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A preliminary study on behavioral aspects in dogs with idiopathic epilepsy

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Abstract: Idiopathic epilepsy is considered the most common chronic neurological disease in dogs, and there is an increasing awareness regarding the behavioral impact of this disease on canine patients. This work aims at showing the potential differences in the behavioral profile and affective state of epileptic and not epileptic dogs, through the Canine Behavioral Assessment and Research Questionnaire (CBARQ) and the judgement bias test (JBT). Thirty dogs were involved: 15 with Idiopathic epilepsy (IE), 10 under treatment with phenobarbital, five not treated; 15 controls. For each dog, the owner completed the CBARQ. Twenty-seven dogs underwent training for the JBT. All data were statistically analyzed. Dogs with IE got a strong tendency for higher scores for excitability (U=70.0; median: 2.3 versus 1.8; p=0.077) and attention-seeking behaviors (U=66.0; median: 2.7 versus 2.2; p=0.053). Moreover, epileptic dogs were less likely to pass the training phase (58.3% versus 86.7%; X2=2.8; p=0.093), but those who passed it completed the JBT similarly to non-epileptic dogs (U=33.0; p=0.618). Although further studies are needed, epileptic dogs in this study showed differences in excitability, anxiety, and trainability compared to control dogs, suggesting a trend for behavioral aspects to be better explored. Being able to recognize and manage them could have a positive impact on the welfare of these animals.

Key Words: behavioral comorbidities; cognitive bias; dog behavior; idiopathic epilepsy.

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Introduction

According to the 'International veterinary epilepsy task force', epilepsy is defined as a disease of the brain characterized by an enduring predisposition to generate epileptic seizures; its diagnosis is based on a history of two or more unprovoked epileptic seizures occurring at least 24 h apart (Fisher et al., 2014). Idiopathic epilepsy (IE) – the most common chronic neurologic disease in dogs (Kearsley-Fleet et al., 2013; Nakamoto et al., 2018; Erlen et al., 2020) is characterized by proven/suspected genetic background or unknown cause and no indication of structural epilepsy (Berendt et al., 2015). In Europe the prevalence of IE in the general dog population is reported to be 0.65%-0.75%, but some breeds, are more affected than others. In studies on Danish Labrador Retrievers and Danish Belgian Shepherds population was found a lifetime prevalence of IE of 3.1 % and 9.4 %, respectively (Berendt et al., 2015).

Seizures in epileptic dogs are identified and classified owing to observations and interpretations of behavior and activity displayed by the affected animal. Historically, research on canine IE focused primarily on diagnosis and seizure management. Historically, research on canine IE focused primarily on diagnosis and seizure management, but recently there has been increased awareness regarding the behavioral impact of IE in dogs (Bhatti et al., 2015; Packer et al., 2019; Finnegan et al., 2020). Although only a few studies have investigated behavioral comorbidities

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in dogs (with different ages, sex and breeds), behavior changes, ranging from 38% to 71%, were observed in studied animals, in the United Kingdom and in the United States of America (Shihab et al., 2011; Levitin et al., 2019). To date, the recorded behavioral changes were cognitive deficit, fear and anxiety, such as a change in trainability, spatial working memory task deficiencies, attachment behavior, impulsivity level, and defensive aggression (Shihab et al., 2011; De Risio et al., 2015; Jokinen et al., 2015; Packer et al., 2016; Packer et al., 2018; Winter et al., 2018; Levitin et al., 2019; Packer et al., 2019; Erath et al., 2020; Watson et al., 2020). Moreover, it is pointed out that epilepsy negatively influences the quality of life (QOL) in dogs of different breeds diagnosed with IE, in the United Kingdom (Wessmann et al., 2016).

The behavioral impact of IE in dogs could be examined analyzing the behavioral profile, which describes aggregation of context specific behaviour (Hart & Miller 1985; Brady et al., 2018) and the affective state, which is the emotional predisposition or moods (Karagiannis et al., 2015).

The Canine Behavioral Assessment and Research Questionnaire (C-BARQ) – a validated questionnaire created to investigate dogs' behavioral profiles (Hsu & Serpell 2003; Barnard et al., 2012) – has also been used to explore behaviors in dogs affected by chronic enteropathies (Marchetti et al., 2021), and neurological diseases, such as syringomyelia and IE (Rutherford et al., 2012; Levitin et al., 2019). In addition, important information on epileptic dogs can also be obtained from the judgement bias test (JBT). This cognitive test investigates the animal's response to a neutral stimulus defining the individual as pessimistic or optimistic (Mendl et al., 2010). JBT has been used in dogs suffering from behavioral problems, such as separation anxiety (Karagiannis et al., 2015), and in dogs with neurologic disorders as syringomyelia (Cockburn et al., 2018). As far as the authors know, JBT has only been used once in canine IE (Hobbs et al., 2020) and the possible correlations between the C-BARQ and JBT in dogs with IE have not been explored yet.

Through these two tools, the CBARQ and the JBT, the present study aims at showing the potential differences in the behavioral profile and affective state of epileptic and not epileptic dogs.

Materials and methods

Subjects and Recruitment

The study was evaluated and approved by the Scientific Ethical Committee for Animal Welfare of the University of Bologna.

Epileptic dogs were recruited during their follow-up visit regarding epilepsy, at the Veterinary Hospital of the University of Bologna and at the Veterinary Hospital I Portoni Rossi, in Bologna, Italy, and at the Veterinary Clinic Santa Croce, Carpi, Italy, whereas the control group was composed by healthy dogs belonging to University of Bologna students. Fifteen epileptic dogs and 15 control subjects were included in the research; the two groups were similar regarding dogs' age and body weight but differed in sex. The control dogs had a mean age of 6.94 years (mean +/- 3.94, a mean weight of 24.60 kg (mean +/- 10.21) and included 11 females (10 spayed) and 4 males (3 neutered). The dogs in the epileptic group had a mean age of 7.00 years old (mean +/- 3.94), a mean weight of 24.06 kg (mean +/- 10.22), and included three spayed females and 12 male (7 neutered).

The complete list of inclusion and exclusion criteria is reported in Table 1.

Table 1. Inclusion and exclusion criteria of the study population.

Inclusion criteria	Exclusion criteria
At least 12 months old	Sever organic disease (other than epilepsy)
Under no drug treatment (except for phenobarbital)	Health problem affect either vision or motility
IE diagnosed with Tier I confidence level (minimum for the epileptic group)	Being pregnant/lactating
Not experienced and seizures in the 14 days prior to the judgement bias test	Severe aggression toward unfamiliar people and/or food related aggression [a]

[a] This study excluded dogs with severe stranger-directed aggression and/or food-related aggressiveness that could strongly impact the cognitive test (in which bowls were used) and the operators' safety.

Questionnaire Design

We used the Italian version of the 100-item validated C-BARQ (Hsu & Serpell 2003), whose items are divided into 7 sections: i) obedience and training, ii) aggression, iii) fear and anxiety, iv) separation-related behaviors, v) excitability, vi) attachment and attention-seeking and vii) miscellaneous (Hsu & Serpell 2003; Barnard et al., 2012). For each item, owners were asked to assign a score on a 5-point Likert scale, according to the frequency or severity of the behavior. In addition, there was a cover letter with general information about the study, but omitting its aim, as to avoid biased questionnaire replies. Owners signed a written informed consent, including a privacy form, prior to the inclusion of their dog in this research.

Cognitive Test Design

JBT was conducted in testing areas with two experimenters and a set-up described in Figure 1. During the JBT, the first experimenter (E1) prepared the bowl behind a barrier, out of sight for the owner and the dog. The second operator (E2) video-recorded the test and recorded the length of time, and the owner held the dog on a leash. During the trial, experimenters looked at the floor, to avoid giving any suggestions to the owner and the dog. The owner, blind to the study aim, was always present during the test because we believed that the bias induced by the presence of the owner was less than the bias induced by the anxiety due to the owner absence. In addition, (Hobbs et al., 2020) reported a data loss due to dog anxiety in their study on JBT, and we wanted to reduce sources of anxiety as much as possible in our dogs. To safeguard dog welfare, the test was stopped any time the experimenters noticed stress and fear signs in the dog. To limit the olfactory prompt, the same bowl for all locations was used.

For the JBT we followed the methodology described by (Mendl et al., 2010), dividing the procedure into a training phase, a test phase, and an exclusion phase. During the training phase, dogs learnt to discriminate between a positive location (P), where the bowl was full of appetizing food, and a negative location (N), where the bowl was empty. P was on the right side for 50% of the dogs. At the beginning of every trial, E1 prepared the bowl and positioned it in one of the two positions. The owner held the dog in the start position, four meters away from the bowl, and released the dog, verbally encouraging the subject for a maximum of two times. The P and the N were proposed in the same order for all the dogs. First, two consecutive P locations followed by two N locations were presented; second, P and N locations were presented with a pseudorandom order (Mendl et al., 2010). Besides, E2 recorded the latency to reach the bowl, to have a baseline speed and verify if the dog could discriminate between the P and the N. Dogs had 30 seconds to visit the bowl, after which the trial was stopped. Dogs were supposed to learn the association between the location and the food reward if, for the preceding three positive trials and the preceding three negative trials, the longest latency to reach P was shorter than any of the latencies to reach

N. Every dog underwent at least 15 trials. The training phase was ended in any case after 50 trials or if the dog displayed stress and/or wanted to leave the testing area. The subjects who succeeded in the training were engaged in the test phase. During this phase, three ambiguous locations, near positive (NP), middle (M) and near negative (NN) were added between the P and N. Each bowl was put in place one at a time and the distance from the starter point and each bowl was four meters, whereas the distance between each bowl was sixty centimeters. The bowl was put in one of the five locations following the same order indicated by Mendl et al. (2010) for all the dogs for a total of 25 trials.

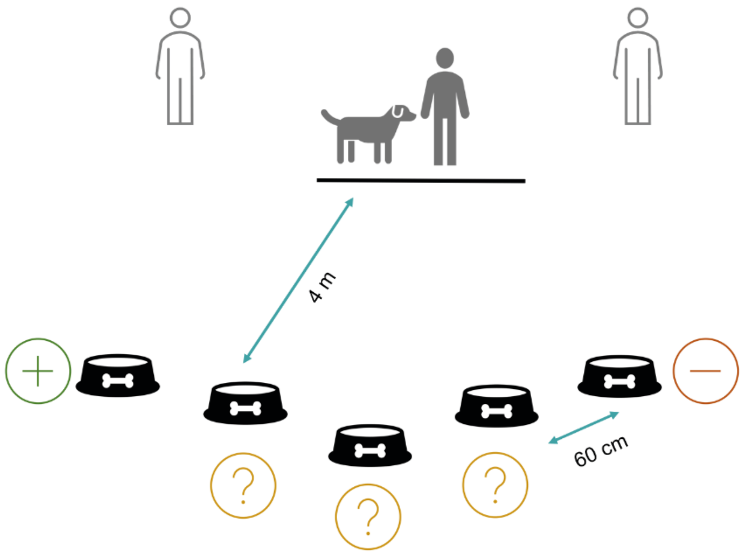

Figure 1. Schematic representation of the judgement bias test (JBT) set up. Positive location (+), negative location (-), ambiguous location (?), barrier (black line), owners (filled in), experimenters (not filled).

During the test, trials were recorded and analyzed with 'Solomon Coder' software to measure the latency to reach locations. The aim of the test trials was to determine how dogs reached these ambiguous locations. Running quickly or more slowly to the ambiguous location could indicate an 'optimistically' biased judgement of the location (related to the anticipation of the food reward), or a 'pessimistically' biased judgement (related to the anticipation of the absence of a food rewarded), respectively. The last trial was the exclusion phase, where we put a bowl without food in P to verify if dogs used or did not use the sense of smell to reach the location.

Data Preparation and Statistical Analysis

The age, gender, and body weight of the epileptic and control group dogs were analyzed with the Mann-Whitney U test. Data from the C-BARQ were examined by calculating a score for each one of 14 behavioral subscales ('Stranger-directed aggression', 'Owner-directed aggression', 'Dog-directed aggression', 'Dog-directed fear', 'Trainability', 'Separation-related problems', 'Attachment/attention-seeking', 'Chasing', 'Stranger-directed fear', 'Non-social fear', 'Touch sensitivity', 'Excitability', 'Energy', 'Dog rivalry') obtained from the seven sections of the C-BARQ. Then, for each one of the 14 subscales the scores were analyzed using the Mann-Whitney U test to determine whether epileptic dogs showed a lower or higher score than the control group. Moreover, with the Mann-Whitney U test, it was checked if there was a difference between the epileptic dogs under treatment with phenobarbital and the epileptic dogs without treatment.

Regarding the JBT, dogs have different baseline running speeds to reach the locations, due to different sizes and motivations toward the food. Therefore, we applied the formula suggested by (Mendl et al., 2010) to convert the raw latency to reach the bowl into a score.

$$Adjusted\,latency = \frac{(mean\ latency\ to\ ambiguous\ location - mean\ latency\ to\ P)}{(mean\ latency\ to\ N - mean\ latency\ to\ P)} * 100$$

This formula returns 0 for P and 100 for N, where a lower score for ambiguous locations indicates a more optimistic score. To compare control and epileptic groups the Mann-Whitney U test was employed. A Chi-Square test was used to assess the association between the condition (control and epileptic, epileptic under treatment and not treated) and the succession of the training phase. Data were analyzed with a commercially available software package and $p<0.05$ was considered significant.

Results

Demography

A total of 30 dogs of various breeds respected the inclusion criteria and were involved in the study. Fifteen of them stood for the epileptic group and the other half, for the control group. Among the epileptic group, 10 of them were under treatment. The characteristic of the population participants at the C-BARQ and at the JBT was described in Table 2.

Table 2. Characteristics of the study population. m: male, f: female, e: entire, n: neutered.

	Participants at the C-BARQ		**Participants at the JBT**	
	Epileptic group	Control group	Epileptic group	Control group
total number	15	15	12	15
under treatment (epileptic dogs)	10	/	8	/
age (years) average-range	7.00 (2-14)	6.94 (1-13)	6.54 (2-14)	6.94 (1-13)
sex (m/f) number	12/3	4/11	10/2	4/11
neutered (e/n) number	5/10	2/13	3/9	2/13
weight (kg) average-range	24.06 (8-37)	24.60 (6-42)	27.45 (10-37)	24.60 (6-42)

For each dog, the owner completed the C-BARQ. The owners of three epileptic dogs (two treated and one under no treatment) withdrew their participation in the JBT because they were afraid that the car travel and/or a test could be stressful for their dogs and adversely affect the occurrence of seizures (Figure 2).

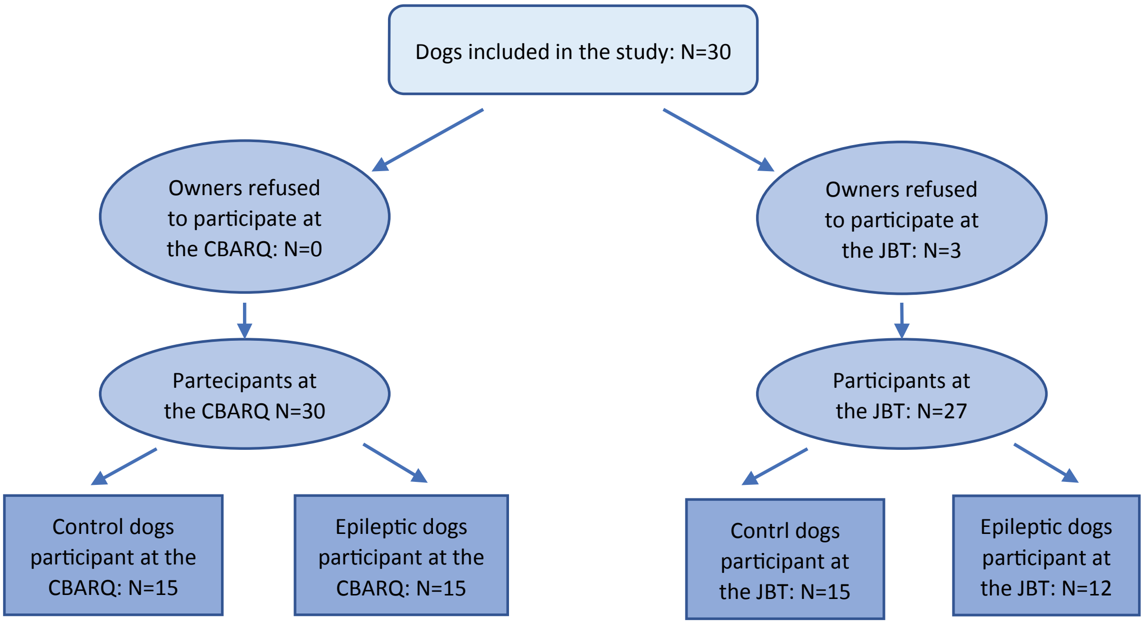

Figure 2. Flow diagram with the enrollment of animals in the CBARQ and in the JBT.

Of the 27 dogs that underwent the training for the JBT test, only 20 passed the training phase. Finally, two control dogs did not get beyond the exclusion phase because they probably used olfactory cues.

C-BARQ

According to the C-BARQ results, dogs with IE recorded a higher score for attention-seeking behaviors (u=66.0; median: 2.7 versus 2.2; p=0.053) and excitability (u=70.0; median: 2.3 versus 1.8; p=0.077), with a significant tendency. For all the other subscale behaviors there was not a significant difference between the epileptic dogs and the control group. Comparing epileptic dogs treated with phenobarbital and those without treatment, the subjects under treatment had a lower touch sensitivity than dogs without treatment (u=9.0; median: 2.0 versus 0.7; p=0.048).

Judgement bias test

According to Figure 3, epileptic dogs were less likely to pass the training phase (58.3% versus 86.7%; x2=2.8; p=0.093). However, the number of trials needed to achieve the discrimination was not significantly different between the epileptic and the control group (see Figure 4).

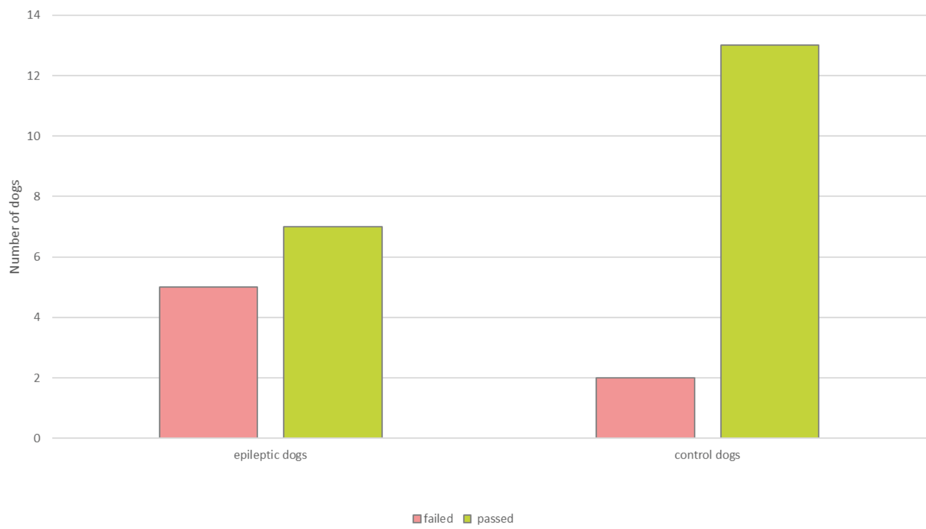

Figure 3. Number of dogs who passed and failed the judgement bias test training phase for the two groups.

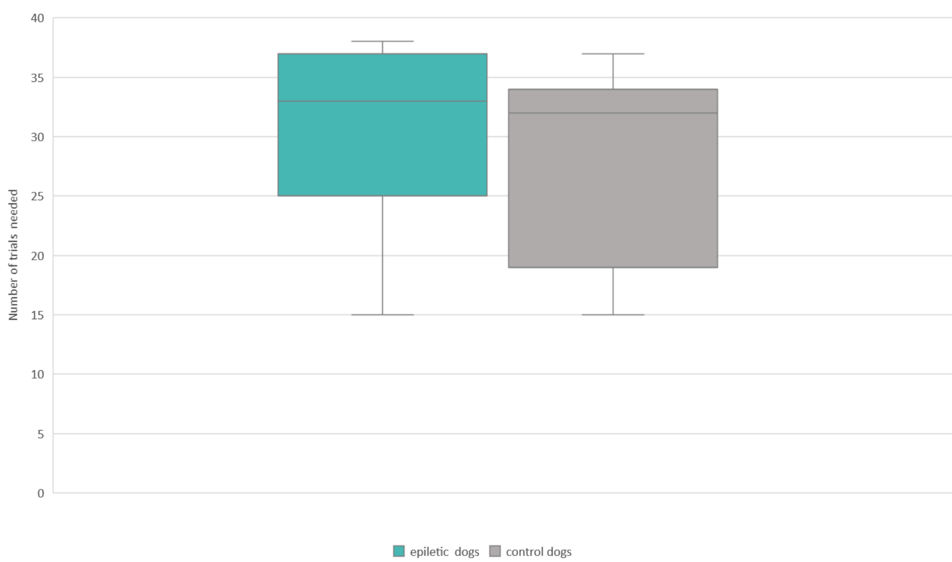

Figure 4. Number of trials needed to discriminate between positive location and negative location during the judgement bias test training phase.

Within the epileptic group, the subjects under treatment did not statistically differ from the untreated dogs regarding the success rate of the training phase (50.0% versus 70.0%; p=0.48). In addition, the scores recorded in the 3 ambiguous locations of the JBT did not statistically differ between the epileptic and the control group (u=33.0; p=0.618) (Table 3). Therefore, we did not find a more pessimistic or optimistic bias in epileptic dogs compared to the control group. We were not able to analyze the difference due to the phenobarbital effect on the JBT because of the low number of epileptic dogs that complete the JBT test (n.7 under treatment vs n.2 not treated).

Table 3. Adjusted score for the 5 locations: positive (P), near positive (NP), middle (M), near negative (NN) and negative (N).

Adjusted latency					
	P	NP	M	NN	N
Control dogs	0.00	33.22	24.38	19.20	100.00
	0.00	5.66	5.97	24.34	100.00
	0.00	3.10	58.34	115.69	100.00
	0.00	1.77	5.01	90.09	100.00
	0.00	-13.42	103.71	12.00	100.00
	0.00	27.17	39.93	71.41	100.00
	0.00	11.34	0.80	5.74	100.00
	0.00	-36.99	-29.48	-24.33	100.00
	0.00	-0.13	37.22	1.71	100.00
	0.00	24.01	40.75	94.36	100.00
	0.00	9.72	13.81	122.96	100.00
Epileptic dogs	0.00	8.19	-0.02	-2.91	100.00
	0.00	0.84	40.77	3.83	100.00
	0.00	13.66	14.52	57.78	100.00
	0.00	15.97	35.59	75.45	100.00
	0.00	51.91	50.40	78.41	100.00
	0.00	-0.57	9.44	53.87	100.00
	0.00	4.26	66.79	89.19	100.00
	0.00	-8.03	41.73	85.94	100.00
	0.00	10.77	48.77	48.04	100.00

Discussion

This preliminary research investigates the behavioral profile and the affective state of epileptic versus control dogs through CBARQ and the JBT.

Regarding the results from the C-BARQ, epileptic dogs have higher scores for excitability and attention-seeking behaviors when compared to non-epileptic dogs. This is in agreement with previous studies, which employed three different questionnaires (11 multiple choice behavioral questions, 77 online questions and a shorter version of the CBARQ). Their results might suggest the presence of behavioral abnormalities in epileptic dogs, most commonly associated with abnormal perception, anxiety, in attention, and excitability/impulsivity. (De Risio et al., 2015; Jokinen et al., 2015; Packer et al., 2016; Levitin et al., 2019). Similarly, in the human population, up to 50% of epileptic patients could display some psychiatric comorbidities and the incidence of these disorders in epileptic patients is 2-3 times higher than in the general population (Kanner 2016; Josephson & Jetté 2017; Salpekar & Mula 2019). The most common psychiatric disorders in human epileptic patients are depression and anxiety, but panic and cognitive disorders are reported as well (Tellez-Zenteno et al., 2007; Berg 2011; Habibi et al., 2016; Kanner et al., 2020). The analogous findings in dogs could play a key role in both owner education and the decision process for drug treatment. According to the 'International Veterinary Epilepsy Task Force', owner education is essential for the treatment of epileptic dogs and behavioral comorbidities can impact owner compliance (Bhatti et al., 2015). Owners and veterinarians should be aware of these comorbidities and their influence on a dog's daily life. Moreover, the medical treatment of these comorbidities may be integrated into the seizure treatment.

An interesting result concerns the lower touch sensitivity found in epileptic dogs under treatment with phenobarbital than in epileptic dogs without treatment. According to the last 'Consensus Statements of the American College of Veterinary International Medicine for seizure management', the phenobarbital is used predominantly as a first-line antiepileptic drug (AED) in dogs (Berendt et al., 2015). Such reduced touch sensitivity might be related to the sedative effect of phenobarbital (Thomas 2000; Tipold et al., 2015), which could play a role in abnormal perception and apathetic behavior.

Regarding results from the JBT, the epileptic group did not show a more pessimistic bias than the control group, despite the first group obtaining higher scores for anxiety-related behavior in the C-BARQ. This partially contrasts with the results of (Mendl et al., 2010; Karagiannis et al., 2015), which report a relationship between separation anxiety and pessimistic bias. This discordance between the JBT and the C-BARQ might be due to the possibility that the most anxious dogs or dogs with a deficit impairment did not take part in the JBT; in fact, we registered a data loss due to the owner decision to not participate in the JBT and dogs that failed to pass his training phase. Particularly, for the epileptic population, three owners were worried that JBT could be stressful for their dogs and declined to participate. This is in line with previous studies reporting epileptic dog owners as being more concerned about stressful episodes than other dog owners (Hobbs et al., 2020; Pergande et al., 2020). Moreover, the data loss in the training phase could have been linked either to a lack of acquisition of spatial discrimination (one dog of the control group and four dogs in the epileptic group) or to a lack of motivation to go to the bowl (one dog of the control group and one dog of the epileptic group). Cognitive deficits have been reported previously in epileptic dogs and both in our and in Hobbs et al., (2020) study, a big data loss in the training phase due to spatial discrimination is considerable and should be considered in future studies. However, future research could take advantage of this result concerning the epileptic dog's difficulty to pass the training test and this phase may be used to analyze cognitive deficits in epileptic dogs.

There are several limitations in our study. Even if a similar number of dogs were included in previous studies on cognitive tests (Mendl et al., 2010; Cockburn et al., 2018), our sample size is surely small and so this should be considered a pilot study and the results should be considered with caution. Also, even if the evidence of sex and neuter status on both cognitive tests and behaviors considered in the C-BARQ is still controversial (Serpell & Hsu 2005; Casey et al., 2021), not having control over the sex of the involved dogs involved be considered a limit as well. These criticalities could be addressed in future research with a bigger sample. However, the JBT might not be the best method to analyze their affective state, it could be used as an adjunctive part of the cognitive test in epileptic dogs.

Conclusions

Owners and veterinarians should be aware that dogs with IE could show behavioral comorbidities (in this study, related to anxiety and cognitive disorders), potentially impacting the daily life of patients and owners. Identifying these comorbidities and treating them, when needed, could add an important part to the treatment of seizures.

Moreover, this preliminary study suggests that future research assessing the affective state of epileptic dogs should use a test that does not involve a cognitive task and does not cause test-induced anxiety, to prevent the risk of data loss.

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Institutional Review Board Statement: The study was evaluated and approved by the Scientific Ethical Committee for Animal Welfare of the University of Bologna (ID 4343).

Informed Consent Statement: Informed consent was obtained from all the owners involved in the study.

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Conflicts of Interest: The authors declare no conflict of interest.

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Studio preliminare su aspetti comportamentali di cani con epilessia idiopatica

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Sintesi

L'epilessia idiopatica è considerata la malattia neurologica cronica più comune nei cani e vi è una crescente consapevolezza riguardo all'impatto comportamentale di questa malattia sui pazienti canini. Questo lavoro mira a mostrare le potenziali differenze nel profilo comportamentale e nello stato affettivo dei cani epilettici e non epilettici, attraverso il Canine Behavioral Assessment and Research Questionnaire (CBARQ) e il Judgement Bias Test (JBT). Sono stati coinvolti 30 cani: 15 con epilessia idiopatica (IE), 10 in trattamento con fenobarbital, cinque non trattati; 15 controlli. Per ogni cane, il proprietario ha compilato il CBARQ. Ventisette cani sono stati addestrati per il JBT. Tutti i dati sono stati analizzati statisticamente. I cani con IE hanno avuto una forte tendenza a punteggi più alti per eccitabilità (U=70,0; mediana: 2,3 contro 1,8; p=0,077) e comportamenti di ricerca di attenzione (U=66,0; mediana: 2,7 contro 2,2; p=0,053). Inoltre, i cani epilettici avevano meno probabilità di superare la fase di addestramento (58,3% contro 86,7%; X2=2,8; p=0,093), ma coloro che l'hanno superata hanno completato il JBT in modo simile ai cani non epilettici (U=33,0; p=0,618). Sebbene siano necessari ulteriori studi, i cani epilettici in questo studio hanno mostrato differenze di eccitabilità, ansia e addestrabilità rispetto ai cani di controllo, suggerendo una tendenza per gli aspetti comportamentali da esplorare meglio. Riuscire a riconoscerli e gestirli potrebbe avere un impatto positivo sul benessere di questi animali.