in zebrafish (Rutkove, unpublished communication). The age-associated decline in EIM parameters, most generally apparent at the oldest ages, is consistent with, but not diagnostic of, sarcopenia. Of course, as we noted earlier, concomitant changes, including cachexia, possibly related to concurrent conditions such as undiagnosed neoplasm or cardiac dysfunction could also play a role. The relatively minor alterations in the cranial tibial in males with age is unclear.

Through our fitted models, we were able to also establish a lower limit of normal for these pelvic limb muscles, as we had done previously in humans and have also provided age-specific lower limits of normal based on the modeled 95% confidence limits (17). The data here could assist in identifying dogs with abnormalities in muscle if the values fall below the lower range

TABLE 2 Lower limit of 95% prediction from the second-degree polynomial regression showing phase reduction with increasing age.

Muscle	Sex	Age (years)						
		1	3	5	7	9	11	13
Gastrocnemius	Female	7.9°	8.4°	8.4°	8.1°	7.6°	6.6°	4.9°
	Male	12.1°	10.1°	8.6°	7.9°	8.0°	8.3°	8.0°
Cranial tibial	Female	7.6°	9.1°	9.4°	9.1°	8.6°	7.7°	5.9°
	Male	9.4°	9.7°	9.5°	9.5°	9.6°	9.6°	8.8°
Caudal sartorius	Female	5.5°	5.3°	4.7°	4.9°	3.4°	2.5°	0.9°
	Male	9.0°	8.5°	7.8°	7.1°	6.4°	5.5°	3.5°
Average of 3 muscles	Female	8.9°	8.5°	7.9°	7.3°	6.7°	6.0°	4.8°
	Male	12.1°	11.3°	10.5°	9.7°	9.2°	8.4°	7.1°

TABLE 3 EIM parameter relationship to age and sex *via* multivariate regression, revealing significant relationships with age for some parameters, but not for sex.

Muscle	EIM measure	Parameter	Estimate	Standard error	*P*-value	Adjusted *R*-squared
Gastrocnemius ($N = 73$)	Phase	Age	−0.347	0.104	0.001	0.125
		Sex	0.558	0.823	0.499	
	Resistance	Age	1.191	0.339	<0.001	0.149
		Sex	−2.888	2.326	0.218	
	Reactance	Age	0.024	0.090	0.78	0.017
		Sex	−0.684	0.666	0.308	
Cranialtibialis ($N = 37$)	Phase	Age	−0.127	0.108	0.251	0.03114
		Sex	1.036	0.859	0.237	
	Resistance	Age	0.829	0.235	0.001	0.252
		Sex	−2.2075	1.805	0.230	
	Reactance	Age	0.116	0.081	0.162	0.0018
		Sex	0.123	0.643	0.849	
Sartorius ($N = 72$)	Phase	Age	−0.1269	0.108	0.251	0.031
		Sex	1.0359	0.859	0.237	
	Resistance	Age	0.829	0.235	0.001	0.252
		Sex	−2.207	1.805	0.230	
	Reactance	Age	0.116	0.081	0.162	0.0018
		Sex	0.123	0.643	0.849	
Average ($N = 73$)	Phase	Age	−0.346	0.091	<0.001	0.170
		Sex	0.918	0.710	0.200	
	Resistance	Age	1.045	0.265	<0.001	0.194
		Sex	−3.027	1.815	0.100	
	Reactance	Age	0.076	0.063	0.24	0.026
		Sex	−0.741	0.495	0.14	

of values. Of note, we do not provide an upper limit of normal, since nearly all muscle disorders would be expected to reduce the phase values, not increase them, with the exception, perhaps of myostatin mutations causing marked muscle hypertrophy as described in Whippets (18, 19).

We performed additional multivariate regression to assess the impact of sex and together on all the major impedance parameters. This analysis further supported the polynomial regression completed separately for the sexes described above but also showed that many of the age effects were mostly related to changes in resistance [which secondarily impacts phase, since phase = arctan (reactance/resistance)] and not reactance. It also suggested that there was no significant impact of sex in this analysis. Finally, our repeatability assessments, although limited, show acceptable values for all three impedance parameters.

TABLE 4 Single evaluator, within-session EIM repeatability assessments.

Muscle	Value	Phase	Resistance	Reactance
Gastrocnemius	Mean percent difference (%)	1.3	1.9	3.8
	Coefficient of variation	0.095	0.0342	0.091
Cranial tibial	Mean percent difference (%)	2.6	1.7	1.2
	Coefficient of variation	0.079	0.035	0.089
Caudal Sartorius	Mean percent difference (%)	2.1	0.98	1.9
	Coefficient of variation	0.090	0.049	0.099

This study had several limitations. First, to some extent these dogs represented a convenience sample of mixed breeds, sexes, and intact or neutered status. There were many Boxers as compared to other breeds because part of data collection was mainly limited to performance dogs present at a dog show. Also, we lacked multiple examiners and day-to-day measurement comparisons for all dogs and thus our repeatability assessments are fairly limited. Clearly, further studies will be needed to fully understand the real-world reproducibility of this technique. Second, while we overall have an acceptable number of animals for such an analysis (seventy-three), as one breaks it down by sex, clearly the numbers are not as large as might be desirable. Thus, the normative lower limits here provided, could only serve as a starting point for an eventually larger database. In addition, it is also possible that a contribution of increasing subcutaneous fat in the oldest dogs could play a role as well. We sought to exclude this possibility by including only dogs with healthy body conditioning scores of 4, 5, or 6, thus any confounding effect from this should have been limited. Nevertheless, it is impossible to exclude a contribution of subcutaneous fat to these results. Furthermore, we only looked at small set of pelvic limb (appendicular) muscles, and did not include any epaxial muscles typically assessed as part of screening of dog health, though there is no reason a similar approach could not be used. In addition, we only report normative values on a single impedance measure at a single frequency; clearly, similar analyses could have been performed separately for the resistance and reactance and at other frequencies. Next, the normative values established here are dependent on the specifics of the electrode array applied, including interelectrode distance and electrode size. Fortunately, the phase values tend to be less effected than other impedance parameters by these variations. In addition, we studied dogs no older than age 13.5 years. While this represents a typical life expectancy for many breeds, clearly extending these assessments to still older dogs would be interesting. Finally, we did not separate out sexually intact from neutered animals in our analyses since this would have further reduced our sample sizes in each category to such a point that the analyses would have lost considerable power.

There are several important applications to this initial work of applying EIM technology to the assessment of dog muscle health. First, it helps demonstrate that EIM has the potential serve as a useful tool to assess canine muscle condition across the ages and can be applied successfully to a number of dogs of various breeds. This could have value in the pet food industry as well as serving as an office tool by veterinarians to assess condition of show dogs or dogs with various medical ailments. Second, it could help in the assessment of dog models of human muscle disease, including dystrophin deficiency (as has already been studied with EIM to some extent (10)), myotubular myopathy (20), and nemaline myopathy (21). Finally, and perhaps most interesting, it could serve as useful tool for studies of aging more broadly, with specific application to human aging. Indeed, it is already currently being applied for this purpose as part of the Dog Aging Project (22).

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by University of Missouri Institutional Animal Care and Use Committee; Protocol # 10077. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

JK and JC contributed to the conception and design of the study. JK, JC, RC, JS, and SL contributed to data collection. SR, SV, and SP performed the data analysis. SR wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

Funding was provided by both the ALS Association TREAT ALS (15-LGCA-249) and University of Missouri, College of Veterinary Medicine Phi Zeta Grant Award.

Acknowledgments

An abstract based on this manuscript has been accepted to the American College of Veterinary Internal Medicine conference.

Conflict of interest

SR has equity in, and serves a consultant and scientific advisor to Myolex, Inc and Haystack Diagnostics, companies that design impedance devices for clinical and research use; he is also a member of the Myolex's Board of Directors. The companies also have an option to license patented impedance technology of which SR is named as an inventor.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Freeman LM. Cachexia and sarcopenia: emerging syndromes of importance in dogs and cats. *J Vet Intern Med.* (2012) 26:3–17. doi: 10.1111/j.1939-1676.2011.00838.x

2. Pagano TB, Wojcik S, Costagliola A, De Biase D, Iovino S, Iovane V, et al. Age related skeletal muscle atrophy and upregulation of autophagy in dogs. *Vet J.* (2015) 206:54–60. doi: 10.1016/j.tvjl.2015.07.005

3. Laflamme D. Developmental and validation of a body condition score system for dogs. *Canine Pract.* (1997) 22:10–5.

4. Nutrition WAAVAG. Muscle Condition Score Chart (2013). Available online at: http://www.wsava.org/nutrition-toolkit

5. Freeman LM, Michel KE, Zanghi BM, Vester Boler BM, Fages J. Evaluation of the use of muscle condition score and ultrasonographic measurements for assessment of muscle mass in dogs. *Am J Vet Res.* (2019) 80:595–600. doi: 10.2460/ajvr.80.6.595

6. Freeman LM, Sutherland-Smith J, Prantil LR, Sato AF, Rush JE, Barton BA. Quantitative assessment of muscle in dogs using a vertebral epaxial muscle score. *Can J Vet Res.* (2017) 81:255–60.

7. Hutchinson D, Sutherland-Smith J, Watson AL, Freeman LM. Assessment of methods of evaluating sarcopenia in old dogs. *Am J Vet Res.* (2012) 73:1794–800. doi: 10.2460/ajvr.73.11.1794

8. Sutherland-Smith J, Hutchinson D, Freeman LM. Comparison of computed tomographic attenuation values for epaxial muscles in old and young dogs. *Am J Vet Res.* (2019) 80:174–7. doi: 10.2460/ajvr.80.2.174

9. Sanchez B, Pacheck A, Rutkove SB. Guidelines to electrode positioning for human and animal electrical impedance myography research. *Sci Rep.* (2016) 6:32615. doi: 10.1038/srep32615

10. Hakim CH, Mijailovic A, Lessa TB, Coates JR, Shin C, Rutkove SB, et al. Non-invasive evaluation of muscle disease in the canine model of duchenne muscular dystrophy by electrical impedance myography. *PLoS ONE.* (2017) 12:e0173557. doi: 10.1371/journal.pone.0173557

11. Rutkove SB. Electrical impedance myography: background, current state, and future directions. *Muscle Nerve.* (2009) 40:936–46. doi: 10.1002/mus.21362

12. Rutkove SB, Sanchez B. Electrical impedance methods in neuromuscular assessment: an overview. *Cold Spring Harb Perspect Med.* (2019) 9:34405. doi: 10.1101/cshperspect.a034405

13. Kolb SJ, Coffey CS, Yankey JW, Krosschell K, Arnold WD, Rutkove SB, et al. Baseline results of the neuronext spinal muscular atrophy infant biomarker study. *Ann Clin Transl Neurol.* (2016) 3:132–45. doi: 10.1002/acn3.283

14. Arnold WD, Taylor RS Li J, Nagy JA, Sanchez B, Rutkove SB. Electrical impedance myography detects age-related muscle change in mice. *PLoS ONE.* (2017) 12:e0185614. doi: 10.1371/journal.pone.0185614

15. Aaron R, Esper GJ, Shiffman CA, Bradonjic K, Lee KS, Rutkove SB. Effects of age on muscle as measured by electrical impedance myography. *Physiol Meas.* (2006) 27:953–9. doi: 10.1088/0967-3334/27/10/002

16. Kortman HG, Wilder SC, Geisbush TR, Narayanaswami P, Rutkove SB. Age- and gender-associated differences in electrical impedance values of skeletal muscle. *Physiol Meas.* (2013) 34:1611–22. doi: 10.1088/0967-3334/34/12/1611

17. Rutkove SB, Fogerson PM, Garmirian LP, Tarulli AW. Reference values for 50-Khz electrical impedance myography. *Muscle Nerve.* (2008) 38:1128–32. doi: 10.1002/mus.21075

18. Mosher DS, Quignon P, Bustamante CD, Sutter NB, Mellersh CS, Parker HG, et al. A Mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genet.* (2007) 3:e79. doi: 10.1371/journal.pgen.0030079

19. Shelton GD, Engvall E. Gross muscle hypertrophy in whippet dogs is caused by a mutation in the myostatin gene. *Neuromuscul Disord.* (2007) 17:721–2. doi: 10.1016/j.nmd.2007.06.008

20. Goddard MA, Mack DL, Czerniecki SM, Kelly VE, Snyder JM, Grange RW, et al. Muscle pathology, limb strength, walking gait, respiratory function and neurological impairment establish disease progression in the PN155k canine model of X-linked myotubular myopathy. *Ann Transl Med.* (2015) 3:262. doi: 10.3978/j.issn.2305-5839.2015.10.31

21. Evans JM, Cox ML, Huska J, Li F, Gaitero L, Guo LT, et al. Exome sequencing reveals a nebulin nonsense mutation in a dog model of nemaline myopathy. *Mamm Genome.* (2016) 27:495–502. doi: 10.1007/s00335-016-9644-9

22. Creevy KE, Akey JM, Kaeberlein M, Promislow DEL. An open science study of ageing in companion dogs. *Nature.* (2022) 602:51–7. doi: 10.1038/s41586-021-04282-9

frontiers | Frontiers in Veterinary Science

TYPE Review
PUBLISHED 26 January 2023
DOI 10.3389/fvets.2023.1037404

OPEN ACCESS

EDITED BY
Fiona May Keir James,
University of Guelph, Canada

REVIEWED BY
Thomas Parmentier,
Montreal University, Canada
Jan Rémi,
Ludwig Maximilian University of Munich, Germany

*CORRESPONDENCE
Marguerite F. Knipe
✉ mfknipe@ucdavis.edu

SPECIALTY SECTION
This article was submitted to Veterinary Neurology and Neurosurgery, a section of the journal Frontiers in Veterinary Science

RECEIVED 05 September 2022
ACCEPTED 03 January 2023
PUBLISHED 26 January 2023

CITATION
Knipe MF, Bush WW, Thomas KE and Williams DC (2023) Periodic discharges in veterinary electroencephalography—A visual review. *Front. Vet. Sci.* 10:1037404. doi: 10.3389/fvets.2023.1037404

Periodic discharges in veterinary electroencephalography—A visual review

Marguerite F. Knipe[1]*, William W. Bush[2], Kristen E. Thomas[2] and D. Colette Williams[3]

[1]Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, Davis, CA, United States, [2]Bush Veterinary Neurology Service, Leesburg, VA, United States, [3]School of Veterinary Medicine, William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, Davis, CA, United States

First described in human EEG over 60 years ago, there are very few examples of periodic discharges in the veterinary literature. They are associated with a wide variety of etiologies, both intracranial and systemic, making interpretation challenging. Whether these patterns are indicative of ictal, interictal, or postictal activity is a matter of debate and may vary depending on the clinical features in an individual patient. Periodic discharges have a repeated waveform occurring at nearly regular intervals, with varying morphology of individual discharges from simple sharp waves or slow waves to more complex events. Amplitudes, frequencies, and morphologies of the discharges can fluctuate, occasionally evolving, or resolving over time. This study presents a visual review of several veterinary cases with periodic discharges on EEG similar to those described in human EEG, and discusses the current known pathophysiology of these discharges.

KEYWORDS

EEG, canine, feline, encephalopathy, epilepsy, seizures, status epilepticus

1. Introduction

In human medicine, electroencephalography (EEG), and particularly continuous EEG (cEEG) has become an essential part of monitoring brain function in critically ill patients, both with and without known brain disease (1, 2). First described in human EEG over 60 years ago (3, 4), periodic discharges are noted with varying frequency depending on the patient population, however, they are being documented with increasing frequency in the continuous critical care EEG recordings (2, 5–7).

The first reported periodic pattern was termed PLEDs (periodic lateralized epileptiform discharges), and these discharges were often noted in association with seizures and acute structural brain disease in humans, particularly stroke (3, 8–11). In 2005, the American Clinical Neurophysiology Society (ACNS) recommended removing the word "epileptiform" from the terminology for periodic discharges to be more strictly descriptive of the waveforms, and to avoid clinical connotations or to suggest etiology (12). Subsequent terminology guidelines upheld and elaborated on standardized descriptions for periodic discharges to facilitate consistency (13–15) (Box 1). This alteration in terminology reflects the research over the years supporting that periodic discharges (PDs) are not always related to seizures (i.e. not "epileptiform"), but are sequelae to a focally or globally injured or otherwise dysfunctional cerebrum (10, 11, 16, 17) from a wide variety of causes, including seizures/status epilepticus (6), systemic metabolic

BOX 1 Definitions (14, 15).

Generalized (G): any bilaterally synchronous and symmetric pattern, even if it has a restricted field (e.g., bifrontal).

Lateralized (L): unilateral OR bilateral but clearly and consistently higher amplitude in one hemisphere (bilateral asymmetric); OR bilateral but with a consistent lead-in from the same side (bilateral asynchronous).

Bilateral Independent (BI): two independent (and therefore asynchronous) lateralized patterns with one in each hemisphere, with both patterns occurring simultaneously, i.e., two independent patterns occurring at the same time (overlapping in time) rather than sequentially (one starting after the other stops).

Unilateral Independent (UI): two independent (and therefore asynchronous) periodic or rhythmic patterns in the same hemisphere, with both patterns occurring simultaneously i.e., two independent patterns occurring at the same time (overlapping in time) rather than sequentially (one starting after the other stops).

Multifocal (Mf): at least three independent lateralized patterns, with at least one in each hemisphere, with all three or more patterns occurring simultaneously.

Periodic: Repetition of a waveform with relatively uniform morphology and duration with a clearly discernible interdischarge-interval between consecutive waveforms and recurrence of the waveform at nearly regular intervals.

Discharges: Waveforms lasting <0.5 s, regardless of number of phases, or waveforms ≥0.5 s with no more than 3 phases. This is as opposed to Bursts, defined as waveforms lasting ≥0.5 s and having at least 4 phases. Discharges and bursts must clearly stand out from the background activity

"Plus" modifiers: additional features causing the PD pattern to be more ictal-appearing than the same PD pattern would without the modifier

- *+F*: superimposed Fast activity as part of the PD pattern and not just background activity
- *+R*: Rhythmic or quasi-rhythmic delta (<4 Hz) activity superimposed over the PD pattern
- *+FR*: both modifiers are present in the PD pattern

Fluctuating: ≥3 changes in frequency, each <1 min length

Evolving: ≥2 changes in frequency in the same direction, e.g., 1 → 1.5 → 2 Hz

disease (1, 18), hypothermia (19), and administration of (20, 21) or withdrawal from (7) anesthetic drugs.

The concept of the "ictal-interictal continuum" (IIC) recognizes that many patients with status epilepticus or acute brain injury from any cause may alternate between ictal and interictal—but encephalopathic—states, and it is within the IIC where many of the rhythmic and periodic patterns lie (2, 10, 22–24).

Veterinary medicine has few reports on EEG beyond describing seizures and ictal patterns (25, 26). In spite of EEG abnormalities determining the highest tier of confidence for diagnosis of idiopathic epilepsy in dogs (27), its use is currently uncommon beyond emergency referral or university practices (28–31). With increased access to digital EEG, its use in monitoring and managing veterinary patients with status epilepticus (SE) or non-convulsive status epilepticus (NCSE) is also increasing (28–30).

This descriptive paper presents visual examples of PDs in veterinary EEG paralleling those described in human EEG, and reviews the human literature on what is known and still unknown about PDs. The goal of this report is to increase recognition and understanding of these periodic patterns on veterinary EEG, especially for monitoring in the critical setting.

2. Case selection

Canine and feline EEG cases from two veterinary facilities (UC Davis, Bush Veterinary Neurology Services—multiple locations) from 2003 to 2021 were retrospectively identified by authors (DCW, WWB, MFK) for presence of periodic discharges on the recording as defined in Box 1. Subdermal needle electrodes were used in all cases, and recording montages and electrode placement for each facility were slightly different (Supplementary Figures 1, 2), with concurrent electrocardiogram (ECG). Recordings were reviewed primarily in each institution's bipolar montage (Supplementary Figures 1, 2) (29, 32); remontaging to view a referential montage was also done to aid localization of events (33). The referential montage utilized 'linked ears' (A1 & A2; Supplementary Figure 1) as the second input.

Cases were chosen exclusively on the visual identification of PDs occurring for 25% or more of the EEG recording. All cases with PDs were clinical patients, for which the EEG was done at the request of the primary clinician for one of three reasons: documenting ictal or interictal abnormalities in a patient with known seizures, evaluating for possible non-convulsive status epilepticus (NCSE), or assessment of cortical function in a patient with stupor or coma. Specific sedation drugs to obtain the EEG were used as clinically needed to assist in electrode placement and dampen muscle artifact. This ranged from no medications (obtunded/stuporous patients), to only whatever antiseizure medications (ASMs) the patient was receiving for management, or specific intravenous (IV) administration of various sedative drugs to obtain a diagnostic EEG recording. Characteristics of the PDs noted were waveform morphology, frequency of the discharges, fluctuation or evolution of either morphology or frequency, and the localization of the discharge, either generalized (GPD) or lateralized (LPD) (Box 1).

3. Results

3.1. Localization/polarity

The prominent feature of visual identification of PDs is the repeated waveform of relatively uniform morphology, prominently standing out from the background, occurring at a regular or nearly regular interval, with a discernible inter-discharge interval present between consecutive waveforms (Box 1). Localization of the PDs was noted as either generalized (GPD) if the discharge was synchronous and symmetric in the hemispheres (Figure 1), or lateralized (LPD) if the discharge localized to one hemisphere or lobe, either exclusively or primarily (Figure 2). GPDs may have a frontal predominance (14), if both right and left sides are similarly affected (Figure 1). LPDs were localized on bipolar montage with instrumental phase reversal, and/or on referential montage by assessing the channels with the highest amplitude (Figure 2). Only GPD or LPD patterns were recognized in our cases: no examples of other described PDs in human medicine were noted [Bilateral Independent (BIPDs), Unilateral Independent (UIPDs), or Multifocal (MfPDs)] (Box 1).

10.3389/fvets.2023.1037404

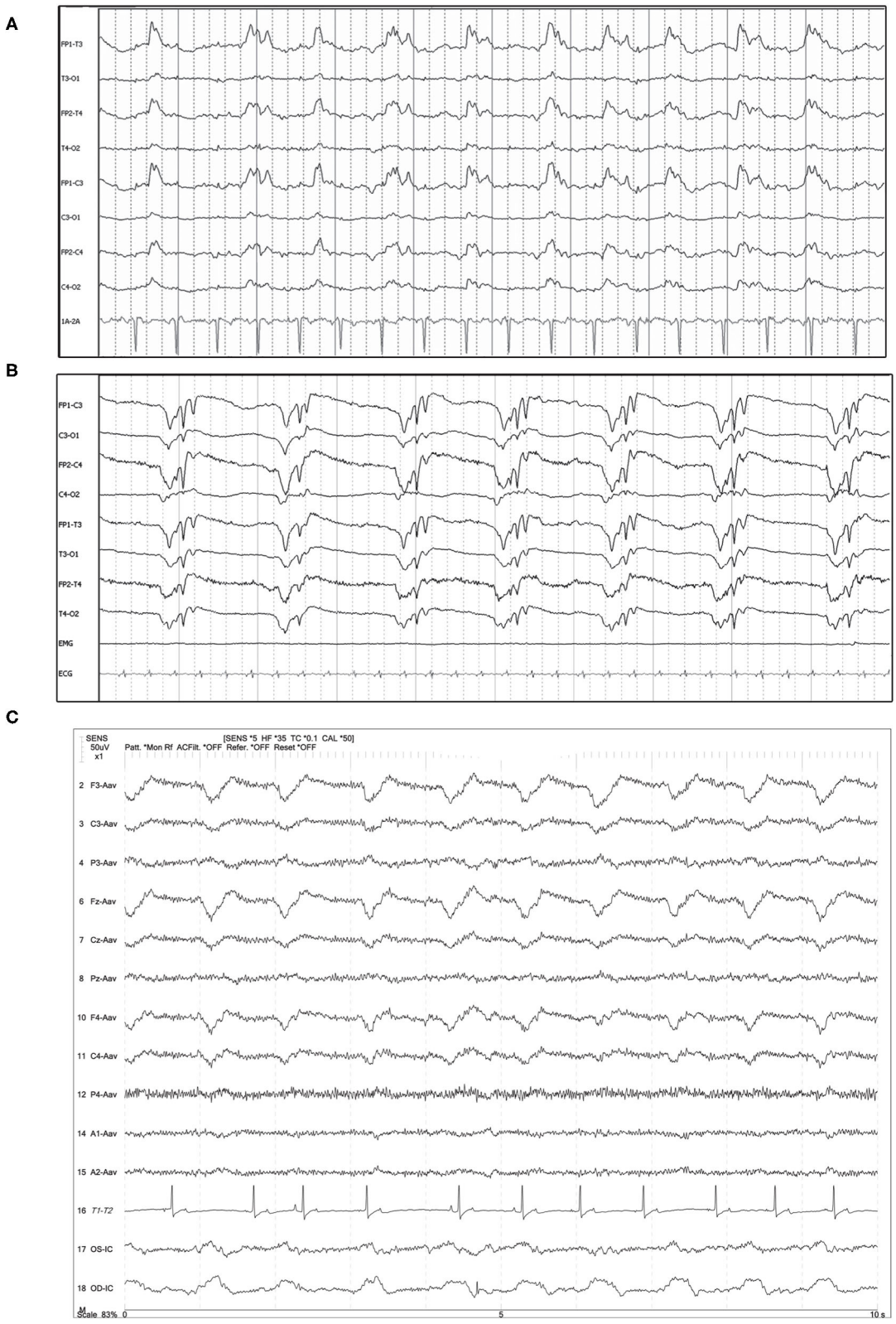


FIGURE 1
Three examples of generalized periodic discharges (GPDs). PDs are noted predominantly frontal and in both hemispheres in each case. **(A)** Ten year old mixed breed dog with several year history of seizures and recent cluster seizures. Polyphasic GPDs, synchronous and bilateral, with slightly higher amplitude in the left hemisphere (Bipolar montage; HF 70 Hz, sensitivity 5 uV/mm). **(B)** Four year old domestic longhair cat with otogenic bacterial encephalitis and seizures. Polyphasic GPDs (Bipolar montage; HF 70 Hz, sensitivity 7 uV/mm). **(C)** Thirteen year old Bichon frise with MUO and recent cluster seizures (Referential montage; HF 35 Hz, sensitivity 5 uV/mm). The amplitude of the discharge is slightly lower in F4 than F3 & Fz, although the timing of the PD is synchronous in the frontal leads. MUO, meningoencephalitis of unknown origin.

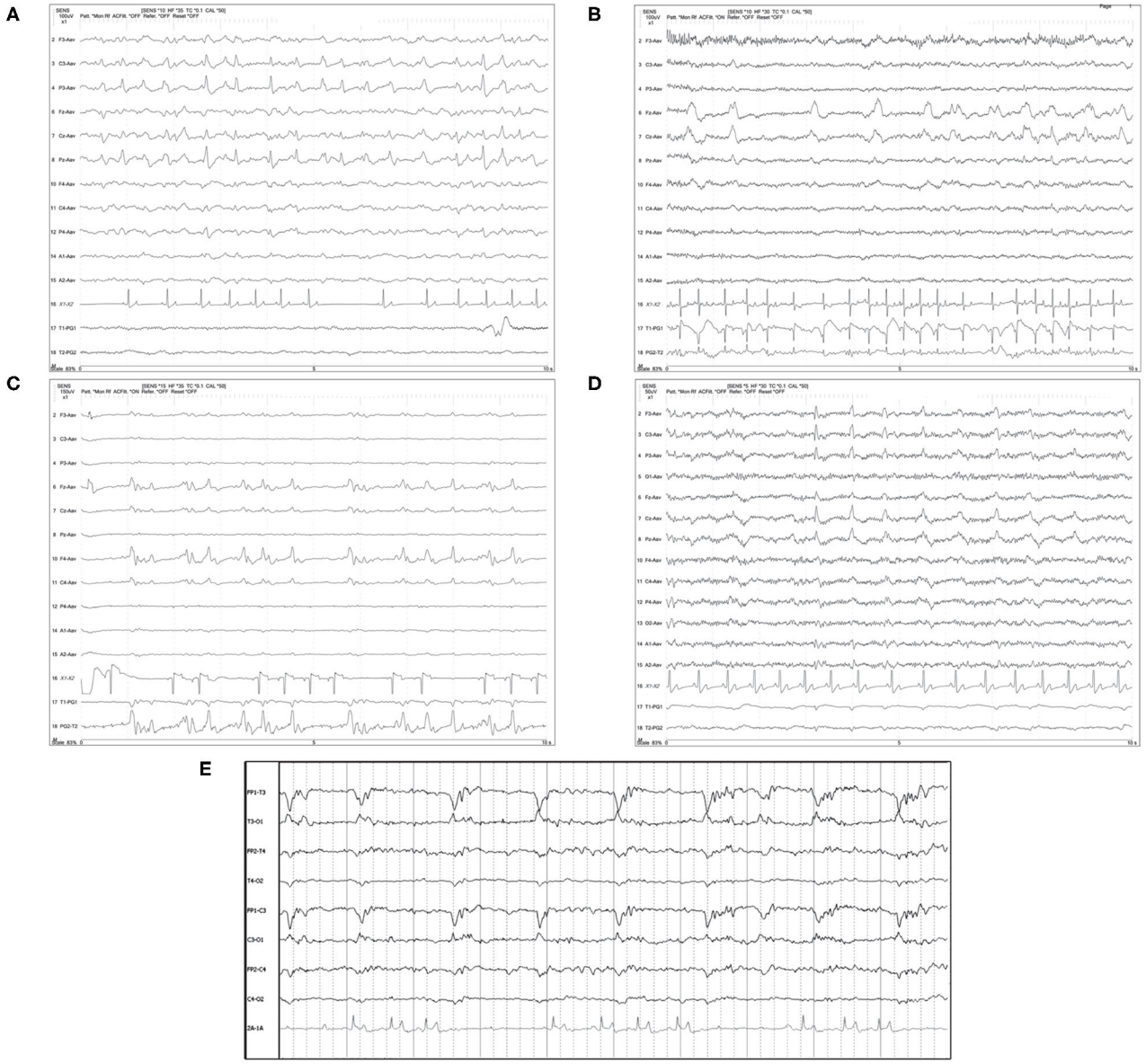


FIGURE 2
Referential **(A–D)** and bipolar **(E)** montages of five cases with lateralized periodic discharges (LPDs). **(A)** Left parietal LPDs in a 12 year old pug with cluster seizures, normal brain MRI (HF 35 Hz, sensitivity 7 uV/mm). **(B)** Frontal midline LPDs in a 14 year old Chihuahua mix with cluster seizures secondary to hypoglycemia and an insulin-secreting tumor (HF 30 Hz, sensitivity 10 uV/mm). **(C)** Right frontal LPDs in an 11 year old miniature Pinscher with recent SE and history of transfrontal craniotomy 6 months previously for right olfactory/frontal lobe meningioma (HF 35 Hz, sensitivity 15 uV/mm). **(D)** Left hemisphere LPDs in an 11 year old Labrador with recent cluster seizures and history of craniotomy 6 months previously for left occipital meningioma (HF 30 Hz, sensitivity 5 uV/mm). **(E)** Left hemisphere polyphasic LPDs (phase reversal at T3 and C3) in an 8 year old soft-coated Wheaton terrier with metastatic neoplasia (HF 35 Hz, sensitivity 5 uV/mm).

3.2. Waveform morphology

Periodic discharge morphology was typically a solitary, static waveform by definition (Figures 1, 2), and there were also instances of paired waveforms, like a doublet or triplet, that had consistent temporal associations with each other (Figure 3). In some cases, morphology of the PD changed: either becoming more polyphasic, or less polyphasic or "simpler." Changes in PD morphology might be noted within the same recording (Figure 4), or on subsequent recheck EEGs (Figure 5), and could change spontaneously (Figure 4), or in response to an intervention, like ASM administration (Figure 6).

3.3. Waveform modifiers

Additional characteristics of PD waveforms, when present in humans, increase the suspicion for association with or development of seizures (Box 1). Fast activity or rhythmic activity (designated "+F" and "+R," respectively), superimposed on

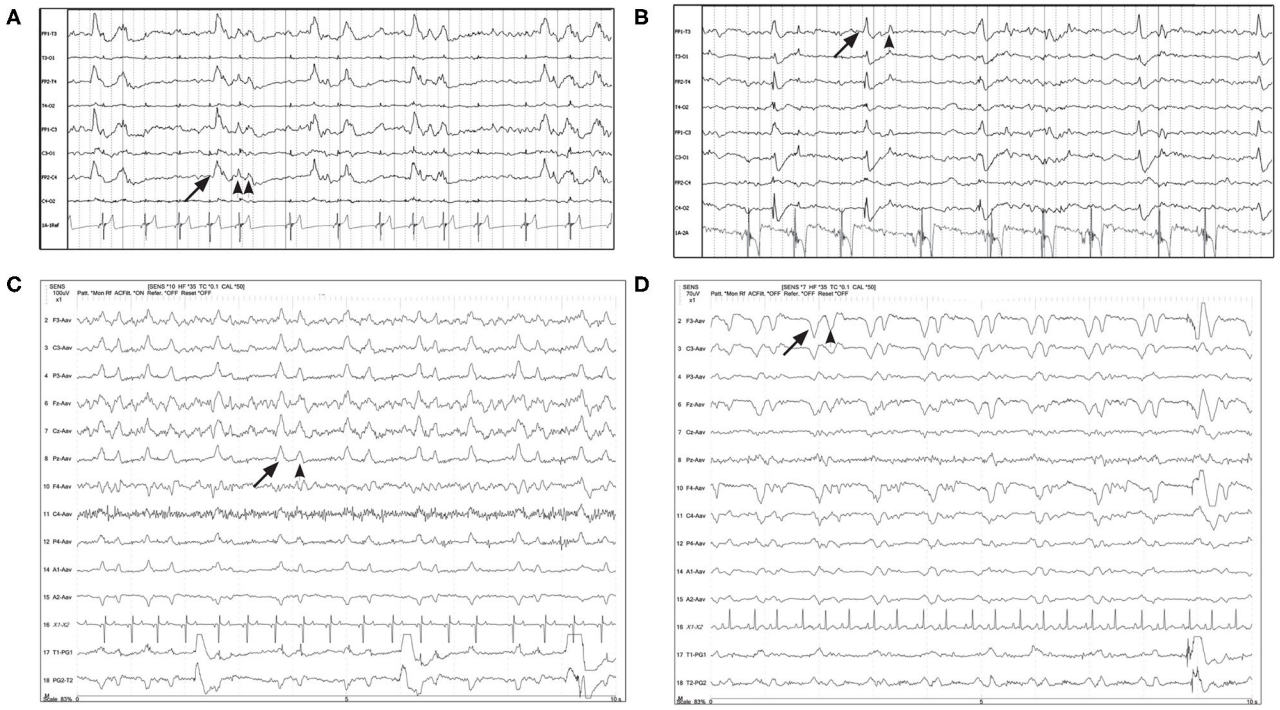


FIGURE 3
Four examples of periodic discharges, where the morphology of the PD (solid arrow) included a second or third waveform (arrowheads), with a consistent temporal relationship to the initial discharge, like a doublet or triplet. **(A)** Eleven year old Chihuahua with SE (Bipolar montage; HF 70 Hz, sensitivity 20 uV/mm). **(B)** Two year old bichon mix with progressive partial seizures and MUO (Bipolar montage; HF 70 Hz, sensitivity 10 uV/mm). **(C)** Three year old terrier mix with cluster seizures, normal brain MRI, likely idiopathic epilepsy (Referential montage; HF 35 Hz, sensitivity 10 uV/mm). **(D)** Five year old Basset hound presented with heat stroke, stuporous, multiple organ dysfunction, no seizures documented (Referential montage; HF 35 Hz, sensitivity 7 uV/mm).

the PDs are correlated with an increased likelihood of seizures in humans (15). In some of our cases, there were PDs with superimposed fast activity that could be considered to have +F features (Figure 7). In the two EEGs depicted in Figure 7, both dogs had recordings done in close temporal proximity to their seizures.

3.4. Waveforms with triphasic morphology

"Triphasic waves" in human EEG are noted as a characteristic GPD with a small upward phase, a large, broad, downward phase, followed by a third larger upward phase (Figure 8 inset), with a relatively low frequency (0.5–2 Hz). Current human nomenclature describes these waveforms as "GPDs with triphasic morphology" (15). Examples of this type of waveform morphology are seen in Figure 8, with underlying etiologies including both metabolic encephalopathy as well as idiopathic epilepsy.

3.5. Frequency

The frequency of the PDs was primarily static for the individual recording (Figures 1–3, 5, 7, 8), and in some instances was fluctuating (Box 1) between higher and lower frequencies (Figure 9), or evolving (Box 1), and evolving PDs were noted to progress to ES or clinical seizures in some cases (Figures 4, 10).

3.6. Response to intervention

If specific intervention during the EEG recording was done, it was typically an IV bolus of an antiseizure medication (ASM) (various drugs based on clinical decision, most commonly a benzodiazepine, phenobarbital, or levetiracetam). When IV ASMs were administered, many cases exhibited changes in the frequency and morphology of the PDs, but the discharges often persisted after drug administration, and in some cases returned to pre-ASM character with time if the medication had a short half-life (Figure 6).

4. Discussion

Even with hundreds of published cases of periodic discharges on EEG, with corresponding imaging, diagnosis, and outcome, there is still much debate in human medicine about the origin and clinical implications of various PDs, and particularly whether or not treatment with anti-seizure medication is indicated (9–11, 16, 17, 24, 34–36). In human medicine, PD frequency is known to change—either increasing or decreasing (8–11)—sliding

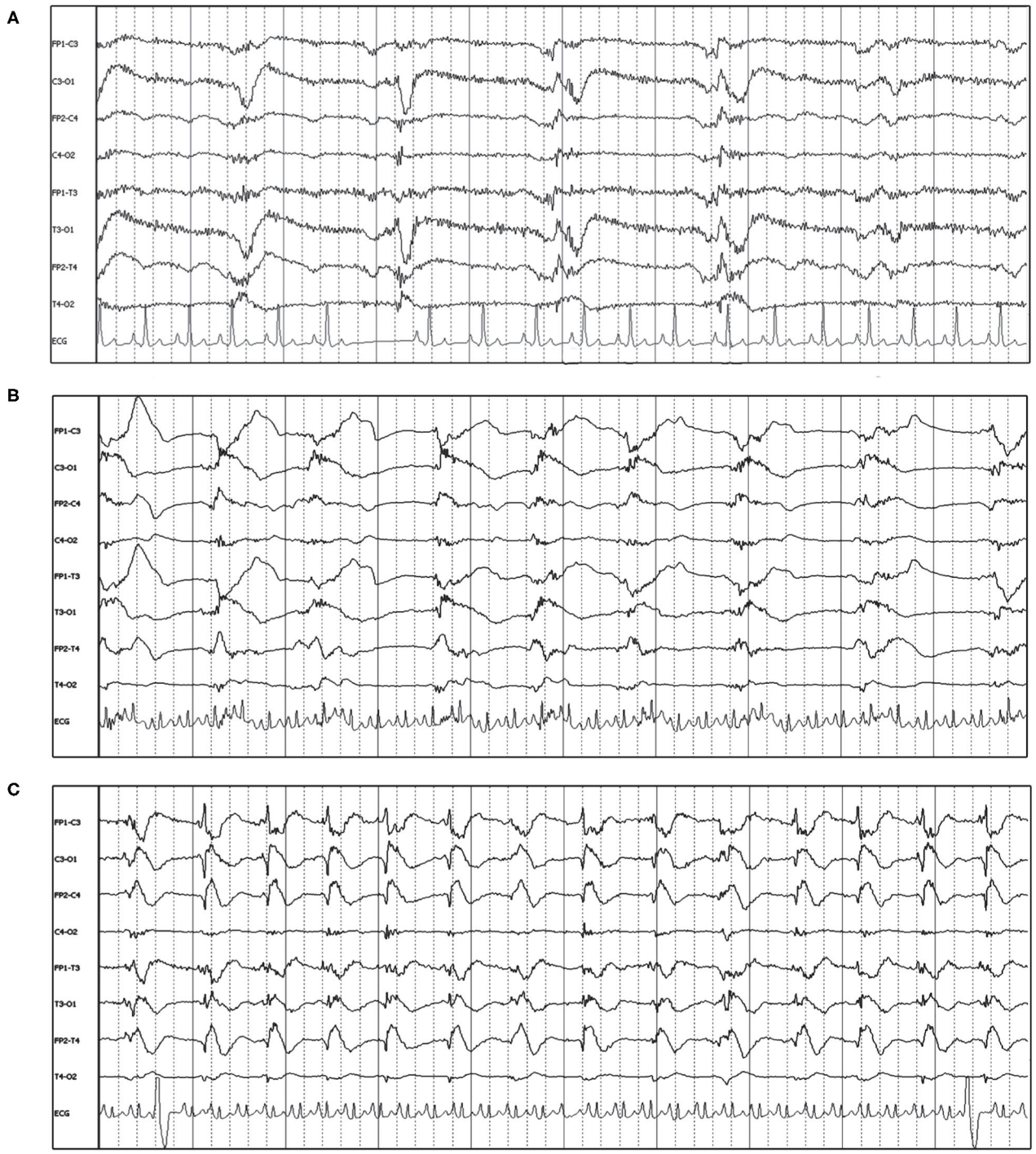


FIGURE 4
EEG epochs over the course of 45 min in a 10 year old Boxer with hypoglycemia and SE demonstrating evolution in PD frequency as well as change in morphology to a more ictal-appearing pattern. Bipolar montage. **(A)** PD frequency is about 0.5 Hz, some with a triphasic morphology. There is no apparent phase reversal, but the discharge has the highest amplitude on the left side, and end-of-chain would localize to O1 (HF 35 Hz, sensitivity 7 uV/mm). **(B)** Frequency increases to almost 1 Hz, along with a change in morphology and increased amplitude—note the change in sensitivity. Discharges are still lateralized (LPDs), with phase reversal noted in the left hemisphere at T3 and C3 (HF 15 Hz, sensitivity 50 uV/mm). **(C)** Frequency of PDs continues to increase to 1.5 Hz, becoming more generalized and developing a spike-wave morphology, and the clinical diagnosis of electrographic seizure (ES) was made (HF 15 Hz, sensitivity 30 uV/mm).

up or down the IIC; PD waveform morphology can change—either spontaneously (11) or in response to intervention like surgery (37) or drugs (20, 21); and PDs are frequently documented to resolve with resolution or improvement of the underlying process (7, 8, 11, 19, 21). The case examples in this report demonstrated all of these characteristics. It is perhaps not surprising that dogs and cats can exhibit pathological periodic patterns comparable to those in people, since these species have been

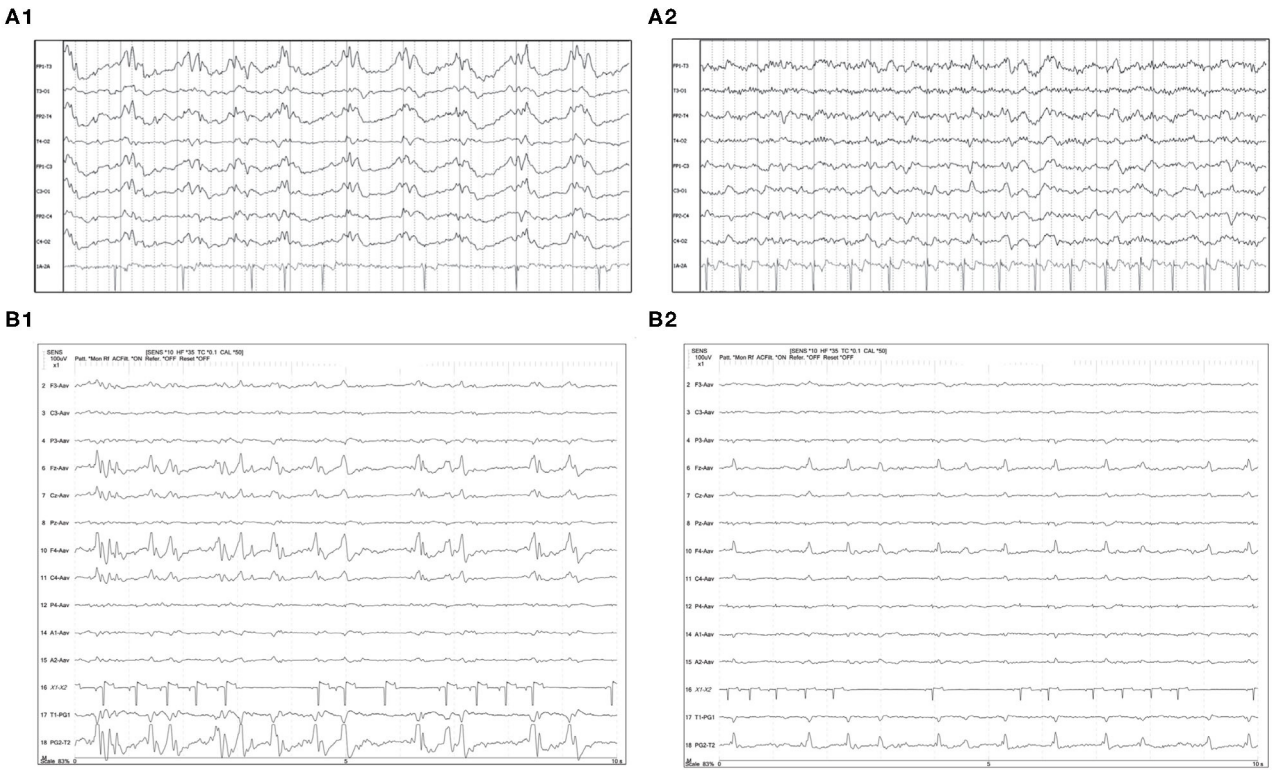


FIGURE 5
Two examples of resolution **(A1, A2)** or apparent improvement **(B1, B2)** of PDs on serial EEGs. **(A1)** Three year old Labrador mix with cluster seizures and MUO. Polyphasic GPDs frequency about 1Hz (bipolar montage; HF 70 Hz, sensitivity 3 uV/mm). **(A2)** Recheck EEG of the same dog **(A1)** on the following day showing resolution of the PDs and apparently normal EEG (bipolar montage; HF 15 Hz, sensitivity 2 uV/mm). **(B1)** Eleven year old Miniature Pinscher with recent SE (same dog as Figure 2C). Polyphasic right frontal LPDs (referential montage; HF 35 Hz, sensitivity 10 uV/mm). **(B2)** Recheck EEG of the same dog **(B1)** on the following day. The right frontal LPDs are still present, but are no longer polyphasic, and have a lower amplitude than noted in **(B1)** (referential montage; HF 35 Hz, sensitivity 10 uV/mm). MUO, meningoencephalitis of unknown origin.

investigated for decades for similarities to humans in functional neuroanatomy (38–40).

Advances in invasive and non-invasive multimodal monitoring have not only increased detection of PDs in critically ill people, they have provided new information about the generators and pathophysiological processes associated with periodic discharges. Periodic discharges were originally thought to be secondary to white matter disease only, but human necropsy studies of patients with PDs on EEG showed cortical and/or subcortical gray matter lesions almost exclusively (41).

The origin of PDs is currently theorized as subsequent to interneuron exhaustion from repeated depolarization. The prolonged recovery of interneurons secondary to a structural lesion or frequent, intense depolarization (e.g., SE) results in disinhibition of adjacent local field potentials, permitting synchronization and generation of large-scale discharges, likely detected as PDs on scalp EEG (24, 42). Recent neural network modeling with disinhibition of synaptic connections as a parameter, reliably reproduces GPDs (43), and altering the patterns of disinhibition or the time delay in the model simulates GPDs with varying morphology (polyphasic, multiple waves). This is interesting pathophysiology to consider, given the variation in the morphology of the PDs both between different patients (Figures 1–3, 5, 6), and changes in PD morphology within the same patient, either spontaneously (Figures 4, 10) or with time and continued treatment (Figure 5). It is likely that the changes in PD morphology over time indicate re-establishment of more normal synaptic connections, and that the patient is "moving in the right direction," down the IIC. In human EEG, the "+F" and "+R" superimposed additional rhythms (Figure 7) impart a higher likelihood of seizures, and the PDs with these additional features are more likely to be on the ictal side of the IIC (15). Therefore, simpler PDs likely indicate a more stable or less epileptogenic cortex, and are likely better for the patient. In case series of human patients with acute causes for PDs, over time, PD frequency decreased and the waveform morphology became less complex and longer in duration (wider waveform) before disappearing (8, 11). Similarly, in our cases where repeat EEGs were done, "simplification" or complete resolution of the PDs was noted (two cases illustrated in Figure 5) as the patient gradually improved clinically.

Visual examples of GPDs with triphasic morphology were specifically highlighted (Figure 8), since the origin, pathophysiology, and naming of this waveform morphology is one of the most debated of all the periodic patterns (44, 45). In human EEG, these previously named "triphasic waves" (TWs) were initially considered pathognomonic for hepatic encephalopathy (HE) (46). Over the years, however, many exceptions were noted, where TWs were present and the underlying process was not HE [toxins/medications (7, 20), immune-mediated encephalitis (47), among many others (44)], and there is no longer an assumption of a metabolic encephalopathy when TWs are noted. A human EEG study (48)

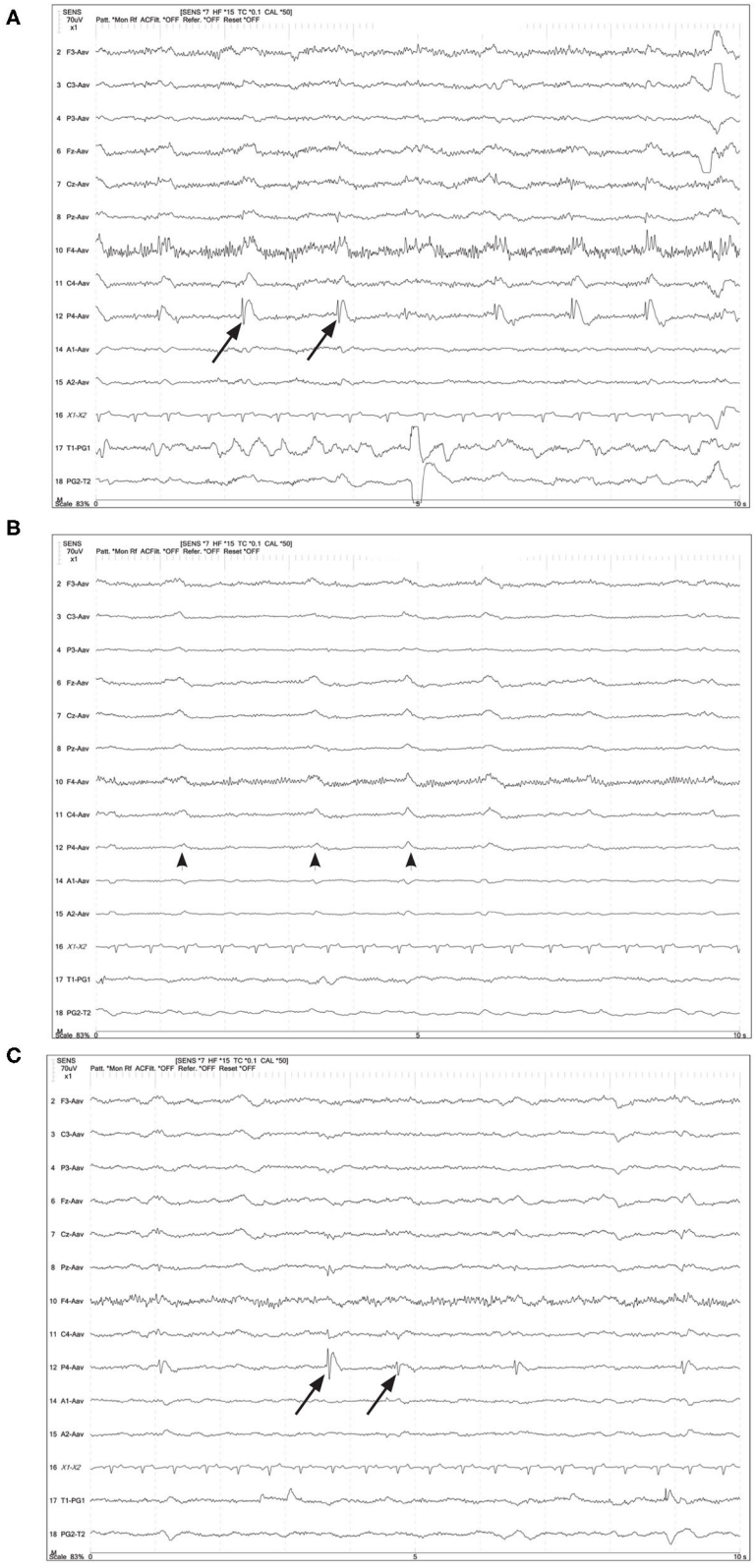


FIGURE 6
Four year old German Shepherd with cluster seizures and HE. Referential montage; HF 15 Hz, sensitivity 5 uV/mm in all panels. **(A)** Right parietal LPDs (arrows) with spike-wave morphology, immediately prior to IV midazolam (MDZ). **(B)** Shortly after IV MDZ, the LPD morphology is changed to more blunted and lower amplitude (arrowheads), but still noted on the right hemisphere and midline chain. **(C)** Ten minutes post-MDZ, the LPDs at P4 (arrows) have returned to the spike-wave morphology.

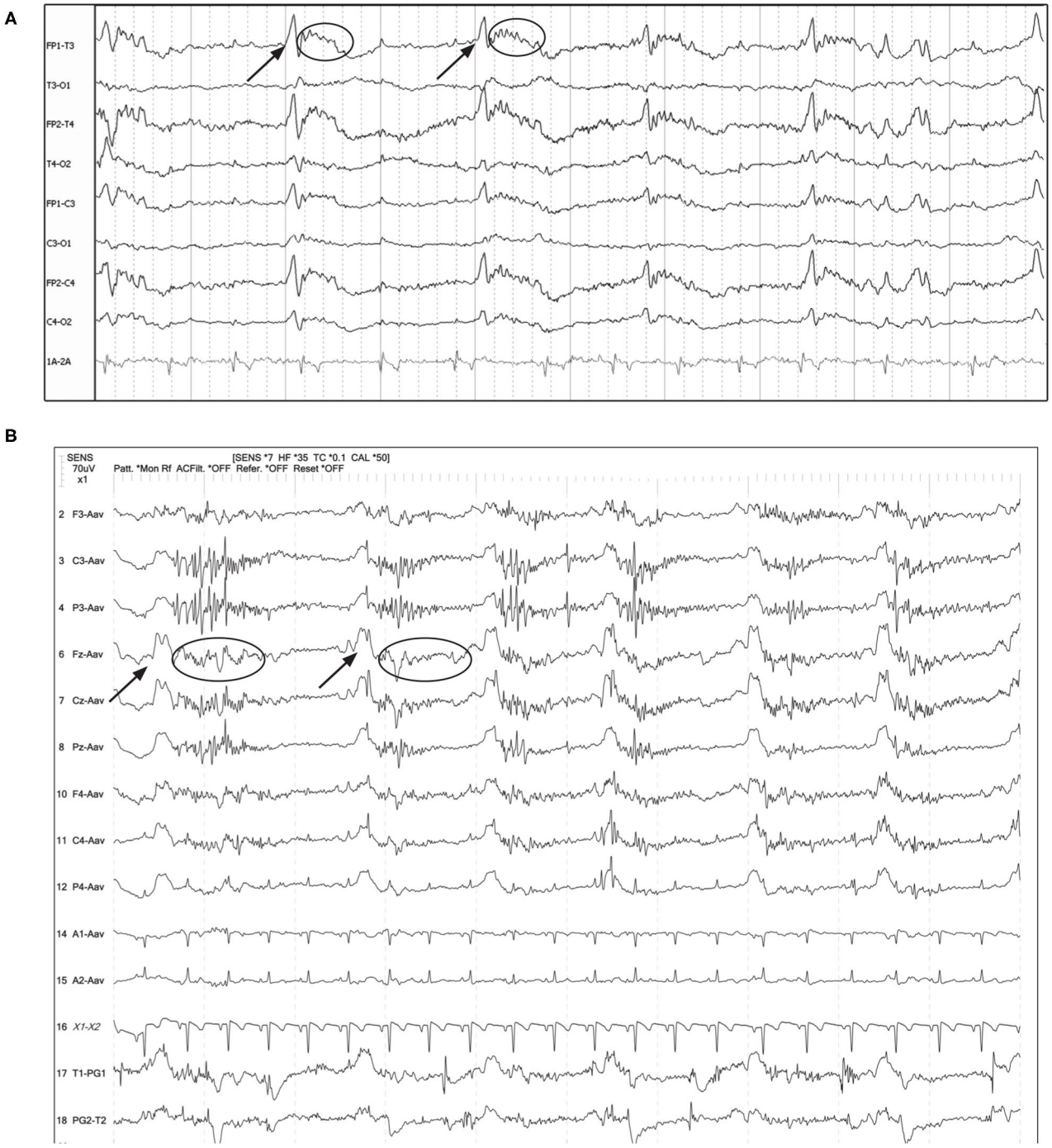


FIGURE 7
Examples of GPDs (arrow) with apparent superimposed fast activity (circle), similar to the "+F" modifier (Box 1). **(A)** Seven year old Swiss Mountain Dog presenting in SE, post-ictal changes noted on MRI, likely idiopathic epilepsy (Bipolar montage; HF 70 Hz, sensitivity 2 uV/mm). **(B)** Two year old French bulldog with cluster seizures and MUO, 10 min post-MDZ IV bolus. A left ear twitch was noted AFTER each PD and fast activity, in the interdischarge interval. ECG artifact is noted in channels 14 & 15 (Referential montage; HF 35 Hz, sensitivity 7 uV/mm). MUO, meningoencephalitis of unknown origin.

evaluating inter-rater agreement on identification of GPDs and TWs found excellent inter-rater agreement for presence of GPDs, but only fair agreement for triphasic morphology. Interestingly, cases with TWs in this human study were less likely to have toxic/metabolic causes for their encephalopathy than those without TWs (48). Similar to the other adjustments in EEG terminology to avoid implying etiology, the current nomenclature describes these waveforms as "GPDs with triphasic morphology" (15), for which a metabolic encephalopathy would be one potential underlying cause (18, 44, 45). In the four cases of TWs illustrated in Figure 8, only one had HE (Figure 8D); the other cases had no systemic metabolic derangements, and seizures only. Conversely, the absence

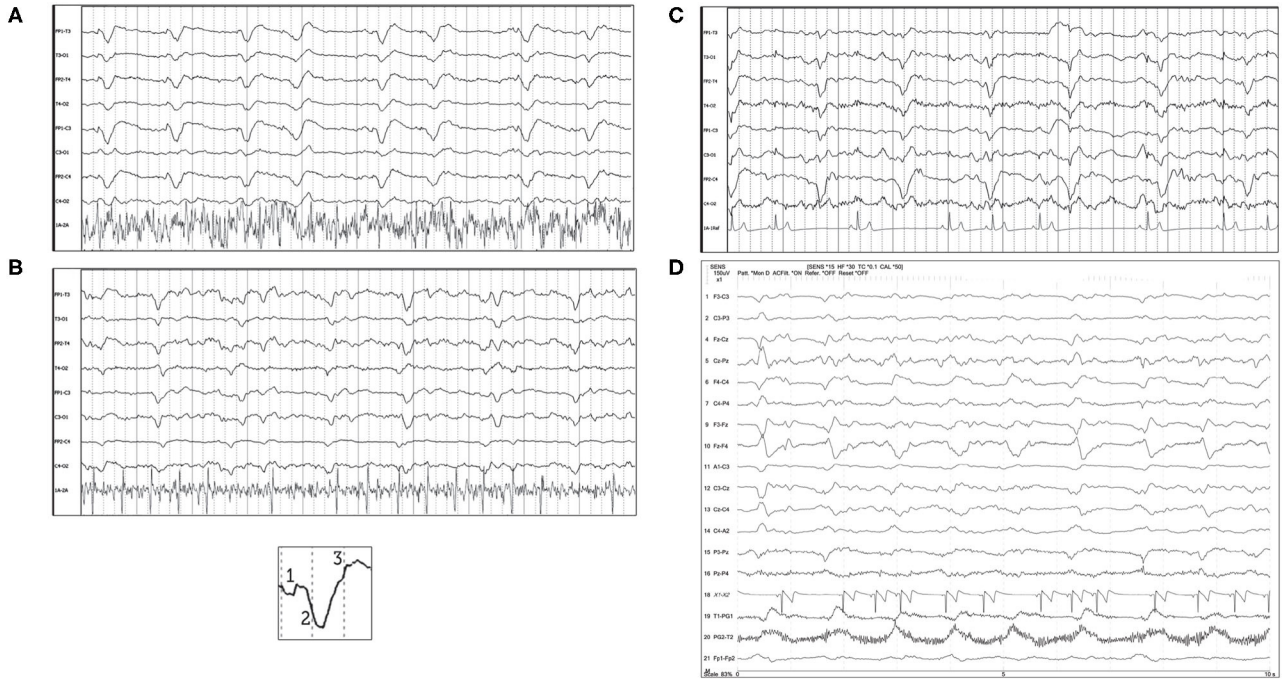


FIGURE 8
Bipolar montages of four cases with GPDs with triphasic morphology. The initial phase in each case is very small, with prominent second and third phases (inset figure). **(A)** Three year old Australian Shepherd mix with seizures and cerebral intra-axial mass on MRI (HF 70 Hz, sensitivity 7 uV/mm). **(B)** Five year old American Staffordshire terrier with cluster seizures, polycythemia (HF 15 Hz, sensitivity 3 uV/mm). **(C)** Five year old Scottish terrier with cluster seizures, likely idiopathic epilepsy (HF 30 Hz, sensitivity 15 uV/mm). **(D)** Four year old wirehair terrier with seizures and HE (HF 30 Hz, sensitivity 15 uV/mm).

of GPDs with triphasic morphology does not exclude metabolic encephalopathy as an underlying disease: Figure 6 is an example of a dog with HE, seizures, and LPDs with a spike-wave morphology and not the prototypical triphasic morphology.

4.1. Frequency

Where a periodic discharge falls on the IIC strongly depends on its frequency. By the ACNS definition (15), any discharge ≥2.5 Hz is more likely to be an ictal pattern, whether a clinical seizure (patient has a synchronous physical manifestation) or solely an electrographic seizure (ES). A periodic discharge of ≤1 Hz is more likely interictal, and PDs with interdischarge intervals between 1 and 2.5 Hz have an increasing predisposition for seizures with the increasing frequency of the discharge (5, 6). In the two figures illustrating evolution of PDs, both the frequency and the complexity of the PDs increased, resulting in ES in one dog (Figure 4) and a clinical seizure in another (Figure 10).

4.2. Indicator or inciter of injury?

While the debate continues about whether PDs should be managed as ictal or interictal, as more is learned about the cellular pathophysiology of brain injury, a new question for consideration is whether PDs are solely sequelae to a cortical/subcortical injury, or are the depolarizations of the large volumes of neurons generating the PDs actually causing additional injury to the brain (5, 24). Some aspects of functional neuroimaging have contributed additional information (49). Magnetoencephalography (MEG) detects magnetic fields associated with current flow in neurons, and can facilitate further understanding of neural networks involved in generating PDs (50). MEG in patients with structural lesions and PDs shows LPDs originating from the interface region between the lesion and the normal brain—the "watershed" zone—not the lesioned brain itself, suggesting reactivity to the insult (50). Focal restricted diffusion on magnetic resonance imaging (rd-MRI) diffusion-weighted imaging (DWI) in patients with seizures results from increased metabolism and cytotoxic edema secondary to the ictal activity (51). A study of DWI and humans with LPDs on EEG with and without seizures, demonstrated rd-MRI only in the patients with seizure patterns and PDs, not in patients with PDs alone, suggesting that PDs alone do not cause restricted diffusion, the ictal activity does (52). Variable DWI changes are described in dogs with known history of clinical seizures (53), but timing of the imaging from the seizures (54) and the necessity of general anesthesia in veterinary patients for MRI likely alter the metabolic changes in the brain and thus the appearance on the imaging.

Witsch et al. (5) utilized invasive multimodal monitoring to show real-time changes in partial pressure of oxygen in interstitial brain tissue (PbtO2) in patients with PDs on EEG. They demonstrated a direct correlation with higher frequency PDs (>2 Hz) and tissue hypoxia, resembling the pathophysiologic changes seen with seizures. Very interestingly, their real-time monitoring of PbtO2 showed a decrease in brain tissue oxygenation that preceded increases in PD frequency, also suggesting that PDs on EEG are reactive to brain injury/hypoxia rather than causal (depending on the frequency).

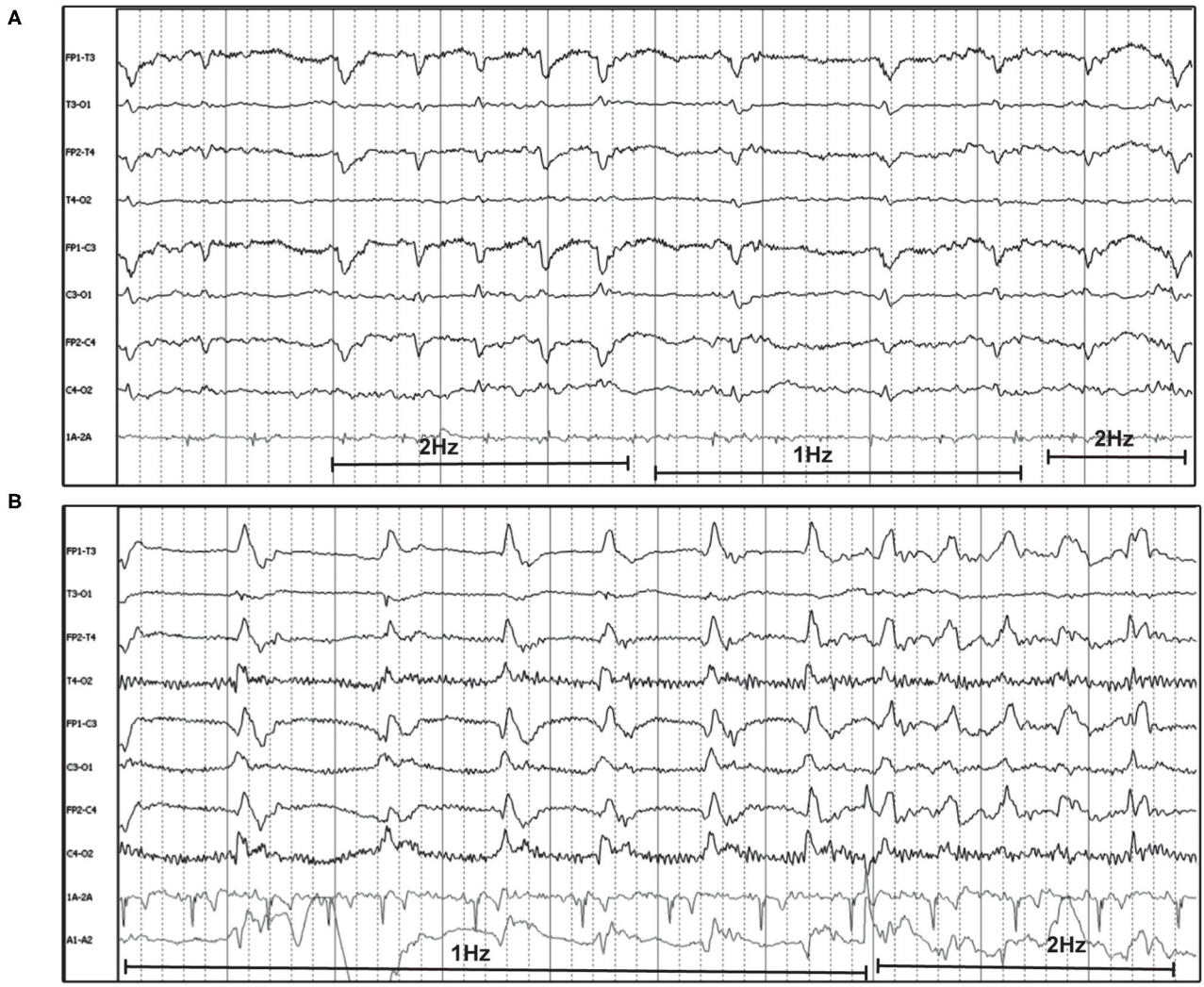


FIGURE 9
Two examples of fluctuating frequency of PDs in bipolar montage. **(A)** Ten year old domestic longhair cat with cluster seizures, unknown etiology (HF 70 Hz, sensitivity 10 uV/mm). **(B)** Two year old beagle with cluster seizures and MUO (HF 15 Hz, sensitivity 7 uV/mm). Only two changes in frequency are seen in this epoch, but frequency continued to fluctuate during the recording.

They acknowledge, however, that brain tissue hypoxia and PDs may also perpetuate a vicious cycle, initiated by hypoxia and exacerbated by higher-frequency PDs (5).

4.3. Outcome

Much of the historical literature on PDs in human medicine is in patients with documented seizures, other brain injury, or severe metabolic derangements (9, 18, 35, 38, 55, 56). Only recently have PDs been regularly identified in the critical care setting (1, 2, 6, 7). One case-controlled study (1) compared outcomes in two groups of patients: one with GPDs on EEG, and another group matched to the first set of patients based on disease etiology and level of consciousness, but without GPDs noted on EEG. GPDs were strongly associated with NCSE, and NCSE was associated with a poor outcome, however, the presence of GPDs alone was not specifically associated with a poorer outcome when compared to the case-controlled patients (1). Historically, in human medicine, PDs in patients with underlying pathology (i.e., not secondary to drugs/toxicity) are most commonly associated with a poor functional outcome or death (9, 35, 55–58).

The goal of this descriptive paper is to present several visual examples of periodic discharges in veterinary patients to increase recognition of these patterns for clinicians in neurology and critical care settings, and to encourage development of a common language for these periodic patterns to facilitate collaborative research. These visually distinctive waveforms are still hotly debated in human medicine, and the more that is known, the less black-and-white the understanding becomes: are PDs ictal or interictal? Should PDs be specifically treated? Does the presence of PDs predict a poor outcome? Are PDs a cause or consequence of brain injury? It seems like the answer to each of these questions is: "It depends…" Characteristics like higher frequency (>2 Hz), more complex waveforms (polyphasic, "+" modifiers), evolution of PDs, and presence of electrographic or clinical seizures are more

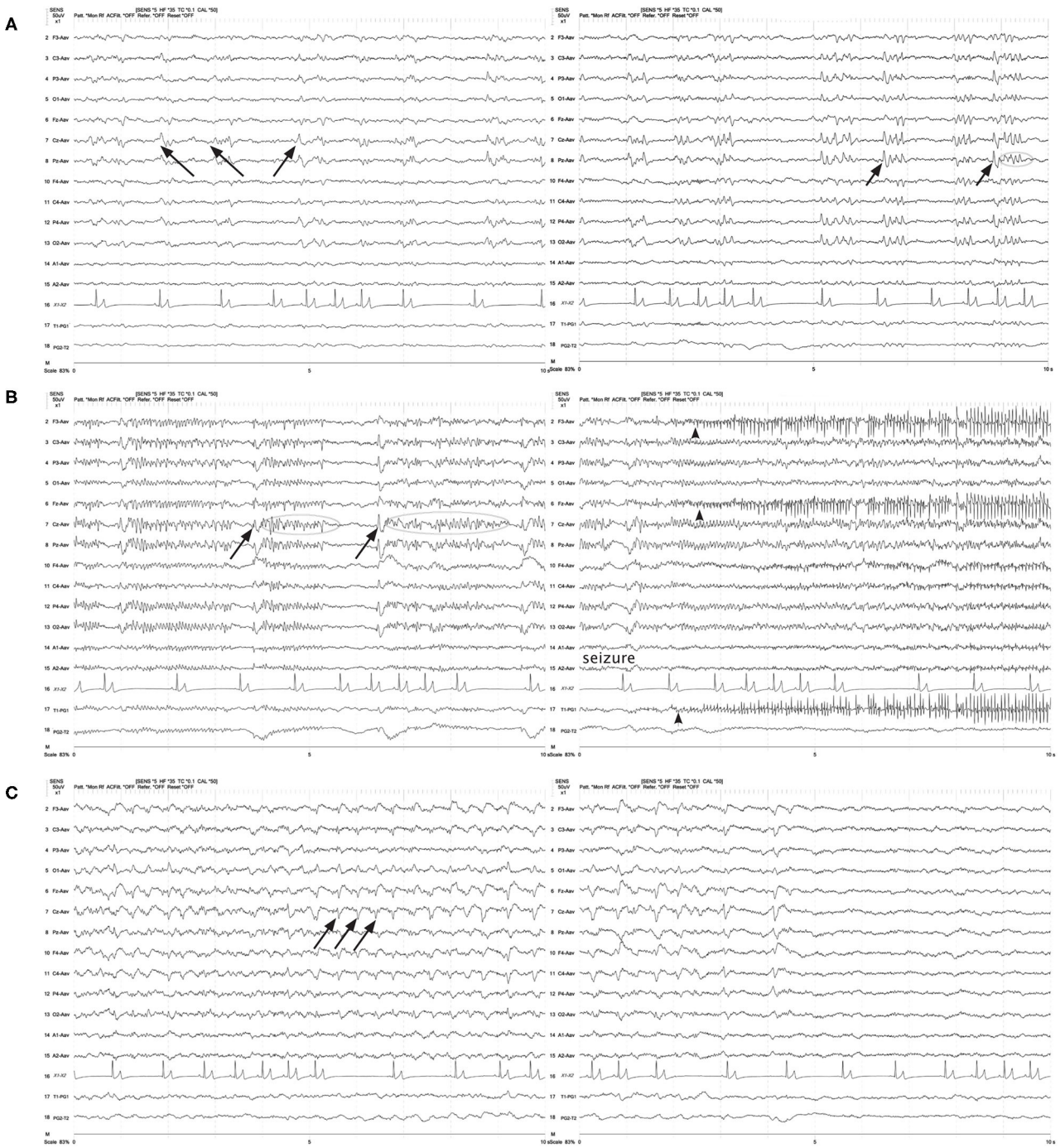


FIGURE 10
Three sets of consecutive EEG epochs in a 12 year old Shepherd mix illustrating evolution of GPDs to a focal seizure, and spontaneous resolution. The dog has a history of nasal aspergillosis and recent cluster seizures. Referential montage HF 35 Hz, sensitivity 5 uV/mm. **(A)** GPDs (arrows) progressively become more polyphasic, then develop bursts (>4 phases, longer than 500 ms) (circle). **(B)** GPDs (arrows) are associated with progressively longer bursts (circles), until a clinical focal seizure involving the left eye and face occurs, lasting 40 s. No pharmacologic intervention was given. Arrowheads indicate the start of electromyography (EMG) artifact from the muscle activity on the left frontal (F3, Fz) and left eye channels. **(C)** After termination of the clinical seizure, the GPDs (arrows) are apparent at a 2–2.5 Hz frequency, until they terminate spontaneously.

supportive of an ictal pattern, while lower frequency (<1 Hz), simpler PDs might be less likely to be associated with seizures, however, a patient's location on the IIC is not static. The presence of PDs on EEG seems to indicate a brain "on the edge:" whether on the edge of further decline or the edge of recovery is dependent on many factors.

This report illustrates that PDs occur in veterinary medicine and have the same features as those in human medicine. Next steps in veterinary medicine will be evaluating if the presence of PDs on EEG is correlated with clinical or electrographic seizure or status epilepticus, specific diagnoses, signalments, and outcomes. Optimally, we would learn which patterns and trends

might benefit from treatment, and if that treatment improves patient outcome.

Author contributions

DW, WB, MK, and KT contributed to the conception of the study. DW, WB, and MK selected EEG cases. KT, MK, and WB organized clinical information. MK wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Acknowledgments

The authors are grateful to Mark M. Stecker for his assistance in interpretation of BVNS EEGs.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1037404/full#supplementary-material

SUPPLEMENTARY FIGURE 1
UC Davis electrode placement and montage.

SUPPLEMENTARY FIGURE 2
Bush Veterinary Neurology Service electrode placement and montage.

References

1. Foreman B, Claassen J, Khaled KA, Jirsch J, Alschuler DM, Wittman J, et al. Generalized periodic discharges in the critically ill: a case-control study of 200 patients. *Neurology.* (2012) 79:951–60. doi: 10.1212/WNL.0b013e3182735cd7

2. Zafar SF, Subramaniam T, Osman G, Herlopian A, Struck AF. Electrographic seizures and ictal-interictal continuum (IIC) patterns in critically ill patients. *Epilepsy Behav.* (2020) 106:107037. doi: 10.1016/j.yebeh.2020.107037

3. Chatrian GE, Shaw C-M, Leffman H. The significance of periodic lateralized epileptiform discharges in EEG: an electrographic, clinical and pathological study. *Electroenceph Clin Neurophysiol.* (1964) 17:177–93. doi: 10.1016/0013-4694(64)90149-X

4. Chatrian GE. An EEG pattern: pseudo-rhythmic recurrent sharp waves. Its relationships to local cerebral anoxia and hypoxia. *Electroenceph Clin Neurophysiol.* (1961) 13:144.

5. Witsch J, Frey H-P, Schmidt JM, Velazquez A, Falo CM, Reznik M, et al. Electroencephalographic periodic discharges and frequency-dependent brain tissue hypoxia in acute brain injury. *JAMA Neurol.* (2017) 74:301–9. doi: 10.1001/jamaneurol.2016.5325

6. Rodriguez Ruiz A, Vlachy J, Lee JW, Gilmore EJ, Ayer T, Haider HA, et al. Association of periodic and rhythmic electroencephalographic patterns with seizures in critically ill patients. *JAMA Neurol.* (2017) 74:181–8. doi: 10.1001/jamaneurol.2016.4990

7. Husari KS, Ritzl EK. Anesthesia-Associated periodic discharges. *J Clin Neurophysiol.* (2022) 39:289–94. doi: 10.1097/WNP.0000000000000779

8. Schwartz MS, Prior PF, Scott DF. The occurrence and evolution in the EEG of a lateralized periodic phenomenon. *Brain.* (1973) 96:613–22. doi: 10.1093/brain/96.3.613

9. Walsh JM, Brenner RP. Periodic lateralized epileptiform discharges – long-term outcome in adults. *Epilepsia.* (1987) 28:533–6. doi: 10.1111/j.1528-1157.1987.tb03684.x

10. Pohlmann-Eden B, Hoch DB, Cochius JI, Chiappa KH. Periodic lateralized epileptiform discharges – a critical review. *J Clin Neurophysiol.* (1996) 13:519–30. doi: 10.1097/00004691-199611000-00007

11. García-Morales I, García MT, Galán-Dávila L, Gómez-Escalonilla C, Saiz-Díaz R, Martínez-Salio A, et al. Periodic lateralized epileptiform discharges: etiology, clinical aspects, seizures, and evolution in 130 patients. *J Clin Neurophysiol.* (2002) 19:172–7. doi: 10.1097/00004691-200203000-00009

12. Hirsch LJ, Brenner RP, Drislane FW, So E, Kaplan PW, Jordan KG, et al. The ACNS subcommittee on research terminology for continuous EEG monitoring: proposed standardized terminology for rhythmic and periodic EEG patterns encountered in critically ill patients, *J Clin Neurophysiol.* (2005) 22:128–35. doi: 10.1097/01.WNP.0000158701.89576.4C

13. Hirsch LJ, LaRoche SM, Gaspard N, Gerard E, Svoronos A, Herman ST, et al. American clinical neurophysiology society's standardized critical care EEG terminology: 2012 version. *J Clin Neurophysiol.* (2013) 30:1–27. doi: 10.1097/WNP.0b013e3182784729

14. Hirsch LJ, Fong MWK, Leitinger M, LaRoche SM, Beniczky S, Abend NS, et al. American clinical neurophysiology society's standardized critical care EEG terminology: 2021 version. *J Clin Neurophysiol.* (2021) 38:1–29. doi: 10.1097/WNP.0000000000000806

15. ACNS. *Standardized Critical Care EEG Terminology Training Module-—2021 Version* (2022). Available online at: https://www.acns.org/research/critical-care-eeg-monitoring-research-consortium-ccemrc/education (accessed July 2022).

16. Lin L, Drislane FW. Lateralized periodic discharges: a literature review. *J Clin Neurophysiol.* (2018) 35:189–98. doi: 10.1097/WNP.0000000000000448

17. Sully KE, Husain AM. Generalized periodic discharges: a topical review. *J Clin Neurophysiol.* (2018) 35:199–207. doi: 10.1097/WNP.0000000000000460

18. Bahamon-Dussan JE, Celesia GG, Grigg-Damberger MM. Prognostic significance of EEG triphasic waves in patients with altered state of consciousness. *J Clin Neurophysiol.* (1989) 6:313–9. doi: 10.1097/00004691-198910000-00002

19. Stecker MM, Cheung AT, Pochettino A, Kent GP, Patterson T, Weiss SJ, et al. Deep hypothermic circulatory arrest: effects of cooling on electroencephalogram and evoked potentials. *Ann Thorac Surg.* (2001) 71:14–21. doi: 10.1016/S0003-4975(00)01592-7

20. Kearse LA Jr, Koski G, Husain MV, Philbin DM, McPeck K. Epileptiform activity during opioid anesthesia. *Electroencephalogr Clin Neurophys.* (1993) 87:374–9. doi: 10.1016/0013-4694(93)90150-T

21. Sonkajärvi E, Rytky S, Alahuhta S, Suominen K, Kumpulainen T, Ohtonen P, et al. Epielptiform and periodic EEG activities induced by rapid sevoflurane anaesthesia induction. *Clin Neurophys.* (2018) 71:638–45. doi: 10.1016/j.clinph.2017.12.037

22. Kalamangalam GR, Pohlmann-Eden B. Ictal-Interictal continuum. *J Clin Neurophysiol.* (2018) 35:274–8. doi: 10.1097/WNP.0000000000000447

23. Koren JP, Herta J, Pirker S, Fürbass F, Hartmann M, Kluge T. Rhythmic and periodic EEG patterns of 'ictal-interictal uncertainty' in critically ill neurological patients. *Clin Neurophys.* (2016) 127:176–181. doi: 10.1016/j.clinph.2015.09.135

24. Rubinos C, Reynolds AS, Claassen J. The ictal-interictal continuum: to treat or not to treat (and how)? *Neurocrit Care.* (2018) 29:3–8. doi: 10.1007/s12028-017-0477-5

25. Wrzosek M, Ives JR, Karczewski M, Dziadkowiak E, Gruszka E. The relationship between epileptiform discharges and background activity in the visual analysis of electroencephalographic examinations in dogs with seizures of different etiologies. *Vet J.* (2017) 222:41–51. doi: 10.1016/j.tvjl.2017.03.003

26. Holliday TA, Williams DC. Interictal paroxysmal discharges in the electroencephalograms of epileptic dogs. *Clin Techniques in Small Animal Pract.* (1998) 13:132–43. doi: 10.1016/S1096-2867(98)80034-0

27. De Risio L, Bhatti S, Munana K, Penderis J, Stein V, Tipold A, et al. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. *BMC Vet Res.* (2015) 11:148. doi: 10.1186/s12917-015-0462-1

28. Raith K, Steinberg T, Fischer A. Continuous electroencephalographic monitoring of status epilepticus in dogs and cats: 10 patients (2004-2005). *J Vet Emerg Crit Care.* (2010) 20:446–55. doi: 10.1111/j.1476-4431.2010.00544.x

29. Cuff DE, Bush WW, Stecker MM, Williams DC. Use of continuous electroencephalography for diagnosis and monitoring of treatment of nonconvulsive status epilepticus in a cat. *J Am Vet Med Assoc.* (2014) 244:708–14. doi: 10.2460/javma.244.6.708

30. Granum LK, Bush WW, William DC, Stecker MM, Weaver CE, Werre SR. Prevalence of electrographic seizure in dogs and cats undergoing electroencephalography and clinical characteristics and outcome for dogs and cats with and without electrographic seizure: 104 cases (2009-2015). *J Am Vet Med Assoc.* (2019) 254:967–73. doi: 10.2460/javma.254.8.967

31. James FMK, Cortez MA, Monteith G, Jokinen TS, Sanders S, Wielaender F, et al. Diagnostic utility of wireless video-electroencephalography in unsedated dogs. *J Vet Intern Med.* (2017) 31:1469–76. doi: 10.1111/jvim.14789

32. Holliday TA, Williams DC. Clinical electroencephalography in dogs. *Vet Neurol Neurosurg.* (1999) 1. Available online at: https://www.vin.com/apputil/content/defaultadv1.aspx?id=3857017&pid=11157 (accessed September 2022).

33. Holliday TA, Williams DC. Advantages of digital electroencephalography in veterinary medicine. *Vet Neurol Neurosurg.* (2001) 3. Available online at: https://www.vin.com/apputil/content/defaultadv1.aspx?pId=11157&catId=29694&id=3859460&ind=21&objTypeID=17 (accessed September 2022).

34. Lothman EW, Bertram EH, Bekenstein JW, Perlin JB. Self-sustaining limbic status epilepticus induced by 'continuous' hippocampal stimulation: electrographic and behavioral characteristics. *Epilepsy Res.* (1989) 3:107–19. doi: 10.1016/0920-1211(89)90038-7

35. Sainju RK, Manganas LN, Gilmore EJ, Petroff OA, Rampal N, Hirsch LJ, et al. Clinical correlates and prognostic significant of lateralized periodic discharges in patients without acute or progressive brain injury: a case-control study. *J Clin Neurophys.* (2015) 32:495–500. doi: 10.1097/WNP.0000000000000206

36. Brenner RP, Schaul N. Periodic EEG patterns: classification, clinical correlation, and pathophysiology. *J Clin Neurophys.* (1990) 7:249–67. doi: 10.1097/00004691-199004000-00007

37. Mora M, Cagle TT, Peartree N, Ebinger K. Lateralized periodic discharges detected and described via intraoperative neuromonitoring. *The Neurodiagnost J.* (2022) 62:81–6. doi: 10.1080/21646821.2022.2060012

38. Bunford N, Andics A, Kis A, Miklosi A, Gacsi M. *Canis familiaris* as a model for non-invasive comparative neuroscience. *Trends Neurosci.* (2017) 40:438–52. doi: 10.1016/j.tins.2017.05.003

39. Gurvitch AM, Ginsburg DA. Types of hypoxic and posthypoxic delta activity in animals and man. *Electroenceph Clin Neurophys.* (1977) 42:297–308. doi: 10.1016/0013-4694(77)90167-5

40. Schaul N. Pathogenesis and significance of abnormal nonepileptiform rhythms in the EEG. *J Clin Neurophys.* (1990) 7:229–48. doi: 10.1097/00004691-199004000-00006

41. Orta DSJ, Chiappa KH, Quiroz AZ, Costello DJ, Cole AJ. Prognostic implications of periodic epileptiform discharges. *Arch Neurol.* (2009) 66:985–91. doi: 10.1001/archneurol.2009.137

42. Kalamangalam GP, Slater JD. Periodic lateralized epileptiform discharges and after discharges: common dynamic mechanisms. *J Clin Neurophys.* (2015) 32:331–40. doi: 10.1097/WNP.0000000000000173

43. Shen Z, Zhang H, Cao Z, Yan L, Zhao Y, Du L, et al. Transition dynamics and optogenetic controls of generalized periodic epileptiform discharges. *Neural Networks.* (2021) 149:1–17. doi: 10.1016/j.neunet.2022.01.022

44. Fernandez-Torre JL, Kaplan PW. Triphasic waves: historical overview of an unresolved mystery. *J Clin Neurophys.* (2021) 38:399–409. doi: 10.1097/WNP.0000000000000809

45. Foreman B. Can we distinguish triphasic waves from other generalized periodic discharges? Do we need to? *J Clin Neurophys.* (2021) 38:362–5. doi: 10.1097/WNP.0000000000000765

46. Bickford RG, Butt RH. Hepatic coma: the electroencephalographic pattern. *J Clin Invest.* (1955) 34:790–9. doi: 10.1172/JCI103134

47. Zhang K, Xu S, Zhou Y, Su T. Case report: triphasic waves in a 9-year-old girl with anti-NMDAR encephalitis. *Front Neurol.* (2022) 13:819209. doi: 10.3389/fneur.2022.819209

48. Foreman B, Mahulikar A, Tadi P, Claassen J, Szaflarski J, Halford JJ, et al. Generalized periodic discharges and "triphasic waves": A blinded evaluation of inter-rater agreement and clinical significance. *Clinical Neurophys.* (2016) 127:1073–80. doi: 10.1016/j.clinph.2015.07.018

49. Herlopian A, Struck AF, Rosenthal E, Westover BM. Neuroimaging correlates of periodic discharges. *J Clin Neurophys.* (2018) 25:279–94. doi: 10.1097/WNP.0000000000000466

50. Shvarts V, Zoltay G, Bowyer SM, Sillgitt A, Moran JE, Mason K, et al. Periodic discharges: insight from magnetoencephalography. *J Clin Neurophys.* (2017) 34:196–207. doi: 10.1097/WNP.0000000000000356

51. Heiniger P, El-Koussy M, Schindler K, Lövblad K, Kiefer C, Oswald H, et al. Diffusion and perfusion MRI for the localization of epileptogenic foci in drug resistant epilepsy. *Neuroradiology.* (2000) 44:475–80. doi: 10.1007/s00234-002-0785-z

52. Narayanan J. Can diffusion-weighted imaging be used as a tool to predict seizures in patients with PLEDs? *Epileptic Disord.* (2016) 18:440–6. doi: 10.1684/epd.2016.0868

53. Nagendran A, McConnell JF, De Risio L, Jose-Lopez R, Quintana RG, Robinson K, et al. Peri-ictal magnetic resonance imaging characteristics in dogs with suspected idiopathic epilepsy. *J Vet Intern Med.* (2021) 35:1008–17. doi: 10.1111/jvim.16058

54. Maseo C, Sánchez-Masian D, Ródenas S, Font C, Morales C, Domínguez E, et al. Prevalence, distribution, and clinical associations of suspected postictal changes on brain magnetic resonance imaging in epileptic dogs. *J Am Vet Med Assoc.* (2022) 260:71–81. doi: 10.2460/javma.21.02.0088

55. Osman G, Rahangdale R, Britton JW, Gilmore EJ, Haider HA, Hantus S, et al. Bilateral independent periodic discharges are associated with electrographic seizures and poor outcome: a case-control study. *Clin Neurophysiol.* (2018) 129:2284–9. doi: 10.1016/j.clinph.2018.07.025

56. Lewis DW, Johnson EL. Prognosis of periodic and rhythmic patterns in adult and pediatric populations. *J Clin Neurophys.* (2018) 35:303–9. doi: 10.1097/WNP.0000000000000442

57. Pedersen GL, Rasmussen SB, Gyllenborg J, Benedek K, Lauritzen M. Prognostic value of periodic electroencephalographic discharges for neurological patients with profound disturbances of consciousness. *Clin Neurophysiol.* (2012) 124:44–51. doi: 10.1016/j.clinph.2012.06.010

58. Chong DJ, Hirsch LJ. Which EEG patterns warrant treatment in the critically ill? Reviewing the evidence for treatment of periodic epileptiform discharges and related patterns. *J Clin Neurophys.* (2005) 22:79–91. doi: 10.1097/01.WNP.0000158699.78529.AF

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 31 March 2023
DOI 10.3389/fvets.2023.1040125

OPEN ACCESS

EDITED BY
Marcin Adam Wrzosek,
Wroclaw University of Environmental and Life Sciences, Poland

REVIEWED BY
Zachary Adam Schakner,
United States Army Corps of Engineers, United States
Colleen Reichmuth,
University of California, Santa Cruz, United States
Peter Cook,
New College, United States in collaboration with reviewer CR

*CORRESPONDENCE
D. Colette Williams
✉ colette.neurophys@gmail.com

SPECIALTY SECTION
This article was submitted to Veterinary Neurology and Neurosurgery, a section of the journal Frontiers in Veterinary Science

RECEIVED 09 September 2022
ACCEPTED 06 February 2023
PUBLISHED 31 March 2023

CITATION
Williams DC, Haulena M, Dennison S, Waugh L, Goldstein T, Nutter F, Bonn BV, Hoard V, Laxer KD, Buckmaster PS, Gulland FMD and Tharp B (2023) Pinniped electroencephalography: Methodology and findings in California sea lions (*Zalophus californianus*). *Front. Vet. Sci.* 10:1040125. doi: 10.3389/fvets.2023.1040125

Pinniped electroencephalography: Methodology and findings in California sea lions (*Zalophus californianus*)

D. Colette Williams[1]*, Martin Haulena[2], Sophie Dennison[3], Lynnette Waugh[4], Tracey Goldstein[5], Felicia Nutter[6], Bill Van Bonn[7], Vanessa Hoard[8], Kenneth D. Laxer[9], Paul S. Buckmaster[10], Frances M. D. Gulland[11,12] and Barry Tharp[13]

[1]Vet EDX, Retired Veterinary Medical Teaching Hospital, University of California, Davis, Davis, CA, United States, [2]The Vancouver Aquarium, Vancouver, BC, Canada, [3]Imaging Solutions, PLLC, Oakton, VA, United States, [4]UC Davis School of Veterinary Medicine, Davis, CA, United States, [5]Zoological Pathology Program, University of Illinois at Urbana-Champaign, Brookfield, IL, United States, [6]Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, United States, [7]A. Watson Armour III Center for Animal Health and Welfare, Animal Care and Science Division, John G. Shedd Aquarium, Chicago, IL, United States, [8]Department of Neurology, The Pacific Marine Mammal Center, Laguna Beach, CA, United States, [9]Sutter Pacific Medical Foundation, San Francisco, CA, United States, [10]Departments of Comparative Medicine and Neurology and Neurological Sciences, Stanford University, Stanford, CA, United States, [11]The Marine Mammal Center, Sausalito, CA, United States, [12]Wildlife Health Center, University of California, Davis, Davis, CA, United States, [13]Emeritus, Department of Neurology, University of California Davis Medical Center, Sacramento, CA, United States

This study was designed to identify abnormalities in the electroencephalograms (EEGs) recorded from stranded California sea lions (*Zalophus californianus*) with suspected *domoic acid (DA) toxicosis*. Recordings from animals presenting for non-neurological issues were also obtained to better understand the normal EEG (background activity and transient events) in this species, as, to date, studies have focused on examining natural sleep in pinnipeds. Most animals were sedated for electrode placement and EEG acquisition with some receiving antiepileptic medications or isoflurane during the procedure. A total of 103 recordings were read and scored from 0 (normal) to 3 (severely abnormal). Epileptiform discharges, consisting of spikes, sharp waves, slow waves, and/or spike waves, were present in all EEGs with scores of 1, 2, or 3. The distribution of these events over the scalp varied. While often generalized, others were lateralized over one hemisphere, bifrontal, bioccipital, and/or bitemporal, while some discharges were multifocal. Findings were different between sea lions and occasionally changed within the EEG on a given sea lion. No clinical seizures were observed during the recording but a few sea lions had findings consistent with electroencephalographic seizures. When available, supporting diagnostic results obtained from magnetic resonance imaging (MRI) and/or necropsy/histopathology were described, as well as the status of those sea lions that recovered and were released with satellite tags.

KEYWORDS

seizures, epilepsy, domoic acid (DA), memory, spatial ability, EEG, hippocampus

Introduction

Credited with being the first to describe the human electroencephalogram (EEG) in 1929, Hans Berger's study (1) was expanded upon with the publication of petit mal (now called absence) epilepsy findings in 1935 (2). Epileptic events consisting of spike-wave discharges at 3 Hz and interictal spikes were described in this report by Gibbs, Davis, and Lennox (2). More studies have been conducted since then with EEG being considered a standard diagnostic tool in human medicine for a variety of disorders, especially epilepsy. This diagnostic technique remains comparatively rare in veterinary medicine with studies available at some academic institutions and large specialty practices but primarily limited to companion animals. Brain wave activity has been recorded from marine mammals in research settings. Several earlier publications describe the use of implanted electrodes to study natural sleep in multiple species of cetaceans (toothed whales and dolphins) (3–5) and pinnipeds (seals and sea lions) (6–9). Recent studies have employed non-invasive methods for recording EEG in the latter (10–12). Sleep background patterns were noted in only one hemisphere when marine mammals were submerged but were bilateral when resting at the surface or, in the case of pinnipeds, hauled out. Other than brief descriptions involving some of the sea lions presented here (13–16), detailed findings of clinical EEGs recorded from *Zalophus californianus* have not been reported.

Recurrent seizures in humans and sea lions have been associated with the consumption of seafood (fish or shellfish) that has ingested certain algal diatoms containing domoic acid (DA). In late 1987, 150 people in Canada ate blue mussels that contained *Pseudo-nitzschia multiseries* that had been collected from nearby Prince Edward Island River (17). Human patients (107 confirmed cases) presented with gastrointestinal symptoms within 24 h or exhibited at least one neurological symptom within 48 h of ingestion. The latter consisted of memory loss, confusion, disorientation, seizures, coma, or cranial nerve palsies. Four people died within 24 days of mussel consumption. Several of those people who were severely affected had anterograde amnesia, resulting in the disease being given the moniker, *amnesic shellfish poisoning*. Seizures were a common finding in severe cases but lessened in frequency over a 2-month period. An 84-year-old male patient went from being disoriented and sleepy the day following shellfish ingestion to comatose with complex partial status epilepticus by day 3 (18). On EEG, he was found to have diffuse background slowing and lateralized periodic discharges (PDs) on the left side and bitemporal independent epileptic events. He was discharged seizure-free 4.5 m later and had a normal EEG 8 m after exposure, although he continued to have severe memory impairment. A year after acute DA exposure, he developed complex partial seizures and was diagnosed with temporal lobe epilepsy. At that time, magnetic resonance imaging (MRI) demonstrated bilateral hippocampal atrophy. An autopsy 2 years later, following his death from pneumonia, revealed sclerosis of both hippocampi and the mesial temporal region.

First, observed in 1998, multiple marine mammal mortality events have been reported along the Western US coastline. California sea lions, long-beaked common dolphins (*Delphinus capensis*), short-beaked common dolphins (*Delphinus delphis*), bottlenose dolphins (*Tursiops truncatus*), gray whales (*Eschrichtius robustus*), and a single northern fur seal (*Callorhinus ursinus*) were all thought to have died after acute DA exposure (19, 20). Northern anchovies (*Engraulis mordax*) were considered the primary vector in the sea lion cases (21). More recently, a large number of sea lions have been stranded with health issues attributed to chronic exposure to DA. In addition to neurological manifestations (13), reproductive failure (22, 23) and a degenerative cardiomyopathy (24) have also been associated with the toxin. Affected sea lions, similar to humans with amnesic shellfish poisoning, have also been shown to have memory deficits (25). There is evidence that some cases may have resulted from exposure *in utero* or neonatally, as DA has been found in amniotic fluid and dam's milk (26). The odd behavior described in sooty shearwaters in 1961 (27), which was the inspiration for Hitchcock's movie, *The Birds*, is thought to have been associated with an earlier *Pseudo-nitzschia* plankton bloom (28, 29). Multiple mass die-offs of brown pelicans and Brandt's cormorants in Monterey Bay, California, in each of the following three decades were determined to be DA-related diseases after finding contaminated anchovies in the stomach contents of affected birds (29).

In this retrospective study, EEG was used in an attempt to identify sea lions with suspected neurological damage subsequent to *DA toxicosis*, a common cause of strandings in this species. Sea lions are exposed to DA by ingestion of fish that have consumed the marine diatom *Pseudo-nitzschia australis*.

Materials and methods

Over a 7-year period (2004–2011), electroencephalographic studies (EEGs) were performed on 120 California sea lions that were rescued by The Marine Mammal Center following stranding along the California coast. Most animals were found between Sonoma County and San Luis Obispo County with the exception of one individual in the Sacramento delta.

Sea lions

Animals were divided into two groups based on signs at presentation. The Neurologically Normal Group consisted of 15 sea lions. There were a total of 88 animals in the group designated as the Suspected DA Toxicosis Group. Notably, 12 sea lions, two that would have been in the neurologically normal group and ten with suspected DA toxicosis, received isoflurane at the beginning of their recordings, making interpretation difficult. These were excluded from the study. Another five, all DA suspects, had unreadable recordings (corrupted files). This brought the total number of sea lions evaluated down from 120 to 103.

Neurologically Normal Group presented with various clinical problems, including malnutrition, leptospirosis, trauma, entanglement, or abscess. Three had no clinical signs and the cause of stranding was unknown. Eight were restrands (they had been previously admitted and tagged before release). Signs associated with central nervous system dysfunction were not

observed in any of these sea lions on admission. This group consisted of 15 animals. With one exception, all animals in this group were young males. They were classified as pups (MP), if less than a year ($N = 5$), yearlings (MY), if 1 to 2 years ($N = 3$), juveniles (MJ), between 2 and 4 years ($N = 3$), and subadults (MS), if 4 to 8 years ($N = 3$). The lone female (FP) in this group was a pup (less than 1 year).

Suspected DA Toxicosis Group presented with neurological signs including, unresponsiveness, seizures, abnormal behaviors (head weaving, scratching, and flipper biting), ataxia, and/or aggressiveness. Blindness was noted in several sea lions, either initially or after time spent in rehabilitation. There were 88 animals in this group: 15 males (1 MP, 4 MY, 8 MJ, and 2 MS), 72 females (1 FP, 10 yearlings [FY], 10 subadults [FS, between 2 and 5 years], 45 adults [FA, >5 years], and 6 unknown ages),[1] and one unknown gender (not recorded). There were 21 restrands in this group.

Several animals, especially those in the latter part of the study, had courses of phenobarbital prior to performing the EEG study. A total of 14 sea lions had additional recordings, one to two repeats. Some involved different sedative/anesthesia combinations or courses of antiepileptic medication between the repeat and the original recording. Those sea lions in which isoflurane was administered at the onset were not scored.

EEG recording

Sea lions were housed at the Marine Mammal Center in individual pens with freshwater or saltwater pools prior to 2009 and saltwater thereafter. They were transported to an enclosure at the surgical facility for EEG recordings at the discretion of the attending veterinarian. Except for two obtunded animals, sea lions received medetomidine at a dose of 0.07 mg/kg IM or dexmedetomidine at 0.35 mg/kg IM for sedation (to facilitate placement of electrodes). A total of 68 individuals were also administered 0.2 mg/kg butorphanol IM at the same time as the a_2 agonist. After observing the effect of the sedatives, at which time the animals were resting quietly, 15 26-gauge platinum needle EEG electrodes (Grass Astromed) were inserted under the skin of the scalp. Due to similarities in skull shape, electrode placement was done based on that routinely used in mesocephalic dogs at the University of California, Davis Veterinary Medical Teaching Hospital. This consisted of three evenly spaced midline electrodes (F_Z, C_Z, and P_Z), four each on the left (F_3, C_3, P_3, O_1) and right (F_4, C_4, P_4, O_2) with all frontal (F), central (C), and parietal (P) electrodes in the same transverse plane and two pairs of lateral electrodes (left and right), dorsal to the pinnae (A_1 and A_2), and caudal but in the same coronal plane (T_5 and T_6) as aural (A) electrodes (Figures 1A, B). A ground electrode (Z) was positioned over the frontal sinus at the midline. Additional electrodes were placed to record the electrooculogram (EOG) and the electrocardiogram (ECG), i.e., the former near the lateral and medial canthus of each eye (in most sea lions, they were not included in highly reactive animals) and the latter on the chest near each pectoral flipper. Electrodes were connected to a mini-input box (Nihon Kohden Inc.) which led to the amplifiers of the Neurofax 2100 digital EEG system (Nihon Kohden Inc.). Electrode impedances were checked and determined acceptable at 10 kΩ or less. The recording bandwidth was set with a time constant of 0.1 s and a high-frequency cutoff value of 70 Hz. Display sensitivities varied between 5 and 20 μV/mm (depending on the amplitude of the EEG signal from each sea lion), and the time base was 10 s/page. Short segments of a 50 μV/mm calibration signal and a biological calibration were recorded to insure proper amplifier function prior to recording. A double banana montage, based on that used in human electroencephalography, was utilized for both display and analysis of the recordings (Figure 1C). Some EEG segments were also reviewed with a referential montage. EEGs varied in length from 10 to 61 min, depending on patient tolerance for the procedure and time constraints. Five animals received midazolam IM and three received lorazepam IM during the EEG recording. Atipamezole at 0.25 mg/kg IM was administered at the end of the procedure to reverse the effects of the a_2 agonists (medetomidine or dexmedetomidine) in 11 sea lions. When additional diagnostic procedures were required, animals were supplemented with isoflurane gas anesthesia with oxygen *via* face mask while recording continued ($N = 22$).

EEG scoring

Most recordings were interpreted independently by two electroencephalographers (DCW, with a background in veterinary EEG, and BT, an expert in human EEG), while the latter recordings, sea lions numbered 9008 and above, were reviewed together. Only those sections following a_2 agonist administration (with or without butorphanol) or not sedated (in those animals deemed too obtunded to give sedative) were scored. A subjective system, based on the amount of abnormal activity present, was used to score each EEG.

0 No abnormal activity was detected, normal.
1 Rare/occasional abnormal activity observed, mild.
2 Abnormal activity alternated with normal EEG, moderate.
3 Abnormal activity abundant, often obliterating the background EEG, severe.

In addition, details regarding the types of epileptiform discharges (spikes, sharp waves, slow waves, and/or spike waves) and their distribution (generalized, lateralized, over a particular brain region, and/or multifocal) of the events were noted. Responses in EEG activity associated with the administration of additional drugs or a change in the state were described.

Related data

Status after treatment was recorded on most sea lions. When available, supporting diagnostic information (MRI and/or

1 Due to differences in maturation, only pup and yearling designations are the same for both sexes. There are no juvenile females, so subadult and adult ages differ between males and females. No adult males (greater than 8 years of age) were present in this study, whereas adult females were the most represented group at 43.7% (45 out of 103).

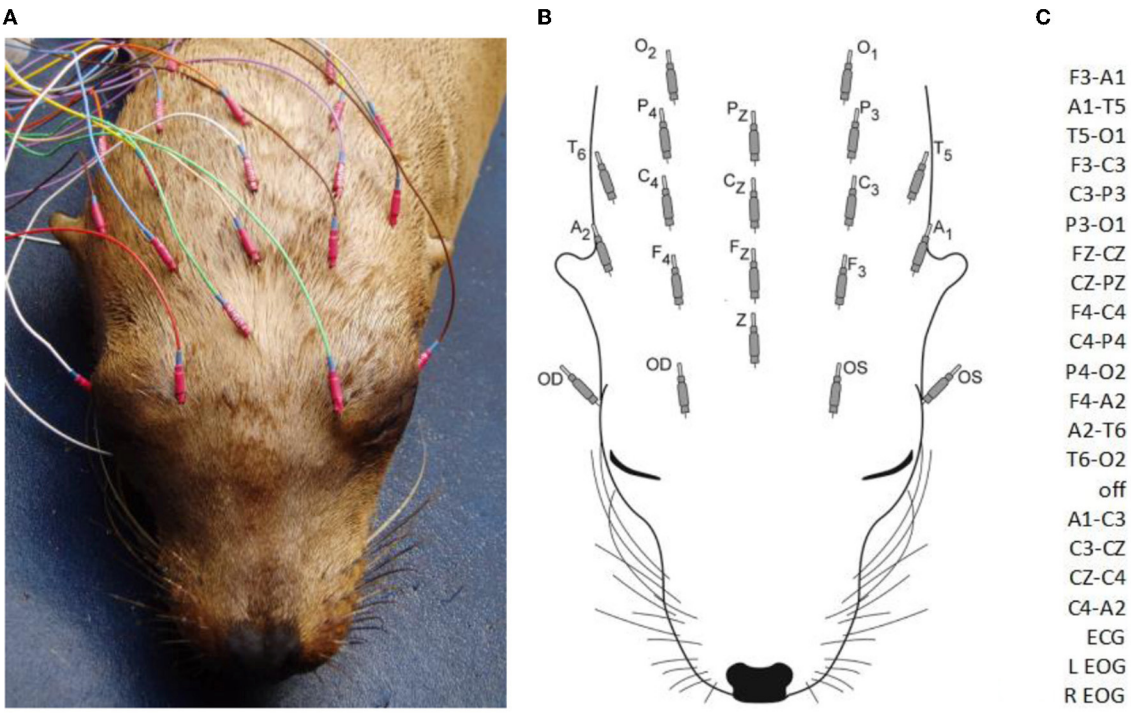


FIGURE 1
A sea lion with subcutaneous electrodes in place for EEG/EOG **(A)**. Only the hubs are visible (the 12 mm needles project rostrally). A diagram of electrodes with designated labels **(B)**. The modified double banana montage utilized **(C)**. F, frontal; C, central; P, parietal; O, occipital; T, temporal; A, aural; odd numbers, left side; even numbers, right side; z, midline; Z, ground; OS, left eye; OD, right eye (ECG not shown).

histology/necropsy) was compared to EEG findings on a given sea lion. These techniques have been described in previous publications (13, 30–32). In addition, those animals that were tagged and released were followed as part of another study (33). Others were evaluated behaviorally to better understand the impact of DA on memory and spatial ability (25).

Results

EEG findings

Some scores differed between readers but not by more than one value. In these instances, the higher of the two scores was used for that sea lion (refer to Supplementary Table).

Neurologically normal group

Artifact was a common finding in these EEGs. It consisted primarily of muscle artifacts (especially in EOG channels), but it also included artifacts associated with eye, head, and generalized movement. Occasional myoclonic jerks were observed during EEG recordings and are described as follows: stereotyped twitches of the eyes, the nose, the head, or the body. Periods of wakefulness and slow-wave sleep (SWS) were recorded and consisted of:

Wakefulness: a mixture of low amplitude (<40 μV) beta activity (>13 Hz) often interspersed with higher (~60 μV) runs of rhythmic alpha activity (8–13 Hz), as shown in Figure 2A. This state was observed primarily only in the latter part of some EEGs (in those sea lions that did not receive general anesthesia and whose recordings were long enough for sedative effects to wear off or were given atipamezole and/or stimulation for reversal).

Slow-Wave Sleep: a background of ongoing large (maximum of 240 μV), random, slow (delta range, <4 Hz) waves and the intermittent appearance of sleep spindles (up to 80 μV, at 12–14 Hz with a duration of 0.5 s or less). This state was symmetrical in both hemispheres. An example is shown in Figure 2B. This state was observed throughout most or all of the EEGs in the majority of sea lions.

No rapid eye movement (REM) sleep was observed in any of these recordings.

In most individuals, the administration of medetomidine/dexmedetomidine was associated with long periods of SWS. The female *Neurologically Normal* sea lion (7158) was an exception, as the entire recording consisted of wakefulness. Other than a suspected increase in myoclonic activity, butorphanol did not appear to influence the EEG. Isoflurane had a profound effect on background activity, occasionally resulting in a burst suppression pattern (Figure 2C).

Scoring in the Neurologically Normal Group was defined, as follows:

0 = 5 sea lions (1 FP, 1 MP, 1 MY, and 2 MS).

1 = 9 sea lions (4 MP, 2 MY, and 3 MJ).

2 = 0 sea lions.

3 = 1 sea lion (MS).

Occasional abnormal slow waves, some high in amplitude (400 μV), were a common finding in animals with an EEG

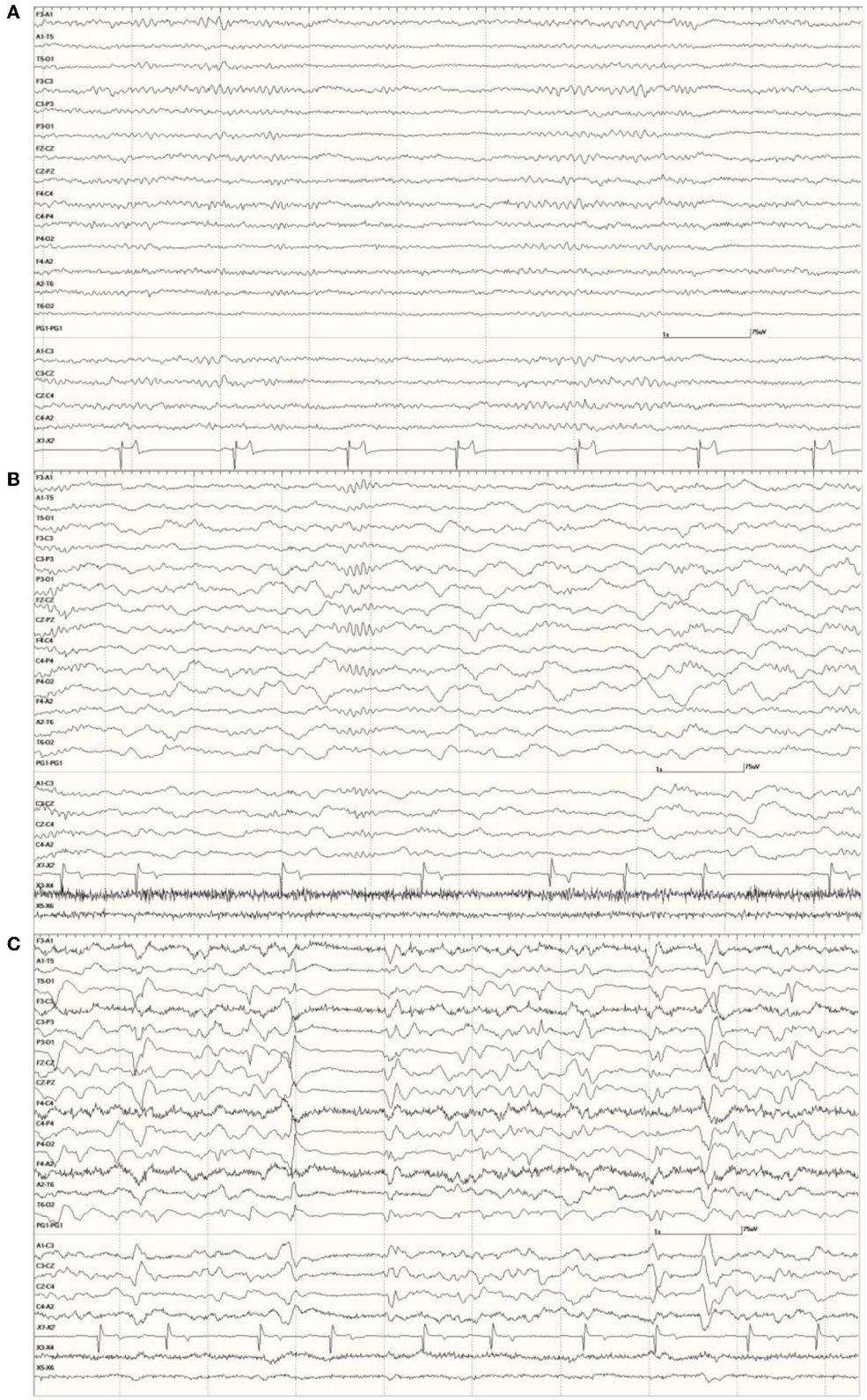


FIGURE 2
Neurologically Normal sea lion (7347) wakefulness EEG pattern with alternating beta activity (>13 Hz, low amplitude at middle and end) and alpha activity (8–13 Hz, medium amplitude at the beginning and between middle and end) **(A)**. Slow wave sleep in a *Neurologically Normal* sea lion (6326) with ongoing delta activity (<4 Hz high amplitude) and a well-developed sleep spindle before the 4 s mark (vertical line) with smaller ones at the beginning and end of this epoch **(B)**. Both had EEG scores of 0. The same sea lion (6326) during isoflurane anesthesia **(C)**. A 1-s suppression period occurred between the 3 and 4 s marks. Multiple sharp (duration 70–200 ms) and slow waves (>200 ms) can be seen. Superimposed muscle artifact (fast, darker, jagged-looking activity) is present bifrontally (F3 and F4).

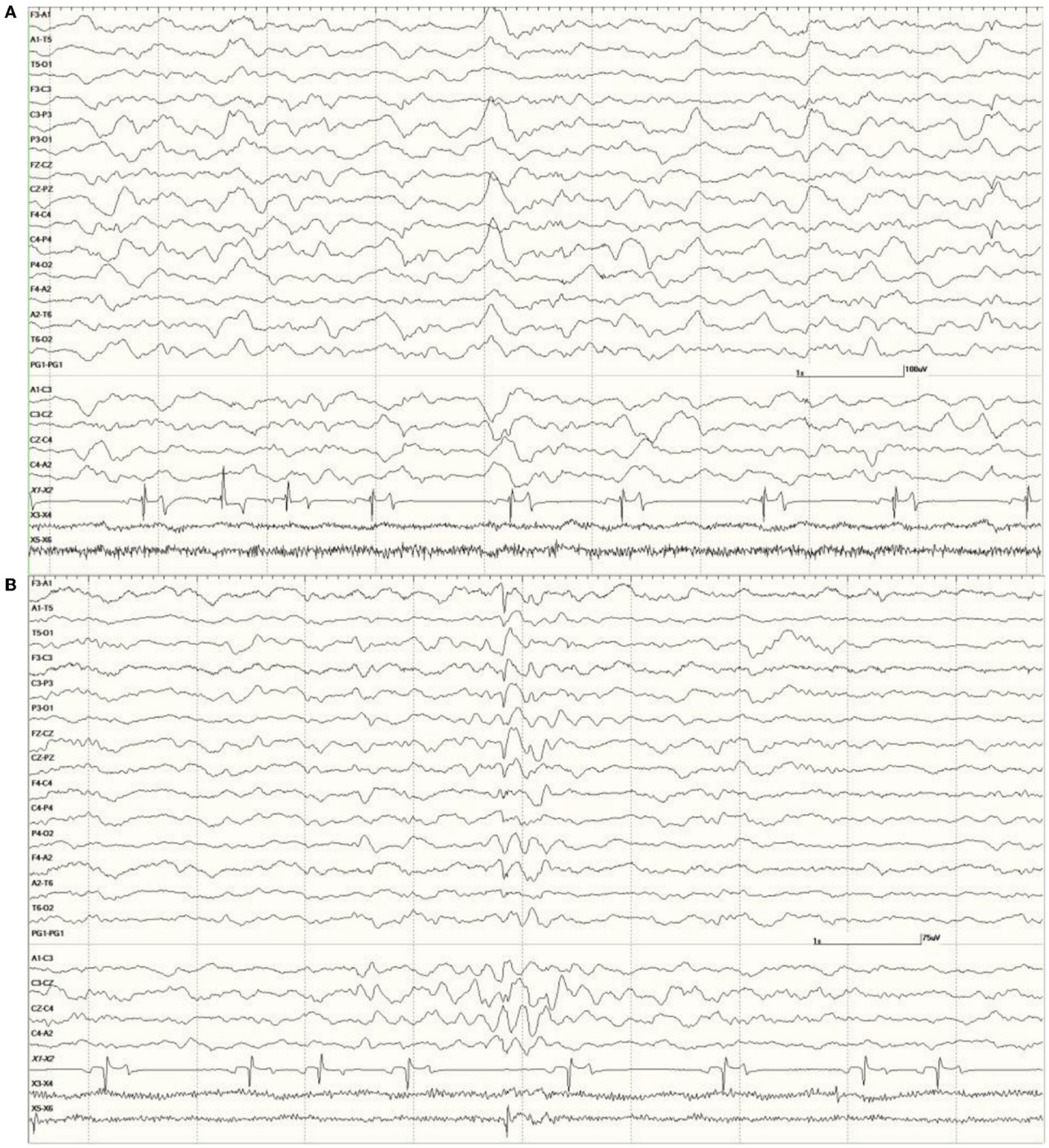


FIGURE 3
Recordings from *Neurologically Normal* sea lions with mild abnormalities (a score of 1). Sea lion 7155 recording with a large slow wave in the middle of the epoch **(A)**. An example of a typical generalized slow wave preceded by a deep positive (downward) deflection at the same time point in sea lion 8488 **(B)**.

score of 1 (Figure 3). In all cases, these slow waves differed from those of SWS in that they were uniform and tended to be generalized. Many were also preceded by a deep positive phase (Figure 3B). The single severely abnormal sea lion (a sub-adult male) in this group (7689), which presented for entanglement and was also a restrand, had continuous slow waves interspersed with sharp waves and spikes throughout the 21-min recording (Figure 4). This may represent an electroencephalographic seizure as no clinical sequelae were apparent in this individual.

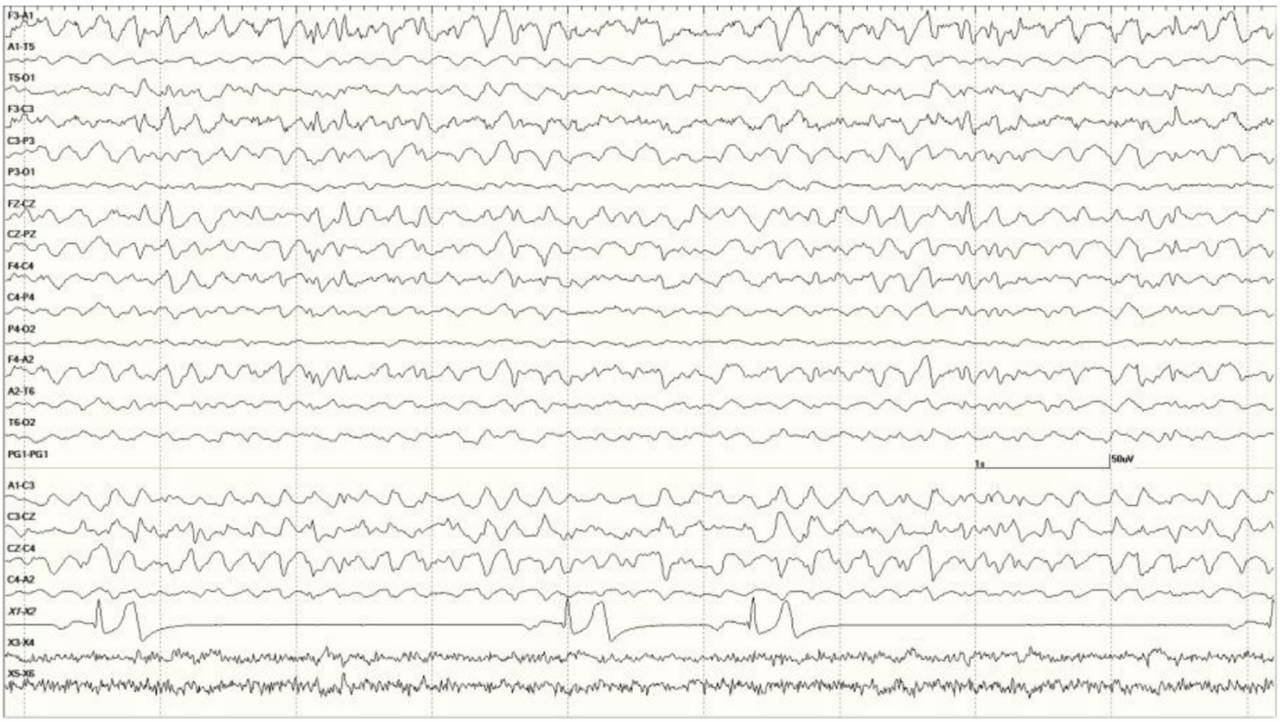


FIGURE 4
Segment of EEG from a sea lion initially thought to be *Neurologically Normal* (7689). Continuous generalized abnormal slow wave activity was present throughout this recording (EEG score = 3). The frequency varied between 4 and 5 Hz and may represent an electroencephalographic seizure (no clinical signs were apparent).

Suspected DA toxicosis cases

Artifact was less frequent in this group of sea lions, as sedation effects tended to be more pronounced. Muscle artifact, when present, was primarily in the EOG channels. When the background was visible, it was primarily that of SWS for most of the recording.

Mixed epileptiform discharges, consisting of
spikes (duration <70 ms),
sharp waves (70–200 ms),
slow waves (> 200 ms), and
spike waves (a spike followed by a slow wave),
were common in the EEGs from this group. Some were single events (Figure 5), but others were bursts of generalized discharges sandwiched between periods of normal EEG (Figure 6). In the most severely affected sea lions, abnormal rhythmic activity was nearly continuous throughout the recording, similar to that seen in Figure 4. Occasionally, the discharges were irregularly periodic in occurrence (referred to quasiperiodic, Figure 7). The abnormal activity was most commonly generalized, but in some animals, epileptiform discharges were worse on one side (unihemispheric), only occasionally extending to the opposite side. Spikes were often multifocal (Figure 8A). All but one sea lion (7160) in this group displayed a mixture of these events. When multiple, slow-wave, and spike-wave bursts varied in frequency from 3 to 5 Hz and were often voltage maximal either frontally or occipitally, though in a few sea lions, it was bitemporal (Figure 8B). An unusual pattern was noted in sea lion 7160 that displayed nearly continuous spike/sharp wave activity for 25 min at which time isoflurane was administered (Figure 9). This recording is suggestive of an electrographic seizure.

In several instances, waking the animal at the end of the recording, in some cases by administering atipamezole and/or stimulating (*via* nose or flipper taps), resulted in a dramatic improvement in the EEG (Figure 10, compared to Figure 5).

As was mentioned for the *Neurologically Normal Group*, isoflurane was associated with dramatic changes in the EEG. Large slow waves, sharp waves, and spikes, similar in appearance to those seen with sedation during SWS, were present with this general anesthesia. These were always generalized, even in those sea lions that had focal or unihemispheric discharges prior to induction (Figure 11, compared to Figure 8A).

The administration of benzodiazepines, midazolam, or lorazepam, during recordings, did not appear to reduce the presence of slow-wave discharges in the EEG but did reduce or eliminate spikes and sharp waves (Figure 12).

Scoring for this group of sea lions was as follows:

0 = 0 sea lions.

1 = 7 sea lions (1 MP, 1 MJ, 2 FS, and 3 FA).

2 = 23 sea lions (4 MY, 2 MJ, 1 MS, 2 FY, 2 FS, and 12 FA).

3 = 58 sea lions (5 MJ, 1 MS, 1 FP, 8 FY, 6 FS, 30 FA, 6 F age unknown, 1 gender and age unknown).

Those sea lions that had repeat recordings after receiving treatment with phenobarbital for 7 to 28 days showed minimal changes in EEG scores, at most, a one-step improvement (e.g., from 3 to 2).

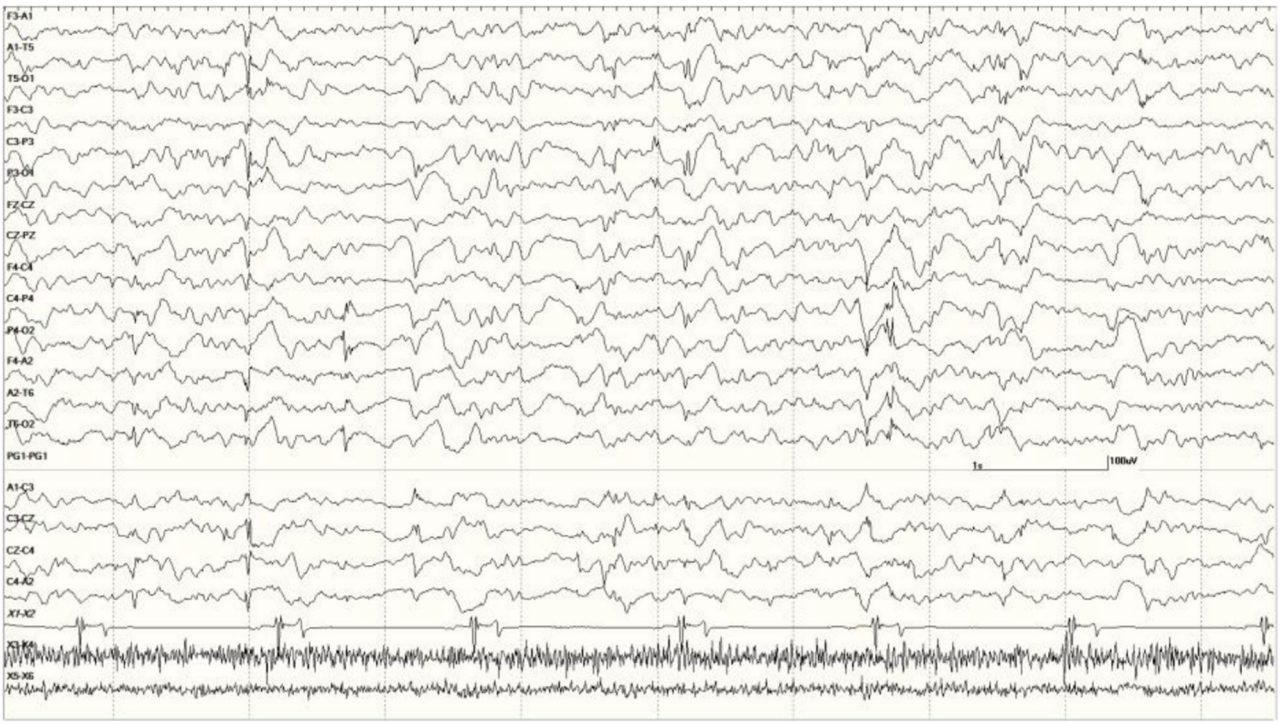


FIGURE 5
A sample of EEG from a *Suspected DA toxicosis* sea lion (8659, a score of 3) during slow-wave sleep. Several generalized large slow waves are apparent in addition to right hemisphere spikes (duration <70 ms, after the 1- and 2-s marks). A spike-wave discharge is shown between the 5- and 6-s marks. Muscle artifact is present in the EOG channels.

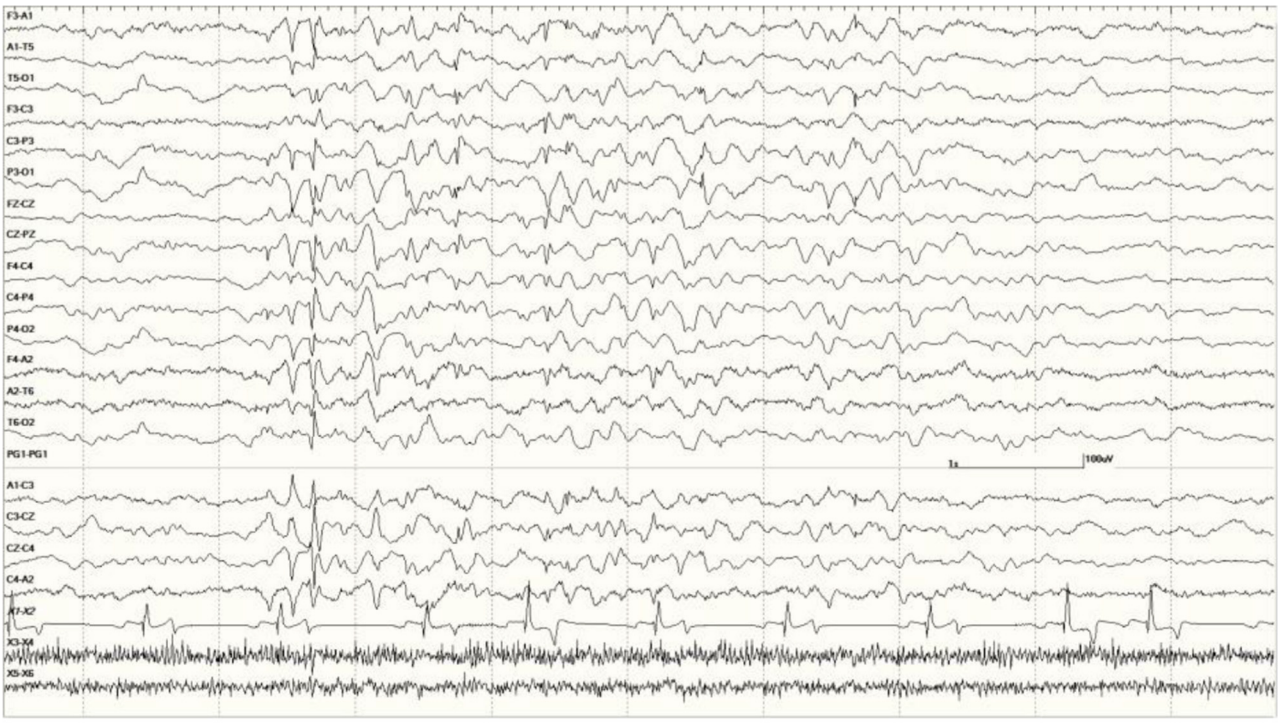


FIGURE 6
Five s burst of generalized abnormal activity consisting of slow waves, spikes, and sharp waves between normal slow-wave sleep background segments in *Suspected DA toxicosis* sea lion 6741 (a score of 3).

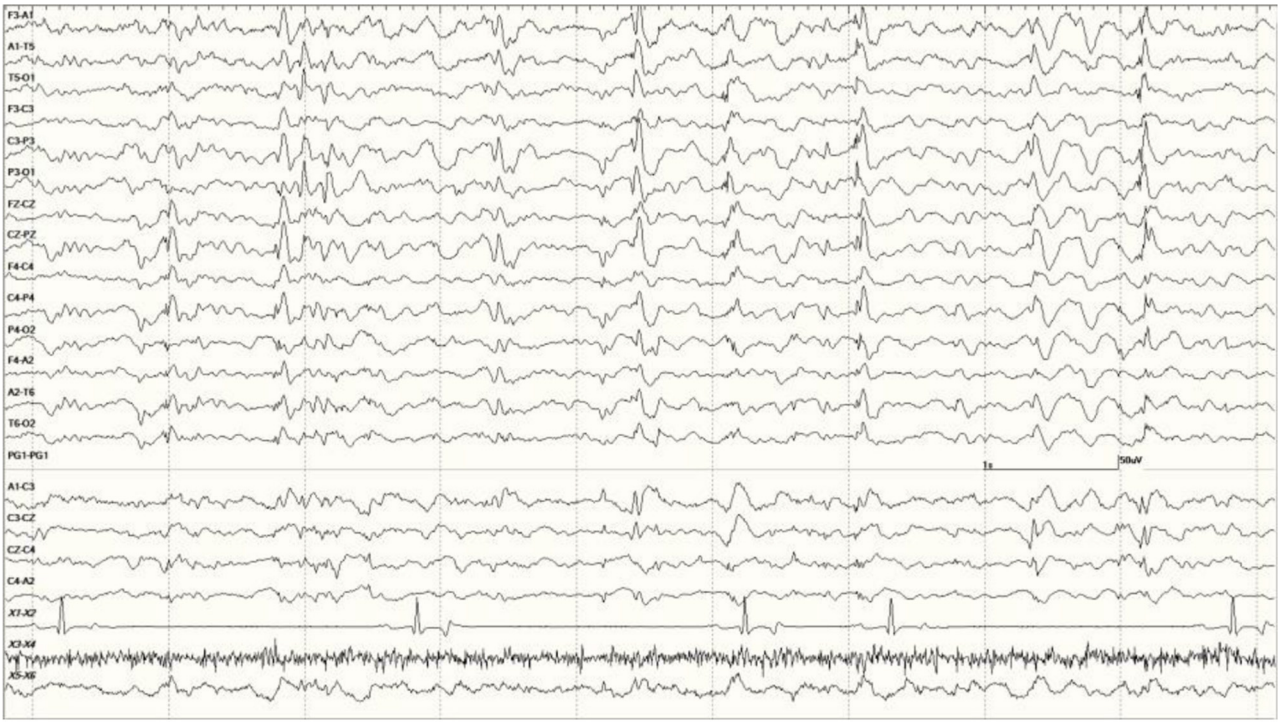


FIGURE 7
Quasiperiodic (irregular intervals) generalized and multifocal discharges in *Suspected DA toxicosis* sea lion 6887 (a score of 3).

Related data

Neurologically normal group

Only two sea lions in this group (8052 and 7689) had MRI studies. The former was read out as normal but no results were reported on the latter. None had necropsy/histological studies. Seven of these sea lions were released after treatment, two died (6326 and 9483), and five were placed at other facilities with 7689 being no status given.

Suspected DA toxicosis cases

In this group, 22 sea lions received MRIs. Findings in one (9724) stated that the hippocampi were normal but white matter lesions were present (Figure 13). The EEG score in this animal was 3. Most ($N = 12$) had unilateral hippocampal (HC) necrosis, with five on the left and seven on the right. Ratings varied on the left with three having mild atrophy (6710, 6859, and 9690), one with severe (6508), and one was not rated (7745). For those with right-sided atrophy, two were moderate (6405 and 6433), three had severe (4589, 6510, and 6707), and two were not rated (8722 and 8883). Six had bilateral HC atrophy with 6887 showing more severe changes on the right than the left. The other cases consisted of two with mild (6667 and 6904) and three with severe bilateral HC atrophy (6740, 6804, and 9821). One each with severe left HC atrophy (6508), severe right-sided atrophy (6510), and severe bilateral HC atrophy (6804) had an EEG score of 1. Another two sea lions (9255 and 9931) in this group had MRI but no results were available.

Brain (primarily hippocampal) necropsy/histology results were provided on 24 sea lions in this group. Various findings are as follows: two no gross HC lesions (9925 and 9938), two perivascular edema (6731 and 8883), one brain necrosis (6365), and one porencephaly (6079; Figure 14). Sea lion 6731 also had signs of a brain hemorrhage. Five had left-sided HC atrophy (two mild [6489 and 6859], one moderate [6318], and two severe [6508 and 6804]) with seven diagnosed with right-sided atrophy (two moderate [6090 and 6405] and five severe [4589, 6510, 6676, 6707, and 6887]). There were two cases of moderate bilateral atrophy (6123 and 6741), one with severe left-sided atrophy and moderate right-sided atrophy (6804) and two with moderate unilateral HC loss (side not mentioned; 6084 and 6290). One case (9574) was described as having diffuse atrophy. One of the sea lions with an incomplete number (805?) had severe left HC atrophy and another (8722) was described with severe loss. A normal left hippocampus (9724) and a severely atrophied left hippocampus (9821) are shown in Figure 15. One adult female without HC changes (9938) was found to have carcinoma throughout the abdominal cavity with enlarged lymph nodes, abnormal kidneys, and multiple liver nodules. Despite an EEG score of 3, this sea lion's brain was grossly normal. On necropsy, sea lion 9724 had mild encephalitis, vaginal carcinoma, myocarditis, and verminous (lungworm) pneumonia. Brain and heart changes were consistent with a protozoal etiology. No evidence of metastatic disease was present, so the carcinoma was likely early stage. On radiography, lead fragments were found in one sea lion (6741) and a 9-mm bullet in another (6804). In both cases, they were lodged below the skin of the neck and appeared to be isolated by fibrous tissue.

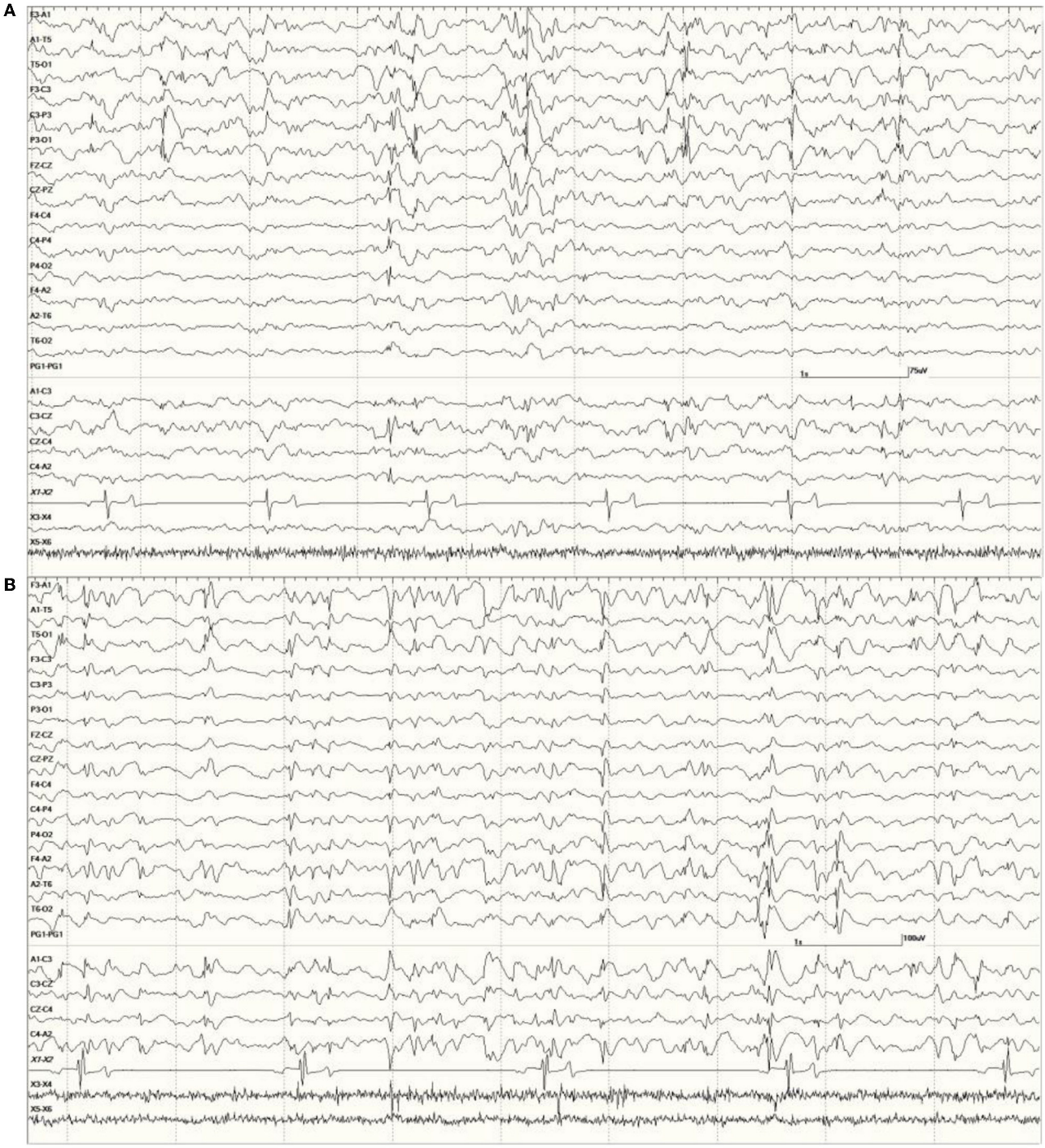


FIGURE 8
Primarily left-sided EEG abnormalities (spikes, sharp waves, and slow waves) with a few spike waves extending into the right hemisphere in *Suspected DA toxicosis* sea lion 6731 **(A)**. A segment of EEG in *Suspected DA toxicosis* sea lion 9935 demonstrating various abnormalities with a bitemporal distribution **(B)**. Both had a score of 3.

For the *suspected DA toxicosis* sea lions, 13 were released, 51 were euthanized, 15 died, and 10 had no status listed. In contrast to the *Neurologically Normal Group*, none were placed. Eight of the released animals were fitted with satellite tags to study their movement following release. One (6904) stayed in Monterey Bay but restranded with seizures and was euthanized. Another (6887) was found to be disoriented on a police car in San Francisco and was also euthanized. Two others in this subset were euthanized with the remaining four animals' statuses unknown.

A summary of all sea lion data included in this study is shown in the Supplementary Table.

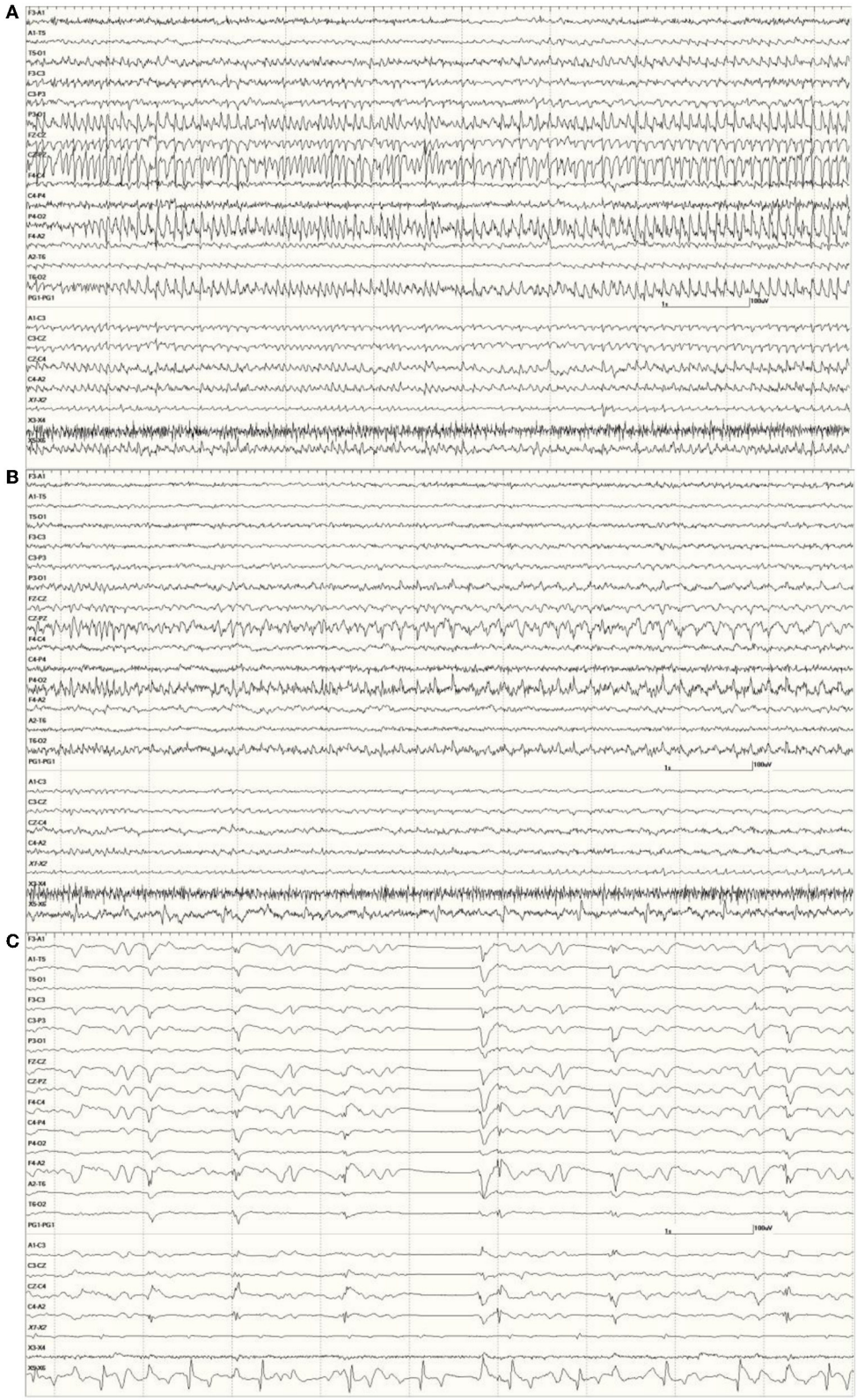


FIGURE 9
An unusual recording in *Suspected DA toxicosis* sea lion 7160 (a score of 3) consisting of continuous spikes **(A)**, spikes devolving into sharp waves **(B)**, both voltage maximum caudally (occipital region). This appears to be an electroencephalographic seizure. Isoflurane administration had profound effects on the EEG pattern **(C)**.

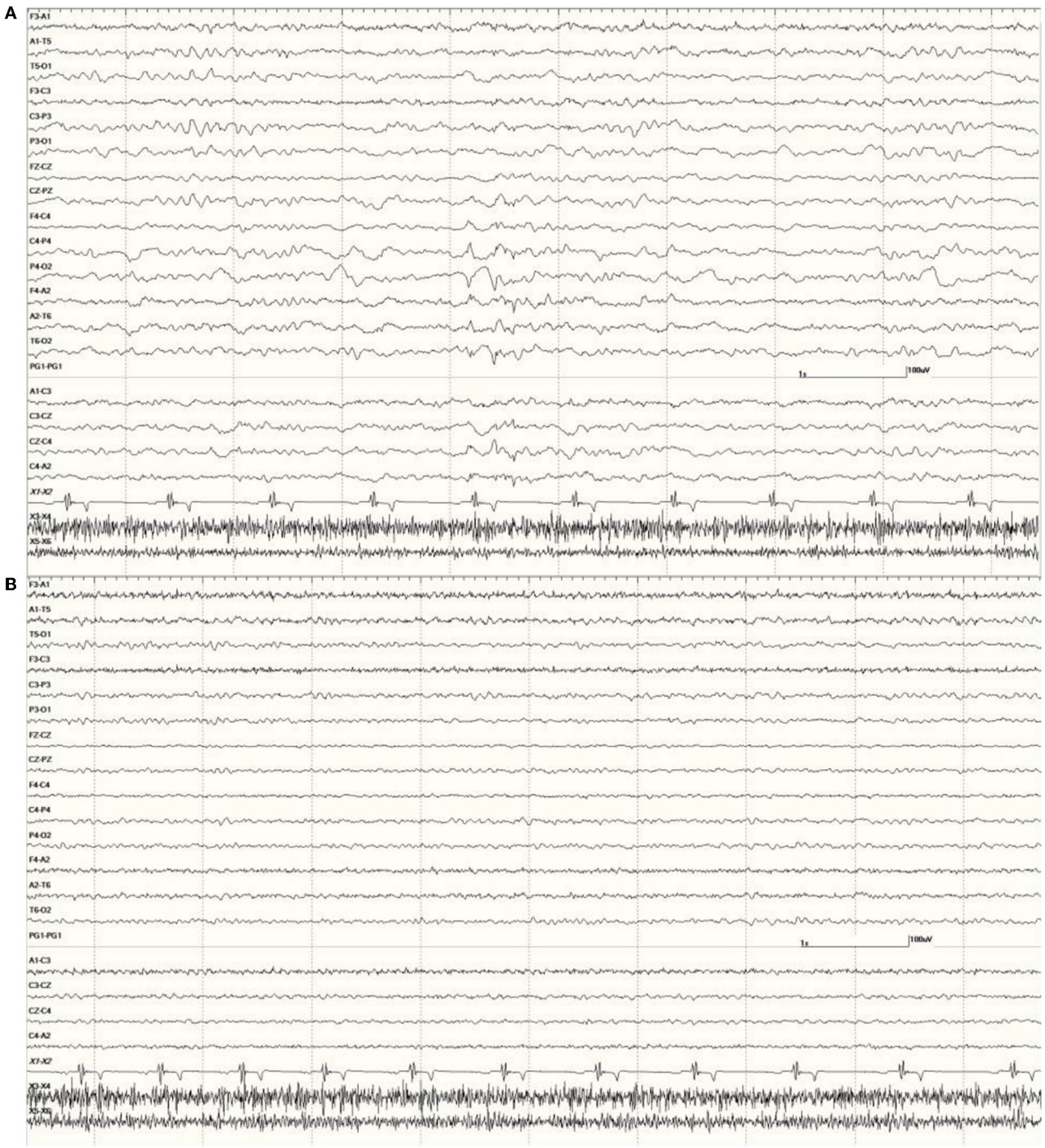


FIGURE 10
Change in abnormal activity associated with state. *Suspected DA toxicosis* sea lion 8659 (same as in Figure 5) near the end of the recording with a right-sided discharge during slow-wave sleep **(A)** and normal wakefulness **(B)**.

Discussion

Recording EEG in free-ranging, stranded California sea lions was possible with the use of a_2 agonists in most cases. As has been reported in cats, dogs, and horses, these drugs produce SWS that has the same characteristics (background and transient events) as that which occurs naturally (34–36). This state has been associated with a greater probability of detecting abnormal activity in an EEG recorded from a patient with epilepsy (37, 38). It holds true across species

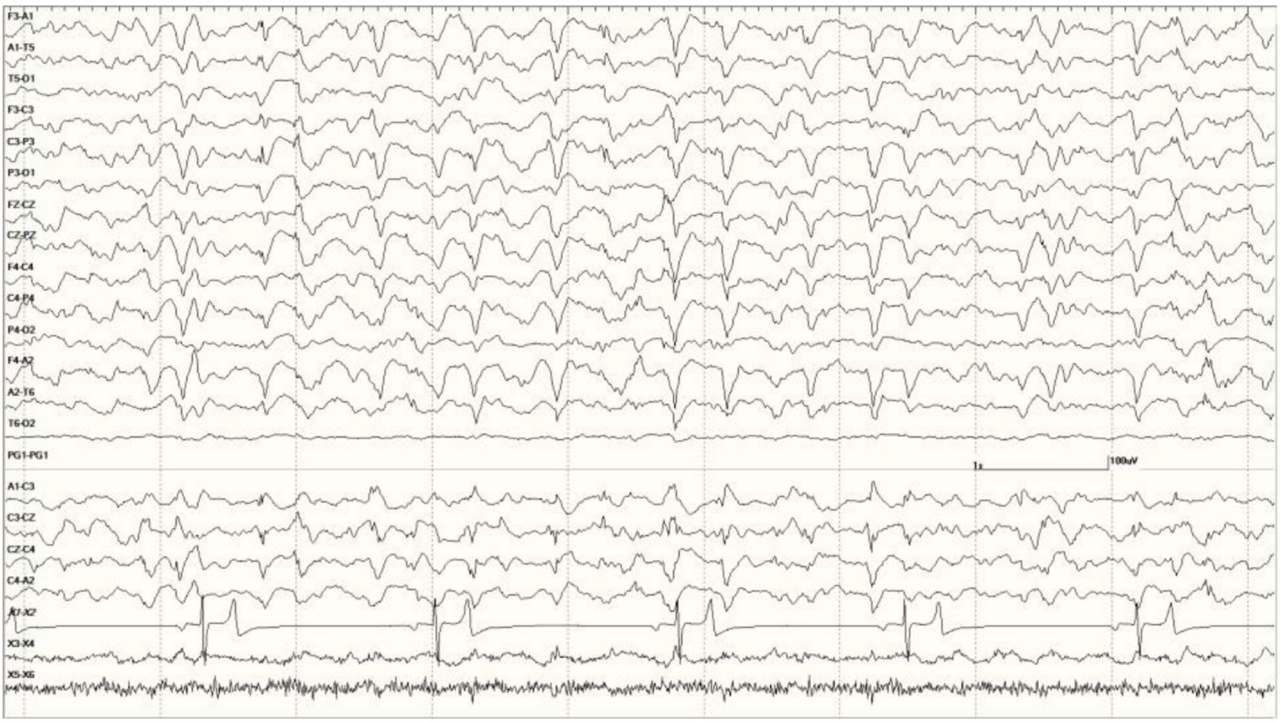


FIGURE 11
Change in EEG associated with isoflurane anesthesia in *Suspected DA toxicosis* sea lion 6731 (compare with Figure 8A).

(39), and sea lions are no exception. Dramatic improvement in EEG recordings (decrease/disappearance of epileptiform activity) was seen following transitions from sleep to wakefulness in all sea lions where state changes occurred. Similar to the findings in horses, isoflurane had confounding effects on the EEG, even in animals determined to be neurologically normal (Figure 2C) (40).

EEG recordings were sensitive, picking up abnormalities in most sea lions during SWS (even those that were not neurologically impaired) but not specific, as similar features were observed in animals diagnosed with porencephaly, cancer, metabolic disease, or white matter lesions. In human medicine, EEG patterns are not pathognomonic for specific disorders. Similar patterns can be associated with a variety of etiologies, cerebral or systemic, such as, cerebral hypoxia, diffuse encephalitis, sepsis, severe metabolic disease, non-convulsive status epilepticus, drug overdose/withdrawal, or hypothermia (41). PDs have been reported with these maladies and were noted in the EEG from the elderly human patient with amnesic shellfish poisoning (18). None of these sea lion EEGs met the PD criteria (refer to Knipe, et al. in this issue), though sea lions 6090, 6667, and 6887 (Figure 7) had occasional quasiperiodic discharges in their EEG recordings.

Multiple studies of the neurological effects of DA have been performed in rats. Some involved DA injections (either into the peritoneum [IP] or directly into the hippocampus) of sublethal doses of the toxin and determination of the time to onset of stereotypic behaviors, such as scratching, lethargy, shaking, tremor, or seizure (42–44). A few also describe the associated electrocorticography (ECoG) findings (43, 44). Latencies to onset of interictal spikes decreased as the dose for those rats hippocampally injected increased with a concurrent increase in the frequency of spikes (43). Clinical seizures were apparent at the 3 highest doses only (100, 200, and 300 pmol). Differences in the display of data make comparisons with the Scallet report difficult, as their analyses were done using fast Fourier transform (FFT) so raw EEG details are lacking (44). They describe significant increases in delta and theta power (voltage squared, in the 1.25–4.50 Hz and 4.75–6.75 Hz frequency bins, respectively) primarily at the higher dose (4.4 mg/kg IP). Theta power in the low-dose (2.2 mg/kg) group only achieved significance 95 min post-injection compared to 30 min for the high-dose group, delta power never did achieve significance in the low-dose group (but did at 35 min for the high-dose group). Higher frequency bands, alpha 1 (7.00–7.50 Hz), alpha 2 (9.75–12.50 Hz), and beta (12.75–18.50 Hz), took longer in both groups to attain significance (75–85 min for high, 110 min for low). Spikes are mentioned but cannot be seen in the figure, as the time base is very compressed (60 s/division instead of the usual 1 s/division).

Early cases of DA toxicosis in sea lions were associated with mass strandings that coincided with algal blooms of *Pseudo-nitzschia australis* (45). This DA exposure was thought to induce damage initially that may later go clinically undetected for some time (a latent period), only to show up eventually as epilepsy (46). This was certainly the case with the human patient described by Cendes who developed complex partial epilepsy 1 year after

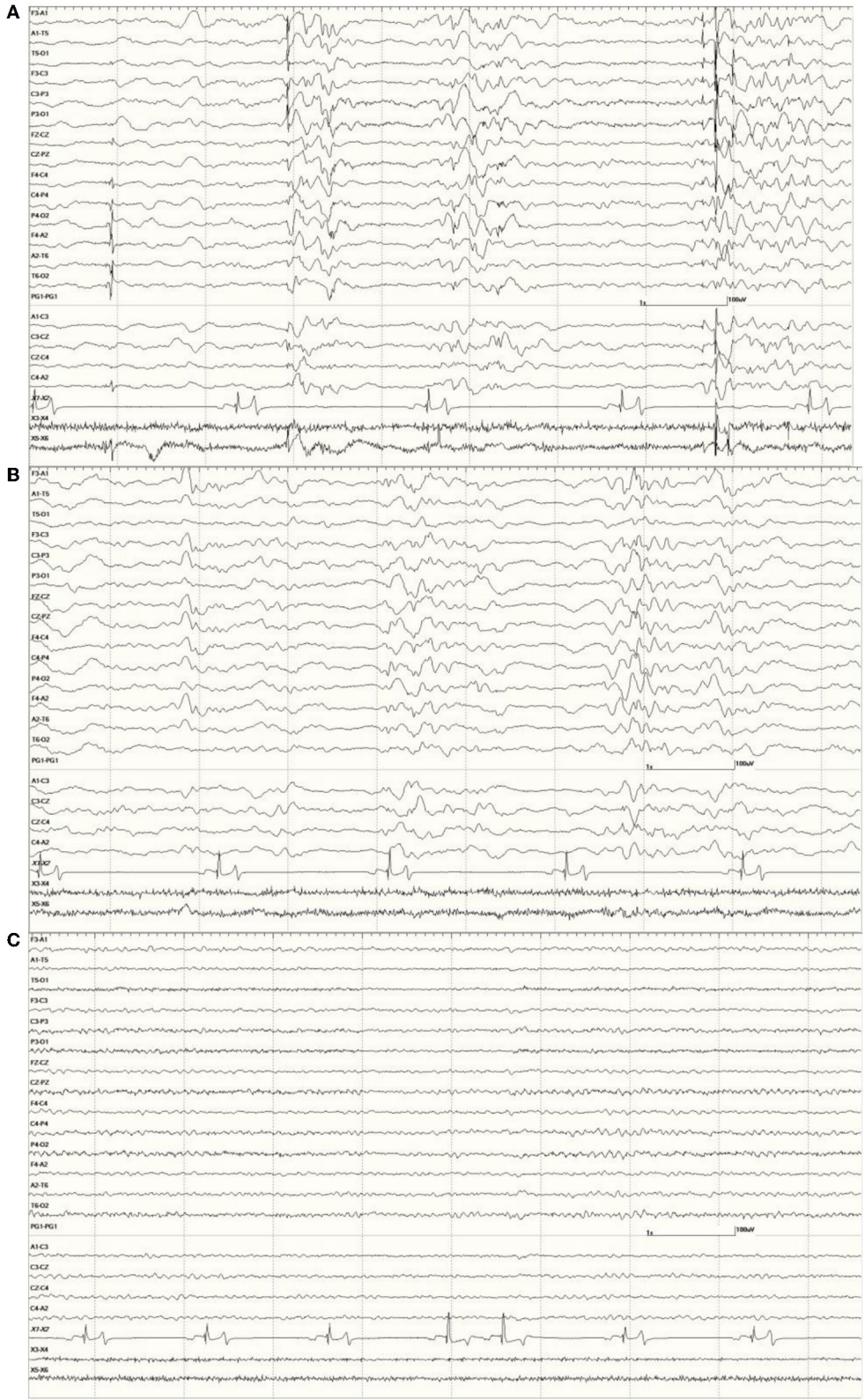


FIGURE 12
The effect of a benzodiazepine on the EEG in sea lion 6667 (a score of 2). Before administration, there are numerous spikes (only the largest one is likely blink artifact) **(A)**. Loss of abnormal fast activity after lorazepam administration **(B)** and disappearance of all abnormalities associated with wakefulness **(C)**.

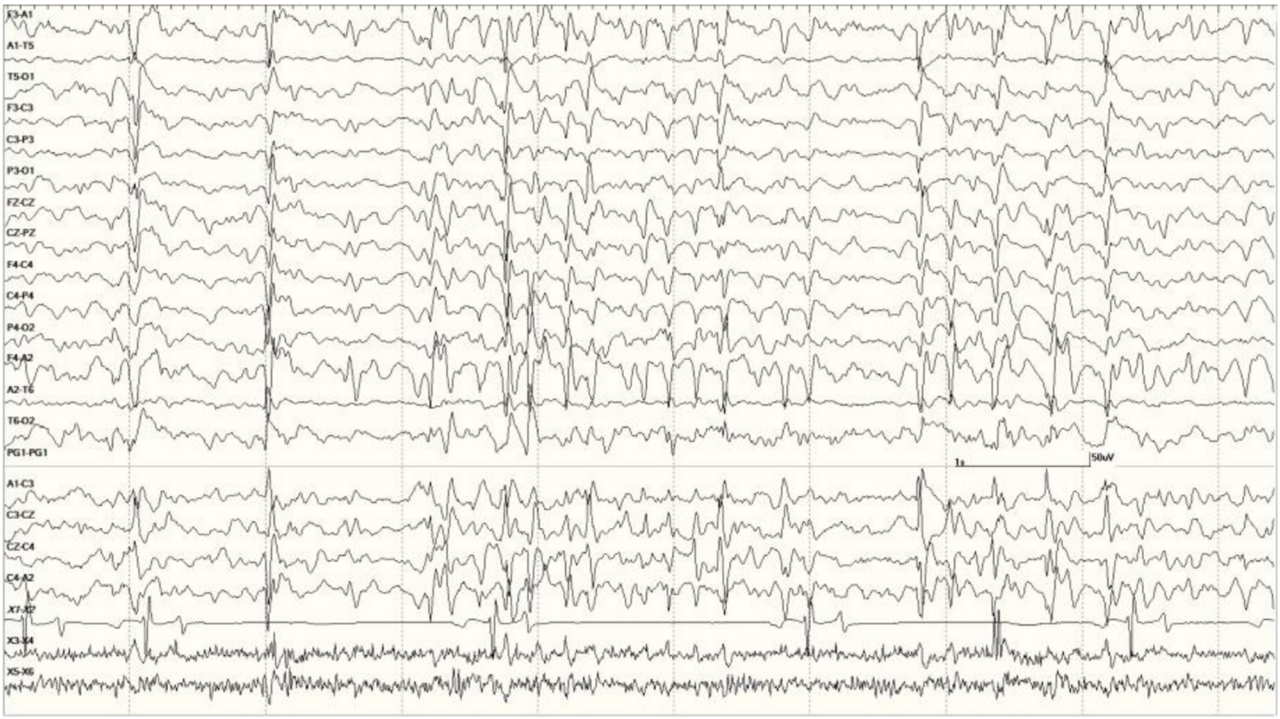


FIGURE 13
Individual generalized events and bursts of sharp and slow waves in *Suspected DA toxicosis* sea lion 9724 (a score of 3). This animal was diagnosed with white matter lesions on MRI but hippocampi were considered normal. Mild encephalitis and cardiomyopathy were found at necropsy consistent with a protozoal etiology.

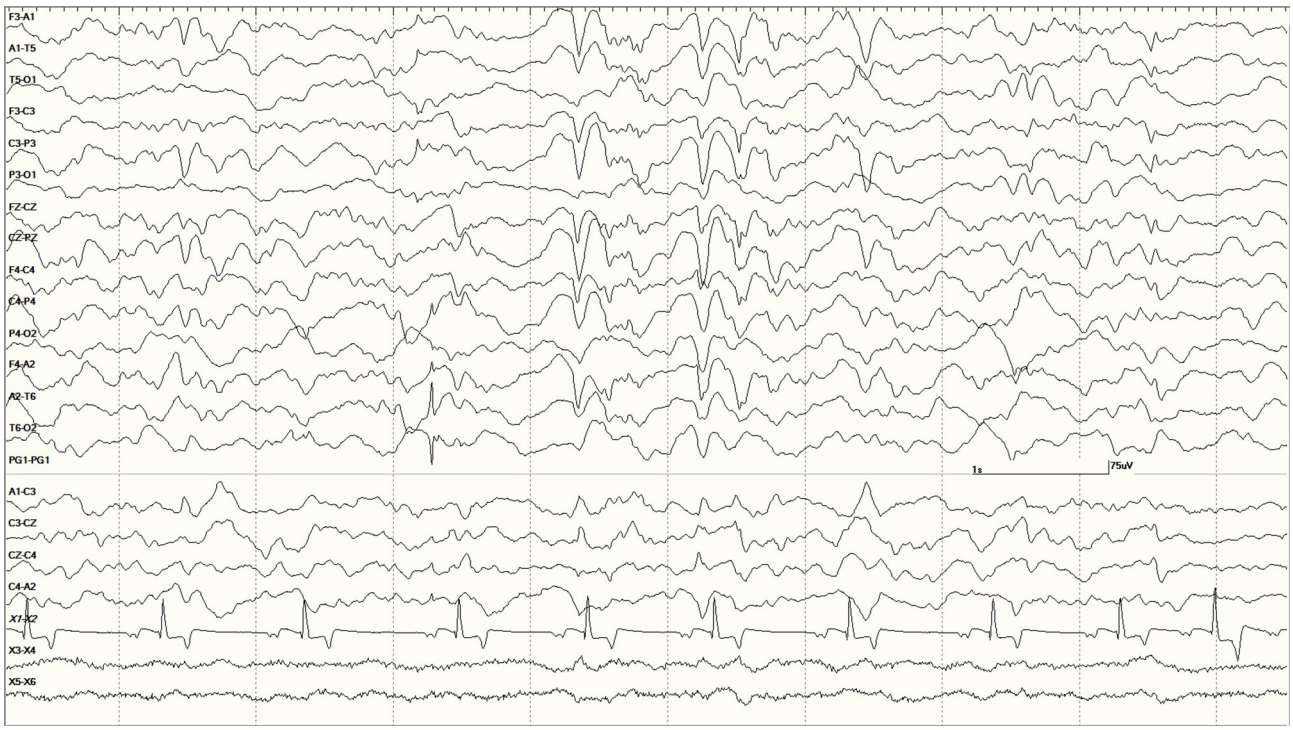


FIGURE 14
Large slow waves and a positive (pointing away) right temporal spike between the 3 and 4 s marks in *Suspected DA toxicosis* sea lion 6079 (a score of 1). This animal was diagnosed with porencephaly at necropsy.

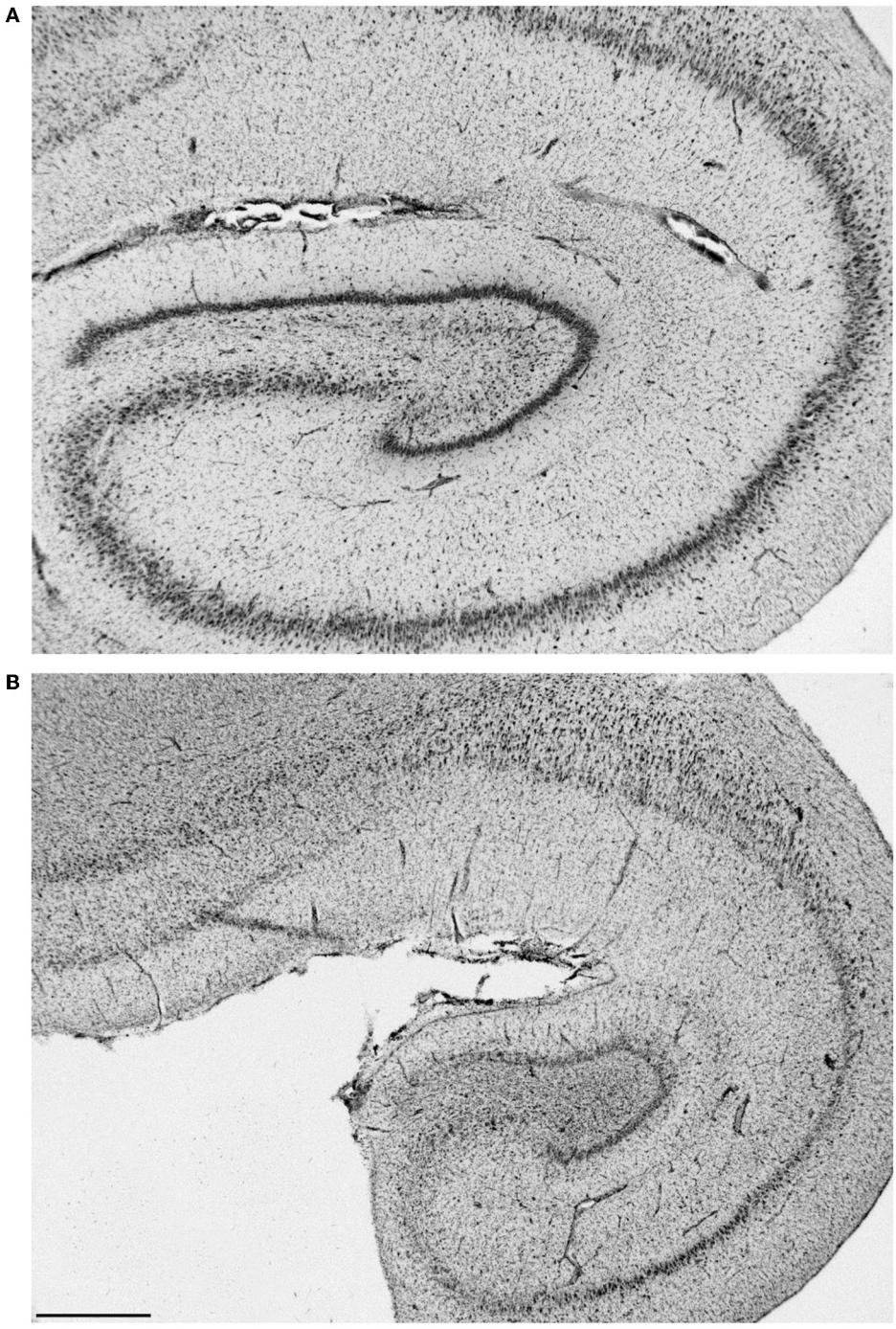


FIGURE 15
Histological section of a normal left hippocampus from sea lion 9724 **(A)**. Hippocampal section from one (9821) with severe bilateral atrophy [**(B)**, left shown]. Bar is 1 mm, the same scale for both.

ingestion of mussels containing DA despite being seizure free for the previous 7.5 m (18). Age susceptibility may have played a role in amnesic shellfish poisoning. Of the 107 confirmed human cases, 49 (46%) were between the ages of 40 and 59 years and 38 (36%) were 60 years or older, with one patient's age unknown (17). An MRI study of 53 sea lion DA toxicosis cases found more severe losses in hippocampal and parahippocampal gyrus volumes in adult animals (47). The authors attributed this to a combination of older animals potentially being more susceptible to the toxin and accumulated damage being induced by the recurrent seizures themselves. This is in contrast to Simeone's report that the developing brain of the fetus/neonate is less likely to be protected from DA effects by virtue of its incompletely formed blood–brain barrier (48). In numerous instances, sea lions exposed to DA *in utero* do not make it to term

(22, 23) and those that do risk further DA exposure by way of the dam's milk (26). The *Neurologically Normal Group* consisted of 40.0% pups (six out of 15) but only two (7158 and 8031) had normal EEGs (a score of 0) and four were 1s. The female pup's (7158) EEG recording did not contain any periods of sleep; therefore, the lack of abnormalities may be state-related. All of these pups could have been exposed to DA with the potential to develop neurological signs over time (even without additional exposure). This age group represented only 2.3% (two of 88) of the *Suspected DA Toxicosis Group* with one receiving a score of 1 and the other with a score of 3. The former was hypoglycemic when admitted, which likely contributed to this sea lion's neurological status. Drawing conclusions about the effect of age requires further study.

Gender appears to be an important factor in this malady, as 81.8% (72 of 88) of the sea lions in the *Suspected DA Toxicosis Group* are females, compared to 6.7% (one in 15) in the *Neurologically Normal Group*. This finding has been attributed to the species' foraging behavior with only females feeding close to rookeries year round where they are more likely to encounter the toxin (46).

Domoic acid preferentially binds to the kainic acid subtype of glutamate receptors prevalent in the CA3 region of the hippocampus (29). It binds both presynaptically and postsynaptically where it induces elevations in intracellular calcium levels, leading to excitotoxic damage such as neuronal loss and astrocytosis. Memory and spatial ability are critical functions of the hippocampus, which can be severely impacted by DA toxicosis/amnesic shellfish poisoning. This brain structure is also involved in the development of some forms of epilepsy. Cats (49), humans (50), and sea lions (29, 32, 46) all have this in common. White matter pathology in the fornix and increases in hippocampal-thalamic connectivity are reportedly similar in both DA toxicosis sea lions and humans with mesial temporal lobe epilepsy (51). In addition to being a sentinel species that can provide valuable information regarding the presence of toxins in the environment, the sea lion may also be an animal model for the most common form of epilepsy in humans. Future sea lion EEG studies can be beneficial in determining the functional aspects of this disorder in conjunction with those involving imaging and pathological findings.

Despite the limitations of this study (no known history prior to stranding [with the exception of restrands]), a lack of truly normal controls (sea lions with no known history of DA exposure or other medical conditions), various missing data points, and minimal follow-up [consisting of telemetry data on the locations of released animals with tags and the status of sea lions placed in other facilities], some valuable knowledge has been gained.

Conclusion

High-quality recordings of normal EEG and a range of abnormal EEG, representing a few different etiologies, were obtained from this population of formerly free-ranging sea lions (those living under natural conditions up until the time they stranded and were treated at a rehabilitation facility). These data can be applied to future studies in this species but can also be used to compare EEG findings across species.

Data availability statement

The datasets presented in this article are not readily available because EEGs are stored on proprietary software. Requests to access the datasets should be directed to DW, colette.neurophys@gmail.com.

Ethics statement

Ethical review and approval was not required for the animal study because the work was authorized under the US Marine Mammal Protection Act by Scientific Research permit no. 932-1489-00.

Author contributions

DW, MH, SD, FG, KL, and BT designed the study. LW, TG, FN, BB, PB, and VH were involved in data acquisition/analysis. DW wrote the manuscript with editing provided by SD, KL, PB, FG, and VH. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to thank Nihon Kohden America, Inc. and Mark Porter for their generous donation of the EEG system used in this study. We also thank John Doval for the diagram depicting the electrode placement used in sea lions. A special thanks to the dedicated staff and volunteers at The Marine Mammal Center who care for these amazing animals.

Conflict of interest

SD is employed by Imaging Solutions, PLLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1040125/full#supplementary-material

References

1. Berger H. Über das elekroenkephalogramm des menschen. *Arch Psychiatr Nervenkr.* (1929) 87:527–70. doi: 10.1007/BF01797193

2. Gibbs FA, Davis H, Lennox WG. The electro-encephalogram in epilepsy and in conditions of impaired consciousness. *Arch Neuro and Psych.* (1935) 34:1133–48. doi: 10.1001/archneurpsyc.1935.02250240002001

3. Mukhametov LM, Supin AY, Polyakova IG. Interhemispheric asymmetry of the electroencephalographic sleep patterns in dolphins. *Brain Res.* (1977) 134:581–4. doi: 10.1016/0006-8993(77)90835-6

4. Lyamin OI, Mukhametov LM, Siegel JM, Nazarenko EA, Polyakova IG, Shpak OV. Unihemispheric slow wave sleep and the state of the eyes in a white whale. *Behav Brain Res.* (2002) 129:125–9. doi: 10.1016/S0166-4328(01)00346-1

5. Lyamin OI, Manger PR, Ridgway SH, Mukhametov LM, Siegel JM. Cetacean sleep: An unusual form of mammalian sleep. *Neurosci Biobehav Rev.* (2008) 32:1451–84. doi: 10.1016/j.neubiorev.2008.05.023

6. Ridgway SH, Harrison RJ, Joyce PL. Sleep and cardiac rhythm in the gray seal. *Sci.* (1975) 187:553–5. doi: 10.1126/science.163484

7. Mukhametov LM, Lyamin OI, Polyakova IG. Interhemispheric asynchrony of the sleep EEG in northern fur seals. *Experientia.* (1985) 41:1034–5. doi: 10.1007/BF01952128

8. Lyamin OI, Chetyrbok IS. Unilateral EEG activation during sleep in the Cape fur seal, *Arctocephalus pusillus. Neurosci Lett.* (1992) 143:263–6. doi: 10.1016/0304-3940(92)90279-G

9. Lyamin OI, Lapierre JL, Kosendo LM, Mukhametov LM, Siegel JM. Electroencephalogram asymmetry and spectral power during sleep in the northern fur seal. *J Sleep Res.* (2008) 17:154–65. doi: 10.1111/j.1365-2869.2008.00639.x

10. Kendall-Bar JM. *Sleeping While Diving: Tools to Detect, Analyze, and Visualize Sleep in Wild Seals*. Santa Cruz: University of California. (2022).

11. Kendall-Bar JM, Mukherji R, Nichols J, Lopez C, Lozano DA, Pitman JK, et al. Eavesdropping on the brain at sea: development of a surface-mounted system to detect weak electrophysiological signals from wild animals. *Anim Biotel.* (2022) 10:1–22. doi: 10.1186/s40317-022-00287-x

12. Kendall-Bar JM, Vyssotski AL, Mukhametov LM, Siegel JM, Lyamin OI. Eye state asymmetry during aquatic unihemispheric slow wave sleep in northern fur seals (*Callorhinus ursinus*). *PLoS ONE.* (2019) 14:e0217025. doi: 10.1371/journal.pone.0217025

13. Goldstein T, Mazet JAK, Zabka TS, Langlois G, Colegrove KM, Silver M, et al. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. *Proc R Soc B.* (2008) 275:267–76. doi: 10.1098/rspb.2007.1221

14. Waugh LF, Williams DC, Greig D, Fravel V, Tharp B, Van Bonn W, et al. Electroencephalography techniques and applications in California sea lions (*Zalophus californianus*). In: *Proceedings of the International Association of Aquatic Animal Medicine.* (2011).

15. Dennison S, Haulena M, Williams DC, Dawson J, Yandell BS, Gulland FM. Determination of a sedative protocol for use in California sea lions (*Zalophus califonianus*) with neurologic abnormalities undergoing electroencephalographic examination. *J Zoo Wildl Med.* (2008) 39:542–547. doi: 10.1638/2007-0077.1

16. Williams DC, Haulena M, Dennison S, Dawson J, Yandell BS, Gulland FMD. Electroencephalographic (EEG) findings associated with domoic acid poisoning in CA sea lions. In: *Proceedings of the International Association of Aquatic Animal Medicine.* (2006).

17. Perl TM, Bedard L, Kosatsky T, Hockin JC, Todd EC, Remis RS. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N Engl J Med.* (1990) 332:1775–80. doi: 10.1056/NEJM199006213222504

18. Cendes F, Andermann F, Carpenter S, Zatorre J, Cashman NR. Temporal lobe epilepsy caused by domoic acid intoxication: evidence for glutamate receptor-mediated excitotoxicity in humans. *Ann Neurol.* (1995) 37:123–6. doi: 10.1002/ana.410370125

19. De La Riva GT, Johnson CK, Gulland FM, Langlois GW, Heyning JE, Rowles TK, et al. Association of an unusual marine mammal mortality event with *Pseudo-nitzschia* spp. *Blooms along the southern California coastline. J Wildl Dis.* (2009) 45:109–121. doi: 10.7589/0090-3558-45.1.109

20. Scholin CA, Gulland F, Doucette GJ, Benson S, Busman M, Chavez FP, et al. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature.* (2000) 403:80–4. doi: 10.1038/47481

21. Lefebvre KA, Powell CL, Busman M, Doucette GJ, Moeller PDR, Silver JB, et al. Detection of domoic acid in Northern anchovies and California sea lions associated with an unusual mortality event. *Nat Toxins.* (1999) 7:85–92. doi: 10.1002/(SICI)1522-7189(199905/06)7:3<85::AID-NT39>3.0.CO;2-Q

22. Brodie EC, Gulland FM, Greig DJ, Hunter M, Jaakola J, Leger JS, et al. Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). *Mar Mamm Sci.* (2006) 22:700–707. doi: 10.1111/j.1748-7692.2006.00045.x

23. Goldstein T, Zabka TS, DeLong RL, Wheeler EA, Ylitalo G, Bargu S, et al. The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus californianus*) on San Miguel Island, California. *J Wildlife Dis.* (2009) 45:91–108. doi: 10.7589/0090-3558-45.1.91

24. Zabka TS, Goldstein T, Cross C, Mueller RW, Kreuder-Johnson C, Gill S, et al. Characterization of a degenerative cardiomyopathy associated with domoic acid toicity in California sea lions (*Zalophus californianus*). *Vet Pathol.* (2009) 46:105–19. doi: 10.1354/vp.46-1-105

25. Cook PF, Reichmuth C, Rouse AA, Libby LA, Dennison SE, Carmichael OT, et al. Algal toxin impairs sea lion memory and hippocampal connectivity, with implications for stranding. *Sci.* (2015) 350:1545–7. doi: 10.1126/science.aac5675

26. Ramsdell JS, Zabka TS. *In utero* domoic acid toxicity: a fetal basis to adult disease in the California sea lion (*Zalophus californianus*). *Mar Drugs.* (2008) 6:262–90. doi: 10.3390/md6020262

27. Trabing W. Seabird invasion hits coastal homes; thousands of birds floundering in the streets. *Santa Cruz Sentinel August.* (1961) 18:1.

28. Bargu S, Silver MW, Ohman MD, Benitez-Nelson CR, Garrison DL. Mystery behind Hitchcock's birds. *Nature Geosci.* (2012) 5:2–3. doi: 10.1038/ngeo1360

29. Ramsdell JS. Neurological disease rises from the ocean to bring model for human epilepsy to life. *Toxins.* (2010) 2:1646–75. doi: 10.3390/toxins2071646

30. Montie EW, Wheeler E, Pussini N, Battey TWK, Barakos J, Dennison S, et al. Magnetic resonance imaging quality and volumes of brain structures from live and postmortem imaging of California sea lions with clinical signs of domoic acid toxicosis. *Dis Aq Org.* (2010) 91:243–56. doi: 10.3354/dao02259

31. Silvagni PA, Lowenstine LJ, Spraker T, Lipscomb TP, Gulland FMD. Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). *Vet Pathol.* (2005) 42:184–91. doi: 10.1354/vp.42-2-184

32. Buckmaster PS, Wen X, Toyoda I, Gulland FMD, Van Bonn W. Hippocampal neuropathology of domoic acid-induced epilepsy in California sea lions (*Zalophus californianus*). *J Comp Neurol.* (2014) 522:1691–706. doi: 10.1002/cne.23509

33. Thomas K, Harvey JT, Goldstein T, Barakos J, Gulland F. Movement, dive behavior, and survival of California sea lions (*Zalophus californianus*) posttreatment for domoic acid toxicosis. *Mar Mamm Sci.* (2010) 26:36–52. doi: 10.1111/j.1748-7692.2009.00314.x

34. Wrzosek M, Nicpon J, Bergamasco L, Sammartano F, Cizinauskas S, Jaggy A. Visual and quantitative electroencephalographic analysis of healthy young cats under medetomidine sedation. *Vet J.* (2009) 180:221–30. doi: 10.1016/j.tvjl.2007.11.003

35. Itamoto K, Taura Y, Wada N, Taga A, Takuma T, Matsumura H, et al. Effect of medetomidine on electroencephalography and use of a quantitative electroencephalograph for evaluation of sedation levels in dogs. *J Vet Med.* (2001) 48:525–35. doi: 10.1046/j.1439-0442.2001.00381.x

36. Williams DC, Aleman M, Tharp B, Fletcher DJ, Kass PH, Steffey EP, et al. Qualitative and quantitative characteristics of the electroencephalogram in normal horses after sedation. *J Vet Intern Med.* (2012) 26:645–53. doi: 10.1111/j.1939-1676.2012.00921.x

37. Binnie CD, Stefan H. Modern electroencephalography: its role in epilepsy management. *Clin Neurophysiol.* (1999) 110:1671–97. doi: 10.1016/S1388-2457(99)00125-X

38. Bazil CW, Malow BA, Sammaritano MR. *Sleep and Epilepsy: The Clinical Spectrum*. Amsterdam: Elsevier Science BV. (2002).

39. Aleman M, Gray LC, Williams DC, Holliday TA, Madigan JE, LeCouteur RA, et al. Juvenile idiopathic epilepsy in Egyptian Arabian foals: 22 cases (1985-2005). *J Vet Intern Med.* (2006) 20:1443–9. doi: 10.1111/j.1939-1676.2006.tb00764.x

40. Williams DC, Aleman M, Brosnan RJ, Fletcher DJ, Holliday TA, Tharp B, et al. Electroencephalogram of healthy horses during inhalation anesthesia. *J Vet Intern Med.* (2016) 30:304–8. doi: 10.1111/jvim.13613

41. Ebersole JS. *Current Practice of Clinical Electroencephalography 4th edition*. Philadelphia: Wolters Kluwer Health. (2014).

42. Sobotka TJ, Brown R, Quander DY, Jackson R, Smith M, Long SA, et al. Domoic acid: neurobehavioral and neurohistological effects of low-dose exposure in rats. *Neurotox and Teratol.* (1996) 18:659–70. doi: 10.1016/S0892-0362(96)00120-1

43. Dakshinamurti K, Sharma SK, Sundaram M. Domoic acid induced seizure activity in rats. *Neurosci Lett.* (1991) 127:193–7. doi: 10.1016/0304-3940(91)90792-R

44. Scallet AC, Kowalke PK, Rountree RL, Thorn BT, Binienda ZK. Electroencephalographic, behavioral, and *c-fos* responses to acute domoic acid exposure. *Neurotox Teratol.* (2004) 26:331–42. doi: 10.1016/j.ntt.2003.10.004

45. Gulland FMD, Haulena M, Fauquier D, Langlois G, Lander ME, Zabka T, et al. Domoic acid toxicity in Californian sea lions (Zalophus californianus): clinical signs, treatment and survival. *Vet Rec.* (2002) 150:475–80. doi: 10.1136/vr.150.15.475

46. Ramsdall JS, Gulland FM. Domoic acid epileptic disease. *Mar Drugs*. (2014) 12:1185–207. doi: 10.3390/md12031185

47. Montie EW, Wheeler E, Pussini N, Battey TWK, Van Bonn W, Gulland F. Magnetic resonance imaging reveals that brain atrophy is more severe in older California sea lions with domoic acid toxicosis. *Harmful Algae*. (2012) 20:19–29. doi: 10.1016/j.hal.2012.07.004

48. Simeone C, Fauquier D, Skidmore J, Cook P, Colegrove K, Gulland F, et al. Clinical signs and mortality of non-released stranded California sea lions housed in display facilities: the suspected role of prior exposure to algal toxins. *Vet Rec*. (2019) 185:304. doi: 10.1136/vr.105371

49. Wagner E, Rosati M, Molin J, Foitzik U, Wahle AM, Fischer A, et al. Hippocampal sclerosis in feline epilepsy. *Brain Pathol*. (2014) 24:607–19. doi: 10.1111/bpa.12147

50. Mathern GW, Adelson PD, Cahan LD, Leite JP. Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited. *Prog Brain Res*. (2002) 135:237–51. doi: 10.1016/S0079-6123(02)35023-4

51. Cook PF, Berns GS, Colegrove K, Johnson S, Gulland F. Postmortem DTI reveals altered hippocampal connectivity in wild sea lions diagnosed with chronic toxicosis from algal exposure. *J Comp Neurol*. (2017) 526:216–28. doi: 10.1002/cne.24317

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 28 April 2023
DOI 10.3389/fvets.2023.1151266

OPEN ACCESS

EDITED BY
Marcin Adam Wrzosek,
Wroclaw University of Environmental and Life Sciences, Poland

REVIEWED BY
Sam Long,
Veterinary Referral Hospital, Australia
Susan Hazel,
University of Adelaide, Australia

*CORRESPONDENCE
Natasha J. Olby
✉ njolby@ncsu.edu

SPECIALTY SECTION
This article was submitted to Veterinary Neurology and Neurosurgery, a section of the journal Frontiers in Veterinary Science

RECEIVED 25 January 2023
ACCEPTED 17 March 2023
PUBLISHED 28 April 2023

CITATION
Mondino A, Catanzariti M, Mateos DM, Khan M, Ludwig C, Kis A, Gruen ME and Olby NJ (2023) Sleep and cognition in aging dogs. A polysomnographic study.
Front. Vet. Sci. 10:1151266.
doi: 10.3389/fvets.2023.1151266

Sleep and cognition in aging dogs. A polysomnographic study

Alejandra Mondino[1], Magaly Catanzariti[2], Diego Martin Mateos[2,3], Michael Khan[1], Claire Ludwig[1], Anna Kis[4], Margaret E. Gruen[1] and Natasha J. Olby[1]*

[1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States, [2]Instituto de Matemática Aplicada del Litoral, Consejo Nacional de Investigaciones Científicas y Técninas, Universidad Nacional del Litoral, Santa Fe, Argentina, [3]Physics Department, Universidad Autónoma de Entre Ríos (UADER), Oro Verde, Entre Ríos, Argentina, [4]Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest, Hungary

Introduction: Sleep is fundamental for cognitive homeostasis, especially in senior populations since clearance of amyloid beta (key in the pathophysiology of Alzheimer's disease) occurs during sleep. Some electroencephalographic characteristics of sleep and wakefulness have been considered a hallmark of dementia. Owners of dogs with canine cognitive dysfunction syndrome (a canine analog to Alzheimer's disease) report that their dogs suffer from difficulty sleeping. The aim of this study was to quantify age-related changes in the sleep-wakefulness cycle macrostructure and electroencephalographic features in senior dogs and to correlate them with their cognitive performance.

Methods: We performed polysomnographic recordings in 28 senior dogs during a 2 h afternoon nap. Percentage of time spent in wakefulness, drowsiness, NREM, and REM sleep, as well as latency to the three sleep states were calculated. Spectral power, coherence, and Lempel Ziv Complexity of the brain oscillations were estimated. Finally, cognitive performance was evaluated by means of the Canine Dementia Scale Questionnaire and a battery of cognitive tests. Correlations between age, cognitive performance and sleep-wakefulness cycle macrostructure and electroencephalographic features were calculated.

Results: Dogs with higher dementia scores and with worse performance in a problem-solving task spent less time in NREM and REM sleep. Additionally, quantitative electroencephalographic analyses showed differences in dogs associated with age or cognitive performance, some of them reflecting shallower sleep in more affected dogs.

Discussion: Polysomnographic recordings in dogs can detect sleep-wakefulness cycle changes associated with dementia. Further studies should evaluate polysomnography's potential clinical use to monitor the progression of canine cognitive dysfunction syndrome.

KEYWORDS

NREM sleep, REM sleep, canine cognitive dysfunction syndrome, quantitative EEG, power spectrum, coherence, complexity

1. Introduction

Sleep is fundamental for cognitive homeostasis as some of its most relevant functions are memory consolidation and learning processing (1–3). Dogs, like humans and other mammals, experience age-related changes in their sleep-wakefulness cycle (4–8). In fact, owners of aging dogs report that sleeplessness is an age-related behavioral change with great impact on the owner-pet relationship (9). While these variations can be attributed to normal

aging, in some cases they may possibly be due to underlying neurodegenerative processes (10, 11).

Humans with Alzheimer's disease (AD), for example, experience sleep disruptions, such as insomnia and sleep fragmentation. These signs can occur early in the course of the disease because brain regions that regulate sleep and circadian rhythms, such as the suprachiasmatic nuclei, are some of the first to be affected (10, 12, 13). Older dogs are prone to develop canine cognitive dysfunction syndrome (CCDS), a disease similar to AD (14–16). This syndrome is characterized by disorientation, memory impairment and difficulty learning, changes in social interactions and house soiling behaviors, increased anxiety, and alteration of the sleep-wakefulness cycle (11, 17–19). Specifically, owners of dogs with CCDS report that their dogs suffer from difficulty sleeping, increased pacing and vocalizations at nighttime, and/or increased sleeping at daytime (11, 20).

While sleep disturbance might be a consequence of these neurodegenerative processes, they can also contribute to the pathophysiology of the disease and to memory impairment resulting in a vicious cycle (13, 21). Clearance of amyloid beta (Aβ) occurs in the brain through the glymphatic system, and this system is primarily active during slow wave sleep (NREM sleep) (22). Therefore, sleep deprivation can increase Aβ deposition in the brain (13, 22–25). While several studies have shown that sleep is disrupted in dogs with CCDS (11, 26), this has been based on owner reports, and no study has looked at the macrostructure or at the electroencephalographic changes of sleep in these dogs. Polysomnography is the gold standard technique to objectively evaluate sleep (27) and a non-invasive technique has been developed in dogs (28). It consists of the simultaneous recording of the EEG, electrical activity of the muscles (EMG) and eye movements (EOG) and can also include the recording of the electrocardiogram (ECG) and respiratory movements.

In addition to the importance of an adequate amount of time dedicated to sleep, the electroencephalographic characteristics of sleep are also associated with Aβ clearance; for example, the reduction of slow oscillations (delta) power is strongly correlated with Aβ deposition in the brain (25). In fact, studies in humans using quantitative electroencephalographic analyses (qEEG) (27) have shown electroencephalographic signatures of dementia during wakefulness and sleep states (29–33). One of the most widely employed qEEG analyses is spectral power, which provides the relative contribution of the frequency components of a signal and reflects the local degree of synchronization of the extracellular potential (34). Spectral power is calculated by means of the fast Fourier transform, and the details of its calculation can be found elsewhere (27). Usually, the frequency spectrum is divided into discrete ranges (bands); in dogs, these are delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), beta (16–30 Hz), and gamma (30–45 Hz) (21). Each band is associated with different behavioral or consciousness states, for example, wakefulness is characterized by higher power in faster oscillations (alpha, beta, and gamma), while NREM sleep or anesthetic states are characterized by higher power in delta oscillations (27, 35, 36). In humans with AD, an increase in delta power and a reduction in the frequency of the peak alpha power has been demonstrated during resting state (37).

Another qEEG analysis frequently used is the spectral coherence, which measures the degree of functional coupling between two cortical areas. Two brain oscillations are completely coherent at a specific frequency if their phase difference and the relationship between the amplitudes at that frequency are constant (27, 38). Coherence can be calculated between two areas within the same brain hemisphere (intrahemispheric) or between areas on the right and left hemispheres (interhemispheric) We have previously shown that, comparable to people with AD (39, 40), during wakefulness, dogs with presumptive diagnosis of CCDS show a reduction of interhemispheric coherence in the high frequency bands (41).

Both power and coherence are measures of linear dynamics, but the complex EEG signal cannot be explained only by them. Non-linear analyses are a more modern method to study the complexity of the system (42). One of the most used in qEEG research is Lempel Ziv Complexity, which evaluates the randomness of finite sequences based on the number of distinct patterns in a signal and the symbolic encoding (43). The LZC analysis is usually performed on the whole signal of a wider range of frequencies, without analyzing different frequency bands. This complexity has been used in the analysis of different neurophysiological signals, for example, to study the EEG signatures of anesthesia, sleep, and altered states of consciousness, as well as brain disorders such as AD or epilepsy (44–47). During wakefulness, people with AD show a reduction in Lempel Ziv Complexity (45), but we have recently found that the opposite is true for dogs with presumptive diagnosis of CCDS (41).

The aim of this study was to describe and quantify age-related changes in the sleep-wakefulness cycle macrostructure and EEG features in senior dogs and to correlate them with their cognitive performance. We predicted that cognitively impaired dogs would sleep less and would have distinct EEG features from dogs without cognitive impairment.

2. Methods

2.1. Study population

All procedures were approved by the North Carolina State University (NCSU) Institutional Animal Care and Use Committee, protocol number: 21-303. Client-owned dogs participating in the longitudinal study of neuro-aging at the North Carolina State University (NCSU), College of Veterinary Medicine were used in this study [described in our previous research (18, 48, 49)]. All owners reviewed and signed an informed consent form prior to participation. To be included, dogs had to be older than the 75% of their expected lifespan, which was calculated using the formula proposed by Greer et al. (50) that takes into consideration the dog's height and weight and can thus be applied in case of mixed-breed dogs as well. Additionally, they had to be free of comorbidities that could impede their ability to perform cognitive tests such as inability to walk or blindness.

Demographic information was collected, and physical, orthopedic, and neurological examinations were conducted for every dog. Height to the dorsal aspect of the scapula (withers) and body weight were measured in order to calculate dogs' expected

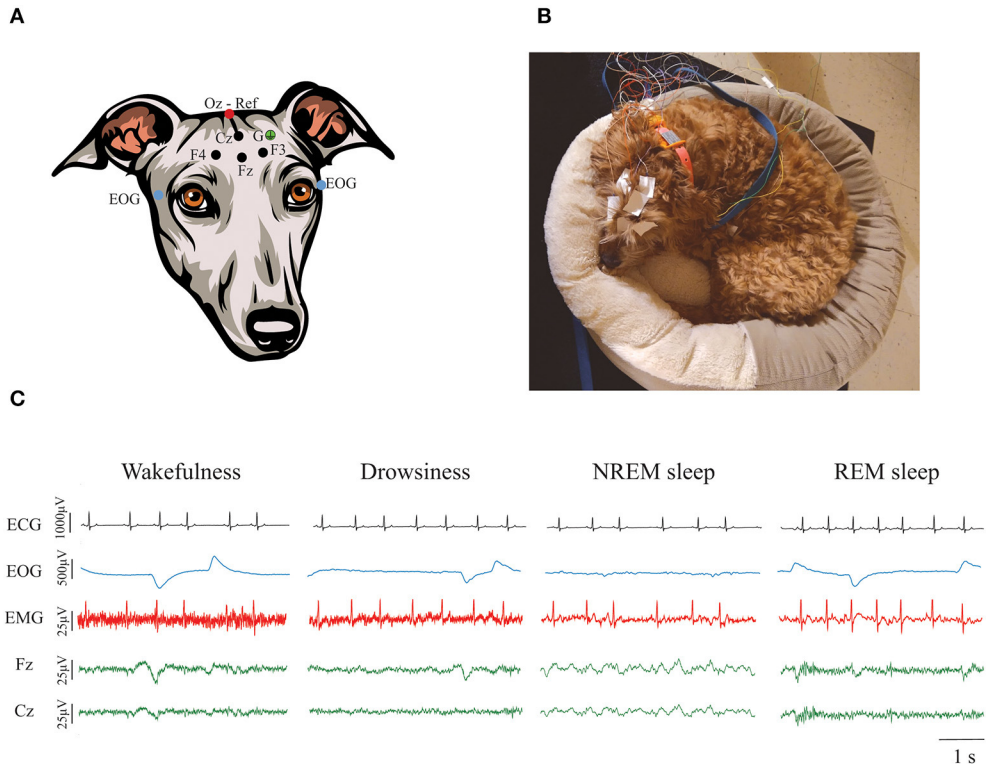


FIGURE 1
Polysomnographic recordings in dogs. **(A)** Electrode locations. Four active EEG electrodes were placed over the midline, left and right frontal areas (Fz, F3, and F4, respectively), and over the vertex (Cz). Those electrodes are represented with a black dot. A reference electrode was placed over the external occipital protuberance (Oz) and it is represented with a red dot. Bipolar EOG signal was recorded by placing two electrodes on the left and right zygomatic arch next to the lateral canthus of each eye (blue dots). A ground electrode (G) was placed over the left temporal muscles (green dot). EMG and ECG were also recorded but it is not shown in the figure. **(B)** Picture of a dog undergoing a polysomnographic recording using their own bed. **(C)** Representative ECG, EOG, EMG, and EEG signals during wakefulness, drowsiness, NREM, and REM sleep. Contamination of the ECG can be observed in the EMG signal.

lifespan. Urinalysis, biochemistry panel and complete blood cell count were also performed to rule out potential comorbidities.

2.2. Polysomnographic studies

Dogs underwent polysomnographic recordings with simultaneous recording of EEG, EOG, EMG, and ECG with a slight modification of the protocol described by Reicher et al. (51). As schematized in Figure 1A, we employed four active EEG electrodes, F3, F4, Fz (left, right, and midline frontal, respectively), and Cz (at the level of the vertex). The electrode location was chosen to evaluate the frontal and parietal cortex, the frontal cortex being particularly important in cognitive decline (52). These electrodes were referenced to Oz, an electrode placed over the external occipital protuberance. Bipolar EOG signal was recorded by placing two electrodes on the left and right zygomatic arch next to the lateral canthus of each eye (F7 and F8, respectively). A ground electrode was placed over the left temporal muscles. For EMG, bipolar signal was obtained by placing two electrodes over the dorsal neck muscles on the left and right side. Finally, one electrode was placed over the fifth intercostal space and referenced to the Cz electrode to record the ECG. We used gold-coated electrodes (Genuine Grass 10 mm Gold Cup, Natus Medical Inc) which were attached to the skin with SAC2 electrode cream (Cadwell Laboratories) after applying a skin preparation and electrode solution (Signa Spray, Parker Laboratories). Recordings were performed with the software Cadwell Easy II software (Cadwell Laboratories). Electrode impedance was checked to be under 20 kΩ before starting the recording. A frequency sample of 400 Hz was used. All signals were bandpass filtered between 0.53 and 70 Hz. A notch filter was also applied during acquisition to remove the 60 Hz power-line noise.

Recordings were performed in a very quiet room, with dim light and white noise reproduced through a laptop computer. Room temperature was maintained at 20°C. Owners were asked to bring their dogs for polysomnography recordings on 2 different days, an adaptation day [to avoid the "first-night-effect" (51)], and a recording day and were instructed to bring their dogs' usual bed or blankets to both recording days, so dogs felt more comfortable to sleep. Figure 1B shows an example of one dog during a polysomnography recording. During the adaptation day we performed a 30 min recording that served to acclimate the dog to the recording room and setup. On the actual testing day, the

dogs had a 2-h polysomnography recording that started between 12:30 and 1:30 PM. This period of time was selected because it has been shown that dogs usually have naps around noon (5, 53, 54). For dogs who became anxious and attempted to leave the room or removed all the electrodes, we stopped the recordings even if the 2 h were not complete. Time between the adaptation and the actual recording day was never more than 2 weeks, and cognitive testing happened in between these days.

States of wakefulness, drowsiness, NREM and REM sleep were manually scored in 3 s epochs in the Spike 2 software (Cambridge Electronic Design) using criteria described previously (28). As shown in Figure 1C, wakefulness was characterized by fast frequency EEG activity, high activity in the EMG, and frequent high-amplitude eye movements. Drowsiness was defined by EEG activity similar to wakefulness, reduced but observable muscle activity in the EMG and decreased amplitude and frequency of eye movements. NREM sleep was characterized by low-frequency and high-amplitude neural oscillations, mainly within the delta frequency band. Muscle activity during NREM sleep was significantly reduced and there were no or sporadic low-amplitude eye movements. Finally, REM sleep was recognized by high frequency activity in the EEG, absence of muscle activity and frequent rapid eye movements that can also be observed as artifacts in the EEG (28). Latency to enter drowsiness, NREM and REM sleep as well as the percentage of the total recording time spent in each behavioral state were calculated. Additionally, sleep efficiency was calculated by adding the time spent in NREM and REM sleep and dividing it by the total time recorded.

2.3. qEEG analyses

Similar to our previous study (41), raw EEG signals from the four EEG electrodes were exported from Spike 2 to MATLAB (version 2022b; The MathWorks Inc). All artifact-free, non-transition epochs were selected for quantitative EEG analysis. Power spectrum was calculated for Fz and Cz electrode locations with a 0.25 Hz resolution between 0.5 and 50 Hz by means of *pwelch* function in MATLAB using the following parameters: 1 s sliding windows with 50% overlap. Power at F3 and F4 were not calculated since we were interested in changes in frontal and parietal cortices and analyzing three different electrodes of the same area was redundant. Total power was computed by summing the power of each 0.25 Hz bin and relative power was calculated by dividing power over total power. Interhemispheric (between F3 and F4) and intrahemispheric (between Fz and Cz) coherence were calculated using the MATLAB *mscohere* function utilizing the same parameters used for power spectrum analysis. Coherence values were then normalized using Fisher's z-transform.

We also evaluated the complexity of the EEG signal by means of Lempel-Ziv complexity for Fz and Cz. To perform the analysis, the signal was pre-processed by applying an order 5 butterworth band-pass filter between the frequencies 1 and 50 Hz. We then used the *lziv_complexity* function from the *AntroPy* package from Python (55).

2.4. Cognitive evaluation

We evaluated cognitive status in these dogs by using an owner-based questionnaire and a battery of cognitive tests. As in our previous studies, the questionnaire utilized was the Canine Dementia Scale (CADES), a validated clinical metrology instrument to capture behavioral changes associated with CCDS. In this questionnaire owners need to specify the frequency with which their dogs experience specific behaviors in four different domains: spatial disorientation, social interactions, sleep-wakefulness cycle and house soiling (11). CADES scores range from 0 (normal) to 95 (severely affected) and allow classification of dogs into four different categories: normal (0–7), mild cognitive impairment (MiCI, 8–23), moderate cognitive impairment (MoCI, 24–44), and severe cognitive impairment (SCI, 45–95).

Cognitive testing was also performed to objectively evaluate different domains of cognition: attention (sustained gaze test), working memory and executive control [inhibitory control and detour (cylinder) tasks]. The detailed procedure for each task has been described by our group elsewhere (18, 56). In brief, the sustained gaze task measures how long the dog holds the gaze with an experimenter who is holding a treat near their face. To evaluate working memory, two red Solo® cups were placed on a mat and a small food treat was hidden under one of them (while the dog is allowed to see where it was placed). The delay (up to 2 min) between placing the treat and allowing the dog to choose one of the cups to retrieve the treat was progressively increased. The upper threshold of time that dogs were able to correctly identify (at least four out of six times) where the treat was hidden was then calculated. In the inhibitory control task, dogs were asked to retrieve a treat from a transparent plastic cylinder that is open on both ends, without touching the outside walls of cylinder. The detour task is similar but adds an additional difficulty—the dog's preferred side for retrieving the treat is now covered, and they need to enter the cylinder from the opposite side (detour) in order to obtain the treat. The outcome measure for these two tests is the percentage of correct trials (retrieving the treat without touching the outer walls of the cylinder or the detour).

2.5. Statistical analysis

We calculated the correlation between the sleep architecture variables (and Lempel Ziv Complexity) and age, CADES score, and performance in each of the cognitive tests by means of Spearman correlation analyses. While fractional lifespan (instead of chronological age) was employed for inclusion criteria, we used chronological age in our analyses because previous studies that have looked at sleep and qEEG analysis in dogs have reported chronological age (28, 51, 57). Statistical significance was adjusted using the Benjamini, Krieger and Yekutieli method (58) with a false discovery rate of 0.05. Adjusted p-value is expressed with a "q." To evaluate the correlation between age and cognitive performance with power and coherence we did a bin to bin spearman correlation analysis and we used the procedure known as Rüger's areas (59) previously employed in polysomnographic studies in dogs (28, 57) to address the issue of multiple comparisons. These areas can be

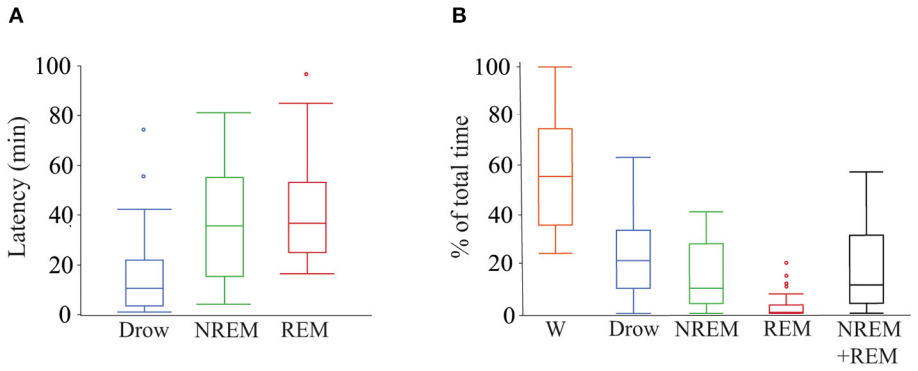


FIGURE 2
Latency to and percentage of time spent in each behavioral state during polysomnography recordings. **(A)** Box plots showing latency in minutes to drowsiness, NREM and REM sleep. Only data from dogs that entered each specific behavioral state was employed (drowsiness $n = 26$, NREM sleep $n = 24$, and REM sleep $n = 15$). **(B)** Box plots showing percentage of total time spent in each behavioral state and the sleep efficiency (sum of time in NREM and REM sleep over total time). All dogs ($n = 28$) were used. Box plots represent the median (central line of the box), the 25 and 75th percentile (lower and upper line of the box). The whiskers represent 1.5 times the interquartile range (1.5*IQR) and outliers are shown with a circle. W, wakefulness; Drow, drowsiness; NREM + REM, sleep efficiency.

defined as a cluster of conventionally significant ($p < 0.05$) results, which are considered significant (or not) as a whole. A Rüger's area is the cluster of all the adjacent, consecutive frequency bins which contain a significant result surrounded by bins containing non-significant results. After defining these areas of significance, the number of frequency bins within the area was counted, and it was inspected to see if at least half of the p-values were lower than 1/2 of the conventional $p = 0.05$ significance level (i.e., below 0.025) and at least 1/3 of them were lower than 1/3 of the conventional $p = 0.05$ (i.e., 0.0167). If both requirements were satisfied, the area as a whole was considered significant.

3. Results

3.1. Demographics

Twenty-eight dogs [13.25 ± 1.57 years old (Range: 10.4–16.2)] were enrolled in this study. Their mean fractional lifespan was 1.06 ± 0.12 (Range 0.81–1.22). Seventeen were spayed females and 11 were castrated males. Seventeen were purebred dogs, and 11 were mix breed. The most represented breed was pit bull terrier with four dogs, followed by Labrador retriever with two dogs. There were also 1 dog of each of the following breeds: Australian shepherd, Basset hound, beagle, Border collie, Brittany spaniel, dachshund, German shorthaired pointer, golden retriever, German shepherd, Irish setter, and Pomeranian.

3.2. Polysomnography recordings

Of the 28 dogs, 17 completed 2 h of recording. Of the remaining 9, five completed at least 1.5 h of recording and the minimum recording time was 1.01 h. Twenty-six dogs entered drowsiness, 24 into NREM sleep and 15 into REM sleep. Percentage of the total recorded time spent in each behavioral state, sleep efficiency and latency to drowsiness, NREM sleep, and REM sleep are shown in Figure 2.

3.3. Cognitive evaluation

Median score in the CADES questionnaire was 18 (Range 0–70). According to their score, eight dogs (28.5%) were classified as normal, eight dogs (28.5%) with mild, four dogs (14.3%) with moderate, and eight dogs (28.5%) with severe cognitive impairment. Every dog completed the sustained gaze, and inhibitory control tasks. Memory task was not performed in three of the dogs because they failed to pass warm-ups (required as a criterion for learning; they did not remember where the treat was even immediately after showing them the location, therefore, these dogs scored 0 for memory) and one dog did not complete the detour task because they lost interest and would no longer engage with the task. For this dog, detour task data were excluded from the analysis. The median sustained gaze time was 17.0 s (range 1.3–60), median memory threshold was 40 s (range 0–120), median percentage of correct trials in inhibitory control was 100 (range 13–100%) and in detour 50 (range 0–100%).

3.4. Correlation between sleep macrostructure and age and cognitive status

As shown in Table 1, older dogs tended to spend more time awake and less time in NREM sleep. However, these correlations did not reach statistical significance after correcting for multiple comparisons. There was also a relationship between cognitive performance and sleep parameters: the CADES score was negatively correlated with time spent in NREM and REM sleep. Dogs with higher CADES scores, also tended to have a higher latency to enter into NREM sleep. Figure 3 shows the hypnogram

10.3389/fvets.2023.1151266

TABLE 1 Correlation between sleep macrostructure and age and cognitive function.

Variable	By variable	Spearman ρ	Raw p-value	q-value
Age	Latency to Drow.	0.229	0.260	0.439
Age	Latency to NREM	0.440	0.031	0.116
Age	Latency to REM	0.091	0.747	0.783
Age	Time in W (%)	0.408	0.020*	0.116
Age	Time in Drow. (%)	−0.373	0.051	0.164
Age	Time in NREM (%)	−0.441	0.019*	0.092
Age	Time in REM (%)	−0.305	0.114	0.295
CADES	Latency to Drow.	0.210	0.304	0.472
CADES	Latency to NREM	0.436	0.033*	0.116
CADES	Latency to REM	0.011	0.969	0.897
CADES	Time in W (%)	0.454	0.015*	0.085
CADES	Time in Drow. (%)	−0.267	0.169	0.331
CADES	Time in NREM (%)	−0.554	<0.001*	**0.020***
CADES	Time in REM (%)	−0.553	0.002*	**0.020***
Memory	Latency to Drow.	−0.197	0.357	0.496
Memory	Latency to NREM	−0.319	0.147	0.331
Memory	Latency to REM	−0.258	0.395	0.529
Memory	Time in W (%)	−0.361	0.077	0.229
Memory	Time in Drow. (%)	0.272	0.189	0.349
Memory	Time in NREM (%)	0.327	0.110	0.295
Memory	Time in REM (%)	0.283	0.170	0.331
Inhibitory control	Latency to Drow.	−0.212	0.298	0.472
Inhibitory control	Latency to NREM	−0.148	0.489	0.613
Inhibitory control	Latency to REM	0.123	0.661	0.755
Inhibitory control	Time in W (%)	0.014	0.942	0.893
Inhibitory control	Time in Drow. (%)	−0.055	0.781	0.798
Inhibitory control	Time in NREM (%)	0.079	0.691	0.767
Inhibitory control	Time in REM (%)	0.111	0.572	0.674
Detour	Latency to Drow.	−0.193	0.355	0.496
Detour	Latency to NREM	−0.525	0.010*	0.065
Detour	Latency to REM	−0.184	0.529	0.642
Detour	Time in W (%)	−0.556	0.003*	**0.020***
Detour	Time in Drow. (%)	0.149	0.457	0.592
Detour	Time in NREM (%)	0.683	<0.001*	**0.019***
Detour	Time in REM (%)	0.596	0.001*	**0.019***
Eye gaze	Latency to Drow.	−0.019	0.926	0.893
Eye gaze	Latency to NREM	−0.305	0.147	0.331
Eye gaze	Latency to REM	−0.343	0.211	0.372
Eye gaze	Time in W (%)	0.193	0.324	0.484
Eye gaze	Time in Drow. (%)	0.041	0.837	0.833
Eye gaze	Time in NREM (%)	−0.071	0.718	0.774
Eye gaze	Time in REM (%)	−0.273	0.160	0.331

Asterisks and bold text indicate significant differences.
q-values represent the corrected p-values using the false discovery rate Benjamini, Krieger, and Yekutieli procedure.
W, Wakefulness; Drow, Drowsiness.

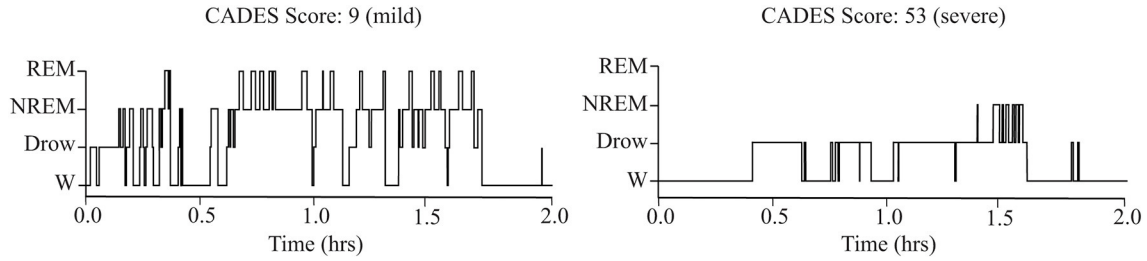


FIGURE 3
Representative hypnograms showing the difference in sleep architecture between a dog with mild **(left)** and severe **(right)** cognitive impairment based on CADES score. W, wakefulness; Drow, Drowsiness.

of two representative dogs, one with a low CADES score (classified as with mild cognitive impairment) and one with a high CADES score (classified as with severe cognitive impairment). This figure clearly illustrates how dogs with higher CADES scores spend less time sleeping. Additionally, performance in the detour task was negatively correlated with time spent in wakefulness and positively correlated with time spent in NREM and REM sleep. Dogs with worse performance also tended to have longer NREM sleep latency. We did not find any association between sleep macrostructure and performance in any of the other cognitive tests.

3.5. Associations between electroencephalographic features, age, and cognitive status

We calculated power, coherence, and Lempel Ziv complexity for every dog, except for one in which Fz, F3 and F4 electrodes were not analyzed because the dog removed those electrodes during the recording. Therefore, in this dog, power and complexity were analyzed only for Cz, and we could not perform coherence analysis. The power spectrum analysis is summarized in Figure 4. There were significant relationships with age, performance on the detour task and memory. For Fz, during drowsiness the power of slow oscillations (1–1.75 Hz) was negatively correlated with age and positively correlated with performance in the detour task. At each of the correlated frequencies, the strength of the correlation was higher for performance at detour task than for age. In addition to this, memory performance was correlated with the power of some frequency bands during REM sleep at Cz: dogs with higher memory scores had higher power of slow oscillations from 1 to 1.75 Hz, and lower power between 15.75 and 19 Hz (within beta frequency band). Also, at Fz, dogs with higher memory scores had lower power of higher oscillations, between 48 and 50 Hz (within gamma frequency band). Interhemispheric (F3–F4) coherence showed some correlations with age and cognitive function. As shown in Figure 5, during wakefulness, older dogs showed higher interhemispheric coherence for frequencies 6.5–13 Hz (within theta and alpha frequency bands). Dogs with higher CADES scores also had higher interhemispheric coherence at those frequencies, but the correlation was only significant between 10.5 and 12 Hz. Similarly, dogs with worse performance at the detour task showed higher interhemispheric coherence between 10.25 and 12 Hz. The strength of these correlations was higher for age than for CADES scores or performance at the detour task. We also found a negative correlation between performance in the sustained gaze task and interhemispheric coherence for slow oscillations up to 3 Hz (within delta frequency band). Furthermore, during REM sleep, older dogs had higher interhemispheric coherence for frequencies between 36.25 and 38 Hz.

Intrahemispheric (Fz-Cz) coherence was correlated with cognitive function, but not with age (Figure 6). During wakefulness, dogs who performed better at the inhibitory control task had lower Fz-Cz coherence for the frequencies between 8 and 14.25 Hz. Similarly, during drowsiness, frequencies between 0.25–6.5 and 7–12.5 Hz were lower for dogs with better performance in the same task. During NREM sleep, there was also a negative correlation between coherence and inhibitory control scores, but for higher frequencies, between 7.75–20.75 and 30.25–44.25 Hz. During REM sleep, an association was found with performance on the detour task, dogs with better performance had higher coherence for frequencies between 10.75 and 16.5 Hz.

We evaluated the complexity of the EEG signal for the electrodes Fz and Cz and evaluated its correlation with age, CADES score, and performance in cognitive tests and we found a negative correlation between memory performance and complexity during REM sleep at Fz (Table 2). Age showed a positive correlation and performance in the detour task showed a negative correlation with complexity during REM sleep, but the significance was lost after multiple comparisons correction. Similarly, we observed that age tended to be positively correlated, while CADES, memory, and performance at the detour task tended to be negatively correlated with complexity during REM sleep at Cz. However, none of these remained significant after multiple comparisons correction (Table 3).

4. Discussion

In this study we evaluated changes in sleep architecture objectively using polysomnography, the gold standard technique used to describe and quantify sleep (27, 28). We were able to obtain artifact-free EEG data using surface (cup) electrodes in elderly dogs while sleeping in the hospital setting. We demonstrated that dogs with higher dementia scores spend less time sleeping in both NREM

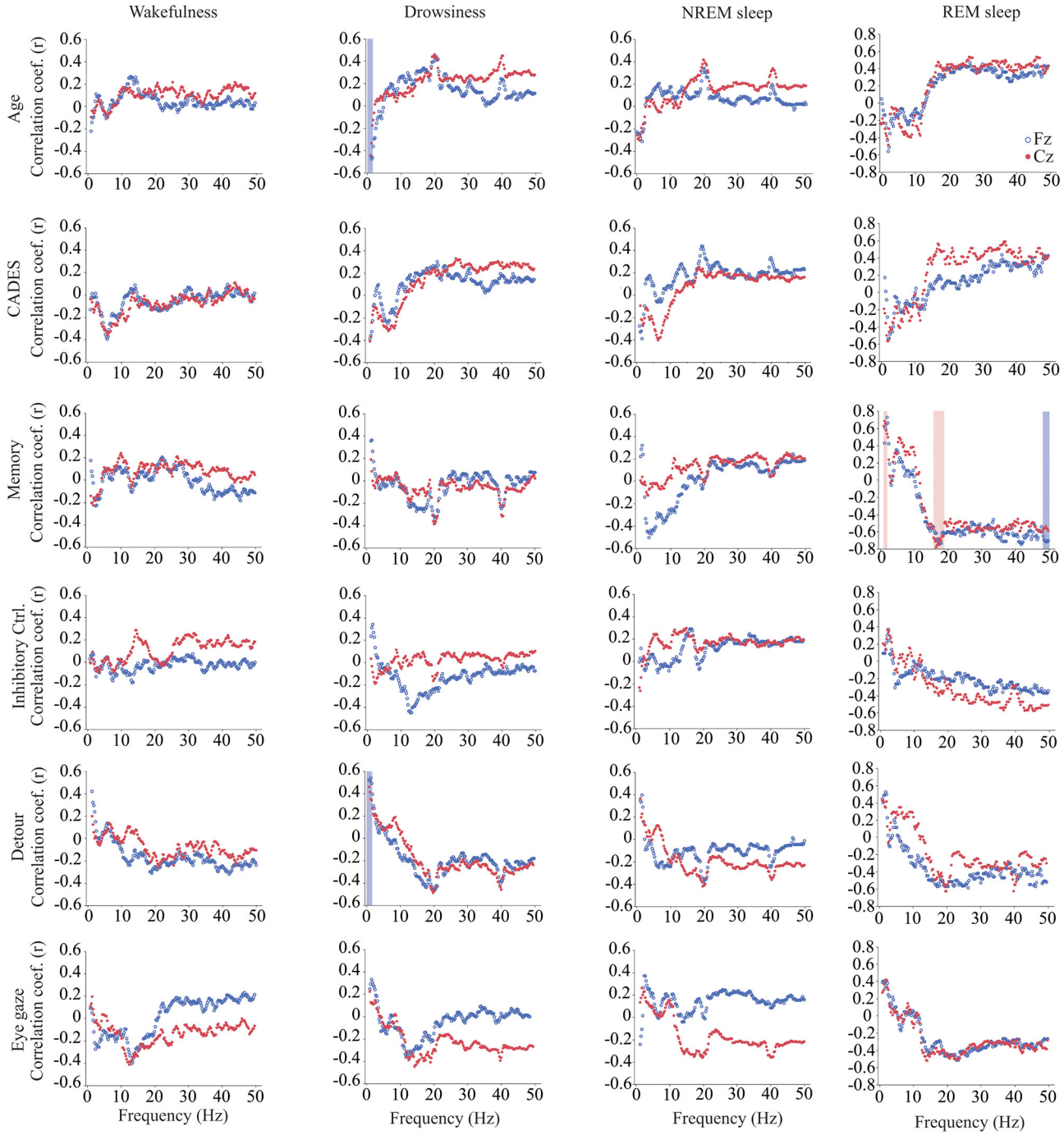


FIGURE 4
Correlations between wakefulness, EEG power and age, CADES score, or performance in cognitive tests. Correlation coefficients for the EEG channels Fz (blue open circle) and Cz (red solid circle) are shown for each frequency bin. Frequencies with significant correlations are indicated with a blue (for Fz) or red (for Cz) shadow box. The analysis was performed only for the dogs that entered each specific behavioral state (wakefulness n = 28, drowsiness n = 26, and NREM sleep n =24).

and REM sleep in the afternoon than dogs with lower scores. This supports owner observations that disruptions of the sleep-wakefulness cycle characterize CCDS (11, 17). Similarly, humans with AD suffer from insomnia and lower sleep quality (60, 61), and a bidirectional relationship between sleep and disease has been proposed (13) in which amyloid burden in the brain disturbs sleep (62–64) while poor sleep promotes amyloid β deposition and impairs memory consolidation (65, 66).

We found that sleeping time was positively associated with the performance in a problem-solving detour task. Studies in humans have demonstrated that sleep plays a critical role in enhancing the skills needed to find novel strategies to solve problems (67, 68). One

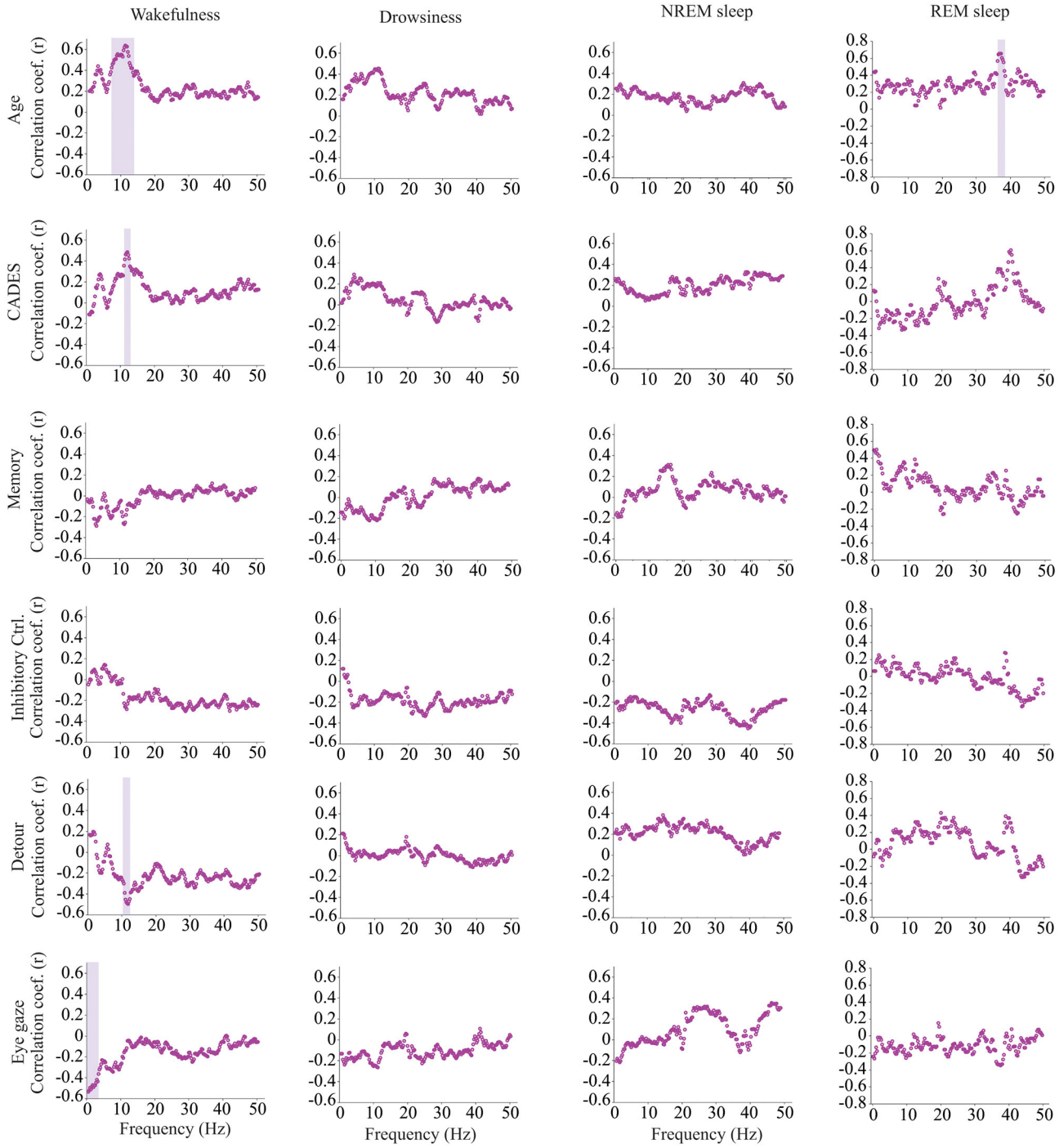


FIGURE 5
Correlations between EEG interhemispheric (F3–F4) coherence and age, CADES score, or performance in cognitive tests. Spearman correlation coefficients are shown for each frequency bin. Frequencies with significant correlations are indicated with a purple shadow box. The analysis included only the dogs that entered each specific behavioral state (wakefulness $n = 28$, drowsiness $n = 26$, and NREM sleep $n = 24$).

study has shown sleep dependent improvement in problem solving tasks, particularly those with higher difficulty (68). In dogs, Kis et al. have shown that sleep is associated with social learning skills (21). In the current study, longer sleep time was associated only with performance at the detour task and not with the other cognitive tasks. Similarly, a previous study on senior dogs (ages 7–14 years) testing the relationship between sleep spindles and cognition found that correlations were specific to, in that case, reversal learning only (69). Some human studies have shown that sleep deprivation can affect particular components of cognition differently (70–72). For example, Tucker et al. observed a larger effect of deprivation induced sleep loss in humans on non-executive rather than executive components of cognition, with working memory being less vulnerable than other domains (71). The main difference

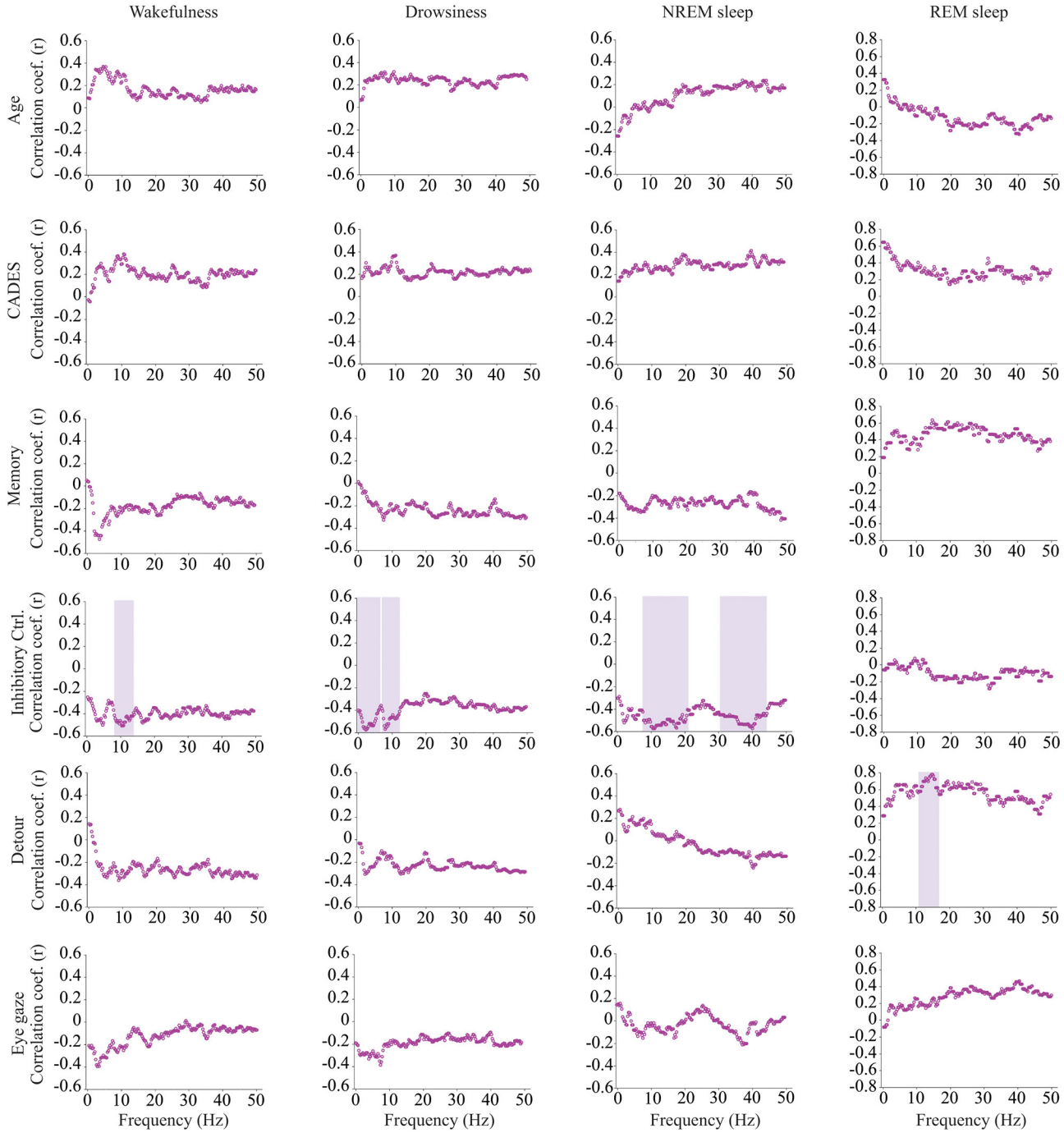


FIGURE 6
Correlations between EEG intrahemispheric (Fz-Cz) coherence and age, CADES score, or performance in cognitive tests. Spearman correlation coefficients are shown for each frequency bin. Frequencies with significant correlations are indicated with a purple shadow box. The analysis included only the dogs that entered each specific behavioral state (wakefulness $n = 28$, drowsiness $n = 26$, and NREM sleep $n = 24$).

between human studies and our study is that they artificially deprive subjects to evaluate the effect of sleep deprivation, and in our study, reduction in sleep occurs naturally, likely due to aging and neurodegeneration. In addition, interindividual differences in the effects of sleep loss on cognition have been shown; some individuals can be more susceptible to attention impairment while others show working memory reduction (73). In dogs, the specific domains of cognition that are impaired by sleep loss are vastly understudied, with a sole paper suggesting that disturbance of NREM sleep affects dogs' socio-cognitive processing (74), but without reporting on the potential interindividual differences. The more challenging nature of the detour task we used, encompassing the interaction of multiple cognitive domains (18) may make this test more susceptible to the effect of sleep loss than other tests. This task requires cognitive stability (the ability to focus) and cognitive flexibility (the ability to adapt behavioral actions due to a change

TABLE 2 Correlation between Lempel Ziv Complexity at Fz and age and cognitive function.

Variable	By variable	Spearman (ρ)	Raw p-value	q-value
Age	LZC W	0.172	0.391	0.708
Age	LZC Drow.	0.291	0.158	0.398
Age	LZC NREM	0.268	0.215	0.433
Age	LZC REM	0.631	0.015*	0.124
CADES	LZC W	0.141	0.483	0.730
CADES	LZC Drow.	0.286	0.165	0.398
CADES	LZC NREM	0.269	0.215	0.433
CADES	LZC REM	0.438	0.117	0.398
Memory	LZC W	−0.036	0.866	0.950
Memory	LZC Drow.	0.025	0.908	0.954
Memory	LZC NREM	−0.178	0.440	0.708
Memory	LZC REM	−0.813	0.001*	**0.031***
Inhibitory control	LZC W	−0.078	0.698	0.887
Inhibitory control	LZC Drow.	−0.119	0.572	0.812
Inhibitory control	LZC NREM	0.002	0.992	0.998
Inhibitory control	LZC REM	−0.228	0.432	0.708
Detour	LZC W	−0.326	0.104	0.398
Detour	LZC Drow.	−0.343	0.100	0.398
Detour	LZC NREM	−0.342	0.119	0.398
Detour	LZC REM	−0.711	0.006*	0.078
Eye gaze	LZC W	0.049	0.806	0.946
Eye gaze	LZC Drow.	−0.087	0.679	0.887
Eye gaze	LZC NREM	−0.049	0.823	0.946
Eye gaze	LZC REM	−0.398	0.159	0.398

Asterisks indicate significant differences and bold text indicates significant differences after the false discovery rate Benjamini, Krieger, and Yekutieli procedure.
q-values represent the corrected p-values.
W, Wakefulness; Drow, Drowsiness; LZC, Lempel Ziv Complexity.

in demands) (75). We have previously shown that the detour task, together with inhibitory control and sustained gaze, is better able to discriminate between dogs with and without cognitive dysfunction than other tests such as the working memory task (18).

4.1. Power and age

Quantification of the EEG signal using power spectrum, coherence and complexity in different behavioral states revealed associations with age and cognitive function (25). As previously mentioned, power spectrum calculates the relative contribution of the frequency components of a signal, and it is a measure of local synchronization. During drowsiness, power of slow oscillations was lower for older dogs and for dogs with worse performance at the detour task. These oscillations are within the delta range and are characteristic of states of diminished levels of consciousness such as NREM sleep and anesthesia (76, 77). Our findings are consistent with the observation in humans (78), rats (79), cats (80), and dogs (28), that delta power declines with aging during NREM sleep, and this is considered a hallmark of reduction in sleep depth (81–83). In our study, the reduction in delta power with age was observed during both drowsiness and NREM sleep, however it only reached statistical significance for drowsiness. Of note, these associations were only observed in the Fz EEG channel, not in the Cz. In humans, it has been observed that frontal brain areas are more sensitive to aging and to response to sleep loss, and while in young subjects there is a predominance of delta waves in the frontal cortex, this predominance is lost with aging (84).

4.2. Power and memory

We identified associations between power and memory scores but not age during REM sleep, the period in which memory consolidation occurs. By contrast, Kis et al. (28) found an age-associated reduction in delta and an increase in alpha and beta power in dogs. However, they studied dogs from 1 to 8 years of

10.3389/fvets.2023.1151266

TABLE 3 Correlation between Lempel Ziv Complexity at Cz and age and cognitive function.

Variable	By variable	Spearman ρ	Raw p-value	q-value
Age	LZC W	0.281	0.147	0.362
Age	LZC Drow.	0.337	0.092	0.362
Age	LZC NREM	0.304	0.148	0.362
Age	LZC REM	0.625	0.017*	0.362
CADES	LZC W	0.124	0.531	0.704
CADES	LZC Drow.	0.312	0.120	0.362
CADES	LZC NREM	0.275	0.193	0.362
CADES	LZC REM	0.572	0.033*	0.362
Memory	LZC W	−0.136	0.518	0.704
Memory	LZC Drow.	−0.056	0.1794	0.934
Memory	LZC NREM	0.010	0.966	1.000
Memory	LZC REM	−0.583	0.047*	0.362
Inhibitory control	LZC W	0.002	0.992	1.000
Inhibitory control	LZC Drow.	0.048	0.816	0.934
Inhibitory control	LZC NREM	0.055	0.798	0.934
Inhibitory control	LZC REM	−0.385	0.174	0.362
Detour	LZC W	−0.232	0.244	0.362
Detour	LZC Drow.	−0.343	0.093	0.362
Detour	LZC NREM	−0.315	0.143	0.362
Detour	LZC REM	−0.376	0.205	0.362
Eye gaze	LZC W	−0.242	0.214	0.362
Eye gaze	LZC Drow.	−0.244	0.230	0.362
Eye gaze	LZC NREM	−0.257	0.225	0.362
Eye gaze	LZC REM	−0.424	0.131	0.362

Asterisks indicate significant differences before the false discovery rate Benjamini, Krieger, and Yekutieli procedure.
q-values represent the corrected p-values.
W, Wakefulness; Drow, Drowsiness; LZC, Lempel Ziv Complexity.

age, without including senior or geriatric dogs. Performance at the memory task was positively correlated with delta power and negatively correlated with power at higher frequencies (beta and gamma). The biological relevance of this finding should be further evaluated in future studies and include dogs across a wider range of ages together in one study. While REM sleep is usually treated as a homogenous state, two different phases have been recognized, tonic and phasic REM sleep. These phases have different roles in information processing and memory consolidation and differ in the power of different oscillations, with higher power of alpha and beta bands during the tonic phase and higher gamma power on the phasic phase (85–87). Studies in dogs are needed to characterize the electroencephalographic features of each REM phase and whether they are associated with cognition and cognitive function.

4.3. Interhemispheric coherence and age

Coherence analyses are a measure of functional coupling between two distant cortical areas. Higher interhemispheric coherence within theta and alpha frequency bands was found in older dogs during wakefulness. This result was unexpected since in people, coherence in theta and alpha band decreases with aging (88, 89). However, those human studies included a wider range of ages, including young, middle age and senior adults, while in our study, we evaluated only senior and geriatric dogs. Our current findings concur with a previous study we performed in a separate cohort of dogs in which we found that dogs at risk of developing CCDS had higher interhemispheric coherence values than normal dogs or dogs with CCDS (41). It is important to note that those dogs at risk were also older than the normal dogs, and differences between groups could also be age-related.

During REM sleep we observed a positive correlation between gamma interhemispheric coherence and age. In the normal individual, absence of gamma coherence characterizes REM sleep (90, 91), and gamma coherence shows its peak during wakefulness. Therefore, higher gamma coherence during REM sleep could be caused by shallower sleep states and frequent arousals and could suggest an age-related reduction in quality sleep in these dogs.

4.4. Interhemispheric coherence and cognition

There was a positive correlation between interhemispheric coherence and CADES score and a negative correlation with performance at the detour task during wakefulness. This was true for frequencies between 10.5 and 12 Hz, some of the same frequencies for which coherence was positively correlated with age. For these frequencies, the strength of the correlations was higher for age than for CADES or performance at the detour task. We have previously demonstrated that cognitive performance decreases with age (18), and therefore, it is possible that age is the main factor driving these coherence differences.

During wakefulness, delta interhemispheric coherence (1–3 Hz) showed a negative correlation with performance at the sustained gaze task, a test that measures sustained attention. Dogs with shorter attention during the task had higher coherence. This mirrors studies in people with dementia that have shown an increase in delta coherence in comparison with healthy age-matched controls (40, 92). In addition to this, patients with attention deficit-hyperactivity disorders have elevated delta coherence in the frontal area of the brain (93).

In our previous study we found that dogs with higher dementia scores had lower interhemispheric coherence in the gamma frequency band during wakefulness, but those results were not replicated in this study. One of the main reasons could be that in our previous study we used needle electrodes that have a much smaller recording surface than the cup electrodes. In fact, Musteata et al. found that values of interhemispheric coherence in dogs are dependent on the type of electrode used (94). A limitation of coherence analyses is that coherence values can be overestimated due to volume conduction in which the conductive properties of the brain, cerebrospinal fluid, skull, and scalp (and the conductive gel used during the recording), cause the neural source information to diffuse before reaching the electrodes (95, 96). Therefore, coherence measurements with cup electrodes might not be completely reliable in dogs, and future studies should evaluate more advances measures of functional connectivity that are able to overcome the volume conduction problem such as the phase lag index (95, 97).

4.5. Intrahemispheric coherence and cognition

Intrahemispheric coherence showed associations with performance in cognitive tasks but not with age or CADES scores. Higher coherence in different frequency bands during wakefulness, drowsiness and NREM sleep were associated with worse performance at the inhibitory control task. In humans with AD, Sankari et al. found an increase in delta, theta, and alpha intrahemispheric coherence during wakefulness (98). Finally, during REM sleep, dogs with better performance at the detour task showed higher intrahemispheric coherence within the alpha and sigma frequency bands. REM sleep in humans is characterized by the occurrence of alpha bursts with high occipito-frontal connectivity (99). It has been proposed that these alpha bursts may contribute to higher information processing during this state, and to the incorporation of external stimuli to dreams (99, 100).

4.6. Complexity and memory

Lempel Ziv Complexity evaluates the randomness of the EEG signal. We did not find any effect of aging on EEG complexity in any of the behavioral states evaluated. Regarding its association with cognitive status, dogs with better performance in memory tasks had a lower EEG complexity during REM sleep. Similarly, dogs with better performance in the detour task had also lower REM sleep complexity, but this result did not achieve statistical significance after multiple comparisons correction. It has been shown that complexity has its maximum value during wakefulness, is reduced during NREM sleep and increases to intermediate values during REM sleep (101, 102). Therefore, higher levels of complexity during REM in dogs with worse cognitive performance can be an indication of increased arousal, or shallower REM sleep.

Together these EEG findings demonstrate that dogs with cognitive impairment spend less time sleeping and show EEG features of shallower sleep than dogs without cognitive impairment. Additionally, changes in power and coherence of specific frequency bands are associated with age or performance at some cognitive tests in aging dogs.

4.7. Limitations

Polysomnographic recordings in this study were completed during afternoon sleep and thus might not reflect the sleep architecture of dogs at nighttime. While there is evidence that at nighttime, dogs sleep more and spend less time in drowsiness and awake after first drowsiness (103), there are no data showing whether day vs. night sleep patterns are differentially affected by age (which would be a limitation to the present study). Thus, we do not know if and to what extent the present results would change if replicated during night-time recordings. Recordings during nighttime require considerably more effort from both the clinical staff and the participating owners, thus afternoon recordings are much more feasible for veterinary settings. Wireless polysomnographic equipment with bandages, Elizabethan collars and or special EEG caps designed to assure that electrodes stay in place (104) were not available for this study. In addition to this, while it has been demonstrated that dogs usually take naps around noon (5, 53), there is individual variability, and for some dogs the time chosen for recordings might not be their preferred nap time. However, the results observed in this study are in line with the sleep difficulties experienced by dogs with CCDS at night, since, as expected (9, 11), dogs with higher CADES scores spent less time sleeping. Future studies could compare these afternoon polysomnographic recordings with easier evaluations of activity at night time such as activity monitors (53) or sleep questionnaires (105). In addition to this, we have already demonstrated that cognitive function decreases with aging (18), and therefore, it is challenging to determine the relative contribution of age and

cognitive impairment on changes in the sleep-wakefulness cycle and the qEEG features.

5. Conclusion

In conclusion, this study evaluates for the first time the correlation between sleep architecture and cognitive performance in aging dogs using polysomnography, the gold standard technique for sleep evaluation. We have demonstrated that dogs with higher CADES scores spent less time in both NREM and REM sleep. Additionally, when evaluating different domains of cognition, problem solving abilities showed a positive correlation with time spent in NREM and REM sleep and a negative correlation with time awake. Finally, we showed that quantitative electroencephalographic analyses during different behavioral states can demonstrate differences in dogs associated with age or cognitive performance which should be further evaluated for its potential clinical use to monitor the progression of CCDS.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee NCSU. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

AM and NJO were responsible for the design, analysis, and primary writing of the manuscript for this study. AK and MEG participated in the design. AM, CL, and MK participated in the data acquisition. DM and MC participated in the analysis of the data. All authors participated in editing and review of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Kady M. Gjessing and Rahna M. Davidson Distinguished Chair in Gerontology, the Sleep Research Society Foundation Grant #04-SRG-21, and the Company of Biologists Grant #DMMTF2205727. AK was supported by National Research, Development and Innovation Fund of Hungary (FK 128242) and the Hungarian Brain Research Program (HBRP) 3.0 NAP.

Acknowledgments

We are grateful with all the owners and the dogs that participated in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Stickgold R. Sleep-dependent memory consolidation. *Nature.* (2005) 437:1272–8. doi: 10.1038/nature04286

2. Mander BA, Winer JR, Walker MP. Sleep and human aging. *Neuron.* (2017) 94:19–36. doi: 10.1016/j.neuron.2017.02.004

3. Lafortune M, Gagnon JF, Martin N, Latreille V, Dube J, Bouchard M, et al. Sleep spindles and rapid eye movement sleep as predictors of next morning cognitive performance in healthy middle-aged and older participants. *J Sleep Res.* (2014) 23:159–67. doi: 10.1111/jsr.12108

4. Bowersox SS, Baker TL, Dement WC. Sleep-wakefulness patterns in the aged cat. *Electroencephalogr Clin Neurophysiol.* (1984) 58:240–52. doi: 10.1016/0013-4694(84)90110-X

5. Takeuchi T, Harada E. Age-related changes in sleep-wake rhythm in dog. *Behav Brain Res.* (2002) 136:193–9. doi: 10.1016/S0166-4328(02)00123-7

6. Bódizs R, Kis A, Gácsi M, Topál J. Sleep in the dog: Comparative, behavioral and translational relevance. *Curr Opin Behav Sci.* (2020) 33:25–33. doi: 10.1016/j.cobeha.2019.12.006

7. Van Gool WA, Mirmiran M. Age-related changes in the sleep pattern of male adult rats. *Brain Res.* (1983) 279:394–8. doi: 10.1016/0006-8993(83)90217-2

8. Cajochen C, Munch M, Knoblauch V, Blatter K, Wirz-Justice A. Age-related changes in the circadian and homeostatic regulation of human sleep. *Chronobiol Int.* (2006) 23:461–74. doi: 10.1080/07420520500545813

9. Landsberg GM, Deporter T, Araujo JA. Clinical signs and management of anxiety, sleeplessness, and cognitive dysfunction in the senior pet. *Vet Clin North Am Small Anim Pract.* (2011) 41:565–90. doi: 10.1016/j.cvsm.2011.03.017

10. Musiek ES, Holtzman DM. Mechanisms linking circadian clocks, sleep, and neurodegeneration. *Science.* (2016) 354:1004–8. doi: 10.1126/science.aah4968

11. Madari A, Farbakova J, Katina S, Smolek T, Novak P, Weissova T, et al. Assessment of severity and progression of canine cognitive dysfunction syndrome using the canine dementia scale (cades). *Appl Anim Behav Sci.* (2015) 171:138–45. doi: 10.1016/j.applanim.2015.08.034

12. Van Erum J, Van Dam D, De Deyn PP. Sleep and Alzheimer's disease: A pivotal role for the suprachiasmatic nucleus. *Sleep Med Rev.* (2018) 40:17–27. doi: 10.1016/j.smrv.2017.07.005

13. Ju YE, Lucey BP, Holtzman DM. Sleep and Alzheimer's disease pathology—A bidirectional relationship. *Nat Rev Neurol.* (2014) 10:115–9. doi: 10.1038/nrneurol.2013.269

14. Mihevc SP, Majdic G. Canine cognitive dysfunction and Alzheimer's disease—Two facets of the same disease? *Front Neurosci.* (2019) 13:604. doi: 10.3389/fnins.2019.00604

15. Rofina JE, van Ederen AM, Toussaint MJ, Secreve M, van der Spek A, van der Meer I, et al. Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer's disease. *Brain Res.* (2006) 1069:216–26. doi: 10.1016/j.brainres.2005.11.021

16. Schütt T, Helboe L, Pedersen LO, Waldemar G, Berendt M, Pedersen JT. Dogs with cognitive dysfunction as a spontaneous model for early Alzheimer's disease: A translational study of neuropathological and inflammatory markers. *J Alzheimers Dis.* (2016) 52:433–49. doi: 10.3233/JAD-151085

17. Dewey CW, Davies ES, Xie H, Wakshlag JJ. Canine cognitive dysfunction: Pathophysiology, diagnosis, and treatment. *Vet Clin North Am Small Anim Pract.* (2019) 49:477–99. doi: 10.1016/j.cvsm.2019.01.013

18. Fefer G, Panek WK, Khan MZ, Singer M, Westermeyer H, Mowat FM, et al. Use of cognitive testing, questionnaires, and plasma biomarkers to quantify cognitive impairment in an aging pet dog population. *J Alzheimers Dis.* (2022) 2022:215562. doi: 10.3233/JAD-215562

19. Salvin HE, McGreevy PD, Sachdev PS, Valenzuela MJ. The Canine Cognitive Dysfunction Rating Scale (CCDR): A data-driven and ecologically relevant assessment tool. *Vet J.* (2011) 188:331–6. doi: 10.1016/j.tvjl.2010.05.014

20. Denenberg S, Liebel F, Rose J. Behavioural and medical differentials of cognitive decline and dementia in dogs and cats. In: G Landsberg, A Madari, N Zilka, editors, *Canine and Feline Dementia Molecular Basis, Diagnostics and Therapy*. Cham: Springer International Publishing AG (2017). p. 13–58. doi: 10.1007/978-3-319-53219-6_2

21. Kis A, Szakadat S, Gacsi M, Kovacs E, Simor P, Torok C, et al. The interrelated effect of sleep and learning in dogs (canis familiaris); An EEG and behavioural study. *Sci Rep.* (2017) 7:41873. doi: 10.1038/srep41873

22. Boespflug EL, Iliff JJ. The emerging relationship between interstitial fluid-cerebrospinal fluid exchange, amyloid-beta, and sleep. *Biol Psychiatry.* (2018) 83:328–36. doi: 10.1016/j.biopsych.2017.11.031

23. Minakawa EN, Miyazaki K, Maruo K, Yagihara H, Fujita H, Wada K, et al. Chronic sleep fragmentation exacerbates amyloid beta deposition in Alzheimer's disease model mice. *Neurosci Lett.* (2017) 653:362–9. doi: 10.1016/j.neulet.2017.05.054

24. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, et al. Sleep drives metabolite clearance from the adult brain. *Science.* (2013) 342:373–7. doi: 10.1126/science.1241224

25. Ju YS, Ooms SJ, Sutphen C, Macauley SL, Zangrilli MA, Jerome G, et al. Slow wave sleep disruption increases cerebrospinal fluid amyloid-beta levels. *Brain.* (2017) 140:2104–11. doi: 10.1093/brain/awx148

26. Bellows J, Colitz CMH, Daristotle L, Ingram DK, Lepine A, Marks SL, et al. Common physical and functional changes associated with aging in dogs. *JAVMA.* (2015) 246:67–75. doi: 10.2460/javma.246.1.67

27. Torterolo P, González J, Castro-Zaballa S, Cavelli M, Mondino A, Pascovich C, et al. Polysomnography in humans and animal models: Basic procedures and analysis. In: E Murillo-Rodrguez, editor, *Methodological Approaches for Sleep and Vigilance Research*. Cambridge, MA: Academic Press (2022). p. 17–32. doi: 10.1016/B978-0-323-85235-7.00010-7

28. Kis A, Szakadat S, Kovacs E, Gacsi M, Simor P, Gombos F, et al. Development of a non-invasive polysomnography technique for dogs (canis familiaris). *Physiol Behav.* (2014) 130:149–56. doi: 10.1016/j.physbeh.2014.04.004

29. Pentilla M, Partanen J, Soinine H, Riekkinen P. Quantitative analysis of occipital EEG in different stages of Alzheimer's disease. *Electroencephalogr Clin Neurophysiol.* (1985) 60:1–6. doi: 10.1016/0013-4694(85)90942-3

30. van der Hiele K, Vein AA, Reijntjes RHAM, Westendorp RGJ, Bollen ELEM, van Buchem MA, et al. EEG correlates in the spectrum of cognitive decline. *Clin Neurophysiol.* (2007) 118:1931–9. doi: 10.1016/j.clinph.2007.05.070

31. Djonlagic I, Aeschbach D, Harrison SL, Dean D, Yaffe K, Ancoli-Israel S, et al. Associations between quantitative sleep EEG and subsequent cognitive decline in older women. *J Sleep Res.* (2019) 28:e12666. doi: 10.1111/jsr.12666

32. Labate D, Foresta FL, Morabito G, Palamara I, Morabito FC. Entropic measures of EEG complexity in Alzheimer's disease through a multivariate multiscale approach. *IEEE Sens J.* (2013) 13:3284–92. doi: 10.1109/JSEN.2013.2271735

33. Horvath A, Szucs A, Csukly G, Sakovics A, Stefanics G, Kamondi A. EEG and ERP biomarkers of Alzheimer's disease: A critical review. *Front Biosci.* (2018) 23:183–220. doi: 10.2741/4587

34. Buzsaki G, Anastassiou CA, Koch C. The origin of extracellular fields and currents—EEG, ECOG, LFP and Spikes. *Nat Rev Neurosci.* (2012) 13:407–20. doi: 10.1038/nrn3241

35. Mondino A, Torterolo P, Vanini G. *Definitions and Measurements of the States of Vigilance. Methodological Approaches for Sleep and Vigilance Research* London: Academic Press; Elsevier (2022). p. 1–16. doi: 10.1016/B978-0-323-85235-7.00002-8

36. Kubicki S, Herrmann WM, Fitche K, Freund G. Reflections on the topics: EEG frequency bands and regulation of vigilance. *Pharmakopsychiat.* (1979) 12:237–45. doi: 10.1055/s-0028-1094615

37. Moretti D, Babiloni C, Binetti G, Cassetta E, Dal Forno G, Ferreric F, et al. Individual analysis of EEG frequency and band power in mild Alzheimer's disease. *Clin Neurophysiol.* (2004) 115:299–308. doi: 10.1016/S1388-2457(03)00345-6

38. Bullock TH, McClune MC. Lateral coherence of the electrocorticogram a new measure of brain synchrony. *Electroencephalogr Clin Neurophysiol.* (1989) 73:479–98. doi: 10.1016/0013-4694(89)90258-7

39. Pogarell O, Teipel SJ, Juckel G, Gootjes L, Moller T, Burger K, et al. EEG coherence reflects regional corpus callosum area in Alzheimer's disease. *J Neurol Neurosurg Psychiatry.* (2005) 76:109–11. doi: 10.1136/jnnp.2004.036566

40. Comi GC, Fornara C, Locatelli T, Medaglini S, Cursi M, Minicucci F, et al. EEG coherence in Alzheimer and multi-infarct dementia. *Arch Gerontol Geriatr.* (1998) 6:91–8. doi: 10.1016/S0167-4943(98)80016-2

41. Mondino A, Gutierrez M, Gonzalez C, Mateos D, Torterolo P, Olby N, et al. Electroencephalographic signatures of dogs with presumptive diagnosis of canine cognitive dysfunction. *Res Vet Sci.* (2022) 150:36–43. doi: 10.1016/j.rvsc.2022.06.024

42. Stam CJ. Nonlinear dynamical analysis of EEG and MEG: Review of an emerging field. *Clin Neurophysiol.* (2005) 116:2266–301. doi: 10.1016/j.clinph.2005.06.011

43. Lempel A, Ziv J. On the complexity of finite sequences. *EEE Trans Inform Theory.* (1976) 22:75–81. doi: 10.1109/TIT.1976.1055501

44. Schartner MM, Carhart-Harris RL, Barrett A B, Seth AK, Muthukumaraswamy SD. Increased spontaneous MEG signal diversity for psychoactive doses of ketamine, LSD and psilocybin. *Sci Rep.* (2017) 7:1–12. doi: 10.1038/srep46421

45. Simons S, Abásolo D. Distance-based lempel–Ziv complexity for the analysis of electroencephalograms in patients with Alzheimer's disease. *Entropy.* (2017) 19:e19030129. doi: 10.3390/e19030129

46. Hudetz AG, Liu X, Pillay S, Boly M, Tononi G. Propofol anesthesia reduces lempel-Ziv complexity of spontaneous brain activity in rats. *Neurosci Lett.* (2016) 628:132–5. doi: 10.1016/j.neulet.2016.06.017

47. Mondino A, González J, Li D, Mateos D, Osorio L, Cavelli M, et al. Urethane anesthesia exhibits neurophysiological correlates of unconsciousness and is distinct from sleep. *BioRxiv.* (2021) 2021:1–43. doi: 10.1101/2021.09.21.461281

48. Panek WK, Murdoch DM, Gruen ME, Mowat FM, Marek RD, Olby NJ. Plasma amyloid beta concentrations in aged and cognitively impaired pet dogs. *Mol Neurobiol.* (2021) 58:483–9. doi: 10.1007/s12035-020-02140-9

49. Panek WK, Gruen ME, Murdoch DM, Marek RD, Stachel AF, Mowat FM, et al. Plasma neurofilament light chain as a translational biomarker of aging and neurodegeneration in dogs. *Mol Neurobiol.* (2020) 57:3143–9. doi: 10.1007/s12035-020-01951-0

50. Greer KA, Canterberry SC, Murphy KE. Statistical analysis regarding the effects of height and weight on life span of the domestic dog. *Res Vet Sci.* (2007) 82:208–14. doi: 10.1016/j.rvsc.2006.06.005

51. Reicher V, Kis A, Simor P, Bodizs R, Gombos F, Gacsi M. Repeated afternoon sleep recordings indicate first-night-effect-like adaptation process in family dogs. *J Sleep Res.* (2020) 29:e12998. doi: 10.1111/jsr.12998

52. Tapp PD, Siwak CT, Gao FQ, Chiou JY, Black SE, Head E, et al. Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging. *J Neurosci.* (2004) 24:8205–13. doi: 10.1523/JNEUROSCI.1339-04.2004

53. Woods HJ, Li MF, Patel UA, Lascelles BDX, Samson DR, Gruen ME. A functional linear modeling approach to sleep-wake cycles in dogs. *Sci Rep.* (2020) 10:22233. doi: 10.1038/s41598-020-79274-2

54. Mondino A, Khan M, Case B, Giovagnoli S, Thomson A, Lascelles BDX, et al. Activity patterns are associated with fractional lifespan, memory, and gait speed in aged dogs. *Sci Rep.* (2023) 13:2588. doi: 10.1038/s41598-023-29181-z

55. Vallat R. *Antropy: Entropy and Complexity of (Eeg) Time-Series in Python. GitHub Repository.* (2022). Available online at: https://github.com/raphaelvallat/antropy

56. Hoel JA, Templeton GB, Fefer G, Case BC, Shah A, Gruen ME, et al. Sustained gaze is a reliable in-home test of attention for aging pet dogs. *Front Vet Sci.* (2021) 8:19135. doi: 10.3389/fvets.2021.819135

57. Reicher V, Kis A, Simor P, Bodizs R, Gacsi M. Interhemispheric asymmetry during NREM sleep in the dog. *Sci Rep.* (2021) 11:18817. doi: 10.1038/s41598-021-98178-3

58. Benjamini Y, Krieger A, Yekutieli D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika.* (2006) 93:491–507. doi: 10.1093/biomet/93.3.491

59. Abt K. Descriptive data analysis: A concept between confirmatory and exploratory data analysis. *Methods Inf Med.* (1987) 26:77–88. doi: 10.1055/s-0038-1635488

60. Moran M, Lynch CA, Walsh C, Coen R, Coakley D, Lawlor BA. Sleep disturbance in mild to moderate Alzheimer's disease. *Sleep Med.* (2005) 6:347–52. doi: 10.1016/j.sleep.2004.12.005

61. Blackwell T, Yaffe K, Laffan A, Ancoli-Israel S, Redline S, Ensrud KE, et al. Associations of objectively and subjectively measured sleep quality with subsequent cognitive decline in older community-dwelling men: The MROS sleep study. *Sleep.* (2014) 37:655–63. doi: 10.5665/sleep.3562

62. Spira AP, Gamaldo AA, An Y, Wu MN, Simonsick EM, Bilgel M, et al. Self-reported sleep and beta-amyloid deposition in community-dwelling older adults. *J Am Med Assoc Neurol.* (2013) 70:1537–43. doi: 10.1001/jamaneurol.2013.4258

63. Brown BM, Rainey-Smith SR, Villemagne VL, Weinborn M, Bucks RS, Sohrabi HR, et al. The relationship between sleep quality and brain amyloid burden. *Sleep.* (2016) 39:1063–8. doi: 10.5665/sleep.5756

64. Duncan MJ, Smith JT, Franklin KM, Beckett TL, Murphy MP, St Clair DK, et al. Effects of aging and genotype on circadian rhythms, sleep, and clock gene expression in appxps1 knock-in mice, a model for Alzheimer's disease. *Exp Neurol.* (2012) 236:249–58. doi: 10.1016/j.expneurol.2012.05.011

65. Sprecher KE, Bendlin BB, Racine AM, Okonkwo OC, Christian BT, Koscik RL, et al. Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. *Neurobiol Aging.* (2015) 36:2568–76. doi: 10.1016/j.neurobiolaging.2015.05.004

66. Spira AP, Yager C, Brandt J, Smith GS, Zhou Y, Mathur A, et al. Objectively measured sleep and beta-amyloid burden in older adults: A pilot study. *SAGE Open Med.* (2014) 2:2050312114546520. doi: 10.1177/2050312114546520

67. van den Berg NH, Pozzobon A, Fang Z, Al-Kuwatli J, Toor B, Ray LB, et al. Sleep enhances consolidation of memory traces for complex problem-solving skills. *Cereb Cortex.* (2022) 32:653–67. doi: 10.1093/cercor/bhab216

68. Sio UN, Monaghan P, Ormerod T. Sleep on it, but only if it is difficult: Effects of sleep on problem solving. *Mem Cognit.* (2013) 41:159–66. doi: 10.3758/s13421-012-0256-7

69. Iotchev IB, Szabo D, Kis A, Kubinyi E. Possible association between spindle frequency and reversal-learning in aged family dogs. *Sci Rep.* (2020) 10:6505. doi: 10.1038/s41598-020-63573-9

70. Satterfield BC, Killgore WDS. Sleep loss, executive function, and decision-making. *Sleep Health.* (2019) 5:339–58. doi: 10.1016/B978-0-12-815373-4.00026-5

71. Tucker MA, Whitney P, Belenky G, Hinson J, Van Dongen HPA. Effects of sleep deprivation on dissociated components of executive functioning. *Sleep.* (2010) 33:47–57. doi: 10.1093/sleep/33.1.47

72. Killgore WD. Effects of sleep deprivation on cognition. *Prog Brain Res.* (2010) 185:105–29. doi: 10.1016/B978-0-444-53702-7.00007-5

73. Van Dongen HPA, Baynard M, Maislin G, Dinges DF. Systematic interindividual differences in neurobehavioral impairment from sleep loss: Evidence of trait-like differential vulnerability. *Sleep.* (2004) 27:423–33. doi: 10.1093/SLEEP/27.3.423

74. Bollo H, Kovacs K, Lefter R, Gombos F, Kubinyi E, Topal J, et al. REM vs. non-rem sleep disturbance specifically affects inter-specific emotion processing in family dogs (canis familiaris). *Sci Rep.* (2020) 10:10492. doi: 10.1038/s41598-020-67092-5

75. Diamond A. Executive functions. *Annu Rev Psychol.* (2013) 64:135–68. doi: 10.1146/annurev-psych-113011-143750

76. Frohlich J, Toker D, Monti MM. Consciousness among delta waves: A paradox? *Brain.* (2021) 144:2257–77. doi: 10.1093/brain/awab095

77. Mashour GA, Hudetz AG. Neural correlates of unconsciousness in large-scale brain networks. *Trends Neurosci.* (2018) 41:150–60. doi: 10.1016/j.tins.2018.01.003

78. Dijk D, Beersma DGM, Van Den Hoofdakker RH. All night spectral analysis of EEG sleep in young adult and middle-aged male subjects. *Neurobiol Aging.* (1989) 10:677–82. doi: 10.1016/0197-4580(89)90004-3

79. Mendelson WB, Bergmann B. EEG delta power during sleep in young and old rats. *Neurobiol Aging.* (1999) 20:669–73. doi: 10.1016/S0197-4580(99)00062-7

80. Bowersox SS, Floyd T, Dement WC. Electroencephalogram during sleep in the cat: Age effects on slow-wave activity. *Sleep.* (1984) 7:380–5. doi: 10.1093/sleep/7.4.380

81. Gulia KK, Kumar VM. Sleep disorders in the elderly: A growing challenge. *Psychogeriatrics.* (2018) 18:155–65. doi: 10.1111/psyg.12319

82. Werth E, Dijk DJ, Acherman P, Borbely A. Dynamics of the sleep EEG after an early evening NAP: Experimental data and simulations. *Am J Physiol Regulat Integr Comparat Physiol.* (1996) 271:501–10. doi: 10.1152/ajpregu.1996.271.3.R501

83. Younes M, Schweitzer PK, Griffin KS, Balshaw R, Walsh JK. Comparing two measures of sleep depth/intensity. *Sleep.* (2020) 43:zsaa127. doi: 10.1093/sleep/zsaa127

84. Munch M, Knoblauch V, Blatter K, Schroder C, Schnitzler C, Krauchi K, et al. The frontal predominance in human EEG delta activity after sleep loss decreases with age. *Eur J Neurosci.* (2004) 20:1402–10. doi: 10.1111/j.1460-9568.2004.03580.x

85. Brankack J, Scheffzuk C, Kukushka VI, Vyssotski AL, Tort AB, Draguhn A. Distinct features of fast oscillations in phasic and tonic rapid eye movement sleep. *J Sleep Res.* (2012) 21:630–3. doi: 10.1111/j.1365-2869.2012.01037.x

86. Simor P, van der Wijk G, Nobili L, Peigneux P. The microstructure of REM sleep: Why phasic and tonic? *Sleep Med Rev.* (2020) 52:101305. doi: 10.1016/j.smrv.2020.101305

87. Simor P, van Der Wijk G, Gombos F, Kovacs I. The paradox of rapid eye movement sleep in the light of oscillatory activity and cortical synchronization during phasic and tonic microstates. *Neuroimage.* (2019) 202:116066. doi: 10.1016/j.neuroimage.2019.116066

88. Vysata O, Kukal J, Prochazka A, Pazdera L, Simko J, Valis M. Age-related changes in EEG coherence. *Neurol Neurochir Pol.* (2014) 48:35–8. doi: 10.1016/j.pjnns.2013.09.001

89. Smit DJ, Boersma M, Schnack HG, Micheloyannis S, Boomsma DI, Hulshoff Pol HE, et al. The brain matures with stronger functional connectivity and decreased randomness of its network. *PLoS ONE.* (2012) 7:e36896. doi: 10.1371/journal.pone.0036896

90. Cavelli M, Castro-Zaballa S, Mondino A, Gonzalez J, Falconi A, Torterolo P. Absence of EEG gamma coherence in a local activated cortical state: A conserved trait of REM sleep. *Translat Brain Rhythmicity.* (2017) 2:1000115. doi: 10.15761/TBR.1000115

91. Cantero JL, Atienza M. The role of neural synchronization in the emergence of cognition across the wake-sleep cycle. *Rev Neurosci.* (2005) 16:69–83. doi: 10.1515/REVNEURO.2005.16.1.69

92. Brunovsky M, Matousek M, Edman A, Cervena K, Krajca V. Objective assessment of the degree of dementia by means of EEG. *Neuropsychobiology.* (2003) 48:19–26. doi: 10.1159/000071824

93. Barry RJ, Clarke A, McCarthy R, Selikowitz M. EEG coherence in attention-deficit/hyperactivity disorder: A comparative study of two DSM-IV types. *Clin Neurophysiol.* (2002) 113:579–85. doi: 10.1016/S1388-2457(02)00036-6

94. Musteata M, Borcea DG, Stefanescu R, Solcan G, Lacatus R. Influence of stainless needle electrodes and silver disk electrodes over the interhemispheric cerebral coherence value in vigil dogs. *Sensors.* (2018) 18:113990. doi: 10.3390/s18113990

95. Peraza LR, Asghar AU, Green G, Halliday DM. Volume conduction effects in brain network inference from electroencephalographic recordings using phase lag index. *J Neurosci Methods.* (2012) 207:189–99. doi: 10.1016/j.jneumeth.2012.04.007

96. Nunez P, Srinivasan R, Westdorp AF, Wijesinghe RS, Tucker DM, Silberstein RB, et al. EEG Coherency I: Statistics, reference electrode, volume conduction, laplacians, cortical imaging, and interpretation at multiple scales electroencephalography and clinical. *Neurophysiology.* (1997) 103:499–515. doi: 10.1016/S0013-4694(97)00066-7

97. Stam CJ, Nolte G, Daffertshofer A. Phase lag index: Assessment of functional connectivity from multi channel EEG and MEG with diminished bias from common sources. *Hum Brain Mapp.* (2007) 28:1178–93. doi: 10.1002/hbm.20346

98. Sankari Z, Adeli H, Adeli A. Intrahemispheric, interhemispheric, and distal EEG coherence in Alzheimer's disease. *Clin Neurophysiol.* (2011) 122:897–906. doi: 10.1016/j.clinph.2010.09.008

99. Cantero JL, Atienza M, Salas R. Human alpha oscillations in wakefulness, drowsiness period, and REM sleep: Different electroencephalographic phenomena within the alpha band. *Neurophysiol Clin.* (2002) 32:54–71. doi: 10.1016/S0987-7053(01)00289-1

100. Burton SA, Harsh JR, Badia P. Cognitive activity in sleep and responsiveness to external stimuli. *Sleep.* (1988) 11:61–8.

101. Abasolo D, Hornero R, Espino P, Escudero J, Gomez C. Electroencephalogram background activity characterization with approximate entropy and auto mutual information in Alzheimer's disease patients. In: *Proceedings of the 29th Annual International Conference of the IEEE EMBS.* Lyon (2007). p. 6191–4. doi: 10.1109/IEMBS.2007.4353769

102. Mateos DM, Gómez-Ramírez J, Rosso OA. Using time causal quantifiers to characterize sleep stages. *Chaos Solitons Fractals.* (2021) 146:110798. doi: 10.1016/j.chaos.2021.110798

103. Bunford N, Reicher V, Kis A, Pogany A, Gombos F, Bodizs R, et al. Differences in pre-sleep activity and sleep location are associated with variability in daytime/nighttime sleep electrophysiology in the domestic dog. *Sci Rep.* (2018) 8:7109. doi: 10.1038/s41598-018-25546-x

104. James FMK, Cortez MA, Monteith G, Jokinen TS, Sanders S, Wielaender F, et al. Diagnostic utility of wireless video-electroencephalography in unsedated dogs. *J Vet Intern Med.* (2017) 31:1469–76. doi: 10.1111/jvim.14789

105. Knazovicky D, Tomas A, Motsinger-Reif A, Lascelles BD. Initial evaluation of nighttime restlessness in a naturally occurring canine model of osteoarthritis pain. *PeerJ.* (2015) 3:1–13. doi: 10.7717/peerj.772

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 29 June 2023
DOI 10.3389/fvets.2023.1150617

OPEN ACCESS

EDITED BY
Daisuke Hasegawa,
Nippon Veterinary and Life Science University, Japan

REVIEWED BY
Colette Williams,
University of California, Davis, United States
Alejandra Mondino,
North Carolina State University, United States
Fiona May Keir James,
University of Guelph, Canada

*CORRESPONDENCE
Fernando Pellegrino
✉ fernando.pellegrino2@gmail.com

RECEIVED 24 January 2023
ACCEPTED 30 May 2023
PUBLISHED 29 June 2023

CITATION
Pellegrino F and Gómez Álvarez CM (2023) Electroencephalographic features of the developing brain in 72 dogs under xylazine sedation: a visual and statistical analysis. *Front. Vet. Sci.* 10:1150617. doi: 10.3389/fvets.2023.1150617

Electroencephalographic features of the developing brain in 72 dogs under xylazine sedation: a visual and statistical analysis

Fernando Pellegrino[1]* and Christian M. Gómez Álvarez[2]

[1]Anatomy Department, Facultad de Veterinaria, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Neurophysiology and Clinical Neurology Service at VetCam Specialists, Valencia, Spain

Electroencephalogram (EEG) is a neurophysiological test, which is widely used in human medicine for epilepsy diagnosis and other neurological disorders. For an adequate interpretation, it is necessary to know the electroencephalogram features for different stages of development. Despite the growing interest in its implementation in veterinary medicine, standardized descriptions of the EEG features of the different stages of brain development in dogs are restricted to studies with limited number of dogs and limited age groups. In this research, the electroencephalographic recording of 72 dogs of different breeds and ages was carried out under xylazine sedation to determine tracing characteristics by visual analysis and through statistical analysis of power spectrum. To establish the EEG features of recordings, 3 essential aspects were selected: (a) the presence or absence of slow waves of 4 to 6–7 Hz; (b) the comparison of the electrical activity recorded in the temporal and dorsal cortex channels; and (c) the visual increase of the alpha activity. Visual analysis on both reference and bipolar montage was performed by the authors and additionally blindly corroborated by two human neurophysiologists. The results allowed us to differentiate 5 age groups: 0–5, 6–11, 12–17, 18–23, and >24 months. Statistical analysis of the power spectrum was performed by analysis of variance (ANOVA) with a completely randomized design (CRD) under factorial arrangement by observing the effect of ages, channels and electroencephalographic rhythms on relative power. The results obtained matched those observed in the visual analysis. According to our results, the characteristics of the EEG corresponding to the adult animal begin to appear at 12 months of age but stabilize after 24 months of age. In this case, the evident differences in the processes of development and maturation of the neopallium and the rhinencephalon play a determining role. Our results differ from those obtained by other authors, probably due to the addition of a deep electrode that facilitates the recording of temporal cortical activity and its deeper rhinencephalic connections.

KEYWORDS

electroencephalogram, developing brain, brain maturation and development, normal electroencephalogram, rhythm, amplitude-frequency characteristics

1. Introduction

Encephalographic recordings in normal adult dogs depending on the state of arousal includes, mainly, 6 to 12-Hz waves (theta and alpha activity type), with a predominant alpha rhythm. This activity may be recognized all over the brain cortex, but mostly, in parietal and occipital regions of mesocephalic and dolichocephalic dogs, and parietal and frontal regions of brachycephalic dogs. The recording amplitude varies among animals, probably depending on the distance between electrodes and the layers of active dipoles, which are proportional to skull's thickness and, therefore, to the distance between the active zone and the recording electrode, but always lower at the frontopolar and temporal areas in all breeds (1).

There is considerable disparity of opinion regarding age-related variations in electroencephalograms (EEG) in dogs, particularly concerning the maturation period. Charles and Fuller reported that the EEG of an 8-week-old puppy is similar to that of an adult dog (2). Petersen et al. (3) described that at 4 weeks of age, the EEG shows a maturity pattern that evolves until remaining constant after 7 weeks of age. Although Fox does not establish in detail the maturation period of the electroencephalographic recording, he states that there exists a significant amplitude decrease between 12 and 16 weeks of age, while the frequency progressively increases until stabilizing at adult age (4–6). Results of inter- and intra-hemispheric coherence studies, according to which all the neocortex takes a similar phase interaction between 6 and 14 weeks of age, seem to support the prior statements (7, 8). Redding claims that EEG maturation develops between 22 and 30 weeks of age, depending on gender and breed (9). Pampiglione's research about brain electrical activity in young dogs indicates that from the 6th month, the recording acquires the features that persist in the adult animal (10). Senba et al. define the immaturity period as the one during which new waveforms and rhythms are observed. According to that criterion, they suggest that the maturation period may take place between 5 and 6 weeks of age, a time when the stabilization of dendritic arborization and an increase of the cortex metabolic rate ensue. However, in the same study, and based on waves amplitude and frequency, they believe maturation may also take place between 20 and 30 weeks of age. Such statements arise from evaluations performed on Beagle dogs, in litters that were followed up from birth to 50 weeks of age (11).

Discrepancies are probably the result of the different criteria when defining the changes found on EEG, during the transition from puppy to adult age. However, from a clinical point of view, EEG maturity has been established in dogs at the age of 6 months, with a predominance of slow waves in the previous stage (12, 13). After the age of 1 year, no research has shown differences in the electroencephalographic tracing between puppies and young or adult dogs, although it has been stated that a decrease in amplitude can be observed in old dogs.

This study's objective was to describe the normal background activity and superimposed transients in dogs of different ages using 10 dorsal subcutaneous electrodes and two deep lateral electrodes that allowed recording the activity of temporal cortex under xylazine sedation. A visual analysis of recordings was achieved by four different researchers and statistical analysis of the relative potency was also done.

Abbreviations: EEG, electroencephalogram; Hz, Hertz; μV, Microvolts.

2. Materials and methods

This is an observational and prospective study. Therefore, live animals were used in this study, and ethical approval was granted from Comisión Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL) and Facultad de Ciencias Veterinarias-Universidad de Buenos Aires. All owners of the animals included in this study, received informed consent, and had approved that their dogs and data would be used for academic research.

2.1. Population

Seventy two healthy dogs with unremarkable neurological and physical exam were included. Neither previous infectious nor neurological diseases were reported. Dogs were included regardless of gender or breed and was based on previous studies indicating that the frequency and electrical characteristics of brain waves are comparable in any breed, although their amplitude is not (9, 14). Dogs with any neurological symptom, any physical exam or blood workout abnormalities were excluded. Hypothetical differences of EEG maturation among dog breeds were not considered, as their structural development is similar; should there exist a difference, it would not depend on the breed, but rather on the training and learning processes that shape intraneuronal synaptic connections (15, 16). To obtain final sample of dogs (72 dogs) we recruited 120 patient who visit our clinic, met the inclusion criteria, and could be distributed among the age groups. Then, 72 dogs (12 for each age group) were selected aleatory through a random number generator from the 120 original dogs recruited. Those patients received in exchange for allow us to do the EEG some compensation as medical attention or vaccines, and tutors signed up an informed consent. The number of dogs was appropriate to ensure a significance level of 5% and a statistical power of at least 80%.

2.2. Electrodes

Both for exploration and reference, 15-mm length stainless steel needle electrodes were used, (Akonic S.A., subdermal needle electrodes) (17). Electromyographic (EMG) monopolar needles (Akonic, S.A.) coated with Teflon, except for their tips, were used to record temporal region activity (T3 and T4). The length of the bare tip was 4 mm; the full length of the needle was 350 mm. This type of electrodes made it possible to go through the muscle and reach the bone, thus avoiding interferences caused by muscle activity. Impedance was measured prior to obtaining the EEGs, always resulting in about 5 kΩ. Cross-impedance or artifacts in recordings that combined subdermal needle electrodes with EMG electrodes (bipolar montage) were not found (1, 18).

2.3. Visual analysis

Visual analysis was performed by the authors and by two human neurophysiologists who received the recording without signalment of dogs to ensure their participation as blind reviewers. The background activity was described (rhythms of similar shape and duration, with regular and recurrent appearance) according to reviewers consensus. In addition, we describe the transient activity present in the recordings (4–6 Hz slow waves, vertex sharp waves, 3 Hz slow waves and K complexes). The criteria to determine the electroencephalographic features of transient activity in the different age groups were: (1) Grapholements were present in at least 75% ($n = 9$) of dogs in each group. (2) Graphoelements repeated with a frequency of at least once per minute, (3) All reviewers agreed on the visual findings.

2.4. Equipment

In order to collect the electroencephalographic recordings, a program specifically designed for computerized electroencephalography and brain mapping reconstruction, with 12 simultaneous recording channels (AKONIC BIO-PC adjusted version 7.0) was used. Technical recording parameters were as follows: common mode rejection >100 db; frequency response of 0.5 to 128 Hz; antialiasing filter: 64 Hz; notch filter for 50 Hz activity was set; high frequency filter (HFF): 70 Hz; low frequency filter (LFF): 0.5 Hz; internal noise < 1 uV; sampling frequency: 256 s; paper speed: 30 mm/s; electrodes impedance: <5 kΩ.

2.5. Animal restraint

Animal restraint was achieved by subcutaneously injecting 0.5 mg/kg of xylazine (Rompun, Bayer Argentina S.A.), based on previous studies (19) and our own experience (1, 18).

2.6. Procedure

2.6.1. Recording

Basic recording was done using reference montage. Active electrodes were placed at the left and right frontopolar (Fp1, and Fp2), frontal (F3, F4), parietal (P3, P4), occipital cortex (O1, O2) and temporal deep electrodes over the sphenoid complex in the ventral aspect of the lateral surface of the cranium with a reference electrode over the rhinal nasal bone. Cz, central electrode (vertex) and Oz, central occipital electrode were placed (see Figure 1). Recording was performed for 30 min (1, 18).

2.7. EEG quantification (mathematical analysis)

2.7.1. Sample editing

For analysis purposes, stationary segments of a 2-s length (epochs) were selected, free from artifacts and transient events,

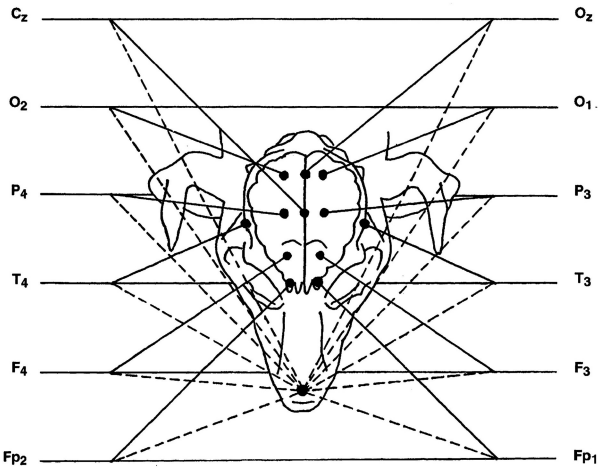


FIGURE 1
Diagram of reference montage, with nasal reference electrode. In the diagram, each channel has two inputs, represented by a solid line and a dashed line. The solid line is the input coming from the active electrode, while the dashed line is the input corresponding to the reference. Cz, Central electrode; O, occipital electrode; P, parietal electrode; T, temporal electrode; F, frontal electrode; Fp, frontopolar electrode; Oz, central occipital electrode. Even and odd numbers correspond to the right and left side, respectively.

averaging results of each epoch. The minimum acceptable time for averaging, from the EEG stationary standpoint, was 30 s (20), therefore, at least 15 epochs were selected for each dog.

2.7.2. Spectral estimate

Fast Fourier transform (FFT) was applied to selected segments. In this way, the usual EEG image is converted into a graph (power spectrum), in which frequency components are organized on the abscissae with their pertaining power on the ordinates; the area below the curve is the absolute power, which is equal to the amplitude squared. In this graph, the dominant frequency components for each channel can be visually defined (20). Frequency components were pooled into classic clinical waves: delta (0.5 to 3.5 Hz), theta (4 to 7.5 Hz), alpha (8 to 13.5 Hz) and beta (from 14 Hz on). Whilst the power spectrum contains all frequency information within a signal period, it is difficult to characterize it in order to make comparisons. As to achieve this goal, a set of parameters or variables was defined, that is to say, numerical values that may be shown in the form of tables or distributed topographically on the spot where the signal was obtained for each channel (numerical maps). In order to characterize the power spectrum, the absolute power (total spectrum area detailed by frequency bands) was taken as a parameter, and from it, the relative power per channel was calculated (frequency components percentage for each channel). The relative power per channel was selected as a parameter since it allows independence from the variability in amplitude caused by morphometric characteristics of each dog (i.e.: skull size, bone thickness, and muscle layer) (9, 14) and by extra-cerebral factors that affect physical electrode features (17). Band relative power

for each channel was calculated by dividing the absolute power of each frequency band for the spectrum total area (20). For the amount of data available to us, relative power showed abnormal behavior (Shapiro-Wilk test $p < 0.05$). Therefore, it was necessary to correct it by applying a Z transformation to normality using a software to scale all measurements with normal distribution, in units proportional to probability in a range from 0 to 1. This allows calculate probability of data observed regardless of initial measurements physical dimensions. Under the same test conditions, this transformed variable exhibited a normal behavior, so statistical analysis was performed based on it (21).

2.8. Statistical analysis of the power spectrum

The impact of age, channels and electroencephalographic rhythms on relative power was analyzed, contrasting their averages. It was determined whether there existed an interaction among these factors and, if so, which ones were involved.

The statistical analysis of the relative power behavior was divided into two stages. During the first stage, due to the lack of previous knowledge on the features of the variables being studied, we evaluated its behavior within a pilot sample in order to estimate the definite sample size that would ensure a sufficient power when reading the tests. In order to broadly define and evaluate the behavior of relative power, we considered that apart from the different ages of individuals, the variability introduced by channels involved and by various electroencephalographic rhythms needed to be isolated. Data was subjected to an analysis of variance (ANOVA) with a completely randomized design (CRD) under factorial arrangement. This arrangement allowed us to compare the effect introduced by the different channels, ages and rhythms, testing their interactions simultaneously. Ages were grouped in four levels (from 0 to 5 months, from 6 to 11 months, from 12 to 23 months, and more than 24 months), each rhythm represented a level (alpha, beta, delta and theta) and each channel represented a level, resulting in a total of 12. The model consisted of a three-level 4 x 4 x 12 factorial arrangement (8). Since the repetitions of observations were made upon the same individuals within each age group, there was a loss of independence between the repeated observations for channels and rhythms. Hence, factorial arrangement had to be corrected into "split-plots" (22, 23), making it possible to correct the lack of independence of rhythm and channel factors. From results obtained in the first stage, it was possible to develop the experimental design for subsequent tests.

In the second stage, due to an interaction among factors (there was no triple interaction), the ANOVA with CRD was respected with an age x rhythm x channel factorial arrangement. Given its minor contribution as a source of variability, beta rhythm was excluded from the analysis. Age was subdivided in comparable space-groups (0 to 5, 6 to 11, 12 to 17, and 18 to 23 months). This new division was adopted considering that, in view of the results of the pilot test and previous biological observations, variability introduced by age should occur during the first development stages. The different channels were rearranged in two levels: one of them, represented the temporal channels; the other one, the dorsal cortex portion channels. The factorial arrangement was then set up as follows: age (four levels), waves (three levels) and channels (two levels). Results were tested according to ANOVA in CRD with a $4 \times 3 \times 2$ factorial arrangement.

Remaining ages were analyzed separately, with the purpose of determining what the changes in power spectrum of adult and elderly animals were. Two groups were formed (24 to 83 months of age and more than 84 months of age). Factorial arrangement for this age group was set up as follows: age (two levels), rhythms (three levels) and channels (two levels). Results were tested according to ANOVA in CRD with a $2 \times 3 \times 2$ factorial arrangement (23). In all cases, a significance level was set at a $= 0.05$.

Finally, all individual values corresponding to absolute power were considered and the average and standard deviation of the sample were calculated for each of the recording channels, in order to elaborate the descriptive statistics of the power spectrum. This procedure was performed for each preselected age group. Amplitude and relative power per channel were calculated from this data (square root of absolute power), obtaining the average and standard deviation as well.

3. Results

3.1. Population

Dogs were divided into six groups of 12 individuals, according to their age: (a) group 1, up to 5 months of age; four German shepherd, three mixed breeds, two French poodles, one Boxer, one Dalmatian, one Yorkshire terrier, eight females, and four males, between 1.5 to 12 Kg of body weight (BW), (b) group 2, from 6 to 11 months of age; three mixed breed, two French poodles, one Great dane, one Cocker spaniel, one Spanish Breton, one Basset hound, one German Shepherd, one Bichon frisse, and one American Pitbull. Seven females and five males, from 4.2 to 31.3 kg of BW; (c) group 3, from 12 to 17 months of age; four mixed breeds, three Cocker spaniels, one Yorkshire terrier, one French poodle, one Beagle, one Airdale terrier and one German shepherd. Seven females and five males between 3.4 and 28.9 Kg of BW; (d) group 4, from 18 to 23 months of age; three mixed breeds, two French poodle, two Doberman pinscher, one Giant schnauzer, one Cocker spaniel, one Yorkshire terrier, one Beagle, one Dogo argentino. Five females and seven males between 3.5 and 38.6 kg of BW (e) group 5, from 24 to 83 months of age; eight mixed breeds, two Siberian Husky, one Dalmatian, one Pointer. Six females and six males between 22.3 kg and 36.8 Kg of BW and (f) group 6, more than 83 months of age, nine mixed breeds, one Shih-tzu, one Boxer, and one French poodle. Eight females and four males between 4.6 and 32.6 Kg of BW. Dogs of groups 1, 2, 3, and 4 had a clinical follow-up for 1 year, with regular physical and neurological examinations every 4 months, without relevant findings suggesting a neurological disease (24).

Dogs from group 1, 2, 3, and 4 included dogs whose central nervous system (CNS) was still in the process of maturing, and the entire brain cortex was also expected to display a similar type of electrical activity. This means that as the dog

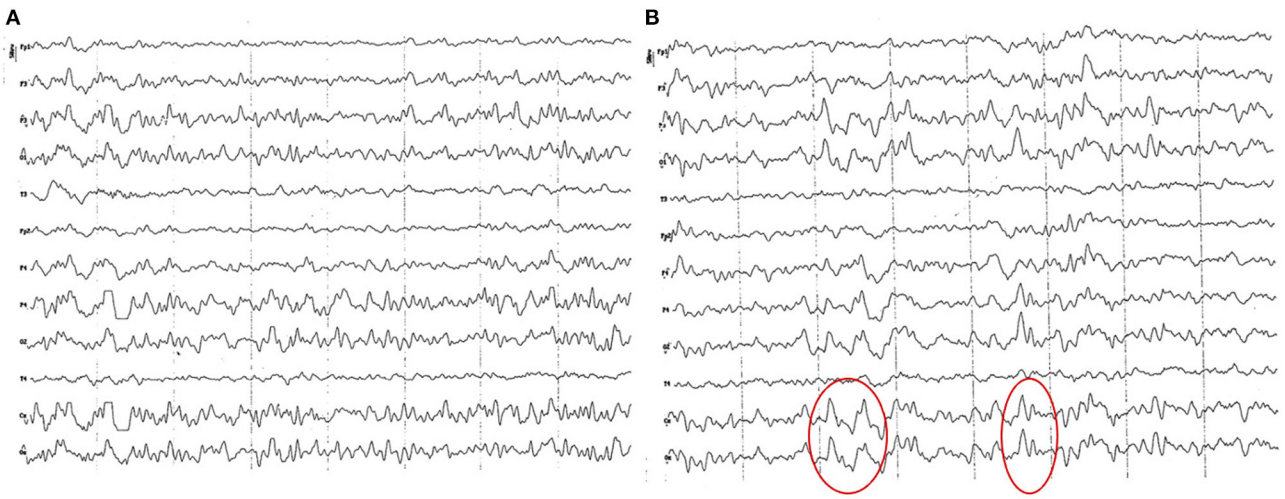


FIGURE 2
EEG (reference montage) corresponding to a female German Shepherd of 1 ½ months of age. Each vertical division represents 1 second and the small vertical bar at the upper left corner represents 50 μV. **(A)** Predominance of slow waves can be observed, showing a very weak outline of superimposed alpha wave, of higher amplitude in the occipito-parietal channels. Temporal (T) and frontopolar (Fp) channels show a very low voltage signal, with a predominant slow activity of 3 to 4 Hz. **(B)** Sharp high voltage waves of the vertex are clearly observed (red circles).

growth electrodes activity recorded seems similar between them regarding frequencies and amplitude. This change is evident particularly at the temporal leads. Dogs from group 5 and 6 included dogs whose CNS was already matured, and the electroencephalogram recording looked stable (9, 11). There is no detailed investigation as to what happens in the EEG after that age, hence the age in the rest of the groups was established arbitrarily.

3.2. Morphology of background activity (visual analysis)

3.2.1. Group 1 (up to 5 months of age)

Background electrical activity shows a mild diffuse disorganization (frequency mixing), with the theta band as the dominant frequency, with an incipient superimposed alpha activity and paroxysmal slow waves (Figure 2A). Higher amplitude was found in the parieto-occipital region. Temporal (T) and frontopolar (Fp) channels feature a low voltage signal, with predominance of slow waves (3–4 Hz), although with a superimposed fast activity (beta 1). Sharp vertex waves of wide voltage are observed relatively frequently (up to 110 uV) (Figure 2B). Semi-periodic paroxysms of slow waves are also observed, which can be differentiated into 2 different types: waves of 4 to 6 Hz, from 50 to 70 uV (Figure 3); and slow waves of 3 Hz, from 20 to 40 uV (Figure 4). Also, an incipient alpha activity was found (activity from 8 to 9 Hz, in at least 50% of dogs, up to 13 Hz) from 40 to 60 uV, particularly evident in the parieto-occipital region, with an onset frequency of once every 10–15 s (see Figure 4). Intermittent tracing asymmetries were found in half of the animals (higher signal amplitude with a 25–30% of difference between hemispheres), 50% of which corresponded to each cerebral hemisphere. No asynchronies were found.

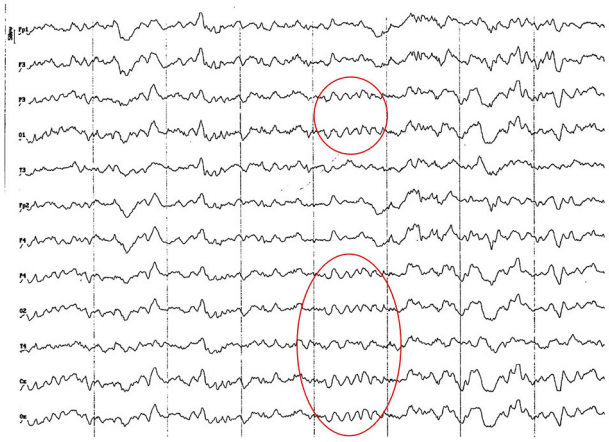


FIGURE 3
EEG (reference montage) corresponding to a 4-month-old female dog of mix breed and 6.5 Kg of body weight. Division 1 seg/50 μV. It is possible to observe one of the varieties of slow waves that appear paroxysmally at this age: 4 to 6 Hz; in this case, 5 Hz, more noticeable in the occipito-parietal regions (red circle).

3.2.2. Group 2 (6 to 11 months of age)

Background electrical activity was similar to that of the previous group, albeit the theta rhythm dominance was more evident, always with incipient superimposed alpha activity and paroxysmal slow wave (Figure 5). Higher amplitude was found in the parieto-occipital region. Acute vertex waves continued to manifest. As in the previous group, paroxysmal slow waves were found, but only in the frequency range of 4 to 7 Hz, and about 60 uV (Figure 6). The alpha activity (8 to 13 Hz) continued to appear paroxysmally, always in the parieto-occipital regions, with an amplitude of 30–60 uV (Figure 7). The most noticeable change in this age group was

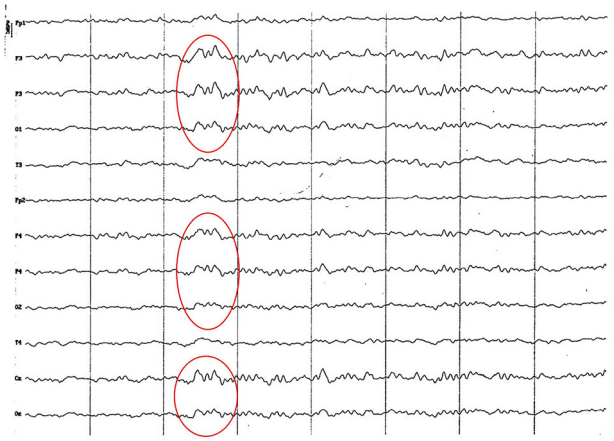


FIGURE 4
EEG (reference montage) corresponding to a mix breed male German Shepherd, of 5 months of age and 8 kg of body weight. Division 1 seg/50 μV. It is possible to observe another type of slow waves that appear paroxysmally at this age: 3 Hz, more noticeable in the dorsal cortex (red circle), except for the Fp channels. In the same tracing, an outline of alpha activity can be observed, which becomes increasingly visible in the occipito-parietal regions.

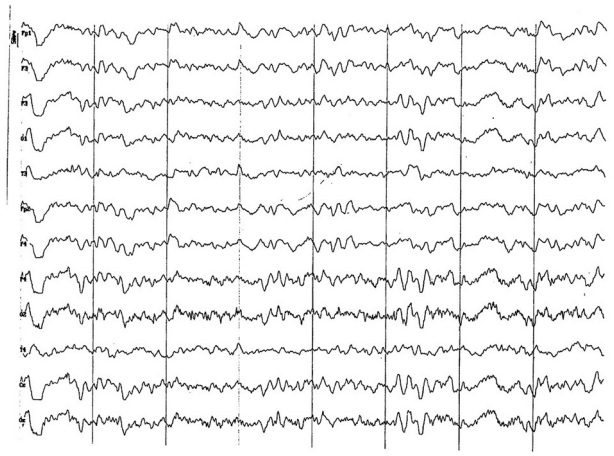


FIGURE 5
EEG (reference montage) corresponding to a male Miniature Poodle of 10 ½ months of age, body weight 13 kg. Division 1 seg/50 μV. In this period (6 to 11 months of age) the predominance of the theta rhythm can be observed, with outlines of superimposed alpha and slow wave paroxysms. Frontopolar (Fp) and temporal (T) channels have significantly increased the voltage of their electrical signal.

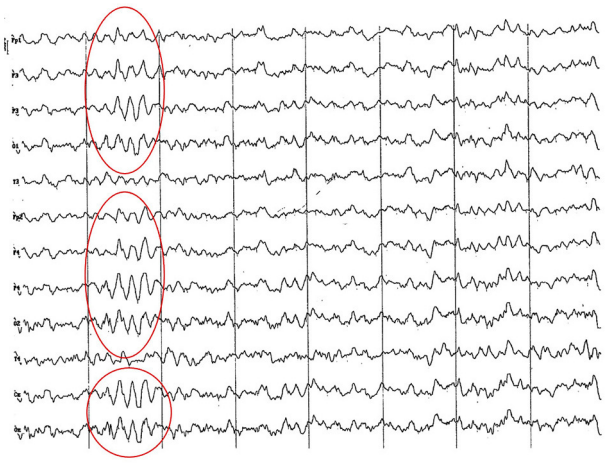


FIGURE 6
EEG (reference montage) corresponding to a female Cocker Spaniel of 8 months of age. It is possible to observe a paroxysm of slow waves of 5 Hz of high amplitude in the entire dorsal cortex (red circle). Note the dominant slow activity in the temporal channels (T) framed in the theta frequency band. Division 1 seg/50 μV.

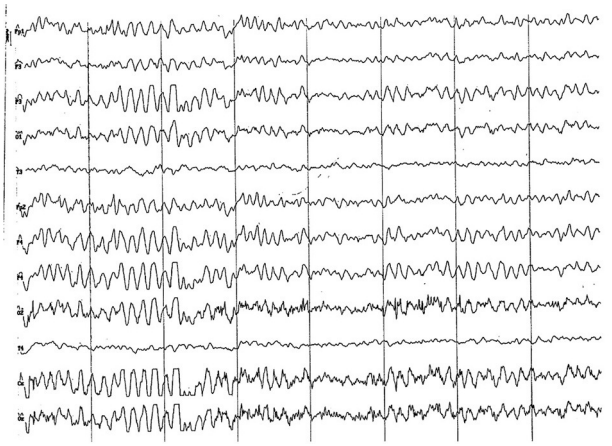


FIGURE 7
EEG (reference montage) corresponding to a female Miniature Poodle of 9 months of age. Frequent paroxysms of an initial alpha rhythm can be observed, with a frequency, in this case, of 8 Hz. Division 1 seg/50 μV.

the substantial amplitude increase in frontopolar channels and, in some animals, in the temporal channels. These channels featured slow dominant activity, mainly contained in the theta band (4 to 5 Hz) (see Figure 6).

3.2.3. Group 3 (12 to 17 months)

The dominant activity in this age group was in the theta frequency band. Nevertheless, an incipient alpha activity started to overlap over this rhythm, particularly in occipital, parietal and frontal regions (Figure 8). At times, this rhythm became the dominant activity. In brachycephalic dogs, the alpha activity was more evident in the fronto-parietal region (Figure 9), while in mesocephalic dogs, in the parieto-occipital region (Figure 10). Paroxysmal slow waves of 4–7 Hz were still observed, but with a smaller amplitude than in the previous groups (25 to 40 uV) and with a less frequent manifestation (Figure 11). The amplitude of the signal recorded in temporal channels and dorsal cortex channels was very similar (see Figure 9), although, temporal leads tracing was significantly slower than previous groups. In this period, a generalized amplitude decrease of the signal recorded begins to be evident.

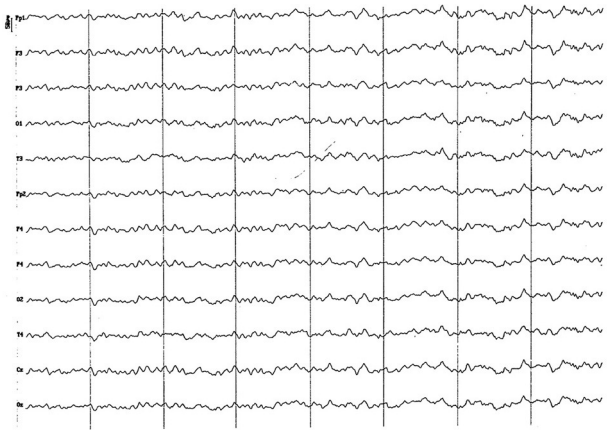


FIGURE 8
EEG (reference montage) corresponding to a 14-month-old female dog, of mix breed of 13 Kg of Body weight. Division 1 seg/50 μV. In this period (12 to 17 months of age), although the dominant frequency is found in the theta band, the alpha rhythm is almost permanently superimposed on it. Although the temporal channels (T) present an amplitude similar to the rest, they show a clearly lower frequency. Generally, the voltage begins to show a decrease regarding previous periods.

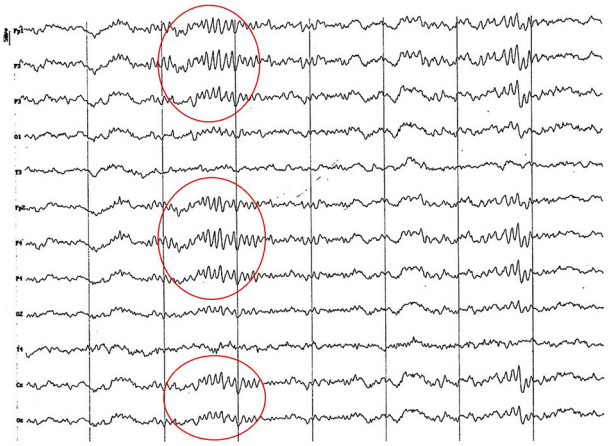


FIGURE 9
Fragment of EEG (reference montage) corresponding to a Yorkshire Terrier of 15 months of age. Division 1 seg/50 μV. In this brachycephalic animal, higher amplitude alpha activity is observed in the fronto-parietal region (red circle).

3.2.4. Group 4 (18 to 23 months of age)

The recording was very similar to that of the previous group. Alpha activity became increasingly important, alternating with theta activity (Figure 12A). Paroxysmal slow wave activity of 4 to 7 Hz, 25 to 40 uV, was maintained (Figure 12B), although it became increasingly more sporadic.

One of the most remarkable characteristics in this group was the generalized decrease in the recording amplitude, which was found in all channels, but much more pronounced in temporal and frontopolar channels. In the first cases, the slow dominant activity prevails, which clearly differentiates them from the rest.

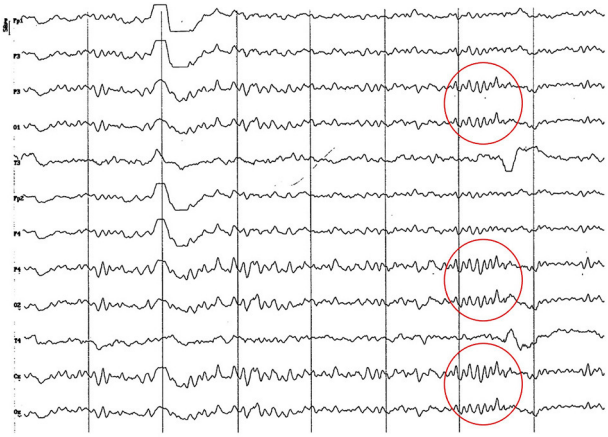


FIGURE 10
Fragment of EEG (reference montage) corresponding to a female Beagle of 17 months of age. In this mesocephalic animal, higher amplitude alpha activity is observed in the occipito-parietal region (red circle). Division 1 seg/50 μV.

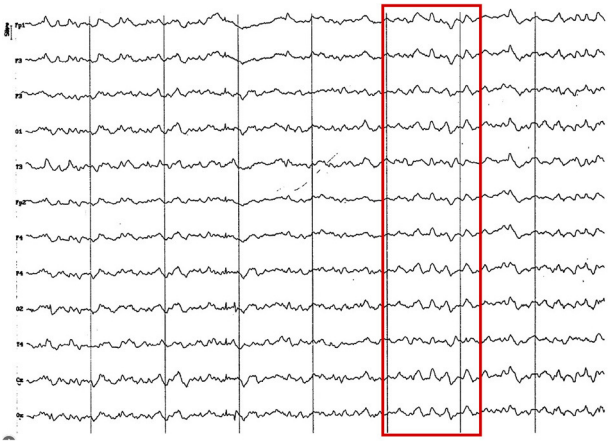


FIGURE 11
EEG (reference montage) corresponding to a 16-month-old female dog of mix breed, body weight 11.5 kg. It is possible to observe the presence of slow waves, in this case 4 Hz of a lower voltage (red square) to previous age periods. Division 1 seg/50 μV.

3.2.5. Group 5 (24 to 83 months of age)

The electrical activity was very similar to that of the previous group. The only difference consisted in the disappearance of the paroxysmal slow wave activity from 4 to 7 Hz, and a slight attenuation of alpha activity (Figure 13). The recording amplitude in all channels continued to show low values. The difference in frequency of the signal recorded in the temporal channels and the rest was not evident, as in the case of the previous groups.

3.2.6. Group 6 (more than 83 months of age)

No major differences were visible in regard to the previous group, except for an apparent increase in the alpha activity in most of the animals recordings (Figure 14). For the first time possible

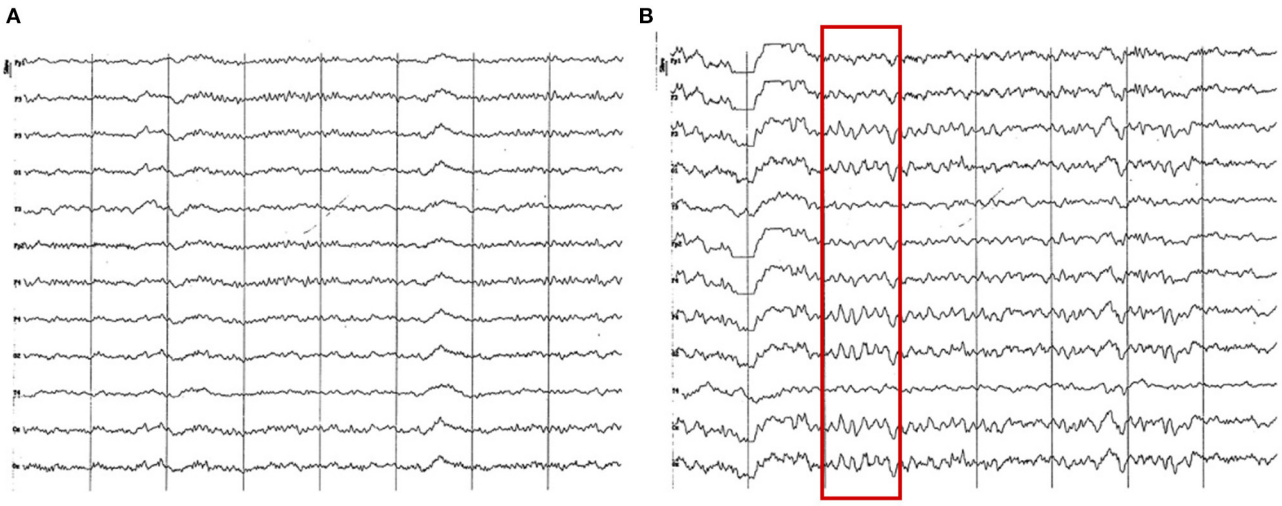


FIGURE 12
EEG (reference montage) corresponding to a Yorkshire Terrier of 23 months of age **(A)**. Female Miniature Poodle of 19 months of age **(B)**. Division 1 seg/50 μV. **(A)** Note the generalized amplitude decrease, despite being a small breed, which is characteristic of this period (18 to 23 months). Also, observe the intermittent dominance of alpha and theta rhythms. **(B)** Slow wave paroxysms can still be observed, in this case of 6 Hz (red square), although much more sporadically than in previous periods.

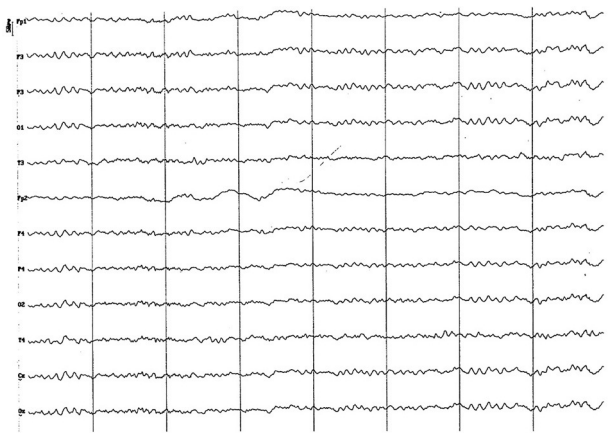


FIGURE 13
EEG (reference montage) corresponding to a 5-year-old female dog, of mix breed and 16 kg of Body weight. Division 1 seg/50 μV. Features of this period are the low amplitude and permanent presence of the alpha rhythm and the similarity of the electrical signal between the temporal (T) and the other channels.

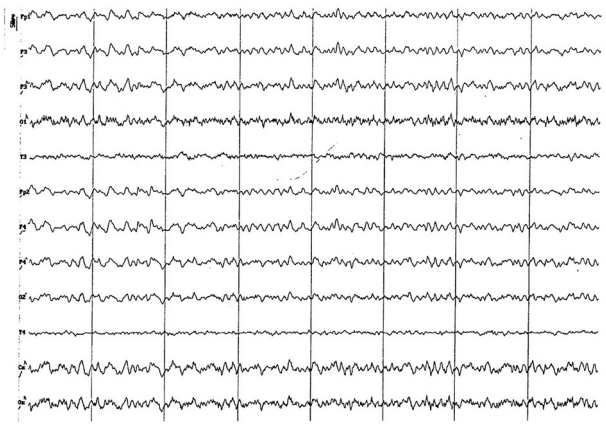


FIGURE 14
EEG (reference montage) corresponding to a 13-year-old male dog of mix breed and 11 kg of body weight. Division 1 seg/50 μV. An apparent visual increase in the alpha rhythm is observed during this period. Once again, the temporal channels (T) show a lower amplitude than the rest of the channels.

pseudo-spindles activity were recorded in temporal channels (Figure 15).

3.3. Power spectrum analysis

3.3.1. First stage

ANOVA for normalized relative power showed the presence of interactions between age and rhythm factors, and between rhythms and channels. The presence of interactions implies that the individual analysis of each considered factor is not valid without taking into account how the factors influence each other. Since there were no triple interactions, one of the factors may be fixed (at any of its levels) to, then, evaluate how the other two interact freely.

Interaction occurred in temporal channels, where relative power of delta rhythm increased, basically, at the expense of the alpha activity decrease and, to a lesser extent, of theta rhythm. Other important conclusions emerged from the previous analysis: (a) the levels assigned to subdivide the age variable proved to be insufficient, as they could have been concealing certain characteristics by being grouped in intervals of different size; (b) channels pertaining the

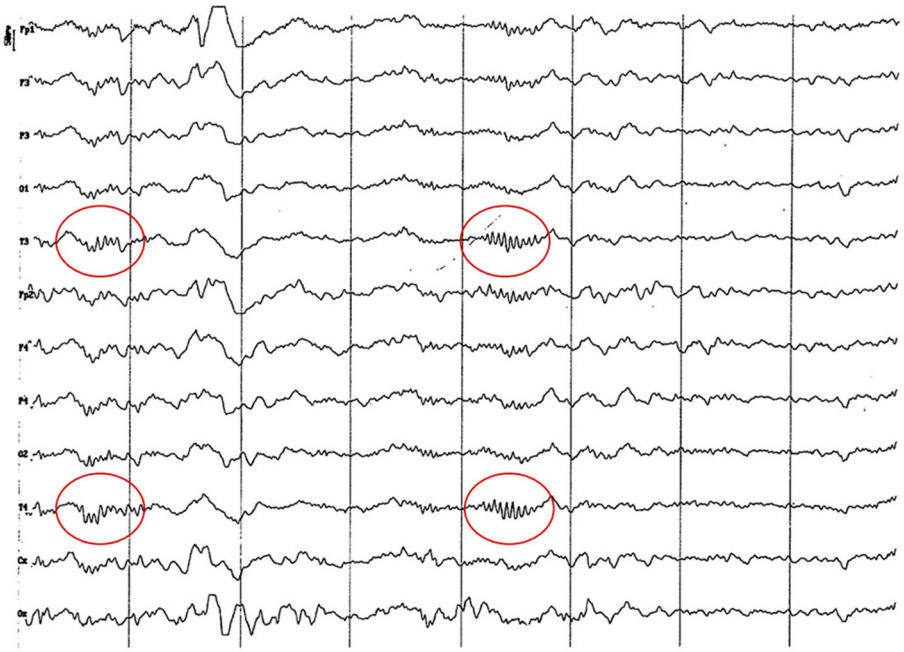


FIGURE 15
EEG (reference mounting) corresponding to an 8-year-old female dog of mix breed 16 kg of body weight. Division 1 seg/50 μV. probably pseudo-spindle activity (10–12 Hz) can be observed (red circle), particularly noticeable in the temporal channels (T).

dorsal portion of cortex (Fp, F, P, O, and central) seemed to have a similar behavior, presenting a strong discrepancy with temporal channels (T); (c) beta rhythm presented an almost constant level throughout all levels of the other factors under study.

3.3.2. Second stage

Beta rhythm was excluded due to its poor participation as a source of variability; age was subdivided into comparable interval groups (0 to 5, 6 to 11, 12 to 17, and 18 to 23 months); channels were regrouped into 2 levels (temporal and dorsal).

ANOVA for normalized relative power for all age groups shows the presence of double interactions. In regard to age/rhythm interaction, comparison of means by general contrast ($p<0.05$) makes it possible to determine that values pertaining delta rhythm are different (higher values) from the average of alpha and theta activity for ages 0 to 5 months, 6 to 11 months, and 18 to 23 months, whereas for the 12 to 17 months group (Supplementary Figure 1), differences are not substantial. On the other hand, theta and alpha activity were different (alpha activity higher than theta activity) from one another (Supplementary Figure 1) for 0 to 5, 6 to 11, and 12 to 17 months groups, but no substantial differences were found in the 18 to 23 months group. The general trend for alpha activity values was upward, as for the theta rhythm values. Delta rhythm behavior stood out, with values showing a downward trend until 17 months of age and then showing an inverse trend. This behavior is understood to be the consequence of amplifiers noise. The relative potency of delta and theta frequencies increases as the amplitude of the tracings decreases.

Age/channel interaction exhibited important differences between dorsal and temporal channels in all ages, except for the 0 to 5 months group, where values obtained for both groups of channels were not different from each other. For age of 6–11 months temporal channels values were higher than dorsal channels. For groups 3 and 4 (12–17 and 18–24 months) dorsal channels values were higher than temporal ones (Supplementary Figure 2).

The rhythm/channels interaction was manifested at the level of delta and alpha frequency bands, whose values showed significant differences between dorsal and temporal channels (Delta frequency was higher in temporal channels and alpha activity was higher in dorsal channels). For theta frequency band, the values obtained for both groups of channels were not different from one another (Supplementary Figure 3).

ANOVA for normalized relative power for age groups 24 to 83 months (adults) and more than 84 months (older dogs) which were analyzed separately, only showed a rhythm effect. There were no double or triple interactions. In other words, in dogs older than 2 years of age, only a variation on the power spectrum composition was found in terms of percentage distribution of electroencephalographic rhythms. The analysis of its behavior by comparison of means (Tukey, $p < 0.05$) indicated that, after 2 years of age, delta rhythm is significantly higher from alpha and theta activity, while the two latter do not differ much between each other (Supplementary Figure 4). We consider this delta behavior as a consequence of an amplifier noise.

Results obtained through visual analysis were consistent with those of the statistical impact analysis that age, channels, and electroencephalographic rhythms have on relative power.

4. Discussion

In order to establish the EEG maturation process through visual analysis, we have selected 3 paramount characteristics: (a) the presence or absence of slow waves from 4 to 6–7 Hz; (b) comparisons of electrical activity recorded in temporal and dorsal cortex channels; and c) visual increase of alpha activity.

In the maturation process of the EEG in dogs, the presence of slow waves of 4 to 6–7 Hz is a key element. The onset of such waves has been described between 5 and 7 weeks of age and their disappearance between 5 and 6 months of age (7, 8, 10, 19, 20). It is likely that the development of cortical neurons and the thalamus-cortical influence produced up to the first month of age (3, 6, 8, 25), but which last longer, up to 10 weeks of age (6–8, 26), is somehow connected to their onset. On the other hand, the strengthening process of ideal synaptic connections and the elimination of unnecessary ones, which occurs approximately up to 4 months of age in dogs (27–29), influences the disappearance of slow waves. Nevertheless, we have found them in all age groups up to 23 months of age. We consider this to the fact that the final postnatal development phase continues for life, and it is characterized by the stability and strengthening of established synapses (27–29).

Our findings suggest that EEGs rhythmic patterns in dogs remain constant and do not present crucial variations from the age of 2 years. From that age onwards, we consider that the brain electrical activity is mature. This includes adult, mature, senior, and geriatric dogs (30). A significant contribution to this fact is that temporal electrodes show a very similar activity to other electrodes. Alpha activity is consolidated as the dominant frequency, while slow waves and differences between the dorsal and temporal channels disappear.

In humans, when maturing, slower frequencies decrease and higher frequencies increase (31). This change from low to high EEG frequencies is a characteristic hallmark of brain maturation (32). We found a two-way interaction during the period between birth and 23 months of age between electroencephalographic rhythms, recording channels and age when analysis of relative power behavior was done. Values of alpha and theta rhythms showed a significant increase from 6 months of age. Alpha activity, although increasing its relative values, was still substantially lower than theta rhythm up to 17 months of age; from this age on, values in both were similar. Delta rhythm values exhibited a progressive decrease until 17 months of age; however, from 18 month of age, they showed a progressive increase. To interpret this phenomenon and correlate it with the visual analysis of the EEG, it is necessary to consider that power spectrum shows all the frequency components of the complex analyzed signal. Therefore, all extracerebral signals affecting the lower end of the relative the power spectrum (e.g., body movements) will be represented in the delta band. Thus, the increased statistical value of delta rhythm had no correlation with visual analysis.

Our assessment about EEGs maturation age differs markedly from those made by other authors, who have established it between 6 and 7.5 months of age (9, 10). This discrepancy is attributable to the use of temporal electrodes, which record the electrical activity of the rhinencephalic cortex. Maturation periods of neopallium and rhinencephalon and their electrical rhythms differences were manifested in the features of electrical activity recorded in temporal and dorsal cortex channels.

In dogs, the development and maturation of rhinencephalic structures have not been cleared yet. However, our findings suggest that it may be established between 6 and 11 months of age when a substantial increase of amplitude in the temporal channels. Statistical analysis shows at this age range significant amplitude differences between the temporal and dorsal channels, with the former ones reaching the highest values.

Through visual analysis of EEG recordings, we identified five development stages in dogs, which coincided with the preselected age groups. Statistical analysis of relative power behavior, particularly with regards to the age/rhythm interaction, support this division.

The period prior to that signaling EEG maturation spans from birth to 23 months of age and can be subdivided into 4 stages, with different features. First stage (0 to 5 months of age) visual analysis is characterized by the mixture of frequencies (predominantly slow ones) and by the low amplitude recorded in frontopolar and temporal channels. However, statistical analysis did not find differences between temporal and dorsal channels. Probably low voltage of Fp channels reduces the average value of dorsal channels, thus matching the average of temporal channels. ANOVA did not find age to be significant factor among the dogs of this group ($p < 0.05$) although some authors have suggested important differences at this stage (2, 7–10, 19, 26). Therefore, there are not differences which validate its division into smaller subgroups. We believe that, for the purpose of clinical application, it is not necessary to subdivide this group, at least in animals older than 45 days, considering that our study did not include any individual under this age.

For second stage (6 to 11 months of age) slow frequencies were still dominant, but theta rhythm is predominant in the visual analysis. The alpha activity began to appear incipiently, particularly in parieto-occipital regions. But the foremost event was the amplitude increase in frontopolar and temporal channels. Statistical analysis supports these observations. We interpret this fact as a maturational process of limbic cortical domains, which is probably closely related to behavioral modifications manifested in this period. This age corresponds to the first stage of behavioral maturation in dogs (33, 34), in which the characteristics concerning the personality and character of the individual begin to be defined.

On visual analysis, the third stage (12 to 17 months of age) showed a very similar pattern to that of the mature adult, yet with differentiating aspects. Theta rhythm was still the dominant frequency, but alpha activity remained frequently superimposed. It was not visually possible to identify the delta rhythm when the animal was arising from sedation and the slow waves of 4 to 6 Hz, although still significantly present, decreased their amplitude considerably. The statistical analysis support these observations since it showed an important decrease of delta rhythm values, matching the theta rhythm values. Alpha activity increased its relative values, but was still notably lower than delta and theta rhythm.

The fourth stage (18 to 23 months of age) was characterized by voltage decrease of the signal recorded and an increase of the alpha activity on visual analysis. Nevertheless, slow waves were still found

intermittently, from 4 to 6–7 Hz with low amplitude. Statistical analysis showed the definite impact of age/rhythm interaction, which remain in the rest of the analyzed ages, becoming one of the hallmarks of the adult animal: alpha activity values match those of theta. In humans, theta rhythm is mainly found in infants, and it continually decreases with age (35). Theta rhythm areas are frequent on posterior regions in children between 7 and 10 years of age. This phenomenon may be a precursor of alpha rhythm in adults, and may be connected to maturation, overlapping areas implied in the generation of a lower frequency alpha rhythm (36).

The impact of the age/channel and rhythm/channel interaction on relative power behavior does not strictly adjust to the 5 age groups described and will be considered separately.

The impact of the age/channel interaction between 12 and 23 months of age showed a substantial change in relative power behavior of temporal and dorsal channels, with this latter reaching the highest values. This fact is due to the generalized amplitude decrease and appears in this group and consolidates from the 24 months of age. This voltage reduction differentially affects temporal channels, probably due to the anatomy of the region where they are placed.

Although the third and fourth stages were very similar to mature dog EEG recording, we considered them different based on the discrepancy between temporal and dorsal cortex channels on the visual analysis. The period between 12 and 23 months of age included the second stage of the behavioral maturation in dogs, in which the consolidation of the features concerning personality and character of the individual is observed (33, 34). It is likely that the synapse neoformation processes (synaptogenesis) and persistent changes in functional properties of neuronal groups (synaptic plasticity) (37), are involved in behavioral changes found between 6 and 23 months of age, and somehow linked to electrical features of the limbic region.

The rhythm/channel interaction analysis indicated that, for any of the studied ages, the composition of the frequency spectrum is different for temporal and dorsal channels. Alpha activity was higher for dorsal channels and delta rhythm was higher for temporal channels. Amplitude average of temporal channels is considerably low for four ages groups. Therefore, delta activity shown by the statistical analysis is artificially high, being theta the dominant frequency band on visual analysis. This finding is coherent with bibliographic information about rhythmic slow electrical activity (RSA), typical of limbic cortical areas. This activity appears modulating not only normal synaptic transfer, but also long-term changes in the synapse strength (7). In addition, this fact matched visual analysis of the signal obtained in temporal channels.

No differences have been found among the EEG of studied animals older than 2 years of age. The oldest group (more than 83 months), with an average of 8.5 and peaks of 11 and 13 years of age, showed no differences with animals of 24 to 83 months of age. Decrease in the recording amplitude in "old" animals stated in the cited bibliography (9) did not represent a specific feature of this group. Voltage decrease occurs substantially from 18 months of age and is probably due to the increase of skull thickness and surrounding muscles. These structural changes subdue the brain's electrical signal. These findings agree with statistical analysis, since from 2 years of age the interaction among analyzed factors disappears, showing the individual effect of the EEG rhythm factor. Thus, for any age older than 2 years, and any channel, alpha and theta activity will have similar contribution to power spectrum. This statistical data was consistent with the visual analysis, in which a similar electrical activity was observed on temporal and dorsal channels from 24 months of age. By the first time we had seen some graphoelements compatible with a possible pseudo-spindle which origin might be the hippocampus and the mechanism underlying this feature still unknown (38, 39). It differentiates from alpha rhythm (Rhythm at 8–13 Hz inclusive during wakefulness over the posterior regions of the head, generally with the higher amplitudes over the occipital areas) and sleep spindles (Train of distinct waves with a frequency at 11–16 Hz with a duration of >0.5 seg and generally with higher amplitude over the central areas) as pseudo-spindles seems to be recorded with higher amplitude at the temporal leads arising from hippocampus and having a slow rhythm (38–40).

All information above refers to the visual analysis of the EEG recording and the statistical analysis of the relative power behavior. However, interhemispheric coherence analysis studies have reported that, even in the period of electrical maturity (2 years of age), differences persist between the signal recorded in temporal and dorsal cortex channels. These differences result from their origin, modulation and transfer over distance (18). Such functional differences are simply the reflection of large structural and morphological differences among isocortex and allocortex. Local nature factors acquire great relevance in the electrical activity of the limbic cortex. This is supported by evidence of the theta hippocampal activity origin, especially in terms of its cellular bases within pyramid neurons from CA1 and CA3, as well as its relation to a septum-induced disinhibitory process (41–43). These features might increase susceptibility of the limbic cortex to some epileptic disorders (44, 45).

5. Conclusions

According to the findings in the present study, maturation of brain bioelectrical activity in dogs is a gradual process and the EEG features of adult animals start showing at 12 months of age, but stabilize after 24 months of age based on the EEG visual analysis and the statistical analysis of spectral power behavior. In this process, the evident differences in the development and maturation of the neopallium and rhinencephalon play a crucial role. The elements to be considered in order to visually identify the EEG maturational periods in dogs are: (a) the presence or absence of slow waves of 4 to 6–7 Hz, (b) homogeneity of signal recorded in the totality of EEG channels and (c) the dominance of alpha rhythm. These features are easily recognizable in the visual analysis of the EEG recording.

It is relevant to know background and normal transient activities of each developmental period in order not to misinterpreted certain graphoelements as abnormal (e.g., slow waves that feature the immature EEG), which would result in wrong decisions regarding diagnosis, prognosis and/or treatment of patients under a brain maturation process with intracranial pathologies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Comisión Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL) Facultad de Ciencias Veterinarias-Universidad de Buenos Aires. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

Acknowledgments

Authors thank Dr. Roberto Sica and Dr. Enrique Delamonica from the Hospital Ramos Mejia Neurology Department Buenos Aires University for their contribution as blinders reviewers of EEG tracings. Authors also thank Dr. Carlos Blanco Statistic from La Universidad de Buenos Aires for his contribution to stablish the statistical analysis of this research.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1150617/full#supplementary-material

References

1. Pellegrino FC, Sica R. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. *Clin Neurophysiol.* (2004) 115:477–87. doi: 10.1016/S1388-2457(03)00347-X

2. Charles MS, Fuller JL. Developmental study of the electroencephalogram of the dog. *Electroencephalogr Clin Neurophysiol.* (1956) 8:645–52. doi: 10.1016/0013-4694(56)90091-8

3. Petersen R, Perri D, Himwich WA. The comparative development of the EEG in rabbit, cat and dog. *Electroencephalogr Clin Neurophysiol.* (1964) 17:557–63. doi: 10.1016/0013-4694(64)90187-7

4. Fox MW. Postnatal development of the EEG in the dog. i introduction and eeg techniques. *J Small Anim Pract.* (1967) 8:71–6. doi: 10.1111/j.1748-5827.1967.tb04528.x

5. Fox MW. Postnatal development of the EEG in the dog. II development of electrical activity. *J Small Anim Pract.* (1967) 8:77–107. doi: 10.1111/j.1748-5827.1967.tb04529.x

6. Fox MW. Postnatal development of the EEG in the dog. III summary and discussion of development of canine EEG. *J Small Anim Pract.* (1967) 8:109–11. doi: 10.1111/j.1748-5827.1967.tb04530.x

7. Lopes da Silva FH, Vos JE, Mooibroeck J, Van Rotterdam A. Relative contribution of intracortical and thalamocortical processes in the generation of the alpha rhytms, revealed by partial coherence analysis. *Electroencephalograph Clin Neurophysiol.* (1980) 50:449–56. doi: 10.1016/0013-4694(80)90011-5

8. Takahashi A, Inada, S. Developmental electrencephalogram of the non-REM sleep in Beagle dogs. *J Vet Sci.* (1986) 48:1115–24. doi: 10.1292/jvms1939.48.1115

9. Redding RW. Canine electroencephalography. In: por Hoerlein BF, editors. *Canine neurology, 3rd. edition.* Philadelphia: W.B. Saunders (1978). p. 150–206.

10. *Pampiglione G. The electrical activity of the brain in the young dog. In: Kellaway P, Petersen I Gonne, editors. Neurological and EEG Correlative Studies in Infancy. Stratton New York and London (1963).*

11. Senba H, Tokuriki M, Sasaki N, Takeuchi A, Usui K. Developmental EEG of the Beagle dogs. *J Vet Sci.* (1984) 46:771–81. doi: 10.1292/jvms1939.46.771

12. Redding RW, Knecht CE. *Atlas of the EEG in the Dog and Cat*. New York, NY: Praeger. (1984). ISBN 0-03-061929-7.

13. Redding RW. Principles of electrophisiological testing. *Prog Vet Neurol.* (1990) 1:66–72.

14. Klemm WR, Hall CL. Subjective and quantitative analyses of the electroencephalogram of anestesized normal dogs: control data for clinical diagnosis. *Am J Vet Res.* (1968) 29:1267–2025.

15. Dehasse J. Epigenèse, émotionnelle et relationnelle du chiot. *Cahiers dÉthologie, Liège.* (1993) 12:443–66.

16. Pageat, P. "Manuel de pathologie comportamentale canine". Le point Vétérinaire, Paris (1995). p. 3–27.

17. Kuijper T. La importancia de los electrodos para EEG. En: Análisis computado del EEG. Eds: Ferrero RGA, Ferrero AL. FADEC. *Laboratorio para el estudio de las funciones cerebrales superiores.* Bs. As. Argentina (1995). p. 424–35.

18. Pellegrino F. Estandarización de los patrones electroencefalográficos de los caninos. *Tesis Doctoral Fac Cs Vet UBA* (2000).

19. Tourai K, Senba, H, Sasaki N, Tokuriki M, Ohashi F, Takeuchi A, Usui K. Developmental EEG of the Beagle dog under xylazine sedation. *J Vet Sci.* (1985) 47:459–63. doi: 10.1292/jvms1939.47.459

20. Roy John E, Prichep LS. *Los principios del análisis neurométrico en EEG y potenciales evocados.* En: Análisis computado del EEG. Eds: Ferrero RGA, Ferrero AL. FADEC. Laboratorio para el estudio de las funciones cerebrales superiores. Bs. As. Argentina (1995). p. 111–35.

21. Norman G, Streiner D. *Bioestadística.* Madrid: Mosby/Doyme libros (1996).

22. Capelletti C. *Elementos de estadística.* Ed. Cesarini. Buenos Aires (1991).

23. Steel RG, Torrie JH. *Bioestadística: Principios y procedimientos. 2nd ed.* McGraw-Hill: México (1992).

24. De Risio L, Bhatti S, Muñana K, Penderis J, Stein V, Tipold A, et al. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. *BMC Vet Res.* (2015) 11:148. doi: 10.1186/s12917-015-0462-1

25. Himwich HE, Fazekas JE. Comparative studies of the metabolism of the brain of infant and adult dogs. *Am J Physiol.* (1941) 132:454–9. doi: 10.1152/ajplegacy.1941.132.2.454

26. Fox MW. *Chap. 4, Structural and functional aspects of CNS development. In: Integrative development of brain and behavior in the dog*. Chicago and London: The Universitiy of Chicago Press (1971). p. 115–76.

27. Arsenault D, Zhang ZW. Developmental remodeling of the lemniscal synapse in the ventral basal thalamus of the mouse. *J Physiol*. (2006) 573:121–32. doi: 10.1113/jphysiol.2006.106542

28. Huttenlocher PR. Morphometric study of human cerebral cortex development. *J Neuropsychol*. (1990) 28:517–27. doi: 10.1016/0028-3932(90)90031-I

29. Watson RE, Desesso JM, Hurtt ME, Cappon GD. Postnatal growth and morphological development of the brain: a species comparison. *Birth Defects Res B Dev Reprod Toxicol*. (2006) 77:471–84. doi: 10.1002/bdrb.20090

30. Bartges J, Boynton B, Vogt AH, Krauter E, Lambrecht K, Svec R, et al. AAHA canine life stage guidelines. *JAAHA*. (2012) 48:1–11. doi: 10.5326/JAAHA-MS-4009

31. Somsen RJM, Klooster BJ, Van der Molen MW, van Leeuwen HM, Licht R. Growth spurts in brain maturation during middle childhood as indexed by EEG power spectra. *Biol Psychol*. (1997) 44:187–209. doi: 10.1016/S0301-0511(96)05218-0

32. Matousek M, Petersén I. Frequency analysis of the EEG in normal children and adolescents. In: *Automation of clinical electroencephalography*. New York, NY: Raven (1973). p. 75–102.

33. Beaver B. Mental lapse aggression syndrome. *J Am Anim Hosp*. (1980) 16:937–9.

34. Beaver B. "*The veterinarians encyclopaedia of animal behavior*". *Iowa State University Press/Ames*. Iowa, U.S.A (1994). p. 173–4.

35. Niedermeyer E. Maturation of the EEG: development of Waking and Sleep Patterns, In: Niedermayer E, Lopes da Silva F, editors. *Electroencephalography*. William y Wilkins. Third edition (1993). p. 167–91.

36. Pulighеddu M, Munck JC, Stam CJ, Verbunt J, de Jongh A, van Dijk BW, et al. Age distribution of MEG spontaneous theta activity in healthy subjects. *Brain Topogr*. (2005) 17:165–75. doi: 10.1007/s10548-005-4449-2

37. O'Donnell P, Pazo JH. Bases biológicas de la generación y transmisión del impulso nervioso. In: por Cingolani HE, Houssay AB, editors. *Fisiología Humana*. El Ateneo, Bs.As: Argentina (1994). p. 1–25.

38. Iotchev IB, Kubinyi E. Shared and unique features of mammalian sleep spindles–insights from new and old animal models. *Biol Rev*. (2021) 96:1021–34. doi: 10.1111/brv.12688

39. Gottesmann C. The transition from slow-wave sleep to paradoxical sleep: evolving facts and concepts of the neurophysiological processes underlying the intermediate stage of sleep. *Neurosci Biobehav Rev*. (1996) 20:367–87. doi: 10.1016/0149-7634(95)00055-0

40. Kane N, Acharya J, Beniczky S, Caboclo L, Finnigan S, Kaplan PW, et al. A revised glossary of terms most commonly used by clinical electroencephalographers and updated proposal for the report format of the EEG findings. revision 2017. *Clin Neurophysiol Pract*. (2017) 2:170. doi: 10.1016/j.cnp.2017.07.002

41. Krnjevic K, Ropert N, Caullo J. Septo hipocampal dis-inhibition. *Brain Res*. (1988) 438:182–92. doi: 10.1016/0006-8993(88)91337-6

42. Riquelme L. Bases neurofisiológicas de las señales bioeléctricas cerebrales. In: Ferrero RGA, Ferrero AL, editors. *Análisis computado del EEG*. FADEC. Bs.As (1995). p. 32–41.

43. Steriade M. Cellular substrates of brain rhytmes. In: Niedermayer E, Lopes da Silva F, editors. *Electroencephalography*. William y Wilkins. Third edition (1993). p. 27.

44. Niedermayer E. Epileptic seizure disorders. In: Niedermayer E, Lopes da Silva F, editors. *Electroencephalography*. William y Wilkins. Third edition (1993). p. 461–564.

45. Prince DA. Basic mechanisms of focal epileptogenesis. In: Avanzini G, Fariello R, Heinemann U, Mutani R, editors. *Epileptogenic and Excitotoxic Mechanisms*. John Libbey, USA (1993). p. 17–26.

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 13 July 2023
DOI 10.3389/fvets.2023.1198134

OPEN ACCESS

EDITED BY
Holger Andreas Volk,
University of Veterinary Medicine Hannover, Germany

REVIEWED BY
Colette Williams,
University of California, Davis, United States
Alejandra Mondino,
North Carolina State University, United States

*CORRESPONDENCE
Fiona M. K. James
✉ jamesf@uoguelph.ca

RECEIVED 31 March 2023
ACCEPTED 16 May 2023
PUBLISHED 13 July 2023

CITATION
Luca J, McCarthy S, Parmentier T, Hazenfratz M, Linden AZ, Gaitero L and James FMK (2023) Survey of electroencephalography usage and techniques for dogs.
Front. Vet. Sci. 10:1198134.
doi: 10.3389/fvets.2023.1198134

Survey of electroencephalography usage and techniques for dogs

Julia Luca[1], Samantha McCarthy[2], Thomas Parmentier[3], Michal Hazenfratz[4], Alex Zur Linden[1], Luis Gaitero[1] and Fiona M. K. James[1]*

[1]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Medical Science, Canadian Academy of Osteopathy, Hamilton, ON, Canada, [3]Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, [4]Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel

Background: Canine epilepsy is a chronic common neurologic condition where seizures may be underreported. Electroencephalography (EEG) is the patient-side test providing an objective diagnostic criterion for seizures and epilepsy. Despite this, EEG is thought to be rarely used in veterinary neurology.

Objectives: This survey study aims to better understand the current canine EEG usage and techniques and barriers in veterinary neurology.

Methods: The online Qualtrics link was distributed via listserv to members of the American College of Veterinary Internal Medicine (ACVIM) Neurology Specialty and the European College of Veterinary Neurology (ECVN), reaching at least 517 veterinary neurology specialists and trainees worldwide.

Results: The survey received a 35% response rate, for a total of 180 participant responses. Fewer than 50% of veterinary neurologists are currently performing EEG and it is performed infrequently. The most common indication was to determine a discrete event diagnosis. Other reasons included monitoring treatment, determining brain death, identifying the type of seizure or epilepsy, localizing foci, sleep disorders, for research purposes, and post-op brain surgery monitorization. Most respondents interpreted their own EEGs. Clinical barriers to the performance of EEG in dogs were mainly equipment availability, insufficient cases, and financial costs to clients.

Conclusion: This survey provides an update on EEG usage and techniques for dogs, identifying commonalities of technique and areas for development as a potential basis for harmonization of canine EEG techniques. A validated and standardized canine EEG protocol is hoped to improve the diagnosis and treatment of canine epilepsy.

KEYWORDS

canine, electroencephalography, epilepsy, survey, EEG technique

Introduction

Epilepsy is the most common neurological condition in dogs affecting 0.6–0.75% of dogs (1–3). Diagnosis and treatment may be limited in veterinary neurology since diagnostic confirmation is based on subjective criteria such as description of episodes, viewing of episodes, physical and neurologic examinations, as well as unremarkable advanced tests like magnetic

resonance imaging (4). None of these provide objective confirmation of seizure events, nor does the caregiver's history. The latter leads to an underreporting of seizure frequency in dogs as episodes may be missed, particularly while the caregiver is away (5).

Electroencephalography (EEG) is a test providing an objective diagnosis of seizures. While brain function can be measured and assessed using multiple methods, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI), and magnetoencephalography (MEG), none of these methods measure brain function in real time at the bedside (6). For this reason, EEG provides a standard for seizure and epilepsy diagnosis (4). The EEG confirmation of seizure (ictal) or interictal activity thus raises the confidence in a diagnosis of canine idiopathic epilepsy to the highest tier, Tier III (4). EEG can differentiate an epilepsy diagnosis from other conditions including episodic or transient paroxysmal disorders (7), behavioral and movement disorders (8–11), or a coma or nonconvulsive seizures (12–17). Despite it being the gold standard test, EEG is thought to be rarely used in veterinary neurology due to various barriers, e.g., labour requirements or cost-effectiveness.

In veterinary medicine, there are not yet standards for EEG usage and technique as there are in human epileptology, making it difficult to compare EEG recordings between dogs, electroencephalographers, and clinics. The last survey examining veterinary EEG usage and technique was conducted over 34 years ago (18). The survey was mailed to 34 neurologists in the United States and Canada, out of which 19 completed and returned the survey (56% response rate). The survey examined questions such as if EEG was being performed, on which species, what type of electrodes were used, what electrode resistance was being used, montage, sensitivity, frequency settings, number of channels used, any other simultaneous recordings (i.e., EKG, respiration, eye movement), usage of photic stimulation, seizure activating procedures, and chemical restraints. At the time, 17/19 respondents reported using EEG in dogs and cats. The most used electrode placement protocol was by Redding and Knecht (1984) (19) using 5 electrodes, (F3, F4, Cz, O1, O2, RF). In the 20th century, EEG machines recorded deflections of a pen on reams of paper (20). Given intervening technological advances and that the survey was completed by such a small group, there is a need to update knowledge of current veterinary EEG practices considering advances in EEG techniques.

The questions arise as to how commonly and by what protocols EEG is currently performed in veterinary neurology. Amongst neurologists, the sense is that EEG is not a commonly performed technique. With a focus on EEG use in dogs, therefore, the hypothesis was that a low proportion of veterinary neurologists use EEG clinically (< 50% of respondents). Further, it was expected to find that the EEG technique has high variability, with the penetrance of any one protocol being less than 20% of those recording EEG routinely amongst respondents. In order to update veterinary EEG literature, the objectives of this study were to understand the current (1) canine EEG usage and its barriers, (2) techniques in veterinary EEG, and (3) the approaches to EEG review.

Methods

This survey study was approved by the University of Guelph Research Ethics Board (REB# 19–11-004). As internal validation, a focus group of Ontario Veterinary College (OVC) clinicians tested and approved the survey before it was distributed. The survey contains 27 questions in total grouped into three themes. Theme One questions regarding usage and its barriers asked about frequency of EEG use, barriers encountered by both clinicians and pet owners. Theme Two questions asked about equipment type, electrode layout, and typical procedures. Finally theme Three asked about typical approaches to EEG review. See Supplementary Datasheet 1 for all survey questions. Survey questions had several different formats including multiple-choice, yes/no options, slider, and free text. Survey questions were presented as a 20-min online Qualtrics survey. This online Qualtrics link was distributed to members of the veterinary neurology specialist community world-wide via professional listservs including members of the American College of Veterinary Internal Medicine (ACVIM) Neurology Specialty and the European College of Veterinary Neurology (ECVN), reaching at least 517 veterinary neurology specialists and trainees worldwide (estimate provided by listserv moderator for January 2021, by private communication). The survey was available for a total of 5 weeks from November 30, 2021, to January 8, 2022. The surveys were completed anonymously, therefore participants were not able to withdraw their data once they completed and submitted the Qualtrics survey. Two authors (JL, FJ) reviewed the responses and for questions with free-text answers grouped them according to commonalities. Simple descriptive statistics were performed on the responses. Discrete data were tested for normality using the Kolmogorov–Smirnov Test with $p = 0.05$. Mean and standard deviation (SD) were reported for normally distributed data, whereas median and interquartile range (IQR) were reported otherwise. For certain questions (Q18 and Q20), the Qualtrics 'slider' question format summarizes the continuous variable output as minimum, maximum, mean, SD, variance, and count, which were reported.

Results

Canine EEG usage and its barriers (questions 1–8, Q1-Q8):

With a 35% response rate, a total of 180 participant responses were recorded. Not all questions were answered by all participants. EEG has been performed at some point by 126/169 (75%) respondents, with 54/123 (44%) respondents performing EEG at the time of survey (Q1, Q2). EEG is most used on an annual basis (70/119, 59%), seen in Figure 1

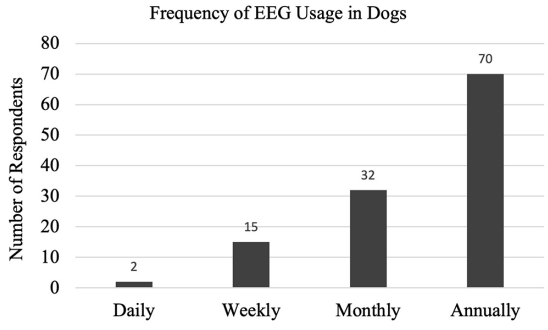


FIGURE 1
(Q6): Frequency of EEG use for survey respondents.

with the relative frequency at which EEG was performed at the time of the survey (Q6). Indications for performing an EEG are listed in Table 1 (Q3). Figure 2 shows the duration of time in years that respondents have performed EEG (Q4) with the majority being 1–5 years (range 0–49 years). Questions 5, 7, and 8 explored barriers to the uptake of EEG capturing free text answers. The commonalities identified in the answers are summarized in Table 2 along with the frequency of their occurrence. Lack of equipment prevented respondents from performing EEG, whereas number of cases needing EEG affected frequency. Cost to client was frequently cited as affecting client compliance (Table 2).

TABLE 1 (Q3): Indications for performing EEG in dogs.

Categories	Examples of Free Text Response	Number of Answers
Discrete Event Diagnosis (I.e Is it a seizure?)	-Behavior (obsessive compulsive) -Movement disorders (paroxysmal dyskinesia, narcolepsy, syncope without cardiac abnormalities)	76 (63%)
Continuous state diagnosis (I.e Is it still having a seizure?)	- Non-convulsive status, status epilepticus, head trauma, other encephalopathy/intoxication, CCD -Coma	41 (35%)
Drug Monitoring Treatment (I.e Are these drugs working?)	-Drug resistant -Seizure under anesthetic	31 (26%)
Do not perform	Do not perform	20 (17%)
Brain Death	Brain Death	14 (12%)
Type of Seizure/Epilepsy	Focal/absence seizure (5)	11 (9%)
Localizing foci	Localizing foci	4 (3%)
Sleep Disorder	NA	2 (2%)
Research/Academia	NA	4 (3%)
Post-op Brain Surgery Monitoring	NA	1 (1%)
TOTAL		119

Techniques in veterinary EEG (Q9-Q23):

A wired EEG machine was used by 97/132 (74%) respondents, the remaining used wireless machines (Q9). Video was not used while recording in 60/119 (50%; Q10). Where video was used, 40% (48/119) of respondents recorded video synchronized with the EEG software while the remaining 9% (11/119) recorded video with a separate system, for example, with a GoPro. Subdermal wire electrodes were the most used 88/134 (66%), followed by steel needle electrodes 31/134 (23%), and skin surface electrodes 15/134 (11%; Q11). The types of skin surface electrodes (15; Q12) were reported to be metal (6), disposable cup electrodes (1), patch (3), CCX chloride electrodes (2), silver coated plastic electrodes with T20 paste (1), and "the ones humans have" (2).

Ninety free text responses were submitted for the number of electrodes in the electrode array (Q13). Between 6 to 32 electrodes are being used including ground and reference (median = 12 electrodes, IQR = 6 electrodes; Q13). To explore the electrode array, four images (maps with electrode nomenclature) were presented for selection (7, 20, 21, 22) or respondents could upload a map that they use (Table 3; Q14, 15, 16). The Holliday and Williams electrode array map was most frequently selected (44/132, 33%), followed by James et al. (31/132, 24%), Tepper and Shores (26/132, 20%) and lastly Pellegrino and Sica (24/132, 18%). The most uploaded image was taken from Wrzosek (4/9 uploads, 44%) (23). Integrity of electrode placement was typically confirmed via

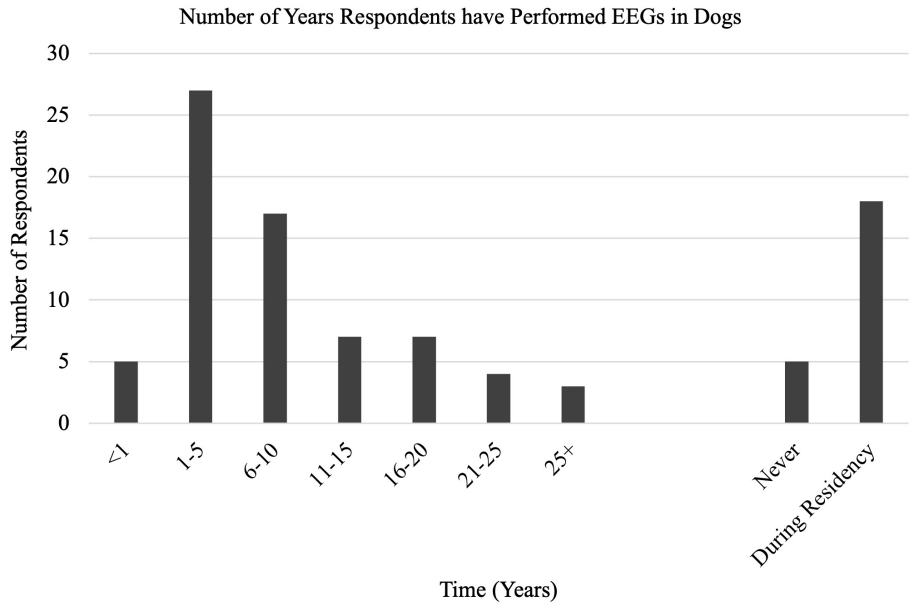


FIGURE 2
(Q4): Years of experience in performing EEG.

TABLE 2 (Q5, 7, 8): Barriers affecting EEG performance in dogs from a veterinarian and owner's perspective.

Barrier Factors	DVM Stopped Performing (Q5)	Affecting DVM frequency (Q7)	Affecting Client's Compliance (Q8)
Lack of Equipment	38 (37%)	20 (16%)	7 (7.5%)
Lack of Training/Experience	20 (19%)	22 (17%)	NA
Financial Cost	12 (12%)	8 (6%)	26 (28%) *Cost to client
Limited Diagnostic Value	15 (14%)	20 (16%)	1 (1%)
Other Barriers	18 (18%)	55 (43%)	NA
	Lack of staff = 4 (4%)	Lack of staff = 9 (7%)	
	Not enough cases needed for EEG = 3 (3%)	Based on # of cases needing EEG = 26 (21%)	
	Lack of time (use & interpretation) = 11 (11%)	Lack of time = 20 (16%)	
Client Com pliance	Lack of client cooperation = 1 (1%)	Lack of client cooperation = 2 (2%)	Total = 22 (24%) Uncooperative dog = 4 (4%)
			Anesthesia or sedation needed = 9 (10%)
			Far drive to specialist = 2 (2%)
			Hospitalization needed = 7 (8%)
Total Responses	104	127	93

TABLE 3 (Q14) Electrode locations maps commonly used for electrode placement on a dog's scalp.

Electrode Location Maps	Frequency of Use	Number of Electrodes (including ground and reference)
Holliday TA, Williams DC. Clinical Electroencephalography in Dogs. Vet Neurol Neurosurg J. 1999;1(1):1–38.	44/132 (33%)	15 electrodes
James FMK, Cortez M, Monteith G, et al. Diagnostic utility of wireless video-electroencephalography in unsedated dogs. J Vet Intern. 2017;31(5):1469-1476.	31/132 (24%)	15 electrodes
Tepper L, Shores A. Electroencephalographic recordings in the canine: effects of low dose medetomidine or dexmedetomidine followed by atipamezole. Open J Vet Med. 2014;04:7-13.	26/132 (20%)	6 electrodes
Pellegrino FC, Sica REP. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. Clinical Neurophysiology. 2004;115:477-487.	24/132 (18%)	14 electrodes
Other (respondents could upload image of map on the following Q15.	9/132 (7%)	
Wrzosek MA. Electroencephalography as a diagnostic technique for canine neurological diseases. J Vet Res. 2016 Jun;60:181–7	4/9 (44%)	
Redding R, Knecht C. Atlas of Electroencephalography in the Dog and Cat. (1984)	1/9 (11%)	
Brauer C, Kästner SBR, Schenk HC, Tünsmeyer J, Tipold A. Electroencephalographic recordings in dogs: Prevention of muscle artifacts and evaluation of two activation techniques in healthy individuals. Res Vet Sci. 2011 Apr;90(2):306–11.	1/9 (11%)	
Holliday TA, Williams DC. Advantages of Digital Electroencephalography in Clinical Veterinary Medicine Part 1. Vet Neurol Neurosurg J. 2001;3(1):11.	1/9 (11%)	
Could not tell based on image submitted	2/9 (22%)	

visual inspection of tracings (45/172, 26%), or electrodes (45/172, 36%). Software measures of impedance were also used: <10 kΩ (35/172, 20%), versus <5 kΩ (27/172, 16%) or < 20 kΩ (6/172, 4%). Integrity of electrode placement was not checked by 14/172 (8%) respondents (Q17).

Restraint protocols during instrumentation were ranked as a proportion of cases that the protocol was used for (with a minimum of 0 and a maximum of 100% for each protocol option). Out of 99 responses the mean proportion of use of sedation was 45% (SD = 36,

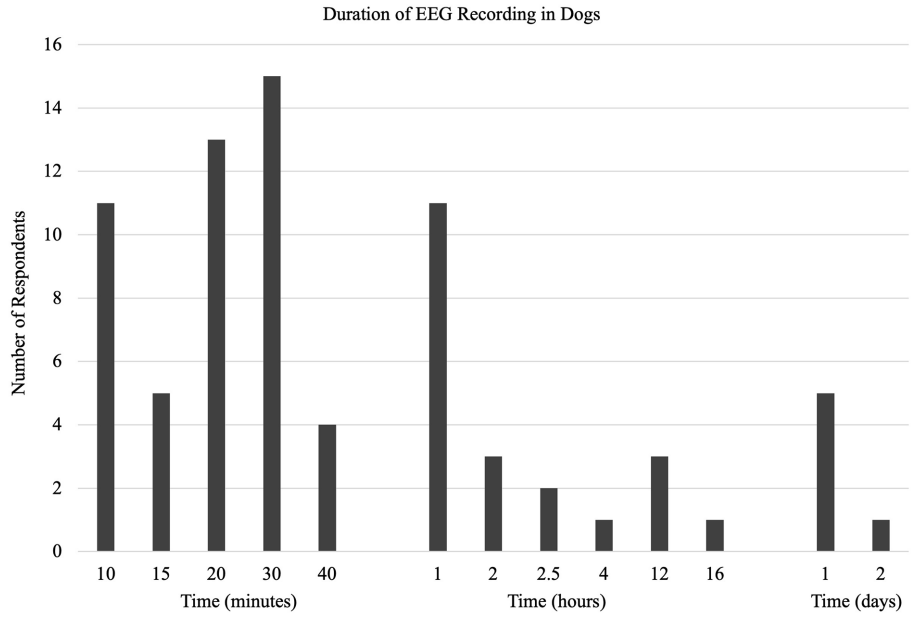


FIGURE 3
(Q23): EEG recording duration.

variance = 1,282), ahead of no restraint protocol 30% (97 responses, SD = 34, variance = 1,171), general anesthesia 17% (97 responses, SD = 27, variance = 729), and unspecified other 7% (97 responses, SD = 23, variance = 540; Q18). Awake recording (92 responses, mean 46%, SD = 42, variance = 1783) had a greater proportion of use than sedated (93 responses, mean 35%, SD = 37, variance = 1,376) or anesthetized (93 responses, mean 12%, SD = 25, variance = 620) recordings (with a minimum of 0 and a maximum of 100% for each protocol option; Q20). Alpha-2 agonists were the most used drugs during both instrumentation (46/100, 46%; Q19) and recording (25/64, 39%; Q21). The other drugs listed in the responses to both questions were used less frequently and included propofol, butorphanol, trazadone, phenobarbitone, isoflurane, acepromazine, benzodiazepines. Rocuronium and ketamine were only reported once.

Thirty-nine percent (37/94) of respondents did not use anything while fixing electrodes in place. The remaining 61% (57/94) described various methods: adhesive/tape - 30/94, 32%; bandage - 18/94, 19%; shaving - 9/94, 10% (Q22). A typical EEG recording ranged from 10 to 2,880 min (48 h) (median = 30 min, IQR = 40 min; Q23) (Figure 3).

Approaches to EEG review (Q24-Q27):

In asking whether respondents interpreted their own EEGs, 60% (56/93) selected 'yes', 28% (26/93) selected 'sometimes', and 12% (11/93) selected 'no' (Q24). A follow-up question asked who, other than the respondent, interpreted EEG (Q25). Sixty-three percent (46/73 responses), indicated that they may also consult a colleague, supervisor, friend, or expert for help with interpretation (Q25). As part of EEG interpretation, software algorithms were used by a minority (11/92, 12%; Q26). Respondents reported using the following software: Persyst (3), Polaris, Cadwell Arc Essentia, NicoletOne, NeuroGuide, and iEEG. Three respondents reported using software but did not provide the manufacturer. Both bipolar and reference montages were used for visual review by a majority (49/92, 53%; Q27). The remaining respondents used either bipolar (15/92, 16%), referential (18/92, 20%), or were unsure (10/92, 11%; Q27).

Discussion

This survey provides an update on EEG usage and techniques for dogs that have evolved since the last survey, over 30 years ago (18). The number of responses (180/517, 35%) represents significant engagement from the veterinary neurology world with a response rate similar to that reported for physician specialist response rates for web-based surveys (24). This support strengthens our conclusions about canine EEG usage and its barriers, techniques in veterinary EEG, and approaches to reviewing EEGs.

Canine EEG usage and its barriers (Q1-Q8)

Even though most respondents have at one point used EEG in dogs (75%), active usage is lower (44%). This supports the hypothesis that fewer than 50% of veterinary neurologists perform EEGs in practice. The low active usage raises the question whether EEG is performed more frequently during the residency training period. The survey examined barriers to the performance of EEGs in dogs. The most common barrier was lack of available EEG equipment (37%), but insufficient cases also decreased the frequency of EEGs (Table 2). Considering the lower active usage of EEG, a further question is whether EEG units are more likely to be found at centres with residency training programs. A deeper exploration of the availability of EEG units and barriers to their acquisition should be the next step if we are to see more frequent use amongst veterinary neurologists.

Another considerable barrier for veterinary neurologists was lack of training and experience in the procedure itself and its

interpretation (19%). This can be addressed by providing advanced continuing education, as well as adjusting learning outcomes within the residency training process. Labour and cost-effectiveness were also factors as respondents were concerned about financial costs associated with EEGs to both the clinic and the pet owners, limited diagnostic value, not enough support staff, and time constraints (Table 2). These factors will likely improve as the body of knowledge advances. As cost effectiveness improves, better client compliance would be expected for consent to an EEG, as the most common barrier was cost (28%, Table 2). Notably the lack of equipment, caseload, and cost are not independent variables. These barriers identify areas for future research and development.

When it is used, EEG is performed annually or less frequently – this cannot be specified due to the limitation of the question format. The most common indication is to determine a discrete event diagnosis – is the dog truly having a seizure or could it be a behavioral or movement disorder? This common indication correlates with the published consensus proposal for diagnosing small animal epilepsy (4), establishing the Tier III confidence level for the diagnosis of idiopathic epilepsy. This indicates broad support for the clinical guidelines of the consensus proposal. Other indications included monitoring treatment, determining brain death, identifying the type of seizure or epilepsy, localizing foci, sleep disorders, for research purposes, and post-op brain surgery monitoring (Table 1) consistent with its use in people (25).

Techniques in veterinary EEG (Q9-Q23)

The survey findings differed from the predicted high variability in EEG technique. There was less variability than expected in EEG techniques and protocols, with the penetrance of many protocols exceeding 20%. Most respondents reported using a wired EEG machine (74%), versus a wireless EEG machine, perhaps reflecting the age and cost of EEG units in use. Wireless machines are a newer technology, particularly those incorporating synchronized video. Video synchronized recording improves the diagnostic utility for people and dogs (7, 26). The survey found that half of clinicians use video with their EEG recording (50%). If the hypothesis regarding the age of existing EEG units is true, repeating this survey in a few years would demonstrate increased wireless video EEG use.

The most used electrodes are the subdermal wire electrodes (66%), followed by steel needle electrodes (23%) and then skin surface electrodes (11%). This difference from the previously reported use of steel needle and skin surface electrodes only highlights technological advances (18). Subdermal wire electrodes, first described in 2005, have the benefits of low maintenance and durability for longer recordings, as well as advanced imaging compatibility (27, 28). While this survey did not investigate the reasons for the uptake of subdermal wire electrodes, we propose that their popularity is due to their low maintenance requirements.

There was a large range in the number of electrodes used, anywhere between 6 to 32 including ground and reference, with an average of 12 electrodes per recording (mean = 12, median = 12, mode = 12). The most popular electrode array out of the 8 maps reported in our survey was the Holliday and Williams 15-electrode array map which was used by only 33% of respondents (20). This low majority explains the variation seen in the number of electrodes. The previous survey reported the Redding and Knecht 5-electrode array map (1984) as the most common (19). This interesting shift may represent a generational change over the intervening 30 years. Of the 8 maps identified in our survey, the Holliday and Williams map is the oldest (1999) and therefore might be expected to have the greatest penetrance (20). This data will be useful to validate, standardize or harmonize electrode placement arrays. There do appear to be commonalities to these maps which could be used for future harmonization.

The survey identified that quality control is an area for future improvement. The majority of respondents (52%) use a visual inspection of the electrodes and tracings. Only 40% measured impedance. Impedance is a quality measure of the connection between the electrode and scalp (29). Although optimal impedance thresholds have yet to be determined in veterinary EEG, considering previous investigations (30) and the standards in people (22) suggests a threshold of 15kΩ is reasonable.

In the last survey, there was no distinction made between chemical restraint for instrumentation or recording periods (18). At the time, 6/9 (67%), used no chemical restraint. As it may be more practical to instrument a dog with chemical restraint, the current survey separated the two periods. Despite separating these two periods, the current survey found considerable variation in restraint approaches. Amongst our respondents, sedation is often used for electrode placement, conversely, recordings are often performed without sedation or anesthesia. The higher frequency of both awake recordings and wired EEG units suggests that some form of physical restraint is used, e.g., confinement in a crate or run in the clinic. With the ascendence of wireless EEG technology, the percentage of awake recordings will be expected to increase as it permits the dogs to behave freely.

Of the pharmaceuticals that were reported in instrumentation and recording, alpha-2 agonists dominate compared to the phenothiazine class 30 years ago. The need to understand the effects of pharmaceutical restraint is visible in recent explorations of the topic (22). The class of pharmaceuticals plays into the indications for the EEG, for example, determining the epileptic or non-epileptic nature of paroxysmal episodes and whether these episodes might be abolished by chemical restraint. Phenothiazines were also listed amongst activation techniques in the 1988 survey. Activation techniques were not investigated in the current survey due to the primary focus on EEG usage and its barriers. Recent discussions of activation techniques, like intermittent photic stimulation and hyperventilation, suggest that a more focused survey and research are required (31, 32).

The significant variation in the approach to fixing electrodes in place indicates an area of need. That a large proportion (44%) of EEGs are done without bandaging of some sort may affect the duration and quality of recordings. This was recognized by the one respondent whose technique included "prayer." That the recording time median and mode were 30 min, but the mean was 3.5 h with a range indicating significant variability, suggested that more work is required to identify the most effective recording period (33). Despite the commonalities in technique, there were some areas that remain open for improvement: number of electrodes, placement map, quality control, and fixation methods. Other technological developments since the previous survey, wireless EEG unit and video recording, may have different indications for use than the standard wired EEG machine. Detection of technology-specific indications was beyond the scope of this survey but would be an interesting area for future research.

Approaches to EEG review (Q24-Q27)

The current and previous surveys differed with respect to EEG review approaches due to technological advances. The old EEG

recordings could not be manipulated post-hoc, which was why the previous survey investigated settings like sensitivity and filters that needed to be adjusted at the time of recording. Nowadays, EEG review software allows adjustments of filters, sensitivity and re-montaging during post-collection review, while some programs even offer automated detection algorithms. In this environment, a high proportion of respondents interpret their own EEG recording without the help software algorithms (60%). Visual inspection rather than software algorithms is the predominant mode for interpretation (87%) likely recognizing that these seizure or spike detection algorithms have yet to be validated for dogs. For visual interpretation, both bipolar and reference montages were used by most respondents (53%) as opposed to reliance on a single montage only, which was a limitation of the pen-and-paper EEG machines. The risk of relying on a single montage for EEG review is an incomplete reconstruction of the three-dimensional cortical potential and is reduced by digital re-montaging post-hoc (34). Furthermore, there is a willingness to seek assistance with interpretation from a more experienced colleague, supervisor, or expert (63%), supporting mentorship and collegiality. It is encouraging to find high levels of self-confidence and collaboration regarding review and interpretation.

Limitations

Similar to the previous survey, this study suffers all the limitations associated with a survey-based design, including respondents being a subset of the target population, incomplete survey responses, response errors, and recall bias. The change in technology limited comparison between the two surveys, meaning that there were slightly different focuses. Administering the survey online and advertising through professional fora extended the survey penetration to the largest audience. Despite 180 respondents, not all questions were answered by all participants. Making the responses anonymous encouraged respondents to provide accurate, honest answers, or even answers that may have presented themselves unfavorably. The converse was that the anonymous responses meant that free text responses could not be linked to earlier responses, e.g., occasional responses reading "see previous answer" (Q14 and Q15), nor could we inquire about career length to normalize timing responses (Q4). The former issue resulted in an unanticipated overlap of results between questions 14 and 15 rendering question 15 less useful, despite initial survey validation. Further, questions 18 and 20 suffered technical glitches with the large response population despite internal preliminary validation; requesting proportions resulted in considerable variation in the results as seen by the large standard deviations and variances. Hindsight also identified at least one question (Q6) where a forced choice limited answers. In retrospect, a larger initial focus group would have identified these issues in the collation of results. To control for recall bias, the survey included images, for example, the electrode map, as well as opportunities for respondents to upload their own images.

Conclusion

EEG techniques for dogs have evolved over the last 30 years. Fewer than 50% of veterinary neurologists are currently performing EEG and it is performed infrequently. Clinical barriers to the performance of EEG in dogs were mainly equipment availability, insufficient cases, and financial costs to clients. These factors are likely interrelated. Clarity of indications and educational support would build confidence in the use of this diagnostic technique. This survey has identified commonalities of technique and several areas for development. These findings will form the basis for harmonization of canine EEG techniques, thus improving its reliability as a diagnostic test. A validated and standardized canine EEG protocol is hoped to improve the diagnosis and treatment of canine epilepsy. Given the functional similarities between human and dog EEG and epilepsy, basic studies of this nature will support significant advancements in canine epilepsy and EEG with translational implications.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

JL, SM, TP, MH, AZ, LG, and FJ: conception, testing, and design. JL and FJ: acquisition and analysis of the data and drafting of the article. JL, SM, TP, MH, AZ, LG, and FJ: revising the article for intellectual content and final approval of completed article.

Funding

We acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC), [funding reference number RGPIN-2021-02606].

Cette recherche a été financée par le Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG), [numéro de référence RGPIN-2021-02606].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1198134/full#supplementary-material

References

1. Kearsley-Fleet L, O'Neill DG, Volk HA, Church DB, Broadbelt DC. Prevalence and risk factors for canine epilepsy of unknown origin in the UK. *Vet Rec*. (2013) 172:338. doi: 10.1136/vr.101133

2. Heske L, Nødtvedt A, Jäderlund KH, Berendt M, Egenvall A. A cohort study of epilepsy among 665,000 insured dogs: incidence, mortality and survival after diagnosis. *Vet J*. (2014) 202:471–6. doi: 10.1016/j.tvjl.2014.09.023

3. Famula TR, Oberbauer AM, Brown KN. Heritability of epileptic seizures in the Belgian tervueren. *J Small Anim Pract*. (1997) 38:349–52. doi: 10.1111/j.1748-5827.1997.tb03483.x

4. De Risio L, Bhatti S, Muñana K, Penderis J, Stein V, Tipold A, et al. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. *BMC Vet Res*. (2015) 11:148. doi: 10.1186/s12917-015-0462-1

5. Ukai M, Parmentier T, Cortez MA, Fischer A, Gaitero L, Lohi H, et al. Seizure frequency discrepancy between subjective and objective ictal electroencephalography data in dogs. *J Vet Intern Med*. (2021) 35:1819–25. doi: 10.1111/jvim.16158

6. Hasegawa D. Diagnostic techniques to detect the epileptogenic zone: pathophysiological and presurgical analysis of epilepsy in dogs and cats. *Vet J*. (2016) 215:64–75. doi: 10.1016/j.tvjl.2016.03.005

7. James FMK, Cortez MA, Monteith G, Jokinen TS, Wielaender F, Fischer A, et al. Diagnostic utility of wireless video-electroencephalography in Unsedated dogs. *J Vet Intern Med*. (2017) 31:1469–76. doi: 10.1111/jvim.14789

8. Poma R, Ochi A, Cortez MA. Absence seizures with myoclonic features in a juvenile Chihuahua dog. *Epileptic Disord*. (2010) 12:138–41. doi: 10.1684/epd.2010.0312

9. Britton JW, Frey LC, Hopp JL, Korb P, Koubeissi MZ, Lievens WE, et al. Electroencephalography (EEG) In: EK St. Louis and LC Frey, editors. *An introductory text and atlas of Normal and abnormal findings in adults, children, and infants [internet]*. Chicago: American Epilepsy Society (2016). 22.

10. Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the international league against epilepsy: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia*. (2017) 58:522–30. doi: 10.1111/epi.13670

11. Wielaender F, James FMK, Cortez MA, Kluger G, Neßler JN, Tipold A, et al. Absence seizures as a feature of juvenile myoclonic epilepsy in Rhodesian ridgeback dogs. *J Vet Intern Med*. (2018) 32:428–32. doi: 10.1111/jvim.14892

12. Serrano S, Hughes D, Chandler K. Use of ketamine for the management of refractory status epilepticus in a dog. *J Vet Intern Med*. (2006) 20:194–7. doi: 10.1111/j.1939-1676.2006.tb02841.x

13. Raith K, Steinberg T, Fischer A. Continuous electroencephalographic monitoring of status epilepticus in dogs and cats: 10 patients (2004-2005). *J Vet Emerg Crit Care (San Antonio)*. (2010) 20:446–55. doi: 10.1111/j.1476-4431.2010.00544.x

14. Cuff DE, Bush WW, Stecker MM, Williams DC. Use of continuous electroencephalography for diagnosis and monitoring of treatment of nonconvulsive status epilepticus in a cat. *J Am Vet Med Assoc*. (2014) 244:708–14. doi: 10.2460/javma.244.6.708

15. Blades Golubovic S, Rossmeisl JH Jr. Status epilepticus in dogs and cats, part 1: etiopathogenesis, epidemiology, and diagnosis. *J Vet Emerg Crit Care (San Antonio)*. (2017) 27:278–87. doi: 10.1111/vec.12605

16. Blades Golubovic S, Rossmeisl JH Jr. Status epilepticus in dogs and cats, part 2: treatment, monitoring, and prognosis. *J Vet Emerg Crit Care (San Antonio)*. (2017) 27:288–300. doi: 10.1111/vec.12604

17. Granum LK, Bush WW, Williams DC, Stecker MM, Weaver CE, Werre SR. Prevalence of electrographic seizure in dogs and cats undergoing electroencephalography and clinical characteristics and outcome for dogs and cats with and without electrographic seizure: 104 cases (2009-2015). *J Am Vet Med Assoc*. (2019) 254:967–73. doi: 10.2460/javma.254.8.967

18. Steiss JE. A survey of current techniques in veterinary electrodiagnostics: EEG, spinal evoked and brainstem auditory evoked potential recording. *Vet Res Commun*. (1988) 12:281–8. doi: 10.1007/BF00343246

19. Redding RW, Knecht CD. *Atlas of electroencephalography in the dog and cat*. Praeger (1984).

20. Holliday TA, Williams DC. Clinical electroencephalography in dogs. *Vet Neurol Neurosurg J*. (1999) 60:181–7. doi: 10.1515/jvetres-2016-0027

21. Pellegrino FC, Sica RE. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. *Clin Neurophysiol*. (2004) 115:477–87. doi: 10.1016/s1388-2457(03)00347-x

22. Tepper LC, Shores A. Electroencephalographic recordings in the canine: effects of low dose Medetomidine or Dexmedetomidine followed by Atipamezole. *Open J Vet Med*. (2014) 4:7–13. doi: 10.4236/ojvm.2014.42002

23. Wrzosek MA. Electroencephalography as a diagnostic technique for canine neurological diseases. *J Vet Res*. (2016) 60:181–7. doi: 10.1515/jvetres-2016-0027

24. Cunningham CT, Quan H, Hemmelgarn B, Noseworthy T, Beck CA, Dixon E, et al. Exploring physician specialist response rates to web-based surveys. *BMC Med Res Methodol*. (2015) 15:32. doi: 10.1186/s12874-015-0016-z

25. Acharya JN, Hani AJ, Thirumala PD, Tsuchida TN. American clinical neurophysiology society guideline 3: a proposal for standard montages to be used in clinical EEG. *J Clin Neurophysiol*. (2016) 33:312–6. doi: 10.1097/WNP.0000000000000317

26. Watemberg N, Tziperman B, Dabby R, Hasan M, Zehavi L, Lerman-Sagie T. Adding video recording increases the diagnostic yield of routine electroencephalograms in children with frequent paroxysmal events. *Epilepsia*. (2005) 46:716–9. doi: 10.1111/j.1528-1167.2005.50004.x

27. Ives JR. New chronic EEG electrode for critical/intensive care unit monitoring. *J Clin Neurophysiol*. (2005) 22:119–23. doi: 10.1097/01.WNP.0000152659.30753.47

28. James FM, Allen DG, Bersenas AM, Grovum WL, Kerr CL, Monteith G, et al. Investigation of the use of three electroencephalographic electrodes for long-term electroencephalographic recording in awake and sedated dogs. *Am J Vet Res*. (2011) 72:384–90. doi: 10.2460/ajvr.72.3.384

29. Kappenman ES, Luck SJ. The effects of electrode impedance on data quality and statistical significance in ERP recordings. *Psychophysiology*. (2010) 47:888–904. doi: 10.1111/j.1469-8986.2010.01009.x

30. Luca J, Hazenfratz M, Monteith G, Sanchez A, Gaitero L, James F. Electrode scalp impedance differences between electroencephalography machines in healthy dogs. *Can J Vet Res*. (2021) 85:309–11.

31. Wielaender F, James FMK, Cortez MA, Kornberg M, Bathen-Noethen A, Flegel T, et al. Generalized myoclonic epilepsy with photosensitivity in juvenile dogs caused by a defective DIRAS family GTPase 1. *Proc Natl Acad Sci USA*. (2017) 114:2669–74. doi: 10.1073/pnas.1614478114

32. Brauer C, Kästner SBR, Schenk HC, Tünsmeyer J, Tipold A. Electroencephalographic recordings in dogs: prevention of muscle artifacts and evaluation of two activation techniques in healthy individuals. *Res Vet Sci*. (2011) 90:306–11. doi: 10.1016/j.rvsc.2010.06.004

33. Parmentier T, Monteith G, Cortez M, Wielaender F, Fischer A, Jokisen TS, et al. Effect of prior general anesthesia or sedation and antiseizure drugs on the diagnostic utility of wireless video electroencephalography in dogs. *J Vet Intern*. (2020) 34:1967–74. doi: 10.1111/jvim.15856

34. Acharya JN, Hani AJ, Thirumala PD, Tsuchida TN. American clinical neurophysiology society guideline 3: a proposal for standard montages to be used in clinical EEG. *J Clin Neurophysiol*. (2016) 33:312–6. doi: 10.1097/WNP.0000000000000317

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 25 July 2023
DOI 10.3389/fvets.2023.1236275

OPEN ACCESS

EDITED BY
Edward (Ned) E. Patterson,
University of Minnesota Twin Cities,
United States

REVIEWED BY
Alejandra Mondino,
North Carolina State University, United States
Sam Long,
Veterinary Referral Hospital, Australia

*CORRESPONDENCE
Daisuke Hasegawa
✉ disk-hsgw@nvlu.ac.jp

†PRESENT ADDRESSES:
Yoshihiko Yu,
Mitaka, Tokyo, Japan

Yuji Hamamoto,
Laboratory of Companion Animal Surgery,
Rakuno Gakuen University, Ebetsu, Japan

RECEIVED 07 June 2023
ACCEPTED 10 July 2023
PUBLISHED 25 July 2023

CITATION
Mizuno S, Asada R, Yu Y, Hamamoto Y and Hasegawa D (2023) Investigation of the effect and availability of ketamine on electroencephalography in cats with temporal lobe epilepsy.
Front. Vet. Sci. 10:1236275.
doi: 10.3389/fvets.2023.1236275

Investigation of the effect and availability of ketamine on electroencephalography in cats with temporal lobe epilepsy

Satoshi Mizuno[1], Rikako Asada[1], Yoshihiko Yu[1†], Yuji Hamamoto[1,2†] and Daisuke Hasegawa[1*]

[1]Laboratory of Veterinary Clinical Neurology, Graduate School of Nippon Veterinary and Life Science University, Musashino, Japan, [2]Veterinary Medical Teaching Hospital, Nippon Veterinary and Life Science University, Musashino, Japan

In recent years, electroencephalography (EEG) in veterinary medicine has become important not only in the diagnosis of epilepsy, but also in determining the epileptogenic focus. In cats, sedation and immobilization, usually with medetomidine or dexmedetomidine, are necessary to place the electrodes and to obtain stable scalp EEG recordings. In this study, we hypothesized that, for cats with temporal lobe epilepsy (TLE), ketamine, a sedative/anesthetic and N-methyl-D-aspartate (NMDA) antagonist that activates the limbic system and is also used to treat refractory status epilepticus in dogs, would induce sufficient sedation and immobilization for EEG, as well as induce interictal epileptiform discharges (IEDs) that are more pronounced than those induced with medetomidine. We obtained EEG recordings from TLE cats and healthy cats administered either ketamine or medetomidine alone (study 1) or ketamine after medetomidine sedation (study 2). In study 1, the frequency of IEDs showed no statistically significant difference between ketamine and medetomidine in both TLE and healthy cats. Seizures were observed in 75% (9/12) cats of the TLE group with ketamine alone. When ketamine was administered after sedation with medetomidine (study 2), 3/18 cats in the TLE group developed generalized tonic-clonic seizure and 1/18 cats showed subclinical seizure activity. However, no seizures were observed in all healthy cats in both study 1 and study 2. Slow wave activity at 2–4 Hz was observed in many individuals after ketamine administration regardless studies and groups, and quantitative analysis in study 2 showed a trend toward increased delta band activities in both groups. While there was no significant difference in the count of IEDs between medetomidine and ketamine, ketamine caused seizures in cats with TLE similar to their habitual seizure type and with a higher seizure frequency. Our results suggest that ketamine may activate epileptiform discharges during EEG recordings. However, caution should be used for cats with TLE.

KEYWORDS

EEG, feline, interictal epileptiform discharge, ketamine, medetomidine, seizure, TLE

1. Introduction

Ketamine (KET) is a noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist that was developed in the 1960s. Corssen et al. (1) reported that the corticothalamic system is suppressed while the limbic system is activated in cats using KET, thus, KET is referred to as a "dissociative anesthetic." In veterinary medicine, KET is generally used for restraint, sedation,

and analgesia. In addition to these applications, KET has been recently used to control refractory (GABAergic drugs-resistant) status epilepticus (RSE) in dogs (2). However, studies on the effects of KET on epilepsy are inconsistent, with reports stating that KET has antiseizure effects (3, 4), while other reports state that KET induces seizure activity (5).

Electroencephalography (EEG) is one of the most important diagnostic tools in epilepsy and has also attracted attention for detecting the epileptogenic zone for both seizure classification and epilepsy surgery (6, 7). However, in the veterinary field, especially in cats, EEG usage is not prevalent. As one factor of this, EEG in cats with high-density coats and hard scalps requires strict sedation and immobilization for electrode placement and stable recording. Some studies have reported the use of sedatives, typically medetomidine (MED) or the enantiomer of MED, dexmedetomidine, to perform scalp EEG in dogs and cats (8–10). MED and dexmedetomidine produce sedative and analgesic effects enough for EEG recording and can be rapidly antagonized by the administration of atipamezole (11, 12). Therefore, MED or dexmedetomidine is preferred for EEG recording in animals. In a canine EEG study with MED, dogs with severe seizures had a predominantly higher incidence of interictal epileptiform discharges (IEDs) than control dogs (13). However, there are cases in which IED cannot be detected in actual EEG measurements, even if the patient has epilepsy, and repeated EEG testing is recommended depending on the case (14). However, because of the burdens associated with EEG in animals, including the administration of sedatives, an agent that is safe and does not interfere with IED detection would be ideal.

In previous studies, we reported familial spontaneous epileptic cats (FSECs) with suspected genetic epilepsy. FSECs have spontaneous focal limbic seizures with or without evolving to generalized tonic-clonic seizures (GTCS), and, therefore, are classified as temporal lobe epilepsy (TLE) (15–21). The epileptogenic zone of FSECs is presumed to exist in the hippocampus or amygdala, which are parts of the limbic system, or both based on the previous evaluations of symptomatogenic, irritative, seizure-onset, structurally abnormal, and functional deficit zones (22).

Although feline EEG using KET was examined in the past (4, 5, 23–26), to our knowledge, there are no reports of such studies conducted in cats with spontaneous TLE. Therefore, we hypothesized that the use of KET, which stimulates the limbic system and inhibits the cortex, in EEG in cats with TLE would increase the number of IEDs. To verify this hypothesis, we conducted scalp EEG in healthy and TLE cats after the sole administration of KET or MED (study 1). Then, according to the results from study 1, we evaluated the effect of KET after sedation with MED on scalp EEG in cats with TLE (study 2).

2. Materials and methods

2.1. Animals

This study, including the care and maintenance of the FSECs colony, healthy, and epileptic cats genetically unrelated to FSECs, was approved by the Animal Care and Use Committee of Nippon Veterinary and Life Science University (accession Nos. 2020K-3, 2021K-2, 2022K-2; the principal investigator is DH).

The study included 24 cats, divided into two groups: the TLE group and the control group. The TLE group included 17 FSECs (Nos. 1–17) and one cat with TLE not related to FSEC strain (No. 18). FSECs show the typical seizure type of feline TLE such as orofacial automatism, salivation, mydriasis, head-turning, and sometimes evolve to GTCS (27). Both physical and neurological examinations were normal. Cat No. 18 had an initial seizure at the age of 50 months and presented focal limbic seizures with or without evolving to GTCS similar to FSECs. Physical and neurologic examinations, complete blood count, serum chemistry, urinalysis, and magnetic resonance imaging showed no abnormalities, and previous EEG revealed IEDs (spikes) in the left anterior temporal region. These cats were observed with seizures for more than 1 year using a video monitoring system. In the TLE group, seizures were observed in cats No. 1 (12 seizures/year), No. 2 (19 seizures/year), No. 4 (1 seizure/year), and No. 18 (14 seizures/year), while other cats had no seizures in the year before inclusion in this study. All cats have not been treated with daily antiepileptic seizure medication; however, they received temporary treatment for severe cluster seizures and/or status epilepticus as appropriate. The TLE group consisted of 12 males and 6 females (3 of which were neutered), and had a median age and body weight of 127 months (range, 94–157) and 3.6 kg (range, 2.5–7.2), respectively. The control group included 6 healthy cats (Nos. 19–24; 2 males and 4 females) without any documented seizures. The control group had a median age and body weight of 94 months (range, 77–107) and 3.6 kg (range, 3.1–6.4), respectively. Signalment and seizure frequency for each individual at the time of study inclusion are summarized in Supplementary Table1.

2.2. Study 1: EEG by sole administration of ketamine or medetomidine

2.2.1. Sedation protocol

Twelve cats (Nos. 1–5 and 18 from the TLE group, and Nos. 19–24 from the control group) were subjected to EEG with the sole administration of either MED or KET on a separate day for each individual. All cats were restricted in feeding and drinking for 12 h before EEG recording. EEG was performed once with MED (Domitor®, Zenoaq, Japan) alone. EEG was also performed once with KET (Ketalar®, Daiichi-Sankyo, Japan) alone, but performed again (i.e., twice) on another day if epileptic seizures occurred or if sufficient EEG recordings could not be obtained, to confirm reproducibility. Each test was performed at least 1 week apart. MED (median dose, 40 μg/kg; range, 30–50) or KET (median dose, 7.5 mg/kg; range, 5.0–13.3) was administered intramuscularly according to the sedation status of each individual. All cats sedated with MED received an intramuscular administration of the MED antagonist atipamezole (Antisedan®, Zenoaq, Japan) after the EEG recording.

2.2.2. EEG recordings

EEG recordings were conducted while the cats were under adequate sedation. After the cats were placed in the prone position, recording needle electrodes were placed subcutaneously on the bilateral frontal (F3/F4), central (C3/C4), temporal (T3/T4), and occipital (O1/O2) regions, as well as three midlines (Fz, Cz, Pz), and the reference and the ground electrodes were placed at the apex of the nose and the neck, respectively (20) (Figure 1). EEGs were measured with a digital EEG unit (Neurofax®EEG-1200, Nihon Kohden, Japan) under following parameters: sampling frequency, 1,000 Hz; sensitivity, 5–10 μV/mm; time constant, 0.1–0.3 s; Hi-cut filter, 60 Hz; and total recording time, 20–30 min. Electrocardiogram was monitored

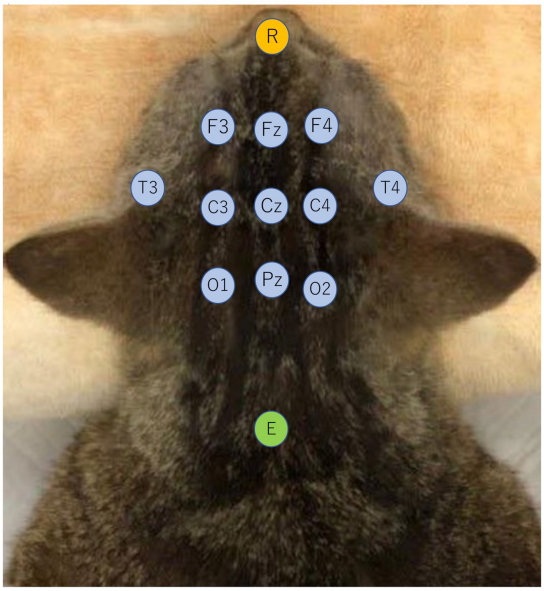


FIGURE 1
Electrode placement viewed from the dorsal aspect of a cat. Recording electrodes are bilateral frontal (F3/F4), central (C3/C4), temporal (T3/T4), occipital (O1/O2), and 3 longitudinal midline electrodes (Fz, Cz, Pz) indicated in light blue. The reference electrode (R) was placed on the dorsal surface of the nasal tip, indicated in orange, and the ground electrode (E) was placed at the level of the axial spinous process, indicated in green.

simultaneously using bipolar electrocardiogram leads in the same EEG unit.

2.2.3. Visual analysis of EEG

In the present study, we define and use "incidence" as "the rate of occurrence" and "frequency" as "Hz."

The recorded EEG montage was set to reference derivation and bipolar derivation, including average reference derivation. Stable recorded periods were randomly selected and evaluated for a total of 5–10 min to assess the incidence of IED per minute. One EEG reading-trained veterinarian (SM) counted IEDs. If the stable recorded periods were less than 5 min, they were excluded from the analysis. If a seizure occurred before sufficient EEG could be recorded, the evaluation of IEDs in the EEG measurements under KET sedation was performed after the seizure had ended. IEDs including spikes, polyspikes, spike-and-waves, and sharp-waves were counted and the region(s) with the highest incidence of IEDs was identified. When an IED was completely synchronized in all derivations, it was counted as one for all regions. The incidence of IEDs (IEDs/min) in each group was calculated, and the presence or absence of seizures after drug administration and their details were recorded. If seizures were observed after the administration of KET, EEG measurements were performed again on another day, and the average of the two measurements was analyzed as the IEDs for that individual. The incidence of IEDs in each group was analyzed by Wilcoxon signed rank test. Statistical analyses were performed using R version 4.1.0 (The R Foundation for Statistical Computing, Vienna, Austria). The significant difference in statistical analysis was defined as $p < 0.05$.

2.3. Study 2: EEG with ketamine administration after medetomidine sedation

2.3.1. Sedation protocol and EEG recording

Based on the results of study 1, we had planned to investigate the potential utility of KET as an EEG activator, i.e., increasing IED or inducing seizure activity, on scalp EEG under MED sedation. Twenty-four cats (Nos. 1–18 from the TLE group and No. 19–24 from the control group) were included. All cats were restricted in feeding and drinking for 12 h before the administration of MED. EEG recording was started after the cat was sedated by intramuscular MED and EEG was recorded for ≥10 min (MED), then KET was administered intravenously and EEG was recorded for another ≥10 min (MED-KET). The median dose of MED until each individual was sufficiently sedated to perform EEG was 30 μg/kg (range, 30–50). The dose of KET was 1.0 mg/kg, however, for cats (Nos. 2 and 18) that developed status epilepticus in study 1, the dose was decreased to 0.25 mg/kg. And for cat No. 3, which was not adequately sedated after 1.0 mg/kg KET administration, an additional dose of 0.5 mg/kg was given. Finally, the median dose of intravenous KET was 1.0 mg/kg (range, 0.25–1.5). After 20–30 min of recording of EEG following KET administration, an adequate dose of atipamezole was administered intramuscularly. The recording conditions of EEG were the same as described in study 1.

2.3.2. Visual analysis of EEG

Montages of the recorded EEG were constructed in the same manner as in study 1. Stable recordings obtained in MED and MED-KET periods were randomly selected and evaluated for each 5–10 min to assess the incidence of IEDs per minute. If the total time of evaluable recordings was <5 min after the administration of KET, the recordings were excluded from the analysis. For counting IEDs and identifying the highest incidence regions, the same methods were used as described in study 1. The incidence of IEDs between MED and MED-KET periods in each group was analyzed by Wilcoxon signed rank test. The significant difference in statistical analysis was defined as $p < 0.05$.

2.3.3. Quantitative analysis of EEG

Quantitative EEG analysis was set to average reference derivation. In both MED and MED-KET periods, a 2 s artifact-free period was visually selected (3 or 4 parts) during each recording period, and background activity was analyzed using a fast Fourier transform (FFT). Spectral bands were 0.5–4.0 Hz for delta band, 4.1–8.0 Hz for theta band, 8.1–13.0 Hz for alpha band, and 13.1–30.0 Hz for beta band. The relative power of the spectral bands was calculated for all leads and averaged. We analyzed each lead in each individual using Wilcoxon signed rank test. The significant difference in statistical analysis was defined as $p < 0.05$.

3. Results

3.1. Study 1: EEG by sole administration of ketamine or medetomidine

EEG recordings were obtained from all 12 cats administered MED only and were performed only once in each cat. On the other hand, EEG recordings of cats with KET administration alone were obtained

for 10/12 cats. Two cats were excluded from the EEG analysis: No. 18 from the TLE group experienced a seizure induced by KET administration, which lasted longer than 30 min (i.e., developed to status epilepticus): and KET administration could not induce sufficient sedation in No. 22 from the control group.

During the EEG recording with MED alone, no TLE or healthy cats showed seizures. However, when KET alone was administered, all cats in the TLE group (6/6) showed clinical seizures, thus, EEG with KET alone was performed twice in all TLE cats (a total of 12 recordings). Seizures were observed in TLE cats in 9 of the 12 recordings (75%), 6 of which progressed to GTCS. In some TLE cats (3/6 cats, 5/12 recordings), seizures did not end within 5 min spontaneously, so antiseizure medications (diazepam, midazolam, levetiracetam) were used to stop the seizure. The seizure signs observed after KET administration overlapped in part with those observed in spontaneous habitual seizures in 5/6 cats (Table 1). On the other hand, there was no cat that showed any seizures in the control group.

After the administration of KET, generalized slow waves of 2–4 Hz were observed intermittently in 4/5 cats of the TLE groups that were available for analysis (Figure 2). Since the number of slow waves tended to decrease with time, the maximum number of slow waves observed in each animal was determined by measuring 1 min from the time the slow waves were first observed. The average incidence of slow waves in the TLE cats was 26.3 cycles/min. Those intermittent slow waves lasted 20–40 min after KET administration. Similarly, intermittent slow waves of 2–5 Hz were observed in 2/5 cats of the control group. Slow waves were measured in the same way as in the TLE group, the average incidence of slow waves in the control group was 26.5 cycles/min observed over 20 min after KET administration.

Compared to the incidence of IED under MED in each individual, the incidence of IED under KET in the TLE group was activated (increased) in 2/5 cats and depressed (decreased) in 3/5 cats (KET: median, 0.70; range, 0.15–2.70; interquartile range (IQR), 0.25–1.05 vs. MED: median, 1.10; range, 0.70–2.00; IQR, 0.80–1.10). The incidence of IED under KET was depressed in all cats of the control group (5/5) (KET: median, 0.00; range, 0.00–0.10; IQR, 0.00–0.10 vs. MED: median, 0.50; range, 0.10–0.50; IQR, 0.20–0.50). There was no significant difference in the incidence of IED between KET and MED in both TLE ($p = 0.63$) and control groups ($p = 0.06$) (Figure 3). The incidence and highest incidence region of IEDs are summarized in Table 2. In the TLE group, the regions with the highest incidence of IEDs were compared between KET and MED, and agreement was found in 3 of 5 cases.

3.2. Study 2: EEG with ketamine administration after medetomidine sedation

EEG records were available for 23/24 cats, with one cat (No. 18 from the TLE group) excluded from the EEG analysis after having a seizure lasting more than 30 min (status epilepticus) after the administration of KET.

All 24 cats had no seizures after MED administration, whereas three cats (Nos. 1, 2, and 18) in the TLE group had GTCS after KET administration. In addition, a subclinical focal seizure activity was observed in another cat (No. 7) of the TLE group (Figure 4). The site of this subclinical seizure occurrence coincided with the site's highest incidence of IEDs under MED and KET. Of 23 cats that were available for analysis after KET administration, 18 cats had slow waves (12/16 from the TLE group, 6/6 from the control group). As in study 1, intermittent generalized slow waves of 1–4 Hz were observed within 1 min after KET administration. Slow waves tended to decrease with time and were measured in the same way as in study 1. The average incidence of slow waves in the TLE group was 43.8 cycles/min, and the average duration was 9.1 min. The average incidence of slow waves and the average duration in the control group were 43.3 cycles/min and 13.2 min, respectively. Changes in EEG findings observed after KET administration are summarized in Table 3.

Compared to the MED period in each individual, the incidence of IEDs of the MED-KET period in the TLE group was activated in 5/17 cats, depressed in 9/17 cats, and unchanged in 3/17 cats (MED: median, 0.80; range, 0.10–3.60; IQR, 0.50–1.10 vs. MED-KET: median, 0.80; range, 0.10–2.60; IQR, 0.20–1.30). In the control group, the incidence of IED was activated in 4/6 cats, depressed in 1/6 cats, and unchanged in 1/6 cats (MED: median, 0.10; range, 0.00–1.20; IQR, 0.03–0.18 vs. MED-KET: median, 0.30; range, 0.00–1.00; IQR, 0.13–0.40). There was no significant difference in the incidence of IED between MED and MED-KET regardless group (TLE group, $p = 0.53$; control group, $p = 0.25$) (Figure 5). The incidence and highest incidence region of IEDs are summarized in Table 4. In the TLE group, agreement of the regions of the highest

TABLE 1 Seizure signs observed during intramuscular ketamine administration and spontaneous seizures in temporal lobe epilepsy group.

Cat (No.)	Ketamine-induced	Spontaneous (habitual seizure)
1	Type 1[a]: orofacial automatism, urination, hypersalivation, mydriasis, GTCS	Looking around, mydriasis, orofacial automatism, head turning, GTCS
	Type 2[a]: orofacial automatism, GTCS	
2	Type 1[a]: looking around, forward leaning posture, mydriasis, GTCS	Looking around, nodding, facial twitching, head turning, GTCS
	Type 2[a]: looking around, orofacial automatism, GTCS	
3	Type 1[a]: mydriasis, orofacial automatism	Orofacial automatism, GTCS
4	Type 1: facial twitching	Mydriasis, urination, head turning, GTCS
5	Type 1[a]: myoclonic seizures	Facial twitching, orofacial automatism, myoclonic seizures, GTCS
18	Type 1[a]: orofacial automatism, GTCS	Looking around, mydriasis, hypersalivation, orofacial automatism, GTCS

GTCS, generalized tonic-clonic seizures.
[a]Ketamine-induced seizures had a similar seizure sign to the spontaneous seizures.

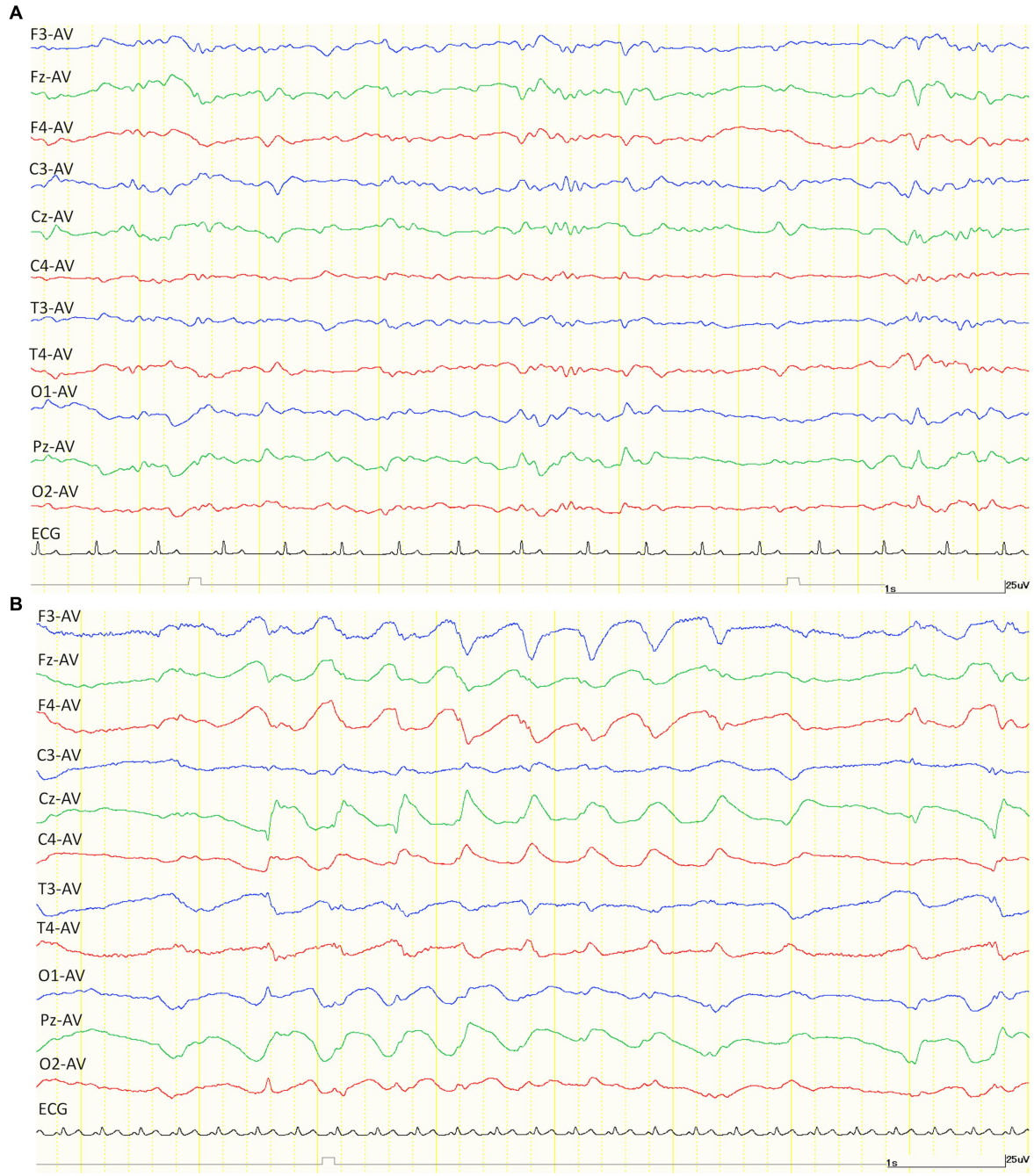


FIGURE 2
Examples of EEG trace after intramuscular administration of medetomidine (30 μg/kg) alone **(A)** and ketamine (7.5 mg/kg) alone **(B)**. Compared to medetomidine **(A)**, the EEG after administration of ketamine showed a generalized slow wave of about 2 Hz. Both EEG montages were set to average (AV) reference derivation. ECG, electrocardiogram.

incidence of IEDs between MED and MED-KET was observed in 10 of 17 cases.

For the quantitative analysis, 17 cats from the TLE group and 6 cats from the control group were included. Cat No. 18 was excluded from the analysis because sufficient EEG activity could not be recorded due to seizure activity. In both groups, there was a trend toward increased delta activity after KET administration when median relative power was compared (Figure 6). Significant differences between MED and MED-KET were observed in all sites, except for the theta band of C3 and the beta of F3, Fz, F4, and C4 in the TLE group, and the theta of F3 and beta of F3 and C4 in the control group (Supplementary Table 2).

4. Discussion

In this study, we investigated the effects of intramuscular KET alone or intravenous KET under MED sedation on scalp EEG in

10.3389/fvets.2023.1236275

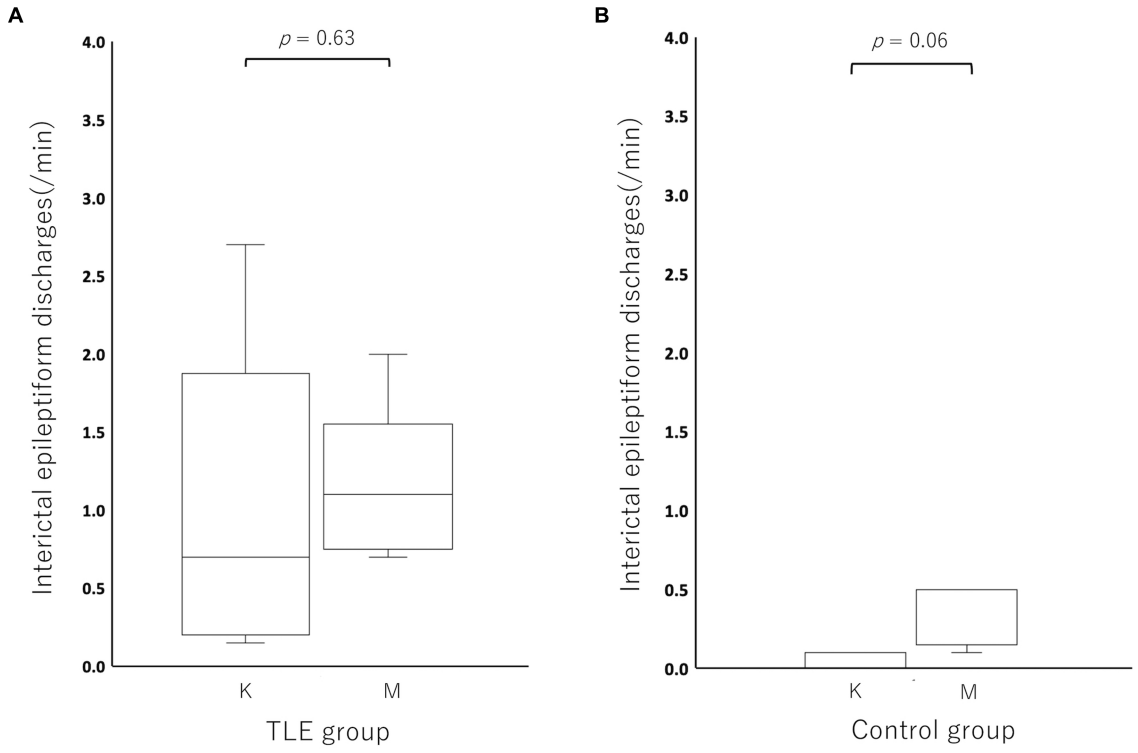


FIGURE 3
Boxplots for comparing the incidence of interictal epileptiform discharges between ketamine and medetomidine in the temporal lobe epilepsy (TLE) group **(A)** and control group **(B)**. There was no significant difference in IEDs between ketamine and medetomidine in the temporal lobe epilepsy group ($p = 0.63$) or in the control group ($p = 0.06$). K, ketamine; M, medetomidine.

TABLE 2 The incidence and the highest incidence region of interictal epileptiform discharges under ketamine or medetomidine.

Cat (No.)	Highest incidence region of IEDs		Incidence of IEDs (/min)	
	Ketamine	Medetomidine	Ketamine	Medetomidine
Temporal lobe epilepsy group				
1	Left temporal	Left temporal	2.7	2.0
2	Left temporal	Left temporal	0.7	1.1
3	Left occipital	Right central	0.25	1.1
4	Left temporal	Right temporal	0.15	0.8
5	Left temporal	Left temporal	1.05	0.7
18	NE	Left temporal	NE	0.8
Control group				
19	Left central	Median central, Left central	0.1	0.2
20	Right central	Left central	0.1	0.5
21	—	Left temporal	0.0	0.1
22	NE	Left frontal	NE	1.0
23	—	Left central	0.0	0.5
24	—	Left central	0.0	0.5

NE, not evaluable; IEDs, interictal epileptiform discharges.

healthy cats and cats with TLE. There were no significant differences in the incidence of IEDs between MED and KET nor between MED and MED-KET. In a previous report, KET suppressed focal seizures in 6 of 16 cats with epileptogenic focus produced by penicillin, and it did not affect IEDs in 10 cats (4). On the other hand, studies using KET in baboons have reported generalized IED activation in the scalp EEG (28). In the present study, the incidence of IEDs tended to be depressed by both KET alone and MED-KET in both control and

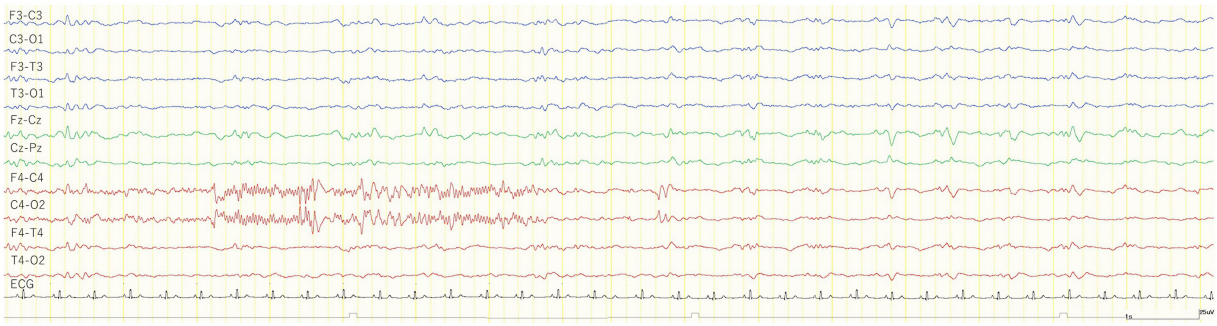


FIGURE 4
EEG trace of a subclinical seizure observed 7 min after the intravenous administration of 1 mg/kg ketamine during medetomidine sedation (cat No. 7). The cat was immobilized and no body movements were observed before and after a subclinical seizure. The recorded EEG montage was set to bipolar derivation. ECG, electrocardiogram.

TABLE 3 Changes in EEG activity after ketamine administration during medetomidine sedation.

Cat (No.)	EEG findings after ketamine administration	Incidence of slow wave (cycles/min)	Duration of slow wave (min)
Temporal lobe epilepsy group			
1	1–2 Hz GSW	18	8
2	No clear change was observed	—	—
3	No clear change was observed	—	—
4	2–3 Hz GSW	72	11
5	2 Hz GSW	23	5
6	2–4 Hz GSW	10	4
7	3 Hz GSW	58	14
8	No clear change was observed	—	—
9	No clear change was observed	—	—
10	2 Hz GSW	32	3
11	1–2 Hz GSW	60	6
12	2 Hz GSW	63	13
13	1 Hz GSW	45	20
14	2–3 Hz GSW	46	10
15	2 Hz GSW	49	8
16	2 Hz GSW	49	7
17	No clear change was observed	—	—
18	NE	—	—
Control group			
19	2 Hz GSW	43	20
20	1–2 Hz GSW	59	12
21	1–2 Hz GSW	47	13
22	2–3 Hz GSW	63	15
23	2 Hz GSW	21	13
24	2–3 Hz GSW	27	6

Incidence of slow wave: the count of waves during the first 1 min after it was observed. Duration of slow wave: the duration time from observation of slow waves to disappearance. NE, not evaluable; GSW, generalized slow wave.

TLE groups. Taking into account the previous study that KET suppressed the corticothalamic system and activated the limbic system (1), the trend in decreasing the incidence of IEDs in the present study may suggest that KET has an inhibitory effect on the corticothalamic system in cats. Although there were no statistically significant differences to support these trends in the present analysis, one factor

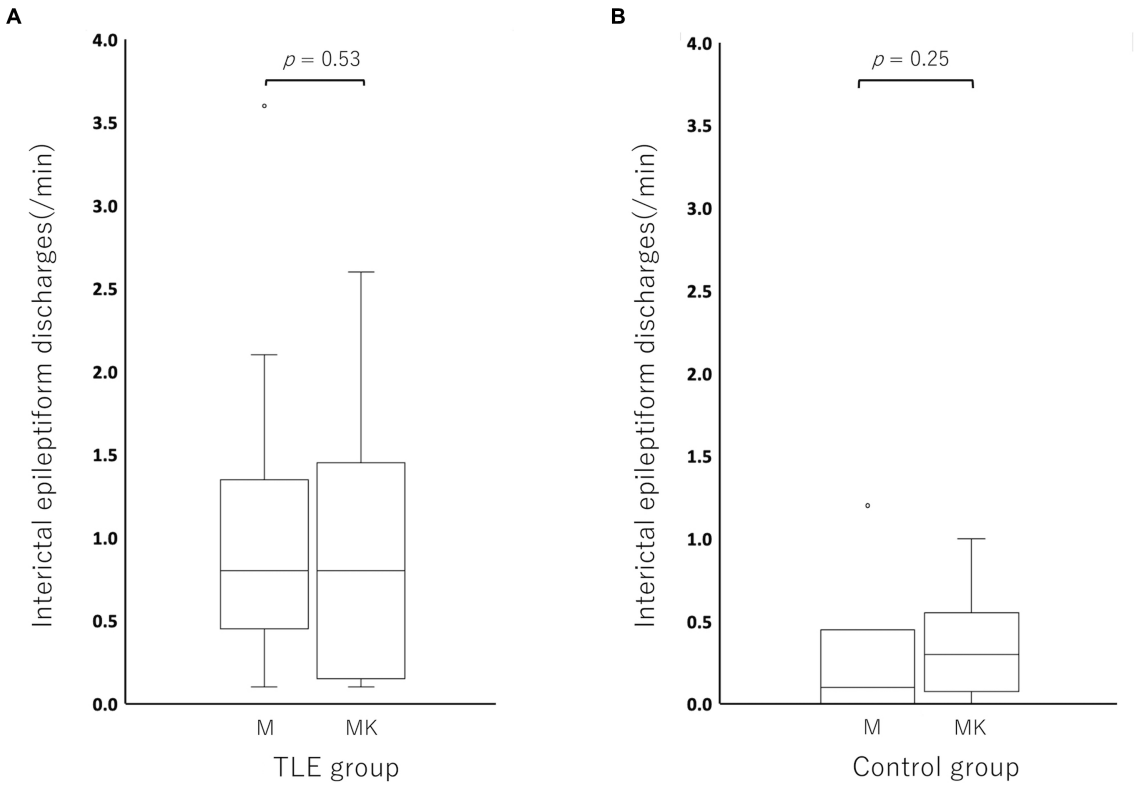


FIGURE 5
Boxplots for comparing the incidence of interictal epileptiform discharge (IED) between medetomidine and ketamine following medetomidine in the temporal lobe epilepsy (TLE) group **(A)** and control group **(B)**. Circles at the top of the box represent outliers. There was no significant difference between medetomidine alone and ketamine following medetomidine in the TLE group ($p = 0.52$), or in the control group ($p = 0.25$). M, medetomidine; MK, ketamine administration following medetomidine sedation.

may be the exclusion of individuals who did not have sufficient EEG records, especially with respect to the administration of KET alone.

In the TLE group, clinical seizures were observed in all cats after the administration of KET alone. The KET-induced seizure in 5 of 6 TLE cats was similar to their habitual seizures, i.e., limbic seizure to generalization (see Table 1 for details). In addition, a subclinical seizure was observed in one TLE cat (No. 7) after the administration of KET following MED sedation, the onset of which coincided with the regions with the highest incidence of IEDs. Although the mechanism by which KET induces seizures has not been elucidated, these facts suggest that KET activated the limbic system, which is the epileptogenic zone of TLE cats in this study. In addition, the three cats (Nos. 1, 2, and 18) that had clinical seizures induced by KET in both studies had a higher seizure frequency (12, 19, and 14 per year, respectively) than the others. Thus, whether the epileptogenic zone is activated with KET administration may depend on seizure frequency.

In the adult human, the detection rate of epileptic discharges is reported to be approximately 50% on the initial examination and increases to approximately 80%–90% with repeated testing (29). However, unlike humans, EEG in cats always requires immobilization using sedatives or anesthetics, and few EEGs are repeated. In addition, a recently developed ambulatory EEG system for dogs and humans may not be adopted for cats due to the nature of felines and the size and weight of the equipment. In this situation, capturing clinical seizure onset during a single scalp EEG is often difficult, depending on the individual's seizure frequency. A previous report suggested that low doses of KET may lower seizure thresholds within minutes of administration in people with a predisposition to focal epilepsy (30). Considering that we were able to capture seizures after KET administration compared to MED alone in the present study, KET administration may be useful for determining the epileptogenic zone.

In both studies of KET administration alone (study 1) and KET administration after MED (study 2), the generalized slow wave was observed in both TLE and control groups. Quantitative analysis showed that the delta band on background activity in both TLE and control groups was significantly increased, while other bands were significantly decreased in most areas. In a study with cats, electrocorticogram changes during KET administration under general anesthesia with ether were reported to occur in a dose-dependent manner, with doses below 5 mg/kg causing generalized beta activity and doses above 5 mg/kg causing alternating bursts of polyspike-slow waves and high-potential synchronous beta waves (4). In humans, low doses of KET were reported to cause the EEG to show fast oscillations in the high beta and low gamma, and that the slow oscillations exhibited by KET were more irregular than the slow oscillations caused by propofol and dexmedetomidine (31). That report also suggested that the beta and gamma oscillations were caused by KET's preference for NMDA receptors on inhibitory interneurons, which, in turn, increased cerebral metabolic rate, cerebral blood flow, and hallucinations (31). However, in the present study, we did not observe beta oscillations, and, in the quantitative analysis of study 2, beta oscillations were clearly reduced and a visually recognizable generalized slowing (2–5 Hz) was observed

10.3389/fvets.2023.1236275

TABLE 4 The incidence and the highest incidence region of interictal epileptiform discharges under medetomidine alone and ketamine following medetomidine.

Cat (No.)	Highest incidence region of IEDs		Incidence of IEDs (/min)	
	Medetomidine	Medetomidine-ketamine	Medetomidine	Medetomidine-ketamine
Temporal lobe epilepsy group				
1	Left temporal	Left temporal	1.8	2.6
2	Left temporal	Right temporal	0.7	0.2
3	Right central	Left temporal	0.8	0.1
4	Right temporal	Right temporal	1.0	1.7
5	Left central	Left central	1.1	0.7
6	Left occipital	Left temporal	1.6	1.6
7	Median frontal	Right central	0.6	1.3
8	Right temporal	Right temporal	1.1	1.0
9	Right temporal	Right temporal	0.5	0.8
10	Left temporal	Left temporal	3.6	1.2
11	Left central	Right temporal	0.4	0.1
12	Right temporal	Right temporal	0.5	0.6
13	Left central	Left central	2.1	1.9
14	Left central	Left temporal	0.3	0.3
15	Right central	Right central	0.1	0.1
16	Right temporal	Right temporal	0.3	0.1
17	Left temporal	Left temporal	1.1	0.9
18	Left temporal	NE	0.9	NE
Control group				
19	Left temporal	Left temporal	0.1	0.4
20	—	Right temporal	0.0	0.1
21	—	—	0.0	0.0
22	Left central	Left central	1.2	1.0
23	Left temporal	Left temporal	0.2	0.4
24	Left frontal	Left temporal, Left frontal	0.1	0.2

NE, not evaluable; IEDs, interictal epileptiform discharges.

in many cats after KET administration. The slow waves observed in study 1 may be due to cortical inhibition by KET, and the lack of fast oscillations as reported in the past (under anesthesia) may be due to insufficient immobilization by KET alone, which may have masked these fast oscillations. The reason why beta oscillations were reduced in study 2 and slow waves were observed in more individuals than in study 1 (6/10 vs. 18/22) may be due to cortical inhibition by KET and the pre-administration of MED. High-voltage and low-frequency activity expressed as delta and theta rhythms have been reported as MED-induced background activity in cats (9). In addition, it has been reported that intraperitoneal administration of MED and KET in rats increases low-frequency bands with only minor changes in the high-frequency bands (32). The KET-induced higher frequency band activity has been reported to be associated with increased cerebral metabolic rate and cerebral blood flow by inhibiting NMDA receptors, as described above, while alpha-2 agonist (MED) has been reported to decrease cerebral blood flow (33). Therefore, fast oscillations in the high beta and low gamma may have been suppressed in the present study.

In previous studies in normal cats or feline experimental models receiving KET, KET was administered under anesthesia, and changes in EEG were investigated (4, 5, 23–26). One of these reports evaluated the effect of KET on IEDs in the penicillin-induced seizure model of cats; however, quantitative evaluation has not been conducted (4). Another study reported that 5 of 22 (23%) healthy cats administered an anesthetic dose of KET (20 mg/kg IM) showed limbic seizures evolving to GTCS (23). However, to the best of our knowledge, there is no report of KET administration in cats with naturally occurring TLE so far. In the present study, we used cats with naturally occurring TLE to assess background activity, the presence of seizure activity, and the quantitative of IEDs under KET-induced sedation. We consider that the results of the present study provide important consideration for the use of KET in cats in the veterinary clinical setting.

We recognize there are several limitations to the present study. First, in the comparison of IEDs between KET and MED, most of the IED counts for KET in the TLE group were conducted in post-ictal EEG measurements because of a seizure induced

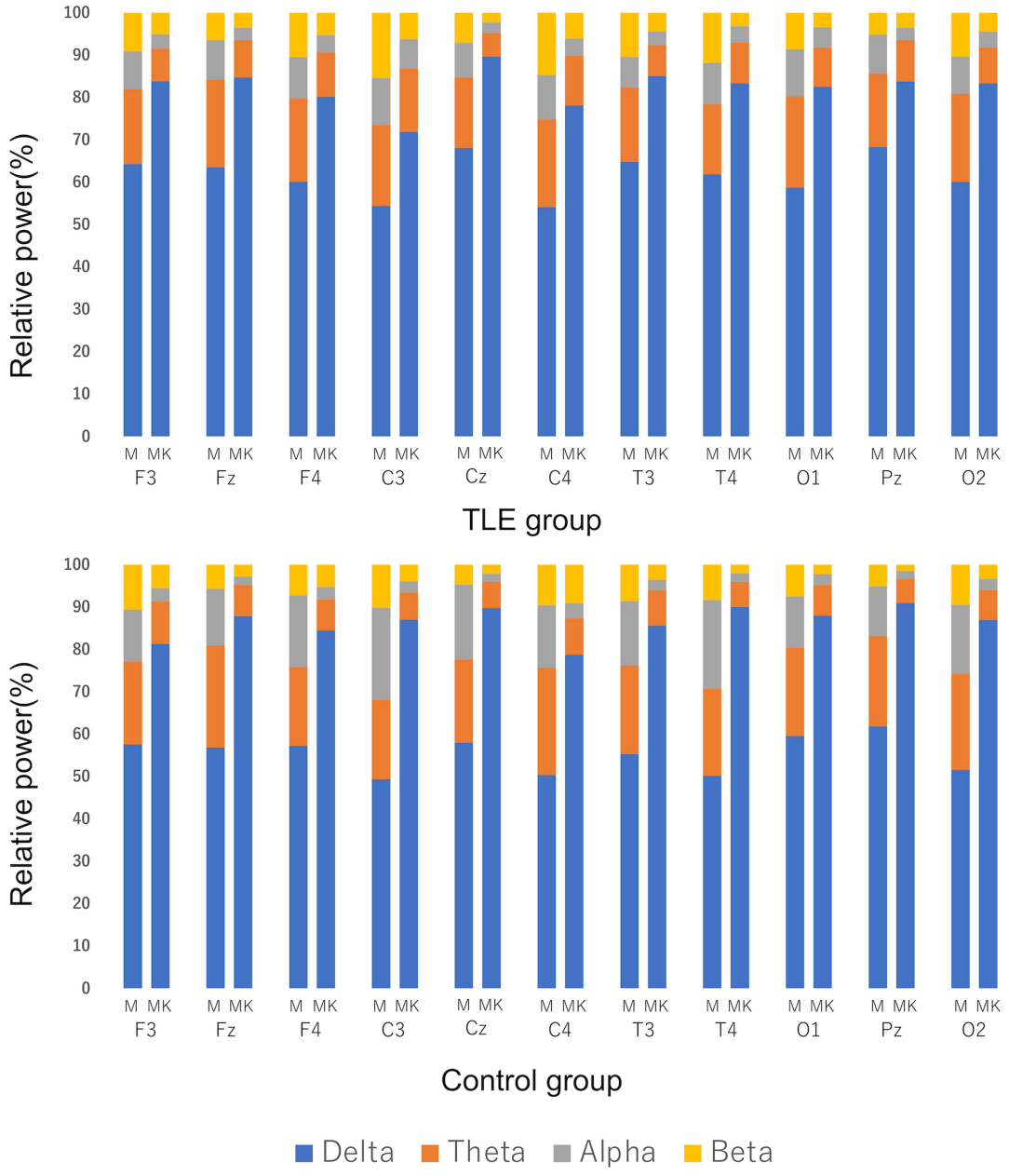


FIGURE 6
Comparison of median relative power using EEG recordings of medetomidine alone (M) and ketamine following medetomidine (MK). Relative power was defined as delta band (0.5–4.0 Hz), theta band (4.1–8.0 Hz), alpha band (8.1–13.0 Hz), and beta band (13.1–30.0 Hz). Vertical lines indicate the percentage of the relative power during M and MK at each site. There was a clear increase in the percentage of delta bands in both the temporal lobe epilepsy (TLE) group and the control group.

immediately after KET administration. It is known that postictal generalized EEG suppression (PGES) occurs immediately after an epileptic seizure (34). PGES are generally observed more frequently in convulsive seizures than in focal seizures, but reports on the frequency of occurrence and duration are very inconsistent (35). Therefore, PGES may have occurred after KET-induced seizures and reduced the number of IED in the present study. Second, we considered the dose variation of agents to obtain sufficient sedation for EEG recording as a possible reason for the variability in the results of this study. Although KET can produce dose-dependent EEG changes as mentioned above (4), the dose of KET alone in this study was at the upper limit of a typical sedation dose because it was intended to sedate sufficiently for measuring EEG and to avoid respiratory depression. Therefore, the large dose variability among individuals may have particularly affected the analysis. Because of individual differences in sedative effects, the administration of KET alone may result in a time lag or inadequate sedation before an EEG measurement can be performed. If the time lag between the administration and EEG measurement was significant, a subclinical seizure may have been missed. To confirm the acute effects of KET immediately after administration, as in this study,

simultaneous administration of a sedative such as MED may be necessary. Alternatively, EEG under intubation should be considered, given the possibility of respiratory depression. When an anesthetic dose of KET was administered, we expected that the reported bursts of polyspike-slow waves (i.e., elevated beta and delta bands) would be observed (4). However, in recent years, it suggested that the administration of KET under appropriate respiratory control might suppress EEG activity (36). Therefore, if appropriate respiratory management is performed after the administration of KET, a remarkable alteration in the power spectrum may be observed. Further investigation of the dose-depend effect of KET on EEG is necessary. Third, the fact that many cats included in the present study were older, and that there was a wide variation in seizure frequency prior to inclusion may have contributed to the variability in the results. An EEG study using two groups of cats divided according to age under sedation with MED reported that older cats had significantly higher relative power in the theta, alpha, and beta bands and lower in the delta band than younger cats (9). Thus, in our MED-KET study, the significant increase in the delta band might have been more pronounced in our older cats than in a young cat population.

In the present study, MED produced more stable sedation than KET alone in cats with TLE, and EEG measurements were feasible. Furthermore, KET is easy to induce seizures in cats with TLE. Therefore single-use KET as a sedative for EEG is not recommended from the results of this study. However, we would suggest that KET may be useful to activate seizure activities on EEG under MED sedation. This will be a critical tool to obtain ictal EEG for detecting the epileptogenic zone (irritative or seizure-onset zones). Although the combination of MED and KET has long been used as an anesthetic induction in veterinary medicine (11), caution will be needed for feline patients with a history of seizures. Recently, KET has received increasing attention in the management of RSE (2). However, our results emphasize that "Do not use ketamine as first line for treatment of status epilepticus (or seizure management)" and "use ketamine after confirmation of GABAergic/benzodiazepine-resistant (refractory) status epilepticus." Whether KET is an anti-seizure or pro-seizure medication, the present results indicate that KET has a proconvulsant feature for interictal cats with TLE and/or high seizure frequency.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study, including care and maintenance of the FSEC colony, healthy, and epileptic cats genetically unrelated to FSECs, was reviewed and approved by the Animal Care and Use Committee of Nippon Veterinary and Life Science University (accession Nos. 2020K-3, 2021K-2, 2022K-2; the principal investigator is DH).

Author contributions

SM, RA, and DH designed this study. SM and RA conducted experiments, data acquisition, and analyses. SM wrote the draft of the manuscript and performed statistics. SM and YY performed data visualization. YH, YY, and DH performed editing of the draft. All authors contributed to the article and approved the submitted version.

Funding

This study was partially supported by the Science Research Promotion Fund (grant numbers 2022-16 and 2023-18) from the Promotion and Mutual Aid Corporation for Private School of Japan (PMAC).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1236275/full#supplementary-material

References

1. Corssen G, Miyasaka M, Domino EF. Changing concepts in pain control during surgery: dissociative anesthesia with CI-581. A progress report. *Anesth Analg.* (1968) 47:746–59. doi: 10.1213/00000539-196811000-00022

2. Roynard P, Bilderback A, Dewey CW. Intravenous ketamine bolus(es) for the treatment of status epilepticus, refractory status epilepticus, and cluster seizures: a retrospective study of 15 dogs. *Front Vet Sci.* (2021) 8:547279. doi: 10.3389/fvets.2021.547279

3. Mccarthy DA, Chen G, Kaump DH, Ensor C. General anesthetic and other pharmacological properties of 2-(O-chlorophenyl)-2-methylamino cyclohexanone HCl (CI-581). *J New Drugs.* (1965) 5:21–33. doi: 10.1002/j.1552-4604.1965.tb00219

4. Celesia GG, Chen RC. Effects of ketamine on EEG activity in cats and monkeys. *Electroencephalogr Clin Neurophysiol.* (1974) 37:345–53. doi: 10.1016/0013-4694(74)90110-2

5. Kayama Y, Iwata K. The EEG, evoked potentials, and single-unit activity during ketamine anesthesia in cats. *Anesthesiology.* (1972) 36:316–28. doi: 10.1097/00000542-197204000-00004

6. Pillai J, Sperling MR. Interictal EEG and the diagnosis of epilepsy. *Epilepsia*. (2006) 1:14–22. doi: 10.1111/j.1528-1167.2006.00654.x

7. Hasegawa D, Asada R, Hamamoto Y, Yu Y, Kuwabara T, Mizoguchi S, et al. Focal cortical resection and hippocampectomy in a cat with drug-resistant structural epilepsy. *Front Vet Sci*. (2021) 8:719455. doi: 10.3389/fvets.2021.719455

8. Ukai M, Hamamoto Y, Yu Y, Fujiwara-Igarashi A, Fujita M, Hasegawa D. Efficacy of zonisamide on interictal electroencephalography in familial spontaneous epileptic cats. *J Feline Med Surg*. (2018) 20:962–7. doi: 10.1177/1098612X17740247

9. Wrzosek M, Nicpon J, Bergamasco L, Sammartano F, Cizinauskas S, Jaggy A. Visual and quantitative electroencephalographic analysis of healthy young and adult cats under medetomidine sedation. *Vet J*. (2009) 180:221–30. doi: 10.1016/j.tvjl.2007.11.003

10. Soison P, Aumarm W, Tiraphut B, Lekcharoensuk C, Lusawat A, Unnwongse K, et al. Efficacy of scalp electroencephalography (EEG) as a supportive diagnostic tool in canine epilepsy using dexmedetomidine. *Thai J Vet Med*. (2015) 45:435–42.

11. Cullen LK. Medetomidine sedation in dogs and cats: a review of its pharmacology, antagonism and dose. *Br Vet J*. (1996) 152:519–35. doi: 10.1016/s0007-1935(96)80005-4

12. Siegenthaler J, Pleyers T, Raillard M, Spadavecchia C, Levionnois OL. Effect of medetomidine, dexmedetomidine, and their reversal with atipamezole on the nociceptive withdrawal reflex in beagles. *Animals*. (2020) 10:1240. doi: 10.3390/ani10071240

13. Utsugi S, Saito M, Sato T, Kunimi M. Relationship between interictal epileptiform discharges under medetomidine sedation and clinical seizures in canine idiopathic epilepsy. *Vet Rec*. (2020) 187:67. doi: 10.1136/vr.104947

14. Krumholz A, Wiebe S, Gronseth G, Shinnar S, Levisohn P, Ting T, et al. Practice parameter: evaluating an apparent unprovoked first seizure in adults (an evidence-based review): report of the quality standards subcommittee of the American Academy of Neurology and the American Epilepsy Society. *Neurology*. (2007) 69:1996–2007. doi: 10.1212/01.wnl.0000285084.93652.43

15. Kuwabara T, Hasegawa D, Ogawa F, Kobayashi M, Fujita M, Suzuki H, et al. A familial spontaneous epileptic feline strain a novel model of idiopathic/genetic epilepsy. *Epilepsy Res*. (2010) 92:85–8. doi: 10.1016/j.eplepsyres.2010.08.010

16. Hamamoto Y, Hasegawa D, Mizoguchi S, Yu Y, Wada M, Kuwabara T, et al. Changes in the interictal and early postictal diffusion and perfusion magnetic resonance parameters in familial spontaneous epileptic cats. *Epilepsy Res*. (2017) 133:76–82. doi: 10.1016/j.eplepsyres.2017.04.015

17. Hasegawa D, Mizoguchi S, Kuwabara T, Hamamoto Y, Ogawa F, Matsuki N, et al. Electroencephalographic features of familial spontaneous epileptic cats. *Epilepsy Res*. (2014) 108:1018–25. doi: 10.1016/j.eplepsyres.2014.05.007

18. Mizoguchi S, Hasegawa D, Hamamoto Y, Yu Y, Kuwabara T, Fujiwara-Igarashi A, et al. Interictal diffusion and perfusion magnetic resonance imaging features of cats with familial spontaneous epilepsy. *Am J Vet Res*. (2017) 78:305–10. doi: 10.2460/ajvr.78.3.305

19. Mizoguchi S, Hasegawa D, Kuwabara T, Hamamoto Y, Ogawa F, Fujiwara A, et al. Magnetic resonance volumetry of the hippocampus in familial spontaneous epileptic cats. *Epilepsy Res*. (2014) 108:1940–4. doi: 10.1016/j.eplepsyres.2014.09.009

20. Yu Y, Hasegawa D, Hamamoto Y, Mizoguchi S, Kuwabara T, Fujiwara-Igarashi A, et al. Neuropathologic features of the hippocampus and amygdala in cats with familial spontaneous epilepsy. *Am J Vet Res*. (2018) 79:324–32. doi: 10.2460/ajvr.79.3.324

21. Hamamoto Y, Hasegawa D, Yu Y, Asada R, Mizoguchi S, Kuwabara T, et al. Statistical structural analysis of familial spontaneous epileptic cats using voxel-based morphometry. *Front Vet Sci*. (2018) 5:172. doi: 10.3389/fvets.2018.00172

22. Hasegawa D. Diagnostic techniques to detect the epileptogenic zone-pathophysiological and presurgical analysis of epilepsy in dogs and cats. *Vet J*. (2016) 215:64–75. doi: 10.1016/j.tvjl.2016.03.005

23. Gourie-Devi M, Cherian L, Shankar SK. Seizures in cats induced by ketamine hydrochloride anaesthesia—a preliminary report. *Indian J Med Res*. (1983) 77:525–8.

24. Weingarten SM. Dissociation of limbic and neocortical EEG patterns in cats under ketamine anesthesia. *J Neurosurg*. (1972) 37:429–33. doi: 10.3171/jns.1972.37.4.0429

25. Black JA, Golden GT, Fariello RG. Ketamine activation of experimental corticoreticular epilepsy. *Neurology*. (1980) 30:315–8. doi: 10.1212/wnl.30.3.315

26. Mori K, Kawamata M, Mitani H, Yamazaki Y, Fujita M. A neurophysiologic study of ketamine anesthesia in the cat. *Anesthesiology*. (1971) 35:373–83. doi: 10.1097/00000542-197110000-00012

27. Kitz S, Thalhammer JG, Glantschnigg U, Wrzosek M, Klang A, Halasz P, et al. Feline temporal lobe epilepsy review of the experimental literature. *J Vet Intern Med*. (2017) 31:633–40. doi: 10.1111/jvim.14699

28. Gowda S, Szabó CÁ. Effects of ketamine on EEG in baboons with genetic generalized epilepsy. *Epilepsy Res*. (2019) 154:50–4. doi: 10.1016/j.eplepsyres.2019.04.008

29. Salinsky M, Kanter R, Dasheiff RM. Effectiveness of multiple EEGs in supporting the diagnosis of epilepsy: an operational curve. *Epilepsia*. (1987) 28:331–4. doi: 10.1111/j.1528-1157.1987.tb03652.x

30. Celesia GG, Chen RC, Bamforth BJ. Effects of ketamine in epilepsy. *Neurology*. (1975) 25:169–72. doi: 10.1212/wnl.25.2.169

31. Purdon PL, Sampson A, Pavone KJ, Brown EN. Clinical electroencephalography for anesthesiologists part I background and basic signatures. *Anesthesiology*. (2015) 123:937–60. doi: 10.1097/ALN.0000000000000841

32. Jang HS, Choi HS, Lee SH, Jang KH, Lee MG. Evaluation of the anaesthetic effects of medetomidine and ketamine in rats and their reversal with atipamezole. *Vet Anaesth Analg*. (2009) 36:319–27. doi: 10.1111/j.1467-2995.2009.00463.x

33. Karlsson BR, Forsman M, Roald OK, Heier MS, Steen PA. Effect of dexmedetomidine, a selective and potent alpha 2-agonist, on cerebral blood flow and oxygen consumption during halothane anesthesia in dogs. *Anesth Analg*. (1990) 71:125–9. doi: 10.1213/00000539-199008000-00003

34. Lhatoo SD, Faulkner HJ, Dembny K, Trippick K, Johnson C, Bird JM. An electroclinical case-control study of sudden unexpected death in epilepsy. *Ann Neurol*. (2010) 68:787–96. doi: 10.1002/ana.22101

35. Bruno E, Richardson MP. Postictal generalized EEG suppression and postictal immobility what do we know? *Epileptic Disord*. (2020) 22:245–51. doi: 10.1684/epd.2020.1158

36. Chang LC, Raty SR, Ortiz J, Bailard NS, Mathew SJ. The emerging use of ketamine for anesthesia and sedation in traumatic brain injuries. *CNS Neurosci Ther*. (2013) 19:390–5. doi: 10.1111/cns.12077

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 08 January 2024
DOI 10.3389/fvets.2023.1240880

OPEN ACCESS

EDITED BY
Catherine Elizabeth Stalin,
University of Glasgow, United Kingdom

REVIEWED BY
Yoshihiko Yu,
Independent Researcher, Mitaka, Japan
Jos Bongers,
University of Glasgow, United Kingdom

*CORRESPONDENCE
Fiona May Keir James
✉ jamesf@uoguelph.ca

RECEIVED 15 June 2023
ACCEPTED 18 December 2023
PUBLISHED 08 January 2024

CITATION
Folkard E, McKenna C, Monteith G, Niel L, Gaitero L and James FMK (2024) Feasibility of in-home electroencephalographic and actigraphy recordings in dogs. *Front. Vet. Sci.* 10:1240880. doi: 10.3389/fvets.2023.1240880

Feasibility of in-home electroencephalographic and actigraphy recordings in dogs

Emily Folkard[1], Charly McKenna[1], Gabrielle Monteith[1], Lee Niel[2], Luis Gaitero[1] and Fiona May Keir James[1]*

[1]Department of Clinical Studies, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, University of Guelph, Guelph, ON, Canada

Introduction: Idiopathic epilepsy is a prevalent neurological disease in dogs. Dogs with epilepsy often present with behavioral comorbidities such as aggression, anxiety, and fear. These behaviors are consistent with pre, post, or interictal behaviors, prodromal changes, seizure-precipitating factors, or absence and focal seizures. The overlap in behavior presentations and lack of objective research methods for quantifying and classifying canine behavior makes determining the cause difficult. Behavioral comorbidities in addition to the task of caring for an epileptic animal have a significant negative impact on dog and caregiver quality of life.

Methods: This pilot study aimed to assess the feasibility of a novel technology combination for behavior classification and epileptic seizure detection for a minimum 24-h recording in the dog's home environment. It was expected that combining electroencephalography (EEG), actigraphy, and questionnaires would be feasible in the majority of trials. A convenience sample of 10 community-owned dogs was instrumented with wireless video-EEG and actigraphy for up to 48 h of recording at their caregiver's home. Three questionnaires (maximum 137 questions) were completed over the recording period by caregivers to describe their dog's everyday behavior and habits.

Results: Six of the 10 included dogs had combined EEG and actigraphy recordings for a minimum of 24 h.

Discussion: This shows that in-home EEG and actigraphy recordings are possible in community-owned dogs and provides a basis for a prospective study examining the same technology combination in a larger sample size.

KEYWORDS

canine epilepsy, idiopathic epilepsy, behavioral comorbidities, questionnaires, electroencephalography, actigraphy

Introduction

Epilepsy is a common neurological disease that dogs present with in veterinary medicine (1). Idiopathic epilepsy (IE), where a cause for the disease cannot be determined, has an estimated prevalence of 0.6% in the general population of companion dogs (1, 2). As seen in people, dogs with epilepsy often experience behavioral changes throughout the course of the disease, including increased comorbid anxiety, aggression, fear, and clinginess (3, 4). Additional behavioral changes as a result of anti-seizure drug usage, include lethargy, lack of motor control, polyuria, and polydipsia (5, 6). Changes in behavior also occur with ictal events. For example, absence or focal seizures experienced in IE are commonly accompanied by altered awareness, lip smacking, facial twitching, and excessive blinking (6). These may be misinterpreted as abnormal behaviors instead of ictal events, contributing to their underreporting (7). Despite the growing recognition of behavioral changes in dogs with epilepsy and the complexity of behavior designation, limited options exist to aid in the classification of these behaviors.

Various investigative tools have been used to enhance our understanding of the impact of epilepsy on canine behavior. Caregiver-reported questionnaires have been used in a variety of contexts including quality of life concerns, seizure semiology, anti-seizure drug side effects, and behavioral changes at various time points throughout the disease (8–13). Although useful for providing initial insight for future investigations, caregiver-reported questionnaires are subjective in nature and prone to recall and observer bias (14). Thus, objective tools are required to provide an accurate depiction of canine behavior within epilepsy.

Electroencephalography (EEG) is instrumental in the diagnosis and treatment of canine epilepsy, with promise for investigation of behavior changes and potential underlying causes. Historically, EEG on sedated or anesthetized animals prevented concurrent analysis of behavior (15–19). Recent ambulatory successes make it the only method with adequate spatial and temporal resolution for accurately diagnosing and classifying seizure types in awake and behaving dogs but only one report of recording in a dog's home environment (20). When EEG is combined with synchronized video recording (vEEG), brain activity captured by EEG can be correlated to behaviors captured on video to aid in objective behavior classification.

As behavior classification using vEEG is time and labor intensive, it would improve clinical and research efficiency to augment EEG with an automated tool. Actigraphy uses accelerometer technology to measure rest and activity levels, and algorithmic analysis of accelerometer data has been used to identify normal behavioral states in dogs such as walking, sleeping, head shaking, and eating (21). Algorithms generated using accelerometer data have successfully identified generalized tonic-clonic (GTC) seizures in dogs, but have been unable to detect non-GTC seizures (21–23). It is possible, then, that combining the ability of EEG to detect and classify all seizure types with the behavior-classifying ability of accelerometers would aid in our understanding and objective classification of behavior within canine epilepsy. The further addition of caregiver-completed behavioral questionnaires would supplement insight into the dog's everyday behavior to aid in distinguishing normal from abnormal behaviors.

This pilot study assessed whether a novel combination of vEEG, actigraphy, and caregiver-reported behavioral questionnaires can be used to collect a minimum 24-h recording in IE and neurotypical (NT) companion dogs at the caregiver's home. Both IE and NT dogs were included to ensure there were no major differences in feasibility between the groups to allow for behavioral comparisons to be made in prospective studies utilizing the data collected in the current study. We predicted that combining vEEG, actigraphy, and questionnaires for a minimum 24-h at-home recording would be a feasible approach to capturing a complete and detailed account of seizure activity and canine behavior to enhance our understanding of canine behavior in epilepsy.

Materials and methods

Subjects

All participants were recruited through the Ontario Veterinary College Health Science Center (OVC HSC) Neurology Service, regional neurology practices, and the research program's social media channels. The study protocol was reviewed and approved by both the Research Ethics Board and the Animal Care Committee at the University of Guelph (REB#22-05-12, AUP#4695). All caregivers provided informed consent.

Inclusion criteria for recruitment were IE and NT dogs between the ages of 2 and 8 years old to help reduce adolescent and geriatric-related behavioral and cognitive differences. IE dogs were required to have been previously diagnosed with minimum tier I IE based on the International Veterinary Epilepsy Task Force (IVETF) recommendations (24). Tier I IE encompasses dogs with (I) a history of two or more unprovoked seizures occurring at least 24 h apart, (II) age at seizure onset between 6 months and 6 years, (III) normal interictal physical and neurological examinations, and (IV) no clinical abnormalities on laboratory tests (including biochemistry profile and complete blood count, with or without fasting bile acids or ammonia, and urinalysis) (24). Aside from tier I IE, both IE and NT participants were required to have an unremarkable medical history with no diagnosed behavioral disorders. Physical and neurological examinations were completed for all dogs by members of the OVC HSC Neurology Service and were required to be unremarkable at the time of study enrolment for inclusion. Dogs were excluded if they were deemed unfit for vEEG instrumentation due to their tolerance for handling.

Instrumentation

Instrumentation for vEEG and actigraphy was completed at the OVC HSC. Following physical and neurological examinations, dogs were sedated, if required, for electrode placement using our standard clinical protocol; dexmedetomidine 10–20 μg/kg intravenous (IV) for sedation with atipamezole 100–200 μg/kg intramuscular (IM) for subsequent reversal. If the dogs required sedation, the official recording start time was extended beyond clinical recovery until normal behavior and mentation was observed.

All vEEGs were conducted using 15 subdermal wire electrodes following our previously described electrode placement protocol, with even numbers denoting right side electrodes and odd numbers denoting left side electrodes (Table 1) (25). Electrodes included reference (R), ground (G), midline (Fz, Cz, Pz), frontal electrodes (F3/F4, F7/F8), central electrodes (C3/C4), temporal electrodes (T3, T4), and occipital electrodes (O1/O2). During instrumentation, electrodes were adjusted to keep impedances below 30 kOhms. Electrodes were secured to the scalp using sticky bandage and connected to leads plugged into the Lifelines Neuro Trackit T4A ambulatory EEG amplifier (Lifelines Neuro Company, Louisville, USA) secured in the dorsal pocket of a harness worn by the dog. Non-adhesive bandage was used to secure the leads in place on top of the dog's head, and a tension loop was created with the leads and non-adhesive bandage to reduce tension on the electrodes (Figure 1). Two hours were given to allow the dog to acclimatize to the equipment before being sent home.

The vEEG amplifier was connected via Bluetooth to a laptop with synchronous webcam video recording. Following instrumentation, caregivers were sent home with the laptop for up

TABLE 1 Electrode placement protocol.

Electrode	Electrode location
R	Midline, between medial canthi
G	Dorsal midline neck, 2–5 cm caudal to occipital protuberance
F7/F8	Zygomatic arch just caudal to the lateral canthus of both eyes
F3/F4/Fz	On the temporal lines causal to the medial canthi and at the midline
C3/C4/Cz	Halway between F and O/P electrodes, in line with T electrodes
O1/O2/Pz	Transverse line between mastoid processes in line with F electrodes
T3/T4	Zygomatic arch, just rostral to the pinnal edge

to 48 h of at-home vEEG monitoring. Caregivers were instructed to keep the webcam trained on the dog as much as possible to allow for behavior and/or ictal event confirmation. Caregivers were advised that the EEG and concurrent video recording could be stopped at any time for any reason at their discretion and were able to arrange a time to return for de-instrumentation at their convenience. Otherwise, recordings were terminated when the dog removed the equipment spontaneously or after 48 h.

The Actigraph GT9X Link (Actigraph, Pensacola, USA) was secured to the EEG amplifier using sticky bandage inside the harness pocket located on the dog's interscapular region (Figure 2). The Actigraph GT9X Link was chosen as it was provided through our collaboration for this project and fit within the harness pocket. To prevent the rotation of the actigraph, the harness was chosen based on body size and adjusted accordingly. The actigraphs were initialized to the proximity setting to allow for both monitoring of activity levels and the dog's proximity to key locations in their environment. Three additional actigraph units were dispensed to caregivers for proximity measures; (I) to be worn by the caregiver on their wrist or hip to monitor the dog's proximity to the caregiver, (II) to be placed at the dog's water bowl to estimate water consumption, and (III) to be placed at the exit door the dog used most frequently to access the outdoors to monitor time spent outside. Once placed at these locations, caregivers were requested not to move the actigraphs until the end of the recording. The actigraph recordings were initialized such that recordings would not commence until after the caregiver had returned home and placed the units in their respective locations and were terminated once the caregiver removed the units from these locations or after 48 h.

Questionnaire design

Using Qualtrics (Qualtrics, Provo, USA), three questionnaires were implemented throughout the study and completed online by the caregiver. The first questionnaire (Q1) (Supplementary material 1) included 137 questions related to the dog's typical behavior and sleep, housing, care, exercise habits, medical history, and seizure signalment (if applicable). Questions

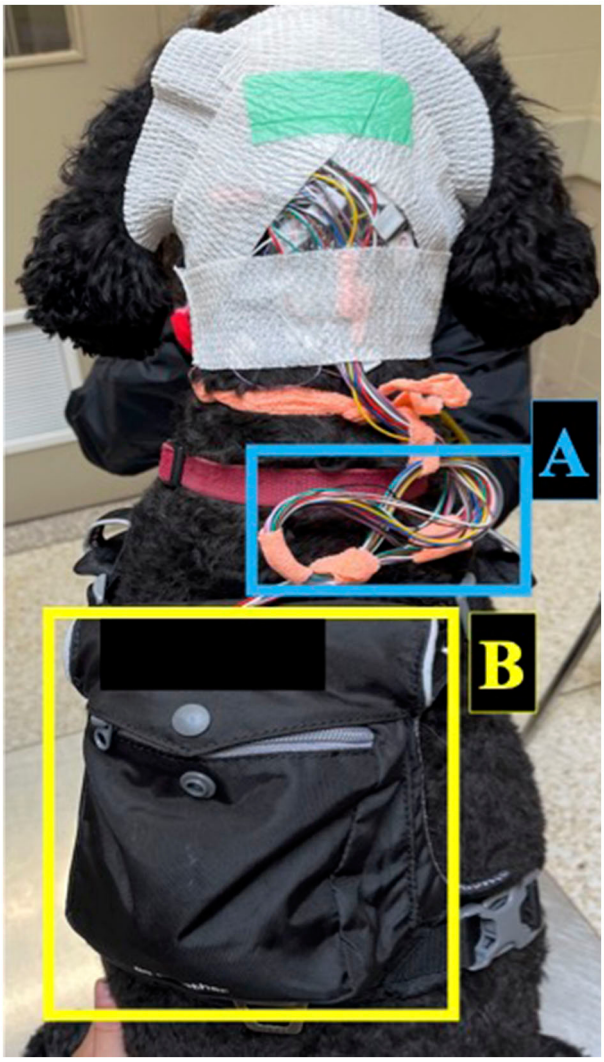


FIGURE 1
Set-up for ambulatory EEG recording in dogs. Electrodes were secured to the scalp using sticky bandage and connected to leads which were secured with non-adhesive bandage. The leads were managed with a tension loop **(A)** and connected to the ambulatory EEG amplifier secured in the dorsal pocket of a harness **(B)**.

pertaining to the dog's behavior and sleep were copied from the previously validated Canine Behavioral Assessment and Research Questionnaire (CBARQ) and Sleep and Night Time Restlessness Evaluation Score (SNoRE), respectively (10, 26). Q1 was completed at the OVC HSC during the acclimatization period.

The second and third questionnaires (Q2 and Q3, respectively; Supplementary material 2) were identical condensed versions of Q1 that were to be completed after every 24 h of recording. If the recording terminated before 24 h or between 24 and 48 h, Q2 or Q2 and Q3 were not completed. Questionnaires 2 and 3 were comprised of 125 questions each.

All 3 questionnaires were completed by outside reviewers prior to enrolment to help ensure the questions were clear and could be completed in a reasonable time (<30 min). Completion rate and time were collected using features embedded in Qualtrics for further analysis.

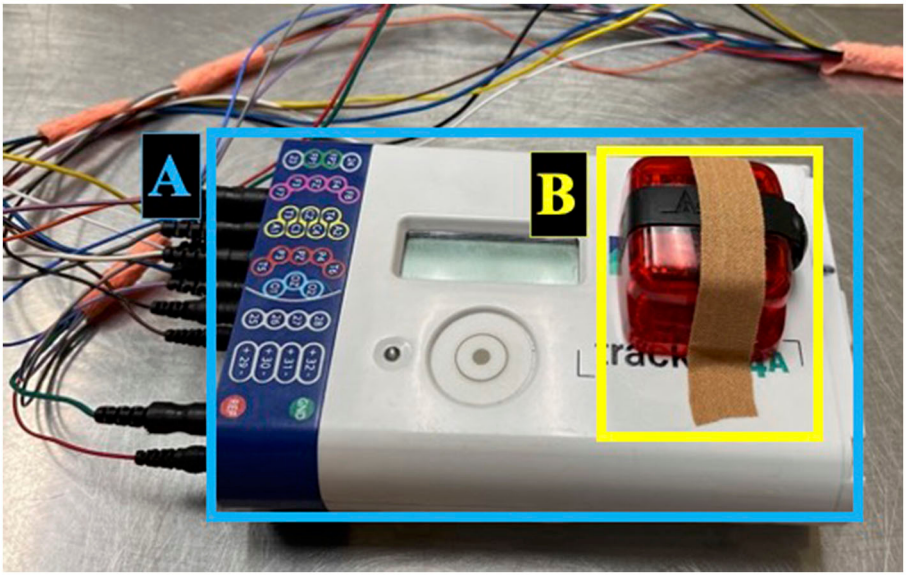


FIGURE 2
Set-up for ambulatory EEG recording in dogs continued. EEG amplifier **(A)** located inside the dorsal pocket of the harness. Actigraph unit secured on the EEG amplifier using sticky bandage **(B)**.

Feasibility criteria

The following feasibility criteria were assessed to determine success or failure for each dog: (I) a >24-h readable EEG and actigraphy recording, (II) a minimum of 2 electrodes remaining in place on each of the right, left, and midline regions, (III) the actigraph remained secure for the duration of the recording, (IV) the dog was able to complete normal tasks with no concerns as reported by the caregiver in questionnaires, and (V) the caregiver was able to complete the questionnaires in reasonable time with no concerns. All feasibility criteria had to be satisfied for a session to be deemed successful.

Statistical analysis

Raw vEEG data was analyzed using Persyst 12 (Persyst Development Corporation, San Diego, USA) and raw actigraphy data was analyzed using Excel 2016 (Microsoft Corporation, Redmond, USA). Simple descriptive statistics were performed on the feasibility outcome measures including EEG and actigraphy recording duration, electrode survival times, and questionnaire completion rate and duration for Q1, Q2, and Q3. Standard error was reported with means to adjust for sample size. A Friedman's rank test was performed to compare the effect of electrode placement location on electrode survival time. A two-sample *t*-test was used to compare the mean EEG recording duration time between sedation and not sedated groups. A Shapiro Wilk test and examination of the residuals confirmed the data was normally distributed. Precision was calculated for measured parameters to determine the prediction limits for the difference in vEEG recording duration between sedated and non-sedated dogs with a power of 85% was calculated as well as the actual power with four and six dogs. All statistical analyses were conducted in R Statistical Software (v4.2.2, R Core Team 2022) and SAS version 9.4 (SAS Institute Inc, Cary, USA).

Results

Six IE and 5 NT dogs were recruited for a total of 11 dogs. One NT dog was excluded due to difficulties with handling during the physical and neurological examinations. Therefore, 6 IE dogs and 4 NT dogs were included for a total of 10 dogs. Participating breeds included six mixed breed dogs and one of each of the following: Border Collie, Standard Poodle, Golden Retriever, and Black Mouth Cur (Supplementary material 3). There were five neutered males and five spayed females with a median age of 5 years (range 2–8 years).

Six of 10 sessions (60%) met all feasibility criteria and were therefore considered to be a success. Five trials terminated at or around 48 h as scheduled. Four trials were terminated prior to 48 h due to spontaneous unscheduled de-instrumentation by the dog and one trial was terminated prior to 48 h due to caregiver concerns of growing discomfort for their dog. There were no caregiver concerns regarding the safety of the biomonitor suite for at-home usage and the possibility of wearing it during seizure activity. The following subsections describe each feasibility outcome in depth.

vEEG

The feasibility of vEEG recordings was determined based on the number of functioning electrodes that remained in place for the duration of the recording. Two electrodes each on the left side, midline, and right side were to be secure and reliably recording for

>24 h for an EEG to be considered successful. All vEEG recordings were of sufficient interpretable quality while the required number of electrodes were in place despite frequent muscle and movement artifacts and occasional electrode "pops." Due to technical issues, a very limited amount of video was captured alongside the EEG recording. This limited video was used to confirm stages of mentation on EEG output but was omitted from further analysis.

Six of 10 dogs required sedation for electrode placement using dexmedetomidine (10–20 ug/kg) and subsequent reversal with atipamezole (100–200 ug/kg). The remaining dogs (4/10) had instrumentation successfully completed without sedation. One sedated dog experienced a clonic seizure during instrumentation and was administered diazepam (0.5 mg/kg IV) as a rescue medicine to stop the episode. Recordings began once clinical recovery and normal behavior and mentation was achieved.

Six out of the 10 EEG recordings were >24 h in duration and were therefore considered successful. The duration of EEG recording in total ranged from 1.90 to 48.27 h, with a median duration of 27.70 h. Of recordings that lasted >24 h, the median recording duration was 46.75 h (range: 27.7–48.27). Recordings that did not reach 24 h had a median recording duration of 2.55 h (range: 1.90–17.42). Recording duration was similar between dogs that were sedated for instrumentation and unsedated dogs, with mean recording durations of 19.20 (standard error: 7.23) and 42.02 h (standard error: 8.85), respectively ($p = 0.08$). Data was normally distributed, confirmed by the Shapiro Wilk test ($p = 0.113$). As the two-sample t-test was underpowered with six unsedated dogs and four sedated dogs, we calculated that sufficient power to detect a potential difference in recording duration between sedated and unsedated dogs would be achieved with eight dogs in each group. Reasons for early recording termination were spontaneous removal of the EEG by the dog ($n = 3$) and caregiver preference ($n = 1$). Notably, there was no damage sustained to the EEG transmitter unit, leads, harness, or actigraph unit during this study. Also, neither ictal nor interictal epileptogenic paroxysmal discharges were recorded in the IE group.

Electrodes were considered lost when the cortical signal captured by the electrode was no longer interpretable due to artifact. The median proportion of electrodes lost by the end of the EEG recording was 40.0% (range: 13.0–64.0%). There was no significant difference in the odds of losing an electrode between sedated and non-sedated dogs [OR = 1.06, 95% CI (0.049–23.16)]. The sample was too small to detect a statistically significant difference in mean electrode survival time between electrode placement locations (Friedman's $p = 0.733$; Figure 3).

Actigraphy

Actigraphy recordings were considered successful if their duration was >24 h and the unit remained securely in place next to the EEG transmitter unit inside the harness pocket on the dog's back.

Six out of 10 actigraphy recordings were >24 h in duration and were therefore considered successful. Actigraphy recording durations ranged from 1.72 to 48.00 h, with a median recording duration of 25.62 h. Of actigraphy recordings that lasted >24 h, the median recording duration was 46.13 h (range: 21.53–48.00) and the recordings that did not last 24 h had a median duration of 5.27 h (range: 1.72–8.92). Actigraphy units remained secure in their original location attached to the EEG transmitter unit on the dog's back in all 10 dogs. Actigraphy recordings in all 10 dogs were terminated near the end of EEG recordings when caregivers moved the units from their homes prior to de-instrumentation.

Questionnaires

All 10 caregivers completed Q1 at the OVC HSC during the acclimatization period with a completion rate of 100%. Completion times ranged from 21.20 to 152.30 min, with a median completion time of 101.47 min.

Q2 and Q3 were completed at home by caregivers after 24 and 48 h of the recording. Caregivers were instructed not to complete the questionnaires if the recording had been terminated before the 24- or 48-h mark. Q2 was completed by 9/10 participants with a completion rate of 100%. Completion times ranged from 12.40 to 2,748.93 min, with a median completion time of 16.5 min. Q3 was completed by 6/10 participants with a completion rate of 100%. The median completion time for Q3 was 21.94 min and ranged from 8.72 to 383.02 min.

Discussion

Three tools commonly used in the investigation of canine epilepsy and behavior are EEG, actigraphy, and caregiver-completed questionnaires. This study demonstrated for the first time that EEG can be successfully recorded in the home environment of companion dogs, although the synchronized video technology proved unreliable. In a further first, this study combined EEG home-recording of dogs with actigraphy for a minimum of 24-h recording in addition to the completion of caregiver-reported behavioral questionnaires. This combination of technologies appeared feasible as median EEG and actigraphy recording times of 27.70 and 25.62 h, respectively, were achieved in 60% of participants. All questionnaires that were attempted by caregivers were completed to 100%, indicating that the surveys were not too onerous for motivated participants.

There was a bimodal "survival time" for the wearable study biomonitors. There was a difference of ∼44 h median recording duration between EEG recordings lasting >24 h and those lasting less. Similarly, the difference in median actigraphy recordings lasting >24 h and those lasting less was ∼37 h. These large differences may be due to canine patient-specific factors such as age, weight, medication status, and/or behavioral characteristics. The influence of these various factors provides the groundwork for a prospective study investigating the correlation between EEG and actigraphy recording length and the dog's behavioral profile, given that four dogs spontaneously de-instrumented themselves. It is noted that the dog's behavior may be impacted by the bulkiness of the equipment thus this factor should be considered in future investigations of behavior. Furthermore, understanding canine patient-specific factors that influence EEG and actigraphy recording length would help determine if these biomonitor

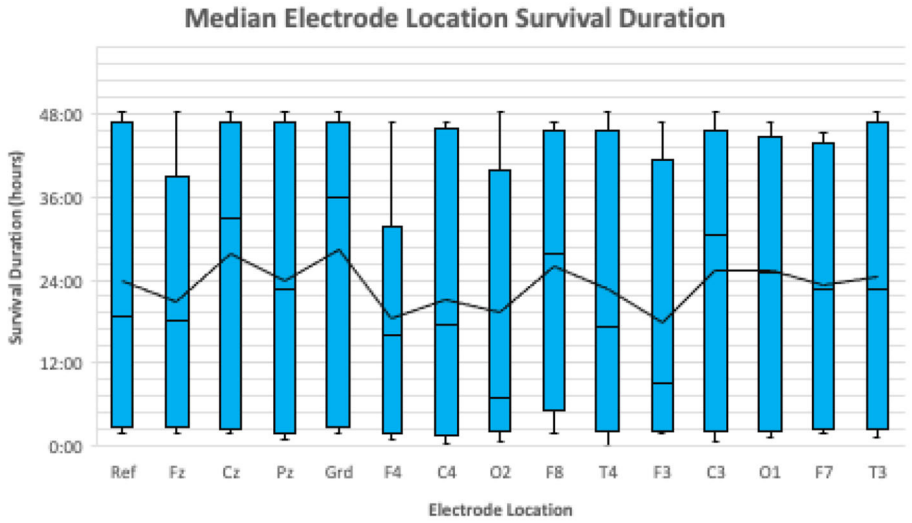


FIGURE 3
A boxplot illustrating the median survival duration of each electrode location for all 10 dogs. The median survival duration for each location is represented by the horizontal black line in each blue box. The mean survival duration for each location is represented by the continuous black line.

technologies are suitable diagnostic options for each patient to optimize clinical efficiency and support personalized patient care. For example, if ictal events or behaviors of interest are not captured within the standard clinical <5-h EEG recording, the option of a longer 24 to 48-h recording could be presented to caregivers as an additional diagnostic measure (21). There is the additional possibility that dogs with more infrequent paroxysmal events might benefit from either a longer recording duration or an in-home recording if it increases the likelihood of capturing an event of interest. Further work is needed to determine the diagnostic yield of, and indications for, in-home EEG recordings.

No significant differences were observed between electrode survival time and electrode placement locations. Veterinary epileptology currently lacks a standardized electrode placement protocol (19). As no electrode location performed significantly better or worse than others, this study cannot provide any suggestions to improve upon the reported electrode placement protocol. As a next step, electrode survival time as a function of electrode location could be investigated in a larger sample size of dogs, while also optimizing source localization techniques. The impact of additional factors such as skull shape, size of dog, and coat length on electrode survival should also be considered when developing an optimal electrode placement protocol.

All actigraphy units remained secure on the EEG transmitter unit in the harness pocket on the dog's back for the duration of the recording. The most suitable accelerometer location for canine epilepsy research has yet to be determined; the two most reported and successful locations are the interscapular region or around the dog's neck (21–23, 27, 28). The current study selected the interscapular region inside the harness pocket for actigraphy placement as the unit was not easily attachable to the dog's collar and to minimize the risk of damage to the unit itself. Establishing the optimal location for actigraphy placement will require comparisons of data quality between locations to determine which placement protocol reduces the movement of the actigraphy itself, as movement or rotation of the unit may interfere with data accuracy (28). As no EEG-confirmed ictal events occurred during the recording period, we were unable to identify actigraphy values correlating to seizure activity. Non-GTC seizures may be more difficult to detect via accelerometry because they are often much less disruptive and convulsive, and the accelerometer may be unable to recognize the variable and relatively minor movements that accompany non-GTC seizures such as excessive blinking or lip licking. The inability to detect non-GTC seizures may result in the underestimation of seizure frequency, which is further compounded by the tendency for caregivers to underreport seizure incidence (7). If ictal events occur in future research, additional actigraphy outcomes such as the feasibility of algorithms to detect and classify GTC and non-GTC seizure activity could be assessed.

A wide range of questionnaire completion times was observed and ranged from 8.72 to 2,748.93 min for Q1, Q2, and Q3, with median completion times of 101.47, 16.5, and 21.94 min, respectively. The questionnaires posed in this research were completed by a small focus group ($n = 5$) of unrelated subjects prior to study initiation to help ensure they could be completed in a reasonable time. Implementing questionnaires of a reasonable length is essential to ensure the quality of the responses does not diminish over time (29). The abnormally long completion times for Q1 may have been a result of the distracting hospital environment and their dog's reaction to instrumentation. In future, this questionnaire could be completed at the caregiver's home prior to instrumentation to minimize distractions. Additional factors affecting completion time for internet-based questionnaires include participant age, experience with internet-based questionnaires, and education, which may have played a role in the longer completion times shown in this study (30). The behavioral data obtained

from these questionnaires were not analyzed for the present study due to the small sample size but will serve as a foundation for a prospective study examining this technology combination in a larger sample size.

The sample size for the present study was intentionally kept small to ensure this technology combination was feasible for at-home recordings before investing significant time and monetary resources in a larger sample size. A prospective study examining the same novel technology combination would require 40 dogs to be sampled from the population to be 80% confident that the estimated proportion of feasible trials is within 10% of the true population proportion.

This feasibility study was limited by several factors. First, the geographic location limited participation as the study design required multiple visits to the OVC HSC, restricting recruitment to local caregivers or caregivers willing to make these visits irrespective of location. This study was also task heavy for caregivers with the multiple visits to the OVC HSC, competition of several questionnaires, and up to 48-h monitoring of both their dog and our equipment. It is worth noting that the demands of the study may have been off-putting for potential trial participants. A frustrating limitation involved the technology itself, resulting in the partial loss of the synchronized video data portion of the vEEGs due to issues with file size and portable computer capacity. The portion of video that was recorded was used to confirm stages of mentation against EEG output, but no additional behavioral observations were able to be made. Lastly, the present study's samples unintentionally consisted of medium and large breed dogs. Weight stipulations were not explicitly stated in the inclusion criteria, as the goal was to recruit IE cases and NT controls. Nonetheless, wireless vEEG devices will need to be smaller to accommodate smaller dog breeds and cats.

The recognition of complex behavioral comorbidities in dogs with epilepsy continues to grow in veterinary research and medicine, although the classification of these behaviors remains a challenge. Many facets of epileptic canine behavior exist, including pre-and post-ictal changes, prodromal changes in behavior, psychosocial behaviors, and behavioral manifestations of absence or focal seizures. These complex behavioral presentations support the growing need for objective behavior classification tools in canine epilepsy. Electroencephalography remains the standard for identifying and classifying seizure types and frequency due to its sufficient spatial and temporal resolution. Combined with synchronized video recording, vEEG becomes useful for behavioral observations and helps classify behavioral events as ictal or non-ictal (20). Actigraphy classifies different states of behavior such as walking, running, and sleeping, and identifies only GTC seizures in dogs (21–23, 27). Caregiver-completed questionnaires describe the dog's typical housing, care, routine, and behaviors, and provides subjective accounts of seizure semiology. Therefore, two objective tools, EEG and actigraphy, could strengthen the data obtained from the more commonly employed subjective questionnaire tool to understand epileptic canine behavior. The end goal of objective behavior classification in epileptic dogs is to improve the diagnosis and treatment of seizures and provide the caregiver with an understanding of how their dog's behavior changes relate to seizures to aid in seizure prediction.

Overall, this study shows that it is possible to record wireless ambulatory EEG in the home environment in addition to actigraphy recordings and caregiver questionnaires. These findings open the door for this combination to act as a research tool to examine behavior in canine epilepsy or as a diagnostic tool for complex presentations. Employing in-home EEG recordings and/or supplementing EEG recordings with actigraphy and the right questionnaires could provide clinicians with a more complete behavioral profile of each dog. In addition, this could aid clinicians in distinguishing behaviors as ictal or non-ictal. Ultimately, this area requires more research as understanding behavior in canine epilepsy is vital to improving clinicians' ability to diagnose and treat seizures while also aiding caregivers to predict seizures more accurately in their dogs.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Research Ethics Board at the University of Guelph. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal studies were approved by the Animal Care Committee and the Research Ethics Board at the University of Guelph. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EF, LN, and FJ: conception. EF, CM, LN, and LG: drafting the grant proposal. EF and CM: experimental logistics. EF and FJ: data collection and drafting the manuscript. EF, GM, and FJ: data analysis. EF, LN, LG, CM, GM, and FJ: revising the manuscript for intellectual content and final approval of the completed manuscript. All authors contributed to the article and approved the submitted version.

Funding

This project was generously funded by the Ontario Veterinary College Pet Trust Fund (#055727 and Pet Trust Equipment Competition W21) and was supported by the Ontario Graduate Scholarship program. We further acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC) [funding reference number RGPIN-2021-02606] and Cette recherche a été financée par le Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG) [numéro de référence RGPIN-2021-02606].

Acknowledgments

Ruby Ng and Stephen Everest are thanked for their assistance with physical and neurological examinations. Grace Kadler was thanked for her assistance with instrumentation. Ashleigh Martin is thanked for her assistance with recruitment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1240880/full#supplementary-material

References

1. Hülsmeyer VI, Fischer A, Mandigers PJJ, DeRisio L, Berendt M, Rusbridge C, et al. International Veterinary Epilepsy Task Force's current understanding of idiopathic epilepsy of genetic or suspected genetic origin in purebred dogs. *BMC Vet Res.* (2015) 11:1–28. doi: 10.1186/s12917-015-0463-0

2. Kearsley-Fleet L, O'Neill DG, Volk HA, Church DB, Brodbelt DC. Prevalence and risk factors for canine epilepsy of unknown origin in the UK. *Vet Rec.* (2013) 172:338. doi: 10.1136/vr.101133

3. Watson F, Rusbridge C, Packer RMA, Casey RA, Heath S, Volk HA, et al. review of treatment options for behavioural manifestations of clinical anxiety as a comorbidity in dogs with idiopathic epilepsy. *Vet J.* (2018) 238:1–9. doi: 10.1016/j.tvjl.2018.06.001

4. Watson F, Packer RMA, Rusbridge C, Volk HA. Behavioural changes in dogs with idiopathic epilepsy. *Vet Rec.* (2020) 186:93–93. doi: 10.1136/vr.105222

5. Chang Y, Mellor DJ, Anderson TJ. Idiopathic epilepsy in dogs: owners' perspectives on management with phenobarbitone and/or potassium bromide. *J Small Anim Pract.* (2006) 47:574–81. doi: 10.1111/j.1748-5827.2006.00203.x

6. Boothe DM, Dewey C, Carpenter DM. Comparison of phenobarbital with bromide as a first-choice antiepileptic drug for treatment of epilepsy in dogs. *J Am Vet Med Assoc.* (2012) 240:1073–83. doi: 10.2460/javma.240.9.1073

7. Ukai M, Parmentier T, Cortez MA, Fischer A, Gaitero L, Lohi H, et al. Seizure frequency discrepancy between subjective and objective ictal electroencephalography data in dogs. *J Vet Intern Med.* (2021) 35:1819–25. doi: 10.1111/jvim.16158

8. Duffy DL, Serpell JA. Predictive validity of a method for evaluating temperament in young guide and service dogs. *Appl Anim Behav Sci.* (2012) 138:99–109. doi: 10.1016/j.applanim.2012.02.011

9. Hobbs SL, Blackwell EJ, Wetz KE, Packer RMA. Owner reported management of interictal anxiety behaviours in canine epilepsy. *Vet Rec.* (2022) 190:e1321. doi: 10.1002/vetr.1321

10. Hsu Y, Serpell JA. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. *J Am Vet Med Assoc.* (2003) 223:1293–300. doi: 10.2460/javma.2003.223.1293

11. Wessmann A, Volk HA, Parkin T, Ortega M, Anderson TJ. Evaluation of quality of life in dogs with idiopathic epilepsy. *J Vet Intern Med.* (2014) 28:510-4. doi: 10.1111/jvim.12328

12. Packer RMA, Volk HA. Epilepsy beyond seizures: a review of the impact of epilepsy and its comorbidities on health-related quality of life in dogs. *Vet Rec.* (2015) 177:306–15. doi: 10.1136/vr.103360

13. Wessmann A, Volk HA, Packer RMA, Ortega M, Anderson TJ. Quality-of-life aspects in idiopathic epilepsy in dogs. *Vet Rec.* (2016) 179:229–229. doi: 10.1136/vr.103355

14. CDC. *A Catalog of Biases in Questionnaires*. (2023). Available online at: https://stacks.cdc.gov/view/cdc/19899 (accessed March 7, 2023).

15. Berendt M, Høgenhaven H, Flagstad A, Dam M. Electroencephalography in dogs with epilepsy: similarities between human and canine findings. *Acta Neurol Scand.* (1999) 99:276–83. doi: 10.1111/j.1600-0404.1999.tb00676.x

16. Bergamasco L, Accatino A, Priano L, Neiger-Aeschbacher G, Cizinauskas S, Jaggy A. Quantitative electroencephalographic findings in beagles anaesthetized with propofol. *Vet J.* (2003) 166:58–66. doi: 10.1016/S1090-0233(02)00254-X

17. Pellegrino FC, Sica REP. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. *Clin Neurophysiol.* (2004) 115:477–87. doi: 10.1016/S1388-2457(03)00347-X

18. Jeserevics J, Viitmaa R, Cizinauskas S, Sainio K, Jokinen TS, Snellman M, et al. Electroencephalography findings in healthy and Finnish Spitz dogs with epilepsy: visual and background quantitative analysis. *J Vet Intern Med.* (2007) 21:1299–306. doi: 10.1111/j.1939-1676.2007.tb01952.x

19. Brauer C, Kästner SBR, Schenk HC, Tünsmeyer J, Tipold A. Electroencephalographic recordings in dogs: prevention of muscle artifacts and evaluation of two activation techniques in healthy individuals. *Res Vet Sci.* (2011) 90:306–11. doi: 10.1016/j.rvsc.2010.06.004

20. James FMK, Cortez MA, Monteith G, Jokinen TS, Sanders S, Wielaender F, et al. Diagnostic utility of wireless video-electroencephalography in unsedated dogs. *J Vet Intern Med.* (2017) 31:1469–76. doi: 10.1111/jvim.14789

21. Uijl I, Álvarez CBG, Bartram D, Dror Y, Holland R, Cook A. External validation of a collar-mounted triaxial accelerometer for second-by-second monitoring of eight behavioural states in dogs. *PLoS ONE.* (2017) 12:e0188481. doi: 10.1371/journal.pone.0188481

22. Muñana KR, Nettifee JA, Griffith EH, Early PJ, Yoder NC. Evaluation of a collar-mounted accelerometer for detecting seizure activity in dogs. *J Vet Intern Med.* (2020) 34:1239–47. doi: 10.1111/jvim.15760

23. Hirashima J, Saito M, Kuriyama T, Akamatsu T, Yokomori M. Detection of generalized tonic-clonic seizures in dogs with a seizure detection system established using acceleration data and the mahalanobis distance: a preliminary study. *Front Vet Sci.* (2022) 9:848604. doi: 10.3389/fvets.2022.848604

24. De Risio L, Bhatti S, Muñana K, Penderis J, Stein V, Tipold A, et al. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. *BMC Vet Res.* (2015) 11:1–11. doi: 10.1186/s12917-015-0462-1

25. James FMK, Allen DG, Bersenas AME, Grovum WL, Kerr CL, Monteith G, et al. Investigation of the use of three electroencephalographic electrodes for long-term electroencephalographic recording in awake and sedated dogs. *Am J Vet Res.* (2011) 72:384–90. doi: 10.2460/ajvr.72.3.384

26. Knazovicky D, Tomas A, Motsinger-Reif A, Lascelles BDX. Initial evaluation of nighttime restlessness in a naturally occurring canine model of osteoarthritis pain. *PeerJ.* (2015) 3:e772. doi: 10.7717/peerj.772

27. Chambers RD, Yoder NC, Carson AB, Junge C, Allen DE, Prescott LM, et al. Deep learning classification of canine behavior using a single collar-mounted accelerometer: real-world validation. *Animals.* (2021) 11:1549. doi: 10.3390/ani11061549

28. Gerencsér L, Vásárhelyi G, Nagy M, Vicsek T, Miklósi A. Identification of behaviour in freely moving dogs (*Canis familiaris*) using inertial sensors. *PLoS ONE.* (2013) 8:e77814. doi: 10.1371/journal.pone.0077814

29. Sharma H. How short or long should be a questionnaire for any research? Researchers dilemma in deciding the appropriate questionnaire length. *Saudi J Anaesth.* (2022) 16:65–8. doi: 10.4103/sja.sja_163_21

30. Yan T, Tourangeau R. Fast times and easy questions: the effects of age, experience and question complexity on web survey response times. *Appl Cogn Psychol.* (2008) 22:51–68. doi: 10.1002/acp.1331

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 26 January 2024
DOI 10.3389/fvets.2024.1326165

OPEN ACCESS

EDITED BY
Marcin Adam Wrzosek,
Wroclaw University of Environmental and Life Sciences, Poland

REVIEWED BY
Mihai Musteata,
Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania
Yoshihiko Yu,
Independent Researcher, Mitaka, Japan
Miyoko Saito,
Azabu University, Japan

*CORRESPONDENCE
Elsa Lyon
✉ elsa.lyon@elyope.com

†These authors have contributed equally to this work and share senior authorship

RECEIVED 22 October 2023
ACCEPTED 08 January 2024
PUBLISHED 26 January 2024

CITATION
Lyon E, Pochat H, Blot S, Troupel T, Van Caenegem N, Besnard S and Escriou C (2024) Use of video-electroencephalography as a first-line examination in veterinary neurology: development and standardization of electroencephalography in unsedated dogs and cats. *Front. Vet. Sci.* 11:1326165. doi: 10.3389/fvets.2024.1326165

Use of video-electroencephalography as a first-line examination in veterinary neurology: development and standardization of electroencephalography in unsedated dogs and cats

Elsa Lyon[1,2,3]*, Hervé Pochat[3], Stéphane Blot[4], Thibaut Troupel[4,5], Nicolas Van Caenegem[4,6], Stéphane Besnard[1†] and Catherine Escriou[2†]

[1]Laboratory Vertex, UFR STAPS Department, UniCaen, Caen, France, [2]Small Animal Internal Medicine Department, Neurology Service, VetAgro Sup, Marcy l'Etoile, France, [3]Amset Medical, Saint-Maur-des-Fossés, France, [4]U955-IMRB, INSERM, Small Animal Internal Medicine Department, Neurology Service, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France, [5]Neurology Service, AniCura CHV Pommery, Reims, France, [6]Neurology Service, AniCura TRIOVet, Rennes, France

Objective: To assess the feasibility and validate the use of video-electroencephalography (EEG) in conscious dogs and cats and to propose guidelines of routine EEG in veterinary clinical practice.

Design: Prospective clinical study.

Data: One hundred and fifty EEG recordings were carried out to validate the clinical adding-value, reproducibility, and guidelines on 140 owned animals. One hundred and one EEGs were performed on dogs and 49 on cats.

Procedures: We compared recordings performed with 8 EEG unwired stud Ag/AgCl electrodes held by elastic straps and 8 EEG wired cup Ag electrodes held by a tailor-made manufactured headset combined with a wired video-EEG device. Electrodes placement was determined according to previously published animal EEG protocols. Physiological sensors, such as electrocardiography, electromyography, and respiratory sensors were added. Stimulation protocols were tested. Quality and interpretability were evaluated.

Results: Headsets and recording procedures appeared suitable for all skull shapes and sizes. Video-EEG recordings were successfully performed without tranquilization or anesthesia except for 9 animals. Median EEG recordings time was 40 min. Impedance remained below 20 kΩ in 99% of dog EEGs and 98% of cat EEGs. Isosynchrony was reported in 6% of the channels. Seventy-five percent of dog EEGs and 83% of cat EEGs were readable for more than 50% (to 100%) of their duration. Successful discrimination of vigilance states from rhythm analysis (wakefulness, drowsiness, and sleepiness) was possible in 99% of dog EEGs and 91% of cat EEGs. Photic driving responses during photic stimulations were observed in 11% of dog EEGs and 85% of cat EEGs. Electroencephalography recordings were directly informative in 32% of the examinations: in 25% EEG abnormalities were associated with clinical signs and 7% concerned EEG abnormalities without clinical symptoms during recording. Thirteen percent of dogs subjected

to photic stimulation exhibited epileptic anomalies. Among 9 EEGs with other history-based stimulations, three displayed epileptic graphoelements.

Conclusions: We have developed a standardized unanesthetized video-EEG procedure easily performed and reproducible in dogs and cats. Qualitative and quantitative technical and medical criteria were evaluated and were in accordance with human EEG recommendations. Moreover, we have demonstrated its relevance and accuracy for diagnostic purposes, providing further arguments for the use of EEG as a first-line neurological functional exploration test.

KEYWORDS

EEG, video-EEG, gel electrode, canine, feline, epilepsy, encephalopathy

1 Introduction

In human medicine, electroencephalography (EEG) and video-EEG are widely and routinely implemented in various fields, including neurology, intensive care (for coma evaluation), neuropediatric, gerontology, and emergency medicine. In particular, EEG is used in epileptology, and its use allows for a finer classification of epilepsies, extending beyond determining seizure types and epilepsy categories to include electro-clinical characterization and the description of epileptic syndromes (1). The EEG recording procedures have been standardized from international and national recommendations, covering aspects such as the numbers and placement of electrodes, duration of examination, recording parameters and settings, choice of stimulation protocols and video analysis for seizure investigations in accordance with the medical context (2–6).

In veterinary neurology, EEG is used confidentially while epilepsy is one of the most common neurological disorder in dogs (7, 8) with an estimated prevalence ranging from 0.6 to 0.75% (8, 9). However, the prevalence of this disease is significantly higher in specific breeds, with reported prevalences of 3.1–33% (10–16) with varying clinical presentation and disease severity (10). Seizures are also common in cats and may account for 2% of reasons for veterinary visits (17) with recurrent seizures representing an estimated prevalence of 0.16% (18). However, feline epilepsy remains poorly characterized and epilepsy of unknown cause is reported in 22% of cats with seizures (19).

Current classifications of seizures and epilepsy in dogs and cats are only based on seizure semiology and epilepsy etiology, respectively (20). Epilepsy includes idiopathic epilepsy with a proven or suspected genetic background or an unknown cause and no indication of structural epilepsy and structural epilepsy, caused by identified cerebral pathology (20). The difficulty to perform easily EEG in veterinary practice is probably the main factor underlying the difference between human and companion animal epilepsy classification. The development of veterinary EEG could significantly enhance the diagnosis, classification and treatment of companion animal epilepsy (21). Recently, there has been a renewed interest in veterinary medicine, with the publications of protocols providing information on electrode positioning on the skull (22–25), suggested electrode types (23), anesthetic protocols (22, 26, 27), the feasibility of recording on animals without anesthesia using video-EEG (24) and interpretation (28). A survey on veterinary neurologists' EEG practices (29) reveals the variability in protocols employed regarding the use of video, recording durations, and assessment of recording quality through impedance measurements. This survey specifically shows a preference for subcutaneous, wired or needle electrodes over surface electrodes. Placement methods may adhere to published protocols (22, 24, 30, 30–34) or individual approaches often involving varying sedation protocols. Additionally, the survey highlights that EEG isn't routinely used by veterinary neurologists, with some performing it less than once a year. Challenges cited include limited access to EEG equipment and insufficient training and experience in conducting and interpreting EEGs.

Our aim was to develop a method and standards for routine EEG examinations in veterinary medicine similar to those used in human patients in unsedated conditions. This involved using cup electrodes or electrode caps and recording sessions lasting 20–30 min during medical visit as mentioned in recommendations (3–6). From a cohort of 230 dogs and cats, in various physiological and pathological contexts, we selected and tested electrodes and positions, developed and validated a method of recording under vigil conditions, without pain, restrain and learning, leading to recommendations and better practices for routine EEG investigations in veterinary medicine. In this article, we present the full methodological section and its validation including the detailed procedure, the evaluation of recording quality, artifact discriminations and illustrations showcasing physiological and pathological EEG patterns.

2 Materials and methods

The Ethics Committee of VetAgro Sup (No. 18) issued a favorable opinion (No. 1966) on the experimental protocol on November 28, 2019.

2.1 General procedures

Electroencephalography recordings were performed in cats and dogs presented to neurology unit at the veterinary campus of VetAgro Sup and École nationale vétérinaire d'Alfort (ENVA). Included animals were either brought by their owners for medical

consultation or hospitalized for a short period due to brain disorders. Unconscious animals (i.e., animals with coma and status epilepticus) were excluded from this study. History, clinical signs, and results of diagnostic investigations were collected. We intentionally avoided using restrictive criteria in this study to demonstrate the method's reproducibility for dogs and cats across various medical contexts and to prevent selection bias.

The recordings were conducted after the consultation, either immediately or by appointment in following days. They were carried out by one of the authors (EL) in the presence of at least the animal's companion (owner or clinician), except for two examinations for which the operator was alone.

The examination rooms were customized to minimize visual, auditory, and olfactory stimulation and to be comfortable. They were clean, equipped with an examination table and a table for placing the acquisition device, chairs, sleeping mats and treats for dogs and cats. It was possible to create darkness in the room for photic stimulation, and a night light provided sufficient illumination light for recording.

Owners were advised to feed and take their animals out before the examination. They could bring their pet's sleeping mate and favorite treats. The recordings were mostly often performed with the dogs lying on the floor, with or without a mat, and the cats in their transport box, often with the top open, and placed on the examination table. Recordings were also operated with the animal on the owner's lap. In case of excessive heat during summer, a refrigerated mat was provided to the animal to prevent polypnea.

2.2 Device

EEG recordings were made using a wired EEG device (Brainbox® 1042 Braintronics BV, Fl. The Netherlands) with EEG software (Coherence® 7.1.3.2037 Natus Europe GMBH, Planegg, Germany). The acquisition settings were sampling frequency per channel 256 Hz, high pass filter 0.3 s, low pass filter 35 Hz, resolution 7 μV/mm, longitudinal and transverse montages. These montages were preferred over the referential montage to avoid contamination of the reference electrode by artifacts, especially cardiac, and to maximize the chances of observing small focal potentials (30, 31). A 50 Hz filter was used to avoid disturbances related to alternating current. These settings are those recommended in human medicine, except for the low-pass filter, which was reduced from 70 to 35 Hz in order to limit muscular artifacts without restricting brain rhythms observation (2–6). This device allowed synchronized video and EEG recording, as well as the configuration and visualization of light protocols.

2.3 Electrodes and cap

Human guidelines recommend the use of surface electrodes for routine EEG, either gold or Ag/AgCl cup gel electrodes or electrode caps (5, 6). As electrode caps are designed for human use, we opted for cup or stud surface electrodes, the latter being equivalent to non-wired cup electrodes. compatible with holding systems adapted to the animal's head. We compared unwired stud Ag/AgCl electrodes (Preborn, M.E.I, La Farlède, France; Figure 1A) and wired cup Ag electrodes (NE-112A, Nihon Kohden®, Tokyo, Japan; Figure 1B). The former was used over an initial period of 18 months, secured to the animals' heads using elastic straps perforated every 1.5 cm, in which the electrodes were inserted and held in place by alligator clips of the electric cables (Figure 1C). The latter was used over a second phase of 14 months, when an electrode EEG cap designed for cats and dogs was available in seven sizes (PetCap®, Elyope, Saint-Maur-des-Fossés, France) (32) (Figures 1D, E).

2.4 Electrode position and skin contact

The placement of the eight electrodes, both on the elastic straps and the PetCap ®, was based on the proposals of Pellegrino and Sica (22) and James et al. (24). The frontal electrodes (Fp1/Fp2) were positioned caudal to the median canthi, on the external edge of the temporal line. The occipital electrodes (O1/O2) were placed on a transverse line between the mastoid processes at an equal distance from the midline as the frontal electrodes. The central electrodes (C3/C4) were placed halfway between the frontal and occipital electrodes in the transversal plan and at an equal distance from the midline as the frontal and occipital electrodes. The temporal electrodes (T3/T4) were placed at the base of the ear, just above the temporal crest (Figure 1F). Our acquisition software allows the use of 2 reference electrodes and calculates the electrical potential difference between them to eliminate the noise. These 2 reference electrodes were placed on the median line at the intersection of diagonals Fp1-C4 and Fp2-C3 for the first reference and at the intersection of diagonals C3-O1 and C4-O2 for the second (Figure 1F). The electrodes were placed on the unshaven head, as close as possible to the skin, parting the hairs. For stud electrodes, elastic straps were placed on the animal's head, then the electrodes were inserted symmetrically into the perforations of the straps. Conductive paste and gel (Ten20®, Weaver and Company, Aurora, CO, USA, and SignaGel® Parker Laboratories, INC. Fairfield, NJ, USA) were then applied between the skin and the electrode. For cup electrodes, the electrodes were pre-inserted in the PetCap ® and coated with Ten20® before placing the headset on the animal and adding the SignaGel®, which reduced intervention time (Figure 1E).

Simultaneously, an electrocardiography (ECG) and respiratory recording were performed, with a thoracic electrode and a movement sensor held by a chest strap positioned behind the animal's front legs. Two electromyography (EMG) surface electrodes were placed on the anterior and dorsal regions of the neck muscles, posterior to the occipital electrodes, secured by the PetCap® (Figures 1E, F).

2.5 Recording

Electroencephalography recordings started after checking the impedance values of the electrodes and monitored in real-time, allowing for electrodes adjustments if necessary.

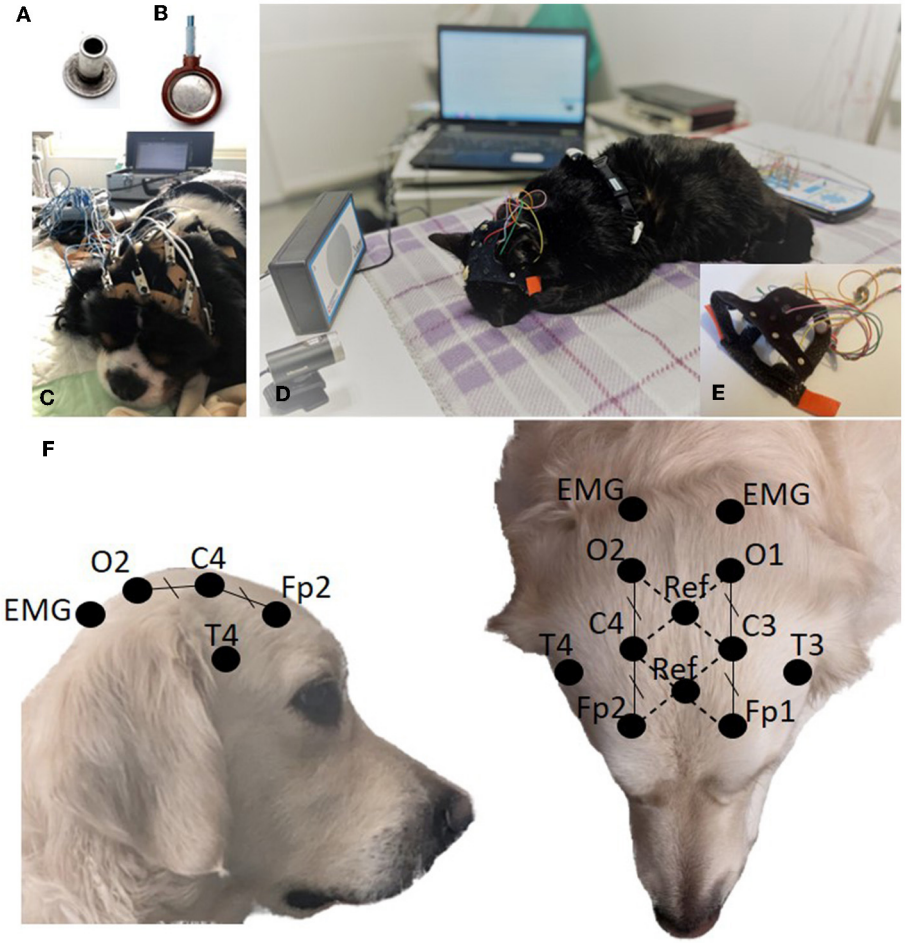


FIGURE 1
Stud electrode **(A)**. Cup electrode **(B)**. EEG acquisition setup using stud electrodes and elastic straps **(C)**. EEG acquisition setup using cup electrodes and PetCap® **(D)** with details of electrodes coated with conductive paste and inserted into the system **(E)**. Electrode positioning **(F)**.

The camera was positioned above and facing the animal and it was repositioned if the animal moved (Figure 1D).

Intermittent Photic Stimulation (IPS) was performed at the beginning of the examination when the animal was lying down in a state of wakefulness. The lamp was positioned at eye level, 30 cm away from the animal and the program followed a program that increased the frequency of light flashes: 3–5–7–10–13–15–17–20–25–30–35–40–45–50 Hz, with 10 s duration and 5 s pauses between frequency changes (Figure 1D). If the patient fell asleep during the photic stimulation, this test was repeated at the end of the EEG examination after the patient woke up. Other stimulations such as noise and meal were carried out based on potential seizure triggers reported by the owner and the clinician.

The animals were not stimulated to promote rest for at least 20 min. If the patient fell asleep during the recording, the waking phase after the nap was recorded for a minimum of 5 min.

The operator annotated the recorded trace with as much information as possible concerning the events that may occur during the examination, in the environment or specific to the patient.

2.6 Interpretation

All recordings were visually reviewed by three authors SBe, CE, and EL during joint reading sessions in order to obtain a consensus. The settings used for interpretation were the same as for acquisition, but could be modulated to aid pattern discrimination. Artifacts, physiological rhythms, and paroxysmal events were listed. Wakefulness was identified on the EEG by visualization of a low voltage fast activity background disturbed by eye and body movement artifacts and muscle contractions (EEG and EMG channels). Drowsiness was identified by visualization of a low voltage fast EEG activity background, with alpha rhythms (8–12 Hz) or theta rhythms (4–7 Hz), fewer eye and body movement artifacts, less muscle tone and more regular breath (respiratory movement sensor). Non-rapid eye movements (Non-REM) sleep was identified by the occurrence of medium and high voltage delta (1–4 Hz) activity and/or sleep spindles (waves with a frequency of 12–16 Hz) in the EEG, no eye and body movement artifacts, regular respiration and decreased muscle tone. REM sleep was identified by visualizing of a low voltage fast activity on EEG, weak amplitude EMG but disturbed during facial or leg twitches and

myoclonic jerks, irregular respiration (respiratory belt) and heart beat (ECG) (33–36). Paroxysmal events noted included spikes, polyspike-complex, spikes-and-slow-waves-complex, polyspikes-and-slow-waves-complex, sharp waves, triphasic waves, and slow waves (37, 38).

2.7 Statistics

In the absence of normal distribution, non-parametric tests were used. Descriptive statistics are presented as median [1st quartile−3rd quartile]. Quantitative variables between groups were compared by the Mann–Whitney–Wilcoxon test (for two groups) or the Kruskall–Wallis test and Dunn-Bonferroni *post-hoc* test (for more than two groups). The distributions of multiple groups were compared using the chi-square test of homogeneity, with some groups aggregated if numbers were insufficient and with Yates' continuity correction if necessary. Results were considered statistically significant at $P < 0.05$. All statistical tests were carried out using R software (4.2.1). The plots were generated using the R package ggplot 2 (39) and Microsoft Excel® (Microsoft Corporation One Microsoft Way, Redmond, WA, USA). We used a ratio expressed in percentage, called "readable percentage" calculated with the time corresponding to the number of 20-second pages readable divided by the total recording duration in minutes. Indeed, if more than half of the 20-second page was uninterpretable due to artifact, i.e., whose amplitude causes overlapping of the recording channels, whatever the montage used (referential or bipolar) at the 7 μV/mm setting, or whose frequency overloads the visualization of physiological rhythms, the page was considered unreadable. In addition, EEG recording was stopped during impedance checks and restarted afterwards. Pages during these impedance checks were also counted as unreadable.

3 Results

3.1 Population

Two hundred and thirty EEGs were investigated between October 2019 and July 2022.

The first 80 recordings were used to develop the protocol, specifically to establish the positioning of the elastic bands for achieving a symmetrical setup during the recordings, validate the electrode positions with PetCap®, choose the types of surface electrodes, select the contact gels and equip the EEG device with video and synchronized photic stimulation lamp.

The following 150 recordings were included in the study involving 140 animals, 101 made on dogs and 49 on cats.

Ninety-four dogs were recorded with 88 dogs recorded once, five dogs twice and one dog, three times. Forty-one dog breeds were represented, 18 dogs were mixed breed. The head conformations of dogs were classified into three categories: dolichocephalic, with a very elongated skull, brachycephalic, with a very flat face, and mesocephalic, close to the primitive type (40) (Table 1). They were 43 female and 51 male dogs, ranging in age from 4 months to 17 years [4.3 years [1.8–7.6]], with weight ranging from 2.6 to 64.6 kg [17.2 kg [8.9–25.45]]. Five dogs were healthy dogs brought in by their owners who were veterinary students involved in the study, while all other dogs were presented by their owners for neurology consultations. Eighty dogs had a history of at least one paroxysmal episode in the 6 previous months, either typical epileptic seizures or less characteristic episodes such as tail chasing, myoclonus, episodic stiffness or ataxia, compulsive licking, fly biting, episodic collapses, trance-like episodes, jaw chattering episodes, episodic drooling, episodic chewing, episodic aggression, episodic polypnea or episodic movement disorders. Nine dogs had a confusional state or signs of vestibular impairment but no paroxysmal event.

TABLE 1 Distribution of dog breeds.

Mesocephalic (68 dogs)
American Staffordshire Terrier, Australian Shepherd (2), Australian Shepherd cross-breed, Basset Hound, Beagle, Beagle cross-breed, Bearded Collie, Beauceron cross-breed, Bernese Mountain dog, Bouvier des Flandres, Brittany Spaniel cross-breed, Bull Terrier cross-breed, Cavalier King Charles (3), Chihuahua (2), Cocker Spaniel, Coton de Tulear, Dutch Shepherd, Eurasier, Great Dan, German Shepherd, German Spitz, Golden Retriever (4), Irish Setter (2), Jack Russell (6), Labrador (4), Malinois (3), Malamute cross-breed, Maltese, Newfoundland cross-breed, Parson Russell Terrier, Rottweiler, Shiba Inu, Siberian Husky (3), Welsh Corgi Pembroke cross-breed, West Highland White Terrier, White Swiss Sheperd, Yorkshire Terrier (6), Yorkshire Terrier cross-breed, indeterminate cross-breed (5).
Brachycephalic (11 dogs)
Continental Bulldog, French Bulldog (6), Boxer, Carlin (2), Lhassa Apso cross-breed.
Dolichocephalic (15 dogs)
Border Collie (6), Border Collie cross-breed (4), Dachshund, Wirehaired Dachshund, Doberman, Italian Greyhound, Podenco.

Forty-six cats were recorded, with 43 cats recorded once and 3 cats twice. Six cat breeds, 1 mix breed and domestic shorthair cats were recorded [Bengal, Birman (2 cats), Devon Rex, Norwegian, Persian, Ragdoll (2 cats), Mix breed Main Coon, Domestic Shorthair (37 cats)]. They were 23 female and 23 male cats, ranging in age from 7 months to 17 years [3.5 years [1.4–7.9]], with weight ranging from 700 to 7.4 kg [4 kg [3.15–4.8]]. Forty cats had a history of at least one paroxysmal event, either typical epileptic seizures or less characteristic episodes such as rolling skin, tail chasing, scratching, episodic aggression, episodic growling, episodic vocalization, compulsive licking, trance-like episodes, episodic stiffness or ataxia, possible REM sleep disorder or episodic movements disorders. Six cats had a confusional state or signs of vestibular impairment but no paroxysmal event.

3.2 Validation of unsedated EEG feasibility

Ninety-four percent (141/150) of the EEG recordings were carried out without the use of sedative medication. Two dogs underwent EEG after Magnetic Resonance Imaging (MRI) or Brainstem Auditory Evoked Response (BAER) and were under sedation, while three cats and one dog received preventive premedication due to their aggressiveness (dexmedetomidine/butorphanol for one cat and gabapentin for the others). Additionally, three restless dogs were sedated to minimize movement and breathing artifacts (dexmedetomidine). The results

presented here pertain to EEG without tranquilization, i.e., 95 EEGs performed on dogs and 46 EEGs performed on cats.

Sixty-five dog EEGs and 28 cat EEGs were performed in the presence of the owner. Treats were used to keep the dogs occupied if they became agitated during the helmet fitting. All dogs kept the EEG system on their head and one cat initially removed the system which was reinstalled. Despite gentle movements, lying down in various position (sternal or lateral recumbency), standing up, or shaking, the electrodes remained attached with good electrode-skin contact and impedances. In some cases, a strip of elastic band (Vetrap™) was applied preventively to secure the mounting system if the animal seemed agitated before recording. No dermatological reactions were reported following the use of Ten20® and SignaGel®. Gel was removed using dry shampoo and, if necessary, supplemented with wet shampoo at home by the owner, like in human use.

Sixty-four dog EEGs were performed using stud electrodes, and 31 dog EEGs were conducted using cup electrodes. Twenty-four cat EEGs were conducted with stud electrodes, and 22 cat EEGs were performed with cup electrodes.

Photic stimulation was achieved for 42 dog EEGs and 27 cat EEGs, as the recording device was not initially equipped for it at the start of the study. Light stimulation was not performed in cases of non-convulsive status epilepticus diagnosed by EEG (4 EEGs).

Other stimulations were carried out according to history provided by owners to trigger seizures, including food (five dogs) and sounds (three dogs and one cat) such as clapping of hands, sound of keys, sound of crumpled paper and sound of crushed plastic bottles.

Median EEG recording time was 40 min for the two species [Dogs: 40 min [30–55]; Cats: 40 min [27–49]] (Figure 2A). Six patients had recording time of <20 min, with 4 due to time constraints related to the functioning of the service, 1 where the diagnosis was immediately established based on EEG findings and 1 due to excessive agitation. Twenty-six animals had a recording lasting more than 60 min, 12 were sleeping deeply, nine were restless and we had to wait for the animal to calm down to have a readable EEG trace, and five others to maximize the chances of recording seizures.

3.3 Validation of the technical quality of the EEG recordings

3.3.1 Impedance

Electroencephalography software gives intervals of values of the impedance measurements (Z) for each electrode: $Z > 100\ k\Omega$ (written >100 on the software), $50 < Z \leq 100\ k\Omega$ (written <100), $20 < Z \leq 50\ k\Omega$ (written <50), $10 < Z \leq 20\ k\Omega$ (written <20), $5 < Z \leq 10\ k\Omega$ (written <10), $Z \leq 5\ k\Omega$ (written <5). All impedance values recorded were ≤50 kΩ (indicate <5 or <10 or <20 or <50 by the software). In dog EEGs, 98.8% (751/760) of the impedances were $\leq 20\ k\Omega$ (indicated <5 or <10 or <20 by the software) and, in cat EEGs, 97.6% (359/368), regardless of the types of electrode used. Impedances $\leq 10\ k\Omega$ (indicated <5 or <10 by the software) were observed in 81 and 85.2% of dog and cat EEGs, with cup electrodes, and in 64.5 and 70.3% with stud electrodes (Figure 3A). Comparing the numbers in the <5 and <10 impedance groups with those in the <20 and <50 groups by electrode type (stud or cup), we concluded that cup electrode impedances are lower than stud electrode impedances (dog EEGs: x-squared = 121.32, df = 1, p <0.001; cat EEGs: x-squared = 10.856, df = 1, p < 0.001; Figure 3A). Impedances are not homogeneous, depending on electrode positioning in dog and cat EEGs (dog EEGs: x-squared = 26.228, df = 14, $p = 0.024$; cat EEGs: x-squared = 33.97, df = 14, $p = 0.002$; Figure 3B).

3.3.2 Montages and isosynchrony

We define "usable channels" as channels without isosynchrony, that appears on the EEG trace as a flat line (Figure 4). For dog EEGs, in the longitudinal montage, 93.4% (478/512) of the channels were usable with stud electrodes, and 94.7% (235/248) were usable with cup electrodes. In the transverse montage, 95.6% (306/320) of the channels were usable with stud electrodes, and 94.8% (147/155) with cup electrodes. For cat EEGs, in the longitudinal montage 83.3% (160/192) of the channels were usable with stud electrodes and 93.2% were usable (164/176) with cup electrodes. In the transverse montage 79.2% (95/120) of the channels were usable with stud electrodes, and 97.3% (107/110) were usable with cup electrodes. By adding the values of the usable channels of dogs and cats in longitudinal montage with cup electrodes (235 + 164 = 399) and dividing by the total number of channel of dogs and cats in longitudinal mounting with cup electrodes (248 + 176 = 424) we obtained the proportion of usable channels which was in percentage 94.1%. The percentage of isosynchrony in the longitudinal montage with cup electrodes for both dog and cat combined was therefore 5.9% (100–94.1%).

The longitudinal montage with eight usable channels was more easily set up in larger dog compared to small dogs (Figure 5A). This difference is less noticeable in cats, as the variation in weight and size is less significant (Figure 5B). The 3 EEGs with only four usable longitudinal channels were performed with stud electrodes on restless animals. One of these EEGs was repeated due to the animal's agitation, and the number of channels was eight during the second recording.

3.3.3 Artifacts

We observed technical artifacts such as electrical environment artifacts, particularly when the amplifier box was positioned on a metal surface, as well as pop artifacts from the electrodes. Additionally, artifacts specific to the use of alligator clips with stud electrodes were observed when they were in contact with each other. We also observed artifacts related to people in the recording environment, including recording of the owner's cardiac pulse, petting the animal and contact with the wires, and movements in the room.

Some physiological artifacts observed were similar to those described in human medicine atlases, such as heartbeat, respiratory movements, muscle contractions and global movements (Figure 4). However, we observed also artifacts specific to dogs and cats, such as licking artifacts, ear movements and tail flapping movements.

Seventy-four point seven percent (71/95) of the dog recordings were readable for more than 50% of their duration up to

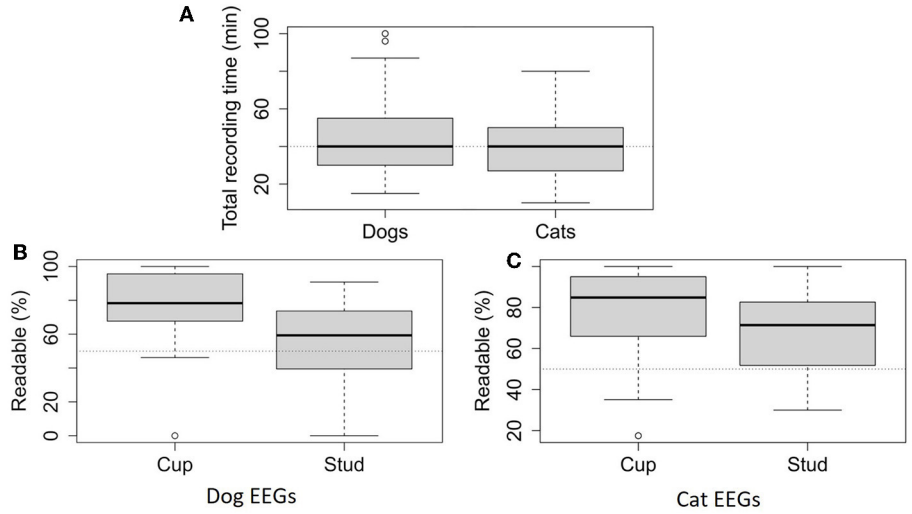


FIGURE 2
Total recording time for dog EEGs (95) and cat EEGs (46) **(A)**. Readable percentage calculated with the 20-second pages that are undisturbed by artifacts for more than half of their duration over the total recording time depending on electrode type, for dog EEGs (64 EEGs with stud electrodes and 31 EEGs with cup electrodes) **(B)** and cat EEGs (24 EEGs with stud electrodes and 22 EEGs with cup electrodes) **(C)**.

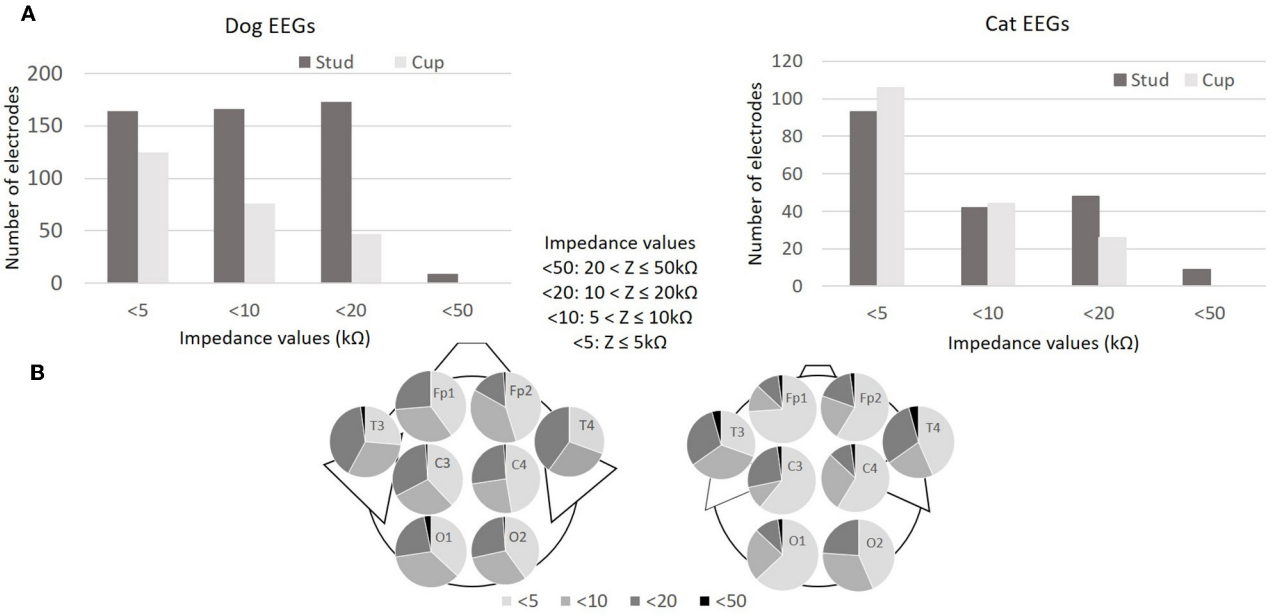


FIGURE 3
Impedance values (kΩ) of the 760 electrodes during the 95 dog EEGs (95x8 electrodes) and impedance values of the 368 electrodes during the 46 cat EEGs (46x8 electrodes) **(A)**. Distribution of impedance values (kΩ) according to electrode position in 95 dog EEGs and 46 cat EEGs **(B)**.

100%, 13.7% (13/95) of the recordings were readable for 25–50% of their duration and 11.6% (11/95) of the recordings were readable for <25% of their duration. In the latter cases, the animal had behavioral problems, or the owners were intrusive, or stimulation techniques such as feeding generated a lot of artifacts. "Readable percentage" below 50% were mainly associated with the use of stud electrodes (20/24). Consequently, "readable percentage" were significantly higher in EEG recordings with cup electrodes than those with stud electrodes [stud electrodes: 59% [40–73]; cup electrodes: 78% [68–96]; $p < 0.001$; Figure 2B].

Nine dogs had 2 registrations, 4 due to their agitation and the others for medical follow-up. The median of readability percentages of the first recordings of agitated dogs was 16% [12–25], while that of the second recordings was 70% [61–75], suggesting the beneficial effect of habituation in some specific cases.

Eighty-two point six percent (38/46) of the cat recordings were readable for more than 50% of their duration up to

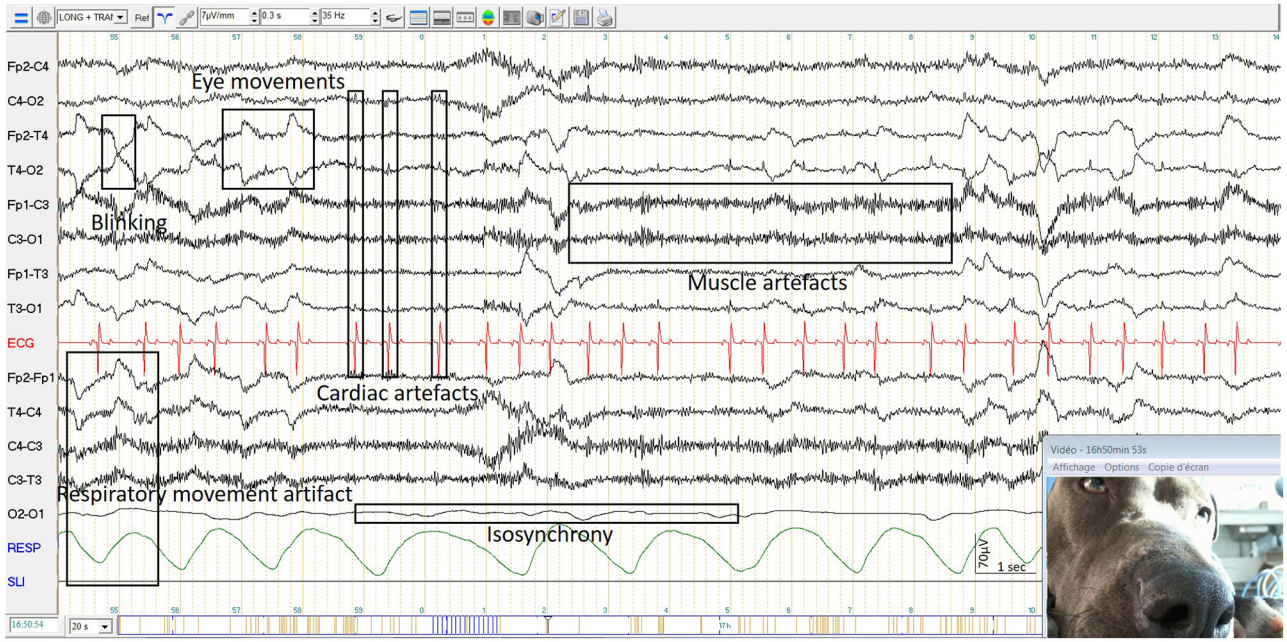


FIGURE 4
Examples of isosynchrony and physiological artifacts in a dog EEG.

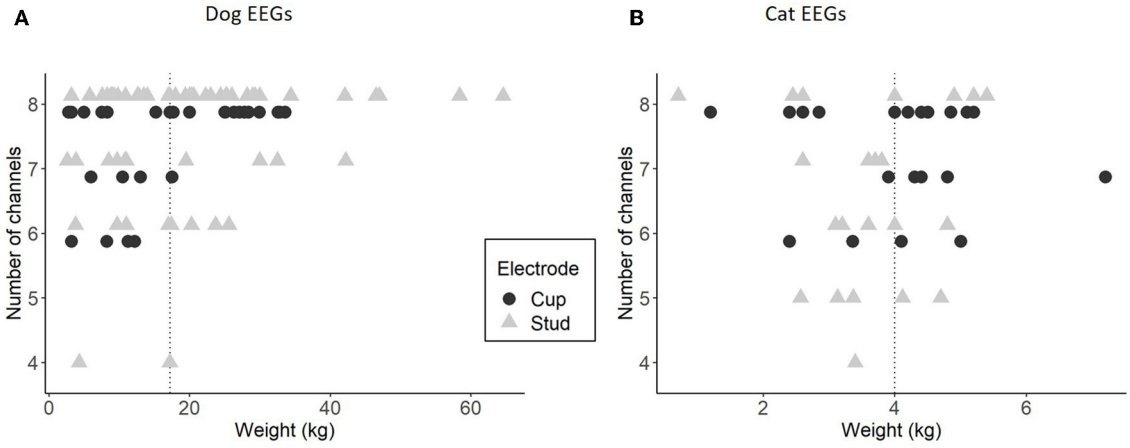


FIGURE 5
Relationship between the weight of the animals and the number of usable channels in longitudinal montage, for dog EEGs with stud (64 EEGs) and cup (31 EEGs) **(A)** and for cat EEGs with stud (24 EEGs) and cup (22 EEGs) **(B)**. The dotted line represents the median of the weights in each species.

100%, 15.2% (7/46) of the recordings were readable for 25–50% of their duration, 2.2% (1/46) of the recordings were readable for <25% of their duration. The main difficulties encountered were related to inappropriate contact between the alligator clips, which is accentuated by the small size of the cats' heads and to the owners' interventions. In cats, we found no significant difference between the "readable percentage" on EEG recordings with stud electrodes and those with cup electrodes [stud electrodes: 71% [52–82]; cup electrodes: 85% [68–94]; $p = 0.0503$; Figure 2C].

3.4 Validation of EEG interpretability

3.4.1 Physiological rhythms

Physiological rhythms were observed in 98.9% (94/95) of dog EEGs including wakefulness rhythms (92/94) (Figures 6A, B), drowsiness (72/94) (Figure 6C), and sleep (46/94) (Figure 6D). For cat EEGs, physiological rhythms were observed in 91.3% (42/46), including wakefulness rhythms (41/42) (Figure 7A), drowsiness (33/42) (Figure 7B), and sleep (9/42) (Figure 7C). No physiological rhythms were observed in five recordings, probably due to the

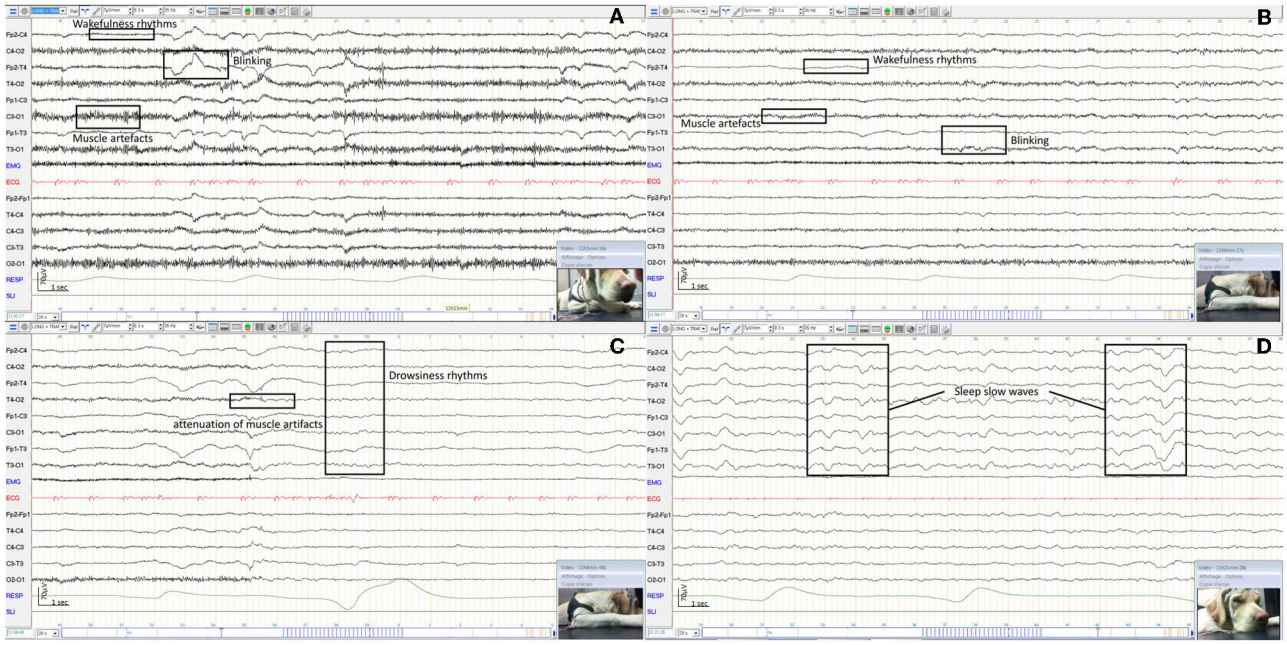


FIGURE 6
EEG of an awake dog showing a rapid low amplitude rhythm and artifacts from blinking and muscle activity with examples in boxes **(A)**. EEG of the same dog as before, awake and calm, showing a rapid low amplitude rhythm with reduced artifacts from blinking and muscle activity with examples in boxes **(B)**. EEG of the same dog as before, dozy, showing a 5 Hz medium amplitude rhythm **(C)**. EEG of the same dog as before, sleepy, showing 1–3 Hz high amplitude rhythms with examples in boxes **(D)**.

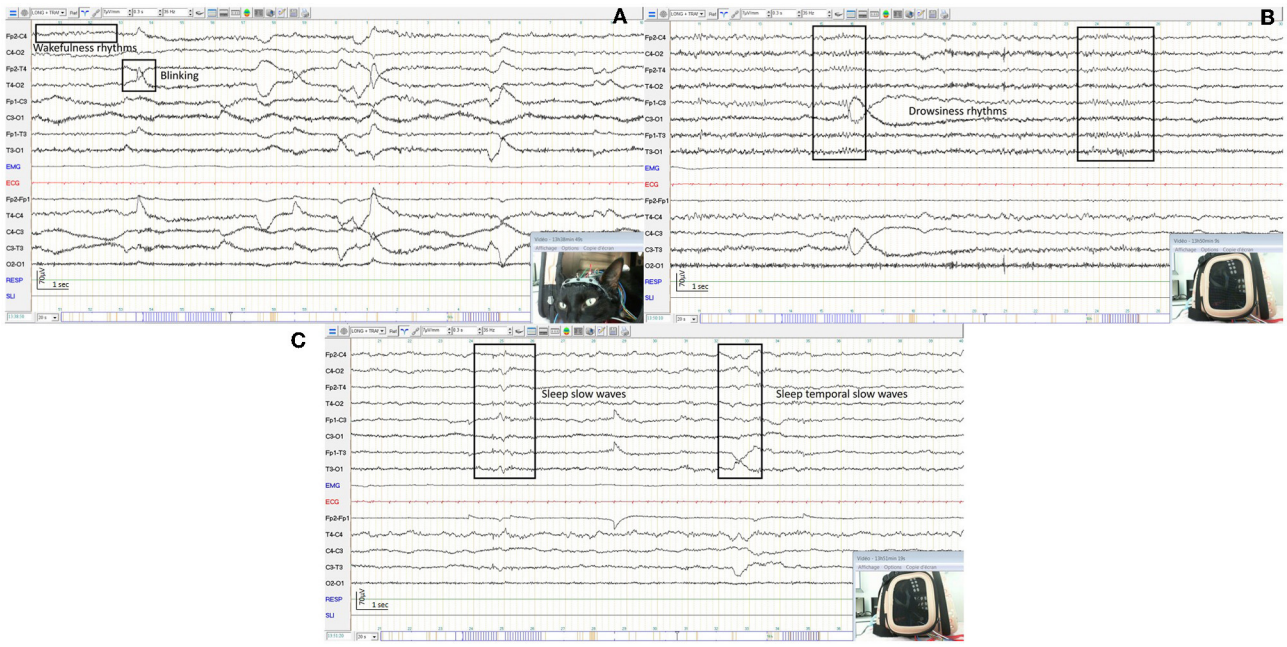
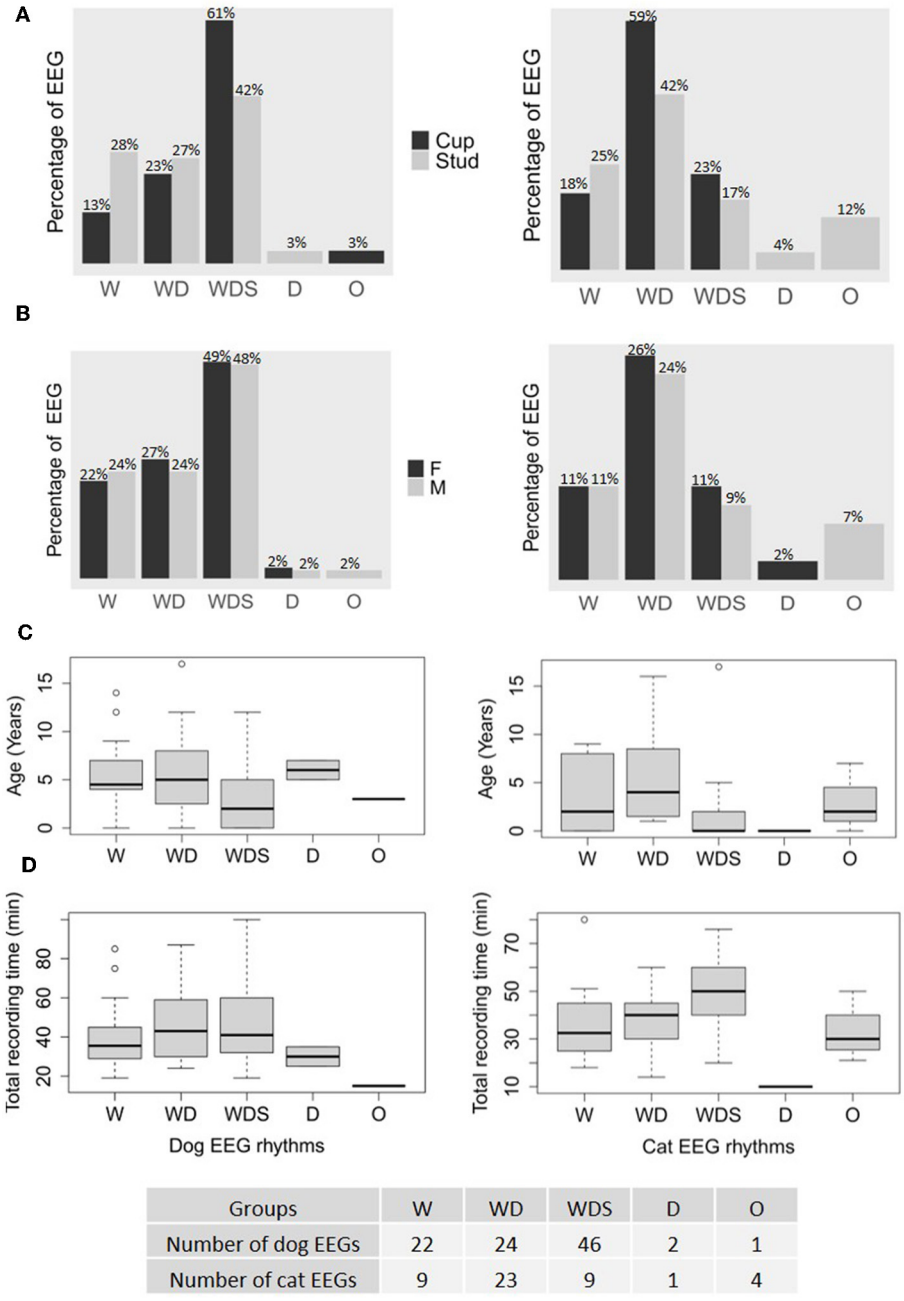


FIGURE 7
EEG of an awake cat showing a rapid low amplitude rhythm and artifacts from blinking and muscle activity with examples in boxes **(A)**. EEG of the same cat as before, dozy, showing a 7 Hz medium amplitude rhythm **(B)**. EEG of the same cat as before, sleepy, showing mixed theta and delta rhythms with examples in boxes **(C)**.

pathology of four animals (diffuse encephalopathy for one dog and one cat, a non-convulsive status epilepticus for two cats), and to technical reason in one cat (intact male with high impedance values possibly due to skin specificity).

Five groups were determined based on the physiological rhythms observed: EEG with wakefulness rhythms only (W), EEG with wakefulness and drowsiness rhythms (WD), EEG with wakefulness, drowsiness and sleeping rhythms (WDS), EEG with

10.3389/fvets.2024.1326165



Groups	W	WD	WDS	D	O
Number of dog EEGs	22	24	46	2	1
Number of cat EEGs	9	23	9	1	4

FIGURE 8
Rhythm distinctions (W, wakefulness only; WD, wakefulness and drowsiness; WDS, wakefulness, drowsiness and sleeping; D, drowsiness only; O, other than physiological states) in the recordings of 95 dogs (on the left) and 46 cats (on the right) detailed according to the type of electrodes used and expressed as a percentage of the total number of recordings made for each kind of electrode, cup and stud **(A)**, their sex **(B)**, their age **(C)**, and the total recording time **(D)**.

drowsiness only (D), and other than physiological rhythms (O). The W group consisted of 22 EEGs from dogs and 9 EEGs from cats, the WD group had 24 dog EEGs and 23 cat EEGs, the WDS group had 46 dog EEGs and 9 cat EEGs, the D group had 2 dog EEGs and 1 cat EEG, and the O group had 1 dog EEG and 4 cat EEGs. The three groups W, WD and WDS were observed in both species of the study regardless of the two types of electrodes (Figure 8A). Dogs were more likely to sleep and cats to doze during recordings and this tendency was more pronounced with cup electrodes (Figure 8A). Physiological rhythms were observed in both males and females (Figure 8B) and they were easier to observe in younger animals compared to older ones (Figure 8C). The ages of dogs in the three groups W, WD and WDS were significantly different (dog EEGs: Kruskall-Wallis chi-squared =

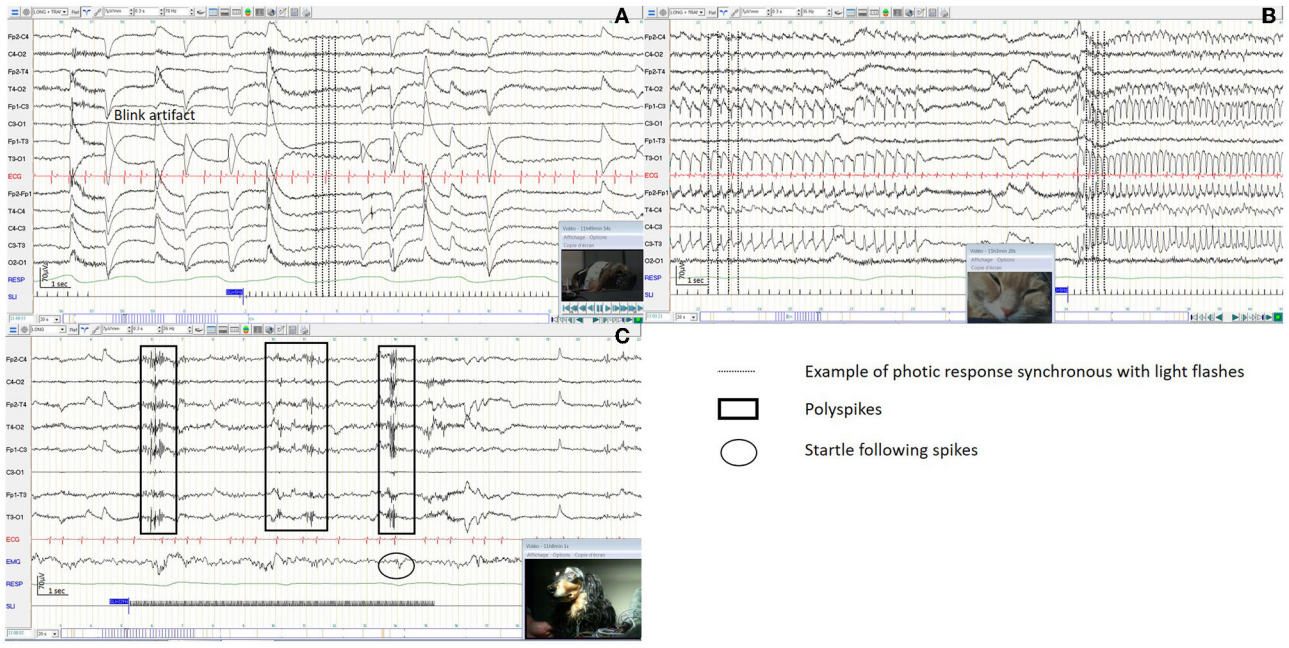


FIGURE 9
Reactivity to intermittent photic stimulation in a dog EEG **(A)**, in a cat EEG **(B)**, with polyspike complexes in a dog EEG **(C)**.

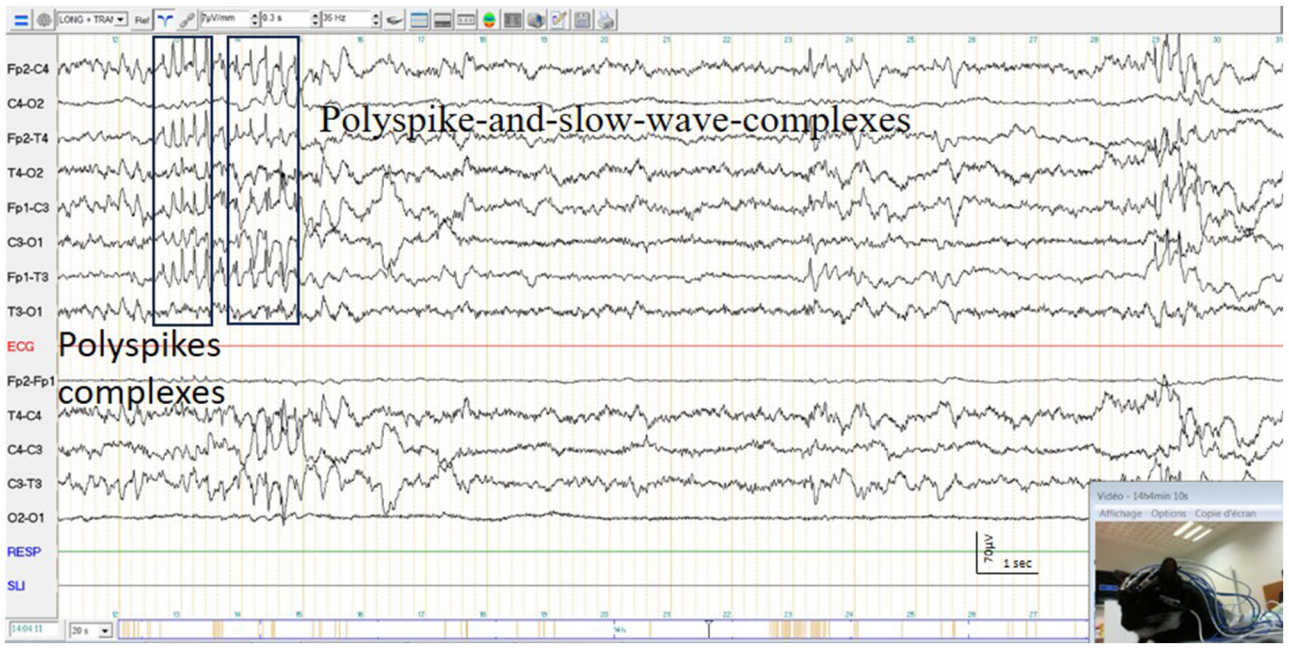


FIGURE 10
Polyspike complexes and polyspike-and-slow-wave-complexes and simultaneous twitching of a cat's face.

8.32, df = 2, $p = 0.02$; cat EEGs: Kruskall-Wallis chi-squared = 6.48, df = 2, $p = 0.04$) with younger animals in the WDS group than in the W group ($p = 0.03$) and in the WD group ($p = 0.03$) in dog EEGs and with younger animals in the WDS group than in the WD group ($p = 0.02$) in cat EEGs (Figure 8C). Median recording time in W groups was 35.5 min [29–45] for dog EEGs and 32.5 min [25–44] for cat EEGs, in WD groups 43 min [30–58] for dog EEGs and 40 min [30–45] for cat EEGs, and in WDS groups was 41 min [33–59] for dog EEGs and 50 min [42–59] for cat EEGs (Figure 8D). The other two groups, D and O, involved only a few animals and were related to their respective disease.

TABLE 2 Correlations between clinical and electrical patterns observed during the EEG according to literature (37).

Clinical signs during EEG \ Electrical events on EEG	Epileptic or seizure patterns	Other electrical events (Periodic discharges and triphasic waves)	No electrical event	Equivocal electrical events (due to artifacts)
Myoclonus (head or body, or limb)	7 dogs 7 cats	1 dog	1 dog 3 cats	1 dog 2 cats
Alterations in consciousness	3 cats	2 dogs 3 cats		
Ptyalism +/- licking	4 dogs 1 cat			
Polypnea + shivering			2 dogs	
No clinical signs	5 dogs 4 cats			

3.4.2 Stimulation tasks

3.4.2.1 Intermittent photic stimulation

Photic response synchronous with light flashes were observed in 11% (5/45) of dog EEGs, with frequencies ranging from 3 to 10 Hz and in 85% (23/27) of cat EEGs, with frequencies ranging from 3 to 30 Hz, without any clinical signs (Figures 9A, B). Epileptic discharges were observed during IPS in 13% (6/45) of the dog EEGs (Figure 9C). For 5 dogs, clinical myoclonus was observed simultaneously with epileptic discharges, while for the remaining dog no clinical manifestation was observed. Four of these six dogs showed mild myoclonus during sleep and epileptic discharges on the EEG trace.

3.4.2.2 Other stimulation tasks

During stimulation with food with five dogs, two dogs presented clinical paroxysmal event including one epileptic and one non-epileptic. During stimulation with sounds with three dogs and one cat, all presented clinical paroxysmal event. On the EEG, epileptic discharges were observed simultaneously with clinical paroxysmal event in one dog and in one cat.

3.4.3 Electro-clinical interpretation

No animal exhibited generalized tonic-clonic epileptic seizure during EEG recordings. However, 37 animals showed symptoms suggesting epileptic seizure, like myoclonus localized on the face, limb or trunk, with a wide range of intensity such as tremors, sudden movements or startles or alterations in consciousness or neurobehavioral signs as shivering and polypnea (41). Among these animals, 75.7% (28/37) exhibited concomitant pathological graphoelements, with 59.5% (22/37) displaying epileptic patterns (37) (Figure 10) and 16.2% (6/37) showing other pathological graphoelements, such as triphasic waves and periodic discharges, suggestive of encephalopathy. Sixteen point two percent (6/37) of animals showed paroxysmal manifestations not followed by EEG abnormalities, ruling out an epileptic cause. Eight point one percent (3/37) of animals exhibited clinical symptoms, but artifacts on the EEG trace hindered interpretation. Additionally, 9 EEGs showed epileptic or seizure patterns without any clinical signs during electrical events.

Among the 136 non control and non-tranquilized animals, 27.2% (37/136) presented clinical signs during the examination and 25% (34/136) of the EEGs provided diagnostic information. Furthermore, 6.6% (9/136) of the EEGs showed epileptic or seizure patterns suggestive of epilepsy without clinical signs (37). Thus, EEG was informative in 31.6% (43/136) of the patients, despite the inclusion of a highly variable population (Table 2).

4 Discussion and conclusion

The aim of this work was to propose a method that would facilitate the use of EEG in veterinary practice for routine use, adapted to specific constraints of the animal while respecting its wellbeing, so as to be able to observe brain function and disorders in the most physiological situation possible. We have succeeded in performing EEG examinations on 150 dogs and cats of various sizes and conformations in a clinical setting, without the need of anesthesia in 94% of cases. The cats were particularly calm during the examination, and the fact that they could remain in their open or covered transport box was beneficial to their relaxation. The presence of the owner and the possibility of lying down in their own basket helped the dogs a lot to relax. For the most agitated animals, the owner sometimes lay down next to his animal or took it on his lap. Managing ambient noise was particularly important, with some preferring silence and others a monotone background noise like that of a discussion. We also took care to be few in number in the room, placing ourselves far from the animal, in a seated, calm position and looking away from the animal and oriented toward the camera screen, being the least interventional as possible and patient.

Our study allowed to describe the performance of EEG without need for anesthesia or sedation, medical training or voluntary recruitment (24, 35, 42–44), in a large sample of dogs and cats. To our knowledge, no study on EEG without anesthesia in healthy or diseased cats has been conducted prior to this one. The use of surface electrodes and the ability to mount them on a holding

system gave us a real advantage over the use of needles in being able to offer this examination to any animal showing signs of encephalic dysfunction, whatever the temperament of the animal and with the full confidence and endorsement of the owner. The latter could even assist with the EEG, reassuring their animal, helping with the setup of the system on animals that exhibited a particularly wary attitude toward the veterinarian, providing insights into the triggering elements of the seizures, and validating the concordance of the seizures observed during the examination and at home.

Surface electrodes are non-invasive, comfortable, provide high-quality EEG examinations and are widely used in human medicine. We therefore opted for surface electrodes rather than needle electrodes, but we had to consider a holding system to enable them to be positioned on the animal's head. This had to be able to ensure symmetrical and constant positioning in relation to the bony relief of the animal's skull, whatever its conformation. We tested 2 holding systems, each compatible with 1 type of surface electrode stud or cup. One consisted of elastic straps placed around the animal's head. These straps were perforated every 1.5 cm, enabling the stud electrode insertion holes to be selected according to the bone relief. The other consisted of a system to be threaded through the animal's head, and offered in 7 sizes to adapt to the varying conformations of the animals. The cup electrodes were fitted with covers so that they could be clipped onto the cap. This enabled us to compare two approaches: the first with a system that allows free placement of the electrodes, but whose installation is tedious, and the second with a more constrained system, but quick installation. The second system is similar to the pre-wired caps available for humans.

We were concerned to obtain good quality examinations to be able to interpret the tracings as accurately as possible. To achieve this, we used evaluation criteria and ensured that the quality of the tracings obtained was equivalent to that of other methods used in veterinary medicine. We also checked that this quality was in line with the recommendations published for the performance of EEGs in human medicine (2–6).

Impedances obtained were below 20 kΩ in 98.8% of dog EEGs and in 97.6% of cat EEGs, with better results with cup electrodes, which obtained impedances below 10 kΩ for over 80% of electrodes. These impedance values align with those reported in literature, ranging from 5 to 30 kΩ for needle electrodes in dogs (22–24, 44–46) and cats (47), and 5 to 15 kΩ for cup electrodes (23, 35) and with human medicine recommendations that impedance values for surface electrodes be below 10 kΩ (4, 6).

The derivations observed in longitudinal montage are determined for each hemisphere between the frontal and central, central and occipital, frontal and temporal and temporal and occipital electrodes, and are referred to as channels. The usable channel rate exceeds 93% for both species, the other channels being affected by isosynchrony linked to the proximity of the electrodes or to gel diffusion between two electrodes. This isosynchrony rate of 5.9% is close to the isosynchrony rate of 5.5% obtained with needle electrodes on dog EEG, with the same observation that isosynchrony was more frequent in smaller dogs (45). We have not found incidence values for isosynchrony in human medicine, but it is well-described. In medical research, dry electrodes were developed to eliminate the need for gel to avoid the problem of isosynchrony and to save time. Regardless of the technologies used, the impedances of these dry electrodes are higher than those of gel electrodes, and for some, especially contactless electrodes, the very weak electrical signal must be amplified directly at the electrode level, which makes them heavier and bulkier (48–50). In human medicine, gel electrodes remain the gold standard (6, 50). Dry electrodes are less studied in veterinary medicine. Polymer electrodes with a silver/silver chloride (Ag/AgCl) coating and gold-plated metal electrodes covered with poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) have been studied as ECG sensors on dogs (51, 52). PressOn^{TM} EEG electrodes and spring-loaded dry EEG electrodes have been used in a unpublished study, but the quality of the EEG signals obtained remains inferior to those recorded by needle electrodes in the same study (53). Hence, we suggest the utilization of gel electrodes in veterinary medicine while closely monitoring the potential advancements that the research and development of appropriate dry electrodes might yield.

We aimed to evaluate the impact of artifacts, which are inevitably more prevalent during EEG recordings without anesthesia. Consequently, we sought to clearly identify these artifacts, assess their extent of influence on the trace, and determine the required examination duration to obtain a 20-minute interpretable trace, in accordance with recommendations for routine EEG in human medicine (6). We have clearly recognized similar technical and physiological artifacts as described in human (38) and veterinary medicine (23, 45), in addition to specific ones originating from the examination situation without anesthesia and stimulation techniques such as food intake. The detailed description of these artifacts remains to be published in a future article. However, despite these artifacts, 75% of dog EEGs and 83% of cat EEGs were readable for more than 50% of their duration, with a median recording time of 40 min in both species. EEG tracings longer than 40 min can therefore provide sufficient data to be medically relevant with minimal impact from artifacts.

The recognition and identification of normal physiological rhythms of wakefulness, drowsiness and sleep is a necessary condition essential to be able to read an EEG. It appeared important for us to be able to clearly identify them using surface electrodes in a context without anesthesia. The physiological rhythms described in the literature (33–36) for stud and cup electrodes were visualized in both species, regardless of conformation, sex, or age. Drowsiness, observed in 84% of dog EEGs and 82% of cat EEGs with cup electrodes, also serves as a marker of good tolerance and wellbeing of the animal during recording.

Routine EEG in human medicine includes the use of two provocative methods for seizure induction: hyperventilation and IPS. Hyperventilation is not possible in animals, as well as in young children, since it requires the patient to voluntarily perform deep breaths. Therefore, we focused on IPS with a protocol of frequency flashes of 3–5–7–10–13–15–17–20–25–30–35–40–45–50 Hz. The IPS protocols suggested in veterinary articles are variable, and no argument favors one over the others (44, 46, 47, 54, 55). Recent recommendations in human medicine suggest performing frequency flashes of 1–2–8–10–15–18–20–25–40–50–60 Hz (6) without us being able to determine if this protocol would be the best for use in pets. It is also indicated that IPS for human

TABLE 3 Protocol recommendations.

Examination facilities
• Unstimulating and peaceful environment, darkness possible • Fed animal, access to a place of disposal before the examination, sleeping place • Involvement of the owner to avoid disturbances and facilitate the relaxation of the animal
Course of the recording
• 45 min minimum • Check impedance <10 kΩ, <20 kΩ accepted • IPS if possible • Interventions limited on the animal • Recording of drowsiness patterns required, sleep if possible • Real-time clinical and EEG events reporting
Equipment
• 8 gel EEG electrodes • Electrodes headset • 2 EMG • 1 ECG • Breathing belt • Video EEG • Synchronized photic stimulation lamp • Wired or unwired device
EEG evaluation and interpretation
• Quality score including impedance, numbers of EEG channels, artifact percentage • Use of a defined and accepted terminology • Description of artifacts, background rhythm, physiological and pathological graphoelements • Electroclinical correlation • Expertise

should be done with both eyes closed and eyes open. However, it is challenging to request this from animals, and manually closing their eyes can lead to movements that generate artifacts. Nevertheless, we have observed that animals naturally close and open their eyes during IPS. Therefore, we found that IPS was a stimulation technique very well-tolerated and easy to use in awake animals.

In the large proportion of cat EEGs (85% of cases), high-amplitude graphoelements were observed synchronously and with the same frequency as the light flashes, identical in appearance to the photic driving described in some humans. This particularity in cats has previously been described during examinations under anesthesia (47), but its significance is unknown, as are its medical implications in cats that do not show this training. In dogs, this phenomenon has been observed in 11% of cases and has also been previous described (56).

Epileptic discharges were observed during IPS in six dogs, associated with myoclonic responses for five dogs. For two dogs, anomalies were only observed during IPS, which justifies its use in dog EEG.

As recommended (6) and since the animals were not anesthetized, we also conducted stimulations known to provoke seizures as reported by the owner, such as noise or meal stimuli. We observed epileptic discharges in one dog EEG following a meal and in one dog and one cat EEGs following noise emission. Our EEG method without anesthesia enables the confirmation of reflex epilepsies, documented in both dogs (7, 57, 58) and cats (59).

Our population included animals with paroxysmal manifestations compatible with epileptic seizures, episodes of dyskinesia or compulsive disorders, and others without paroxysmal manifestations but with confusional states or vestibular involvement. Within this large population, 31.6% of EEG recordings allowed for the establishment or clarification of diagnoses in different ways: by establishing a correlation between clinical symptoms and electrical anomalies observed on the EEG, by demonstrating the absence of electrical abnormalities during clinical paroxysmal event, or by revealing interictal epileptic discharges. One study reports a diagnostic EEG rate under ambulatory conditions without anesthesia of 68% (43/63) in a population of dogs with a history of paroxysmal events (43). However, comparison of our results is difficult because we included animals with a history of paroxysmal events, some of whom were treated with anti-epileptic drugs, and animals with encephalopathy or vestibular disorders. Some studies report epileptic discharges in up to 50% of healthy dogs under anesthesia (27, 54). In our study, none of the five dogs in the control group exhibited any abnormalities on the EEG. This highlights the need to use standardized protocols, overcoming the variability associated with anesthetic protocols electrode and criteria for interpreting EEG to perform examinations on both healthy and diseased animals. These results have led us to develop the first guidelines for routine EEG recording (Table 3) in an easy, fast and reproducible manner. Thanks to this methodology, routine EEG, performed non-invasively and without anesthesia, could be offered before MRI and cerebrospinal fluid analysis as recommended by an International Veterinary Epilepsy Task Force Consensus report (21). It can enable diagnosis as well as appropriate therapeutic management of cerebral dysfunction and stabilization of the animal's condition, allowing for a comprehensive investigation of any structural causes under better conditions.

Our study is a promising step toward the widespread use of EEG in common practice for neurological diagnosis with an easy, non-invasive protocol using surface electrodes and no anesthesia. This protocol is particularly suitable for dogs and cats, for which EEG in clinical practice is not at all developed and would enable electro-clinical characterization of epilepsies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by the Ethics Committee of VetAgro Sup (No. 18). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing. HP: Funding acquisition, Project administration, Resources, Supervision, Writing—review

& editing. SBl: Investigation, Methodology, Writing—review & editing. TT: Investigation, Methodology, Writing—review & editing. NV: Investigation, Methodology, Writing—review & editing. SBe: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing—review & editing. CE: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing—review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We would like to thank the University of Caen and VetAgro Sup for the support of this work and Amset Medical for EEG equipment.

Conflict of interest

EL was employed by AMSET MEDICAL. EL is the patent applicant No. FR3101533.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia*. (2017) 58:512–21. doi: 10.1111/epi.13709

2. Nuwer MR, Comi G, Emerson R, Fuglsang-Frederiksen A, Guérit JM, Hinrichs H, et al. IFCN standards for digital recording of clinical EEG. *Electroencephalogr Clin Neurophysiol*. (1998) 106:259–61. doi: 10.1016/S0013-4694(97)00106-5

3. Flink R, Pedersen B, Guekht AB, Malmgren K, Michelucci R, Neville B, et al. Guidelines for the use of EEG methodology in the diagnosis of epilepsy. *Acta Neurol Scand*. (2002) 106:1–7. doi: 10.1034/j.1600-0404.2002.01361.x

4. American Clinical Neurophysiology Society. Guideline 2: Minimum technical standards for pediatric electoencephalography. *J Clin Neurophysiol Off Publ Am Electroencephalogr Soc*. (2006) 23:92–6. doi: 10.1097/00004691-200604000-00003

5. André-Obadia N, Sauleau P, Cheliout-Heraut F, Convers P, Debs R, Eisermann M, et al. Recommandations françaises sur l'électroencéphalogramme. *Neurophysiol Clin Neurophysiol*. (2014) 44:515–612. doi: 10.1016/j.neucli.2014.10.001

6. Peltola ME, Leitinger M, Halford JJ, Vinayan KP, Kobayashi K, Pressler RM, et al. Routine and sleep EEG: minimum recording standards of the International Federation of Clinical Neurophysiology and the International League Against Epilepsy. *Epilepsia*. (2023) 64:602–18. doi: 10.1111/epi.17448

7. Thomas WB. Idiopathic epilepsy in dogs. *Vet Clin North Am Small Anim Pract*. (2000) 30:183–206. doi: 10.1016/S0195-5616(00)50009-6

8. Heske L, Nødtvedt A, Jäderlund KH, Berendt M, Egenvall A. A cohort study of epilepsy among 665,000 insured dogs: incidence, mortality and survival after diagnosis. *Vet J*. (2014) 202:471–6. doi: 10.1016/j.tvjl.2014.09.023

9. Kearsley-Fleet L, O'Neill DG, Volk HA, Church DB, Brodbelt DC. Prevalence and risk factors for canine epilepsy of unknown origin in the UK. *Vet Rec*. (2013) 172:338. doi: 10.1136/vr.101133

10. Hülsmeyer VI, Fischer A, Mandigers PJJ, DeRisio L, Berendt M, Rusbridge C, et al. International Veterinary Epilepsy Task Force's current understanding of idiopathic epilepsy of genetic or suspected genetic origin in purebred dogs. *BMC Vet Res*. (2015) 11:175. doi: 10.1186/s12917-015-0463-0

11. Berendt M, Gulløv CH, Christensen SLK, Gudmundsdottir H, Gredal H, Fredholm M, et al. Prevalence and characteristics of epilepsy in the Belgian shepherd variants Groenendael and Tervueren born in Denmark 1995–2004. *Acta Vet Scand*. (2008) 50:51. doi: 10.1186/1751-0147-50-51

12. Berendt M, Gulløv CH, Fredholm M. Focal epilepsy in the Belgian shepherd: evidence for simple Mendelian inheritance. *J Small Anim Pract*. (2009) 50:655–61. doi: 10.1111/j.1748-5827.2009.00849.x

13. Famula TR, Oberbauer AM, Brown KN. Heritability of epileptic seizures in the Belgian tervueren. *J Small Anim Pract*. (1997) 38:349–52. doi: 10.1111/j.1748-5827.1997.tb03483.x

14. Hülsmeyer V, Zimmermann R, Brauer C, Sauter-Louis C, Fischer A. Epilepsy in Border Collies: clinical manifestation, outcome, and mode of inheritance. *J Vet Intern Med*. (2010) 24:171–8. doi: 10.1111/j.1939-1676.2009.0438.x

15. Berendt M, Gredal H, Pedersen LG, Alban L, Alving J. A cross-sectional study of epilepsy in Danish Labrador Retrievers: prevalence and selected risk factors. *J Vet Intern Med*. (2002) 16:262–8. doi: 10.1111/j.1939-1676.2002.tb02367.x

16. Casal ML, Munuve RM, Janis MA, Werner P, Henthorn PS. Epilepsy in Irish Wolfhounds. *J Vet Intern Med*. (2006) 20:131–5. doi: 10.1111/j.1939-1676.2006.tb02832.x

17. Schriefl S, Steinberg TA, Matiasek K, Ossig A, Fenske N, Fischer A. Etiologic classification of seizures, signalment, clinical signs, and outcome in cats with seizure disorders: 91 cases (2000-2004). *J Am Vet Med Assoc*. (2008) 233:1591–7. doi: 10.2460/javma.233.10.1591

18. O'Neill DG, Phillipps SA, Egan JR, Brodbelt D, Church DB, Volk HA. Epidemiology of recurrent seizure disorders and epilepsy in cats under primary veterinary care in the United Kingdom. *J Vet Intern Med*. (2020) 34:2582–94. doi: 10.1111/jvim.15881

19. Wahle AM, Brühschwein A, Matiasek K, Putschbach K, Wagner E, Mueller RS, et al. Clinical characterization of epilepsy of unknown cause in cats. *J Vet Intern Med*. (2014) 28:182–8. doi: 10.1111/jvim.12250

20. Berendt M, Farquhar RG, Mandigers PJJ, Pakozdy A, Bhatti SFM, De Risio L, et al. International veterinary epilepsy task force consensus report on epilepsy definition, classification and terminology in companion animals. *BMC Vet Res*. (2015) 11:182. doi: 10.1186/s12917-015-0461-2

21. De Risio L, Bhatti S, Muñana K, Penderis J, Stein V, Tipold A, et al. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. *BMC Vet Res*. (2015) 11:148. doi: 10.1186/s12917-015-0462-1

22. Pellegrino F, Sica R. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol*. (2004) 115:477–87. doi: 10.1016/S1388-2457(03)00347-X

23. James FMK, Allen DG, Bersenas AME, Grovum WL, Kerr CL, Monteith G, et al. Investigation of the use of three electroencephalographic electrodes for long-term electroencephalographic recording in awake and sedated dogs. *Am J Vet Res*. (2011) 72:384–90. doi: 10.2460/ajvr.72.3.384

24. James FMK, Cortez MA, Monteith G, Jokinen TS, Sanders S, Wielaender F, et al. Diagnostic utility of wireless video-electroencephalography in unsedated dogs. *J Vet Intern Med*. (2017) 31:1469–76. doi: 10.1111/jvim.14789

25. Hasegawa D. Diagnostic techniques to detect the epileptogenic zone: pathophysiological and presurgical analysis of epilepsy in dogs and cats. *Vet J*. (2016) 215:64–75. doi: 10.1016/j.tvjl.2016.03.005

26. Tourai K, Senba H, Sasaki N, Tokuriki M, Ohashi F, Takeuchi A, et al. Developmental EEG of the beagle dog under xylazine sedation. *Nihon Juigaku Zasshi Jpn J Vet Sci*. (1985) 47:459–63. doi: 10.1292/jvms1939.47.459

27. Utsugi S, Saito M, Sato T, Kunimi M. Relationship between interictal epileptiform discharges under medetomidine sedation and clinical seizures in canine idiopathic epilepsy. *Vet Rec*. (2019) 187:67. doi: 10.1136/vr.104947

28. Berendt M, Høgenhaven H, Flagstad A, Dam M. Electroencephalography in dogs with epilepsy: similarities between human and canine findings. *Acta Neurol Scand*. (1999) 99:276–83. doi: 10.1111/j.1600-0404.1999.tb00676.x

29. Luca J, McCarthy S, Parmentier T, Hazenfratz M, Linden AZ, Gaitero L, et al. Survey of electroencephalography usage and techniques for dogs. *Front Vet Sci*. (2023) 10:1198134. doi: 10.3389/fvets.2023.1198134

30. Acharya JN, Hani AJ, Thirumala PD, Tsuchida TN. American Clinical Neurophysiology Society Guideline 3: a proposal for standard montages to be used in clinical EEG. *J Clin Neurophysiol*. (2016) 33:312. doi: 10.1097/WNP.0000000000000317

31. Acharya JN, Acharya VJ. Overview of EEG montages and principles of localization. *J Clin Neurophysiol*. (2019) 36:325. doi: 10.1097/WNP.0000000000000538

32. Lyon E. *Dispositif de recueil des signaux électrophysiologiques pour la tête d'un animal. France Patent FR3101533* (2021).

33. Lucas EA, Powell EW, Murphree OD. Baseline sleep-wake patterns in the pointer dog. *Physiol Behav*. (1977) 19:285–91. doi: 10.1016/0031-9384(77)90340-7

34. Wauquier A, Verheyen JL, Van Den Broeck WAE, Janssen PAJ. Visual and computer-based analysis of 24 h sleep-waking patterns in the dog. *Electroencephalogr Clin Neurophysiol*. (1979) 46:33–48. doi: 10.1016/0013-4694(79)90047-6

35. Kis A, Szakadát S, Kovács E, Gácsi M, Simor P, Gombos F, et al. Development of a non-invasive polysomnography technique for dogs (*Canis familiaris*). *Physiol Behav*. (2014) 130:149–56. doi: 10.1016/j.physbeh.2014.04.004

36. Bódizs R, Kis A, Gácsi M, Topál J. Sleep in the dog: comparative, behavioral and translational relevance. *Curr Opin Behav Sci*. (2020) 33:25–33. doi: 10.1016/j.cobeha.2019.12.006

37. Kane N, Acharya J, Beniczky S, Caboclo L, Finnigan S, Kaplan PW, et al. A revised glossary of terms most commonly used by clinical electroencephalographers and updated proposal for the report format of the EEG findings. Revision 2017. *Clin Neurophysiol Pract*. (2017) 2:170–85. doi: 10.1016/j.cnp.2017.07.002

38. Gelisse P, Crespel A. *Atlas of Electroencephalography—Volume 1 : Awake and Sleep EEG. 3ED : Activation Procedures and Artifacts*. John Libbey Eurotext.

39. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag (2016).

40. Ekenstedt KJ, Crosse KR, Risselada M. Canine brachycephaly: anatomy, pathology, genetics and welfare. *J Comp Pathol*. (2020) 176:109–15. doi: 10.1016/j.jcpa.2020.02.008

41. Packer R, Berendt M, Bhatti S, Charalambous M, Cizinauskas S, De Risio L, et al. Inter-observer agreement of canine and feline paroxysmal event semiology and classification by veterinary neurology specialists and non-specialists. *BMC Vet Res*. (2015) 11:39. doi: 10.1186/s12917-015-0356-2

42. Kujala MV, Törnqvist H, Somppi S, Hänninen L, Krause CM, Vainio O, et al. Reactivity of dogs' brain oscillations to visual stimuli measured with non-invasive electroencephalography. *PLoS ONE*. (2013) 8:e61818. doi: 10.1371/journal.pone.0061818

43. Parmentier T, Monteith G, Cortez MA, Wielaender F, Fischer A, Jokinen TS, et al. Effect of prior general anesthesia or sedation and antiseizure drugs on the diagnostic utility of wireless video electroencephalography in dogs. *J Vet Intern Med*. (2020) 34:1967–74. doi: 10.1111/jvim.15856

44. Wielaender F, Sarviaho R, James F, Hytönen MK, Cortez MA, Kluger G, et al. Generalized myoclonic epilepsy with photosensitivity in juvenile dogs caused by a defective DIRAS family GTPase 1. *Proc Natl Acad Sci USA*. (2017) 114:2669–74. doi: 10.1073/pnas.1614478114

45. Cauduro A, Dondi M, Favole P, Opreni M, Simonetto LA, Lorenzo V. Artifacts during short-term interictal electroencephalographic recording in dogs. *J Am Anim Hosp Assoc*. (2017) 53:80–9. doi: 10.5326/JAAHA-MS-6486

46. Brauer C, Kästner SBR, Rohn K, Schenk HC, Tünsmeyer J, Tipold A. Electroencephalographic recordings in dogs suffering from idiopathic and symptomatic epilepsy: diagnostic value of interictal short time EEG protocols supplemented by two activation techniques. *Vet J*. (2012) 193:185–92. doi: 10.1016/j.tvjl.2011.10.006

47. Brauer C, Kästner SBR, Kulka AM, Tipold A. Activation procedures in the electroencephalograms of healthy and epileptic cats under propofol anaesthesia. *Vet Rec*. (2012) 170:360. doi: 10.1136/vr.100113

48. Godin DT, Parker PA, Scott RN. Noise characteristics of stainless-steel surface electrodes. *Med Biol Eng Comput*. (1991) 29:585–90. doi: 10.1007/BF02446089

49. Searle A, Kirkup L. A direct comparison of wet, dry and insulating bioelectric recording electrodes. *Physiol Meas*. (2000) 21:271. doi: 10.1088/0967-3334/21/2/307

50. Lopez-Gordo MA, Sanchez-Morillo D, Valle FP. Dry EEG electrodes. *Sensors*. (2014) 14:12847–70. doi: 10.3390/s140712847

51. Virtanen J, Leivo J, Vehkaoja A, Somppi S, Tornqvist H, Fiedler P, et al. Dry contact electrodes performance in canine ECG. In: *ACI'18: Proceedings of the Fifth International Conference on Animal-Computer Interaction. 2018 Dec 4–6*. Atlanta, GA; New York, NY: ACM (2018). p. 1–5.

52. Brugarolas R, Latif T, Dieffenderfer J, Walker K, Yuschak S, Sherman BL, et al. Wearable heart rate sensor systems for wireless canine health monitoring. *IEEE Sens J*. (2016) 16:3454–64. doi: 10.1109/JSEN.2015.2485210

53. Hazenfratz M. *Feasibility of Dry Surface Electrodes for Electroencephalography (EEG) in Dogs*. (Thesis), University of Guelph, Guelph, ON, Canada (2021).

54. Wrzosek M, Ives JR, Karczewski M, Dziadkowiak E, Gruszka E. The relationship between epileptiform discharges and background activity in the visual analysis of electroencephalographic examinations in dogs with seizures of different etiologies. *Vet J Lond Engl*. (2017) 222:41–51. doi: 10.1016/j.tvjl.2017.03.003

55. Holliday TA, Cunningham JG, Gutnick MJ. Comparative clinical and electroencephalographic studies of canine epilepsy. *Epilepsia*. (1970) 11:281–92. doi: 10.1111/j.1528-1157.1970.tb03892.x

56. Holliday TA, Williams C. Clinical electroencephalography in dogs. *Vet Neurol Neurosurg J*. (1999) 1:1.

57. Shell L, Scariano R, Rishniw M. Features of stimulus-specific seizures in dogs with reflex epilepsy: 43 cases (2000–2014). *J Am Vet Med Assoc*. (2017) 250:75–8. doi: 10.2460/javma.250.1.75

58. Brocal J, Lowrie M, Wamsley G, Cauduro A, Mandigers P, Gutierrez-Quintana R, et al. Epileptic seizures triggered by eating in dogs. *J Vet Intern Med*. (2020) 34:1231–8. doi: 10.1111/jvim.15773

59. Lowrie M, Bessant C, Harvey RJ, Sparkes A, Garosi L. Audiogenic reflex seizures in cats. *J Feline Med Surg*. (2016) 18:328–36. doi: 10.1177/1098612X15582080

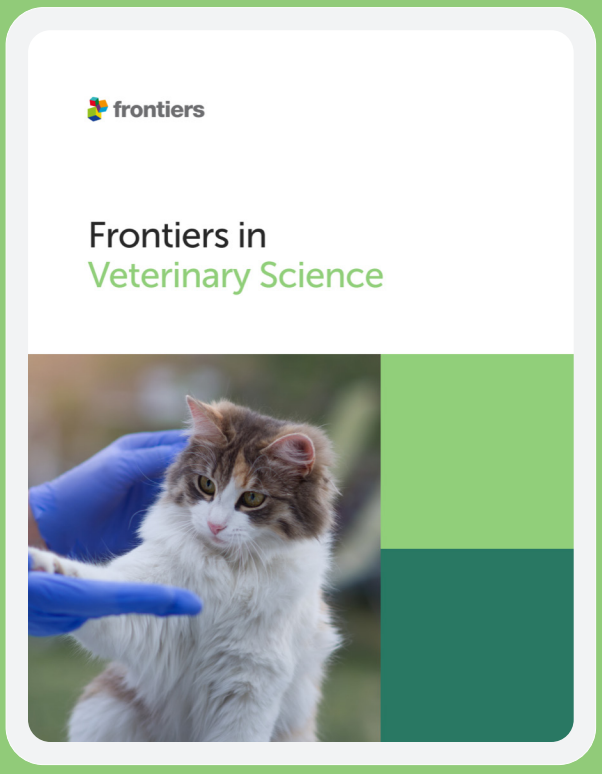
Frontiers in Veterinary Science

Transforms how we investigate and improve animal health

The third most-cited veterinary science journal, bridging animal and human health with a comparative approach to medical challenges. It explores innovative biotechnology and therapy for improved health outcomes.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact