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**Physiological reactivity to spontaneously occurring seizure activity in dogs with epilepsy and their carers**

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**Abstract**

There is a complex bidirectional relationship between stress and epilepsy. Stressful stimuli and subsequent cortisol release act as a trigger for seizure activity in some individuals with epilepsy, and seizure activity itself may act as a stressor to the affected individual. Epilepsy is the most common chronic neurological condition in domestic dogs and requires chronic management by their human carers, impacting upon the quality of life of both dog and carer. Seizures occur unpredictably and may be stressful for carers to witness and manage. In the present study we investigated the role of seizure activity as a stressor, measuring the effect of spontaneously occurring seizure activity in dogs with epilepsy upon their own cortisol levels and that of their carers. Furthermore, we tested whether individual differences in HPA reactivity were associated with owner personality characteristics and the quality of the dog–carer relationship. Saliva samples were obtained from sixteen dog-carer dyads in the home setting 20 and 40 minutes post-seizure, and at time-matched points on the following (non-seizure) day. Significant differences in cortisol levels were found in dogs at 40 minutes post-seizure (265.1% increase), and at 20 minutes post-seizure in their carers (40.5% increase). No associations were found between cortisol reactivity and the strength of the dog-carer bond. Carers with higher neuroticism scores exhibited higher cortisol levels at both post-seizure sampling points. As there was a gender bias in the carer sample (15/16 were female), and there are known sex differences in cortisol reactivity in response to psychological stress, the conclusions of this study may be limited to female carers. These findings are the first to objectively demonstrate the acutely stressful effects of seizures in dogs with epilepsy and their carers.

## 1. Introduction

Epilepsy is a disease of the brain characterised by an enduring predisposition to generate epileptic seizures, practically applied as having ≥two unprovoked epileptic seizures >24 h apart [1]. Epilepsy is the most common chronic neurological condition in domestic dogs, estimated to affect 0.6-.075% of dogs [2, 3]. For many dogs with epilepsy, no cause can be found for their seizures (e.g. gross neuroanatomical or neuropathological abnormalities nor other relevant underlying diseases), and as such are diagnosed with 'idiopathic' epilepsy (IE), which is thought to be of a predominantly genetic origin [4, 5]. Age of onset of IE is most commonly between 6 months and 6 years [6, 7] and the condition is usually lifelong, in some cases requiring constant medication [8, 9].

Management of dogs with IE often requires a long-term commitment by caregivers, including medicating their dog several times per day (which is associated with an ongoing financial burden) and continually monitoring their pets for side-effects of these medications, in addition to acute events such as seizures resulting in emergency vet visits at unpredictable times [10-13]. Seizures can be unpredictable and appear uncontrollable, and may be stressful for carers to witness and manage. Negative effects of caring for an individual with epilepsy are seen in both the carers of dogs and people with epilepsy. The unpredictability of seizures can lead to excessive apprehensiveness in the parents of children with epilepsy, resulting in a deterioration of parental quality of life [14]. Parents of children with epilepsy are also at an increased chance of developing post-traumatic stress disorder, anxiety and depression compared to parents of children with other chronic illnesses or non-caregivers, due to unpredictability of seizures and the restrictions imposed upon their lives by epilepsy [15-17].

To date, little research has objectively explored the potentially negative consequences of caring for an animal with a chronic disorder such as epilepsy. Previous interview-based studies have found that some canine caregivers with a 'humanistic' orientation would describe their love for their dog to be similar to that of a parents for a child, and would consider their dogs as children [18]. It is therefore conceivable that witnessing seizure activity in their dog would have an impact upon a carer's own

psychological stress levels and stress physiology, and that relationship quality between dog and carer may influence the degree of impact. We anticipate that caregivers with high-quality relationships with their dogs will have more pronounced stress responses when their dogs have a seizure than caregivers with poor or lower quality relationships.

In this study, we examine the interactions between hypothalamic-pituitary-adrenal (HPA) axis activation in dogs with epilepsy and their carers following seizure activity. The HPA axis is activated by physiological as well as psychological stressors. Stress, as measured by cortisol, does not necessarily reflect negative experiences, as the term may imply in ordinary language [19]. Cortisol levels have been found to increase during a variety of experiences in the context of positive or negative affect, including exercise, sexual behaviour, and in anticipation of punishment or reward [20]. As such, increased HPA activity alone should not be considered a proxy for stress arising from aversive experiences, and may simply reflect physiological arousal. [19]. Cortisol has frequently been used as a physiological measure in dogs to explore the effect of a variety of stressful situations, including the impact of simulated thunderstorms on thunderstorm-phobic dogs [21], the impact of housing and exercise [22] and petting by humans in an animal shelter [23]. Salivary cortisol has proven to be a robust and convenient biomarker in both people and dogs, highly correlated with plasma cortisol values in both species [24, 25]. Saliva collection is relatively noninvasive and although animal handling is required, saliva collection is tolerated well by most dogs, and if the collection procedure takes <4 min there is no handling effect on the cortisol concentration of that sample [26]. Saliva sampling is also not technically challenging, allowing people such as carers to be easily trained to collect samples with sufficient instruction [27].

We hypothesized that the cortisol levels of dogs with epilepsy would increase in response to a spontaneous seizure, and that individual differences in this response should be related to the (i) carer-perceived severity and (ii) length of the seizure. In addition, we hypothesized that their carer's cortisol levels would increase in response to witnessing and managing their dog's seizure, and that individual

differences in this response should be related to the carer's (i) personality characteristics, and (ii) the quality of the dog–carer relationship.

## 2. Materials and methods

### 2.1 Study design

To explore these hypotheses, we drew on a sample of dog–carer pairs, selected because the dog was diagnosed with IE. Saliva samples were collected from dogs and carers after the dog had experienced a seizure. These post-seizure samples were compared to 'baseline' time-matched samples, 24 hours after the post-seizure samples were taken, to account for daily rhythms in cortisol release. In humans, cortisol is imprinted with a diurnal rhythm, with peak cortisol levels being measured at around 0700h falling in the late evening; however, evidence of diurnal cortisol rhythms in dogs are mixed, with conflicting evidence of their presence from previous studies [28-30]. Samples were collected by the carer which reduced the potential stress of collection by an unfamiliar person [31, 32], and allowed for sampling of an unpredictable event and corresponding baseline values without the researcher needing to be present. In addition, the samples were taken within the home environment which avoids increases in cortisol associated with travelling to, and novelty of an experimental setting [29].

### 2.2 Recruitment of participants

Sixteen dyads of dogs with IE and their caregivers were recruited by contacting clients whose dogs were previously diagnosed with IE at the Queen Mother Hospital for Animals (Hertfordshire, England), and advertising the study on social media, including carer support groups for dogs with epilepsy. Dogs were eligible for inclusion in this study if they had met the criteria for Tier I diagnostic certainty, as described by the International Veterinary Epilepsy Task Force (IVEFT) [7]. This includes a history of two or more unprovoked epileptic seizures occurring at least 24 h apart, age at epileptic seizure onset of between six months and six years, unremarkable inter-ictal physical and neurological

examination, and no significant abnormalities on minimum data base blood tests and urinalysis. Carers were only included in the study if they (i) were not taking glucocorticoid-containing medication, and (ii) were not affected by any known endocrine disorder. If criteria were met and both the dog and carer, upon accepting invitation to take part in the study they were sent a study pack by post containing the necessary materials and instructions. Forty dog-carer dyads were recruited, of which sixteen had experienced a seizure and returned samples within the study period. No financial or other incentive was offered in exchange for participation. All protocols were approved by the RVC Ethics and Welfare Committee URN 2014 1273. All carers consented to their involvement in the study via a consent form within the study pack.

### 2.3 Collection of whole saliva

Saliva samples were collected from each member of the dyad by the carer 20 and 40 minutes after the seizure, as well as at matching time points on the following control non-seizure day (24 hours after the initial seizure, unless a seizure occurred in the intervening period, where the carer was asked to wait a further 24 hours to collect these samples). Overall, eight samples were collected from each dog-carer dyad. Carers received a pictorial instruction manual in their study pack to explain how to effectively collect saliva samples. Saliva was collected from the dogs using a plain cotton swab stored in a Salivette tube (Sarstedt, Germany), The swab was placed in the cheek for at least 2 minutes, with chewing of the cotton allowed, but no further external stimulation for salivation (e.g. citric acid). Carer's saliva was collected in the same way; chewing on the swab for 2 minutes until saturated. Participants were requested not to feed their dog or to eat/drink themselves (except water) for the time between the seizure starting and the samples being collected (20 minutes). As the seizure could not be predicted in most cases, this period could not be extended; however, on submission of their samples, all owners were asked to report whether they or their dog had eaten in the hour prior to sample collection [33], to exclude contaminated samples from analysis. Carers were instructed to refrigerate tubes immediately after samples were taken, and to send them back to the study center in pre-paid Next Day Delivery envelopes with a freezer pack within 24 hours of collection. On arrival at the study

center the samples were centrifuged, saliva swab removed, and the isolated saliva sample was stored at −80 °C until the day of the assay.

### *2.4 Cortisol EIA assay*

Saliva samples were measured for cortisol using a commercially available, high sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, UK) as described previously [34, 35]. Interassay and intraassay coefficients of variance for this assay were 11% and 9%, respectively. All samples were assayed for cortisol according to the manufacturer's instructions without modification as described (http://www.salimetrics.com/).

### *2.5 Questionnaire*

Alongside the saliva samples, carers were asked to complete a paper questionnaire. Section (1) consisted of questions regarding their dog (e.g. age, sex, neuter status, breed, weight), and their dogs epilepsy, including age at first seizure, diagnostic tests carried out, seizure frequency/per month, type of seizures experienced, seizure phenotype (history of cluster seizures and/or status epilepticus) and treatment history. Section (2) consisted of questions regarding the carer (e.g. age, gender), how long they have lived with their dog, other pets owned, and medical history (to screen for endocrine disorders and the use of steroid-based medications). Section (3) explored the carer-dog bond using the The Monash Dog Owner Relationship scale (MDORS), a multi-dimensional questionnaire to assess human-companion dog relationships [36]. The MDORS consists of 28 items that make up three sub-scales, (i) Dog–Owner Interaction (DOC), (ii) Perceived Emotional Closeness (PEC), and (iii) Perceived Costs (PC). Scores for each sub-scale and an overall score were calculated for each dyad. Section (4) explored the personality of the carer, using the Five Factor Personality (FFP) test [37]; a model used to describe human personality. The test consisted of 41 closed questions on carer personality which made up five subscales: Openness, Neuroticism, Extroversion, Conscientiousness and Agreeableness. All questions were listed as statements to which the carers rated how much they agreed, on an interval scale of 1-5 from disagree strongly – agree strongly. The final section of the questionnaire, Section (5) requested the carer to give an account of the seizure that they collected

saliva samples following. The carer reported the time of the seizure, how long it lasted (in minutes: 0-1 minute, 2-3 minutes, 4-5 minutes, >5 minutes), if there was a perceivable 'trigger' and if the carer was aware the seizure was about to occur before it started. They then completed tick boxes detailing aspects of seizure semiology (e.g. whether the dog collapsed, if there were motor signs e.g. paddling movements, or autonomic signs e.g. urination, defecation, excessive salivation) for the investigators to ascertain the seizure type (N.B. only dogs experiencing generalised seizures were included in the analyses). Carers were then asked to rate the severity of the seizure on a scale of 1-7 (very mild-very severe), and report how fully recovered their dog was at the 20 minute sample and 40 minute sample (completely recovered – back to normal, partially recovered – more than 50%, not recovered – less than 50%).

### 2.6 Data handling and statistics

All analyses of were carried out in SPSS Statistics 22 (IBM). The Shapiro-Wilk test was used to test all data for normality, and then the appropriate parametric or non-parametric test was selected. The Wilcoxon was used to compare the differences between cortisol levels in samples taken 20 and 40 minutes after the seizure and time-matched non seizure day samples. Where insufficient saliva samples led to missing data points in the paired datasets, those dogs or carers were excluded from statistical comparison (20 or 40 minutes post seizure) for that individual test. Bivariate spearman's rank correlations were used to examine the relationship between carer's percentage difference in cortisol level between the seizure and time-matched non-seizure samples and their MDORS scores and FFP scores. Bivariate spearman's rank correlations were used to examine the relationship between the dog's percentage difference in cortisol level between the seizure and time-matched non-seizure samples and the reported severity of the seizure (on a scale of 1-7). Kruskall-Wallis tests were used to examine the relationship between the dog and carer's percentage difference in cortisol level between the seizure and time-matched non-seizure samples and the reported length (in minutes) of the seizure. In all tests $p<0.05$ was considered to be significant and data was presented as mean ($\pm$ SD) for normally distributed data, or median ($25^{th}$-$75^{th}$ percentile) for non-normally distributed data.

## 3  Results

### 3.1 Canine demographics

Data were collected from sixteen dog-carer pairs during the study period. The majority of the dogs were purebred (n=14/16), over half were male (56.3%, n=9) and the majority of dogs were neutered (75.0%, n=12). The mean weight (kg) ± SD was 22.94 ± 10.21 and the mean age (months) ± SD was 72.50 ± 28.45. All dogs met tier I certainty of the International Veterinary Epilepsy Taskforce's criteria for the diagnosis of idiopathic epilepsy [7], and all dogs were being treated with anti-epileptic drugs (AEDs). Further information regarding patient clinical history, epilepsy phenotype and treatment are available in the supplementary materials.

### 3.2 Carer demographics

The majority of carers were female (15/16), with a mean age (years) ± SD of 46.25 ± 11.32. The mean age of acquisition of their current dog with epilepsy (months) ± SD was 2.55 ± 2.44, and they had owned their dog for a mean length (months) ± SD of 70.00 ± 28.92 months.

### 3.3 Carer personality and dog-carer bond

The mean MDORS score for sub-scale I (DOC) was 4.07 (+/- 0.47), for sub-scale II (PEC) was 4.07 (+/- 0.62) and for sub-scale III (PC) was 4.13 (+/- 0.71) (see Table 1 for comparison with other recent studies).

Table 1. Descriptive statistics for the three subscales of the MDORS in the present study and comparison studies

| Subscale | Current study (mean +/- SD, range) | Meyer and Forkman, 2014 (mean +/- SD, range) | Rohlf et al, 2015 (mean +/- SD, range) |
|---|---|---|---|
| Emotional closeness | 4.07 +/- 0.6 2.8-5.0 | 3.7 +/- 0.7 1.9-5.0 | 3.9 +/- 0.6 1.9-5.0 |
| Dog–owner interaction | 4.07 +/- 0.5 3.1-4.9 | 3.8 +/- 0.5 1.4-5.0 | 3.9 +/- 0.6 1.3-5.0 |
| Perceived costs | 4.1 +/- 0.7 2.2-5.0 | 4.1 +/- 5.0 2.0-5.0 | 1.8 +/- 0.6 1.0-4.4 |

The median FFP score for openness was 23 (21.5-29.0), for neuroticism was 20.5 (18.25-22.75), for extraversion was 30.5 (26.25-35.0), for conscientious 44.0 (35.0-45.0) and for agreeableness was 29.5 (23.0-31.0).

### 3.4 Seizure characteristics

All dogs experienced a generalised seizure with (n=5) or without (n=11) initial focal seizure activity. None of the seizures experienced were considered to be status epilepticus (where seizure activity continues for >5 minutes or there is incomplete recovery of consciousness between two seizures[5]).The most common time of day for the seizure to occur was between 4-8am (31.3%, n=5), followed by 4-8pm (25.0%, n=4), 12-4pm (18.8%, n=3), 8-12pm (12.5%, n=2) and 12-4am (12.5%, n=2). No seizures were reported to occur between 8-12am.

At 20 minutes post-seizure, the majority of dogs had not yet fully recovered (56.3%=partially recovered, 18.8%=not recovered), and at 40 minutes 43.8% were still described as partially recovered, and 6.3% not recovered. The most common post-ictal signs at 20 minutes post-seizure were agitation and ataxia ('clumsy') (Table 1). At 40 minutes the prevalence of all post-ictal signs had reduced with the exception of sleepiness which had increased (Table 2).

Table 2. Presence of post-ictal signs at 20 and 40 minute post-seizure sampling times

| Post-ictal sign | 20 minutes post-seizure | 40 minutes post-seizure |
|---|---|---|
| Aggressive | 6.3% (n=1) | 0.0% (n=0) |
| Agitated | 56.3% (n=9) | 12.5% (n=2) |
| Clumsy | 68.8% (n=1) | 18.8% (n=3) |
| Clingy | 25.0% (n=4) | 18.8% (n=3) |
| Hiding | 0.0% (n=0) | 0.0% (n=0) |
| Sleepy | 31.3% (n=5) | 37.4% (n=6) |
| Staring | 12.5% (n=2) | 6.3% (n=1) |
| Fly-catching | 0.0% (n=0) | 0.0% (n=0) |
| Sniffing | 6.3% (n=1) | 0.0% (n=0) |
| Blind | 6.3% (n=1) | 6.3% (n=1) |
| Fearful | 18.8% (n=3) | 0.0% (n=0) |

### 3.6 Effect of seizure activity on cortisol level

There was a significant effect of seizure activity upon canine salivary cortisol levels, with a Wilcoxon test demonstrating a significant increase in cortisol levels 40 minutes after a seizure, compared to a time-matched non-seizure day (p=0.017, Table 3). In contrast, no difference was seen between the 20 minute post-seizure sample and its time-matched non-seizure day sample (p>0.05). The median percentage change in cortisol levels between the post-seizure samples and time-matched non-seizure samples was 531.60% at 20 minutes, and 265.13% at 40 minutes.

There was also a significant effect of seizure activity upon carer salivary cortisol levels, with a Wilcoxon test demonstrating a significant increase in cortisol levels 20 minutes after a seizure, compared to a time-matched non-seizure day (p<0.05, Table 3). In contrast, no difference was seen between the 40 minute post-seizure sample and its time-matched non-seizure day sample (p>0.05).

The median percentage change in cortisol levels between the post-seizure samples and time-matched non-seizure samples was 40.53% at 20 minutes, and 138.21% at 40 minutes.

Table 3. Median (25th-75th percentiles) cortisol levels (µg/dL) from 20 minutes and 40 minutes post-seizure samples and at time-matched non-seizure samples in dogs and their carers. *n*= number of samples available for each parameter.

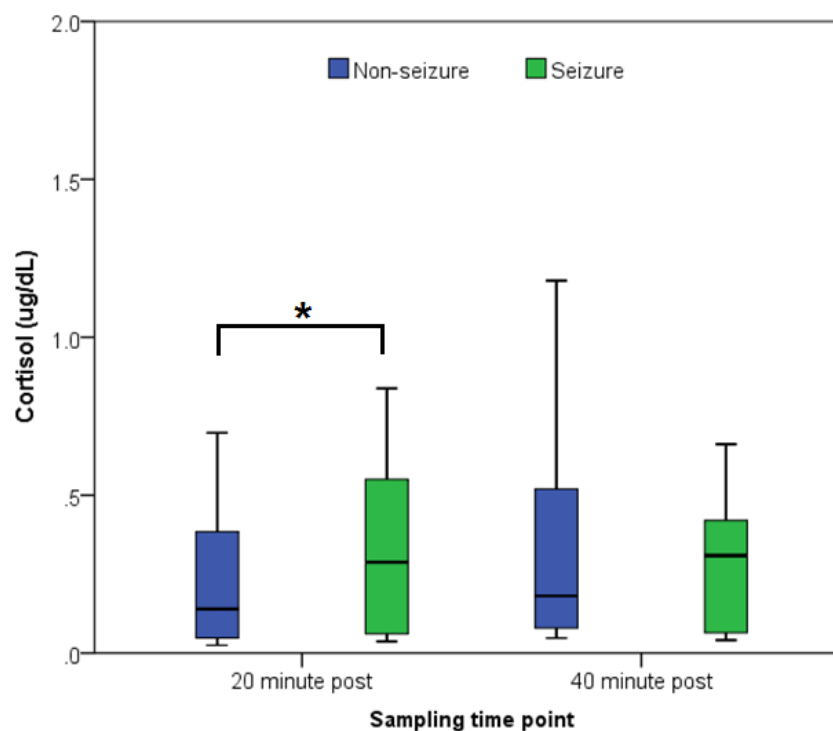| | Cortisol levels (µg/dL) | | | | | Cortisol levels (µg/dL) | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Subject | Matched non-seizure sample (20 minutes) *n* | Post seizure sample (20 minutes) *n* | W | p | Median % difference (20 minutes) | Matched non-seizure sample (40 minutes) *n* | Post seizure sample (40 minutes) *N* | W | p | Median % difference (40 minutes) |
| Dog | 0.119 (0.045-0.183) 14 | 0.200 (0.119-1.264) 11 | 16.0 | 0.131 | 531.6 (6.3-7286.5) | 0.105 (0.027-0.234) 11 | 0.281 (0.137-0.578) 12 | 4.0 | 0.017 | 265.1 (265.3-941.3) |
| Carer | 0.160 (0.065-0.394) 16 | 0.458 (0.086-0.636) 16 | 28.0 | 0.039 | 40.5 (-10.5-102.6) | 0.181 (0.074-0.647) 13 | 0.374 (0.079-0.495) 16 | 29.0 | 0.249 | 138.2 (-26.8-228.8) |

N.B. These raw values for canine samples can be compared to the results of a recent meta-analysis of canine salivary cortisol, where a concentration range of 0.00 to 33.79 µg/dLwas established (median: 0.15 µg/dL; mean: 0.45 µg/dL) [29]. For human salivary cortisol samples, the reference range at 8am is 0.13-0.97 µg/dL and at 10pm is <0.22 [38]. Note that time of sample collection differed between participants due to the unpredictability of seizure activity.

Figure 1. Boxplots of the median and interquartile ranges of (A) canine and (B) carer cortisol levels at 20 and 40 min post-seizure on a seizure day, and at time-matched points on a non-seizure day. Canine cortisol was significantly higher 40 minutes after a seizure compared to the matched-time point the next non-seizure day. Carer cortisol was significantly higher 20 minutes after their dog had a seizure compared to the matched-time point the next non-seizure day. Significant differences are highlighted as * (p<0.05)

(A)
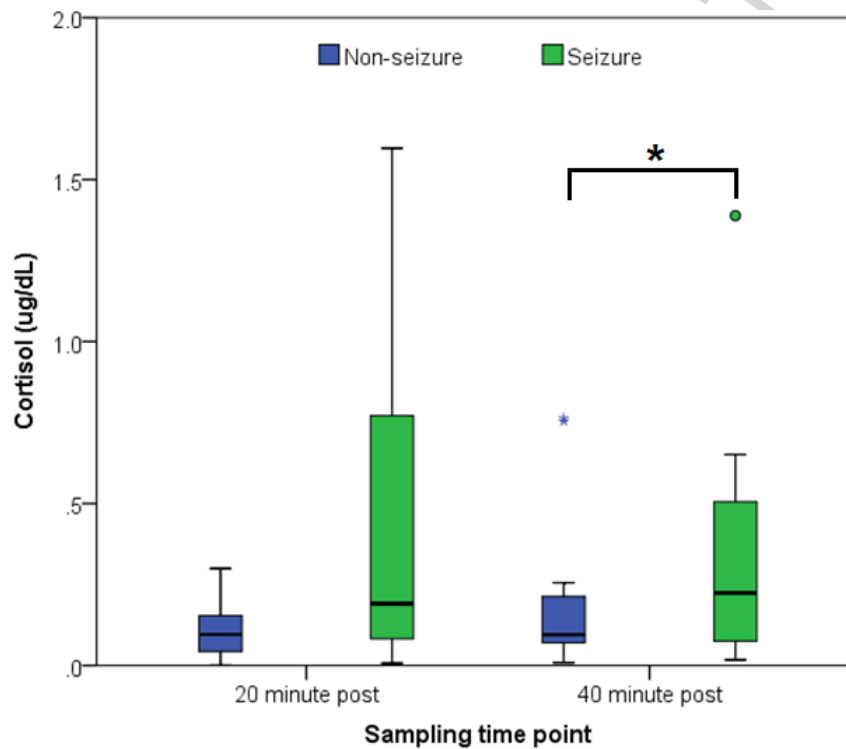


(B)

There was no association between the percentage change in cortisol levels between carer and dog samples at any sampling point (p>0.05). There was no association between absolute cortisol values for any of the carer or dog samples and the time of day the samples were taken (p>0.05).

### 3.7 Individual differences in HPA responses to seizure-related stress

There was no association between carer-reported seizure severity or length and the dog or carer's percentage difference in cortisol level between the seizure and time-matched non-seizure samples at 20 minutes (>0.05.) The majority of carers (75%, n=12) could not tell their dog was about to have a seizure before it occurred, knowledge of which did not influence the percentage difference in cortisol level between the seizure and time-matched non-seizure samples (p=0.64). No carers reported a perceivable trigger for their dog's seizure and thus this factor could not be explored. No association was found between the gender of the dog (sex*neuter status) and cortisol levels at any of the four sampling points (p>0.05).

### 3.8 Impact of dog-carer relationship on HPA reactivity

There was no correlation between any of the three MDORS subscales and the percentage change in carer or dog cortisol levels between time-matched samples and 20 minutes post-seizure or 40 minutes post-seizure samples (p>0.05).

### 3.9 Impact of carer personality

There was no correlation between any of the FFP scores and the percentage change in carer cortisol levels at 20 minutes post-seizure or 40 minutes post-seizure (p>0.05). When absolute cortisol values (μg/dL) were correlated with FFP scores, significant positive correlations were found between FFP neuroticism scores and carer cortisol levels at 20 minutes (r=0.51 p=0.043) and 40 minutes post-seizure (r=0.52 p=0.041).

## 4. Discussion

There is a complex relationship between stress and epilepsy, with stress and subsequent cortisol release potentially acting as a trigger for seizures [39, 40], and seizures themselves acting as a stressor upon the body. Spontaneous seizure activity in dogs with IE was found to have a marked effect upon the HPA reactivity of both the dog affected by the seizure and their carer. The median increase in cortisol 20 minutes following the seizure was substantial (531.6% in dogs, 40.5% in their carer), and remained high 40 minutes following the seizure (265.1% in dogs, 138.2% in their carer). Significant differences between time-matched non seizure and post-seizure samples were found at 40 minutes post-seizure in dogs, and 20 minutes post-seizure in carers. Seizure-associated stress in dogs is likely to be due to both physical and psychological stressors associated with seizures. Initial stress may be associated with physiological arousal caused by seizure activity, with muscle contractions associated with uncontrolled motor signs (e.g. tonic-clonic 'paddling movements' reported in 15/16 dogs) potentially mimicking exercise. However, a recent meta-analysis showed that there were no significant effects of exercise within 3 hours of sampling, or taking part in physical activity within 1 hours of sampling [29] on salivary cortisol levels. As such, physiological stress may not be a major contributor to the increase in cortisol in post-seizure dogs, and this may explain the lack of significant increase in cortisol at 20 minutes post-seizure. The significant elevation at 40 minutes post seizure may be caused by psychological stressors in the post-ictal phase. As all dogs included in this study experienced generalised seizures, it is assumed that the seizure activity was associated with a loss of consciousness, followed by regaining of consciousness in the post-ictal phase. Lack of awareness during the ictal phase may protect dogs from the psychological distress of the seizure [41]. In contrast, once consciousness has been regained, psychological stress associated with the post-ictal phase may partly explain the significant result seen at 40 minutes post-seizure. Post-ictal signs including agitation were commonly reported at both sampling points, and indeed post-ictal anxiety is common in the period shortly after a seizure in people with epilepsy [42], with one study finding that 45% of patients reported anxiety in the first 72 hours after a seizure [43].

In carers, significant differences in cortisol levels were observed at 20 minutes post-seizure, but not 40 minutes post-seizure. This initial peak in cortisol may be associated with the initial psychological stress of seizure onset, which is often unpredictable; indeed, three quarters of carers could not tell that their dog's seizure was about to occur before it began. This initial stress may have diminished by 40 minutes post-seizure, once the carer was more certain that their dog had survived the seizure episode. Seizures have been described as 'frightening' by parents of children with epilepsy, with uncertainty during the event regarding both their child's survival and the degree of damage resulting from the seizure [44]. All of the seizures sampled following in this study were <5 minutes in length. Studying the effect of status epilepticus, where seizures extend >5 minutes may be of interest, as this is considered the most life-threatening form of seizure, potentially lead to irreversible neuronal injury and further systemic complications [45]. As such, a more pronounced stress response may be observed in the carers of affected dogs.

No correlation was seen between the dog and carer's cortisol levels at either sampling point, which was also found in a study of psychological characteristics of the human–dog relationship [46]. A complex variety of factors affect cortisol levels in dogs and humans, which may explain this lack of correlation. In a recent meta-analysis, no effects of breed, body weight, function (e.g. pet vs. working etc) or coat colour on cortisol concentration have been found; however, effects of gender (with higher levels in intact female dogs) and age (with lower levels in puppies <6 months old) were found (Cobb et al., 2016). In this study, neither age nor gender had a significant association with cortisol levels; however, this may be due to the relatively low sample size. Due to sample size limitations, we had limited power in examining the number of relationships that we were interested in. It is possible that some of the non-significant results were due to inadequate power; however, the primary hypotheses of the study were supported with the available data.

Much research is undertaken demonstrating the positive benefits of companion animals as stress reducers, with measures including blood pressure, cortisol and heart rate reduced following positive interactions with animals when faced with stressful situations [47-49]. To date, little research has

objectively studied companion animals as a negative stressor, despite the potential negative impact of caring for an animal with health or behavioural disorders. There is increasing focus in canine epilepsy on the quality of life of both dogs and their carers [12, 13, 41]. This study demonstrates that seizures pose a potentially chronic stressor upon dogs with recurrent seizure activity and their carers. Further studies are required to quantify the impact of recurrent seizures as a chronic stressor, as well as the acute effects on the stress response as demonstrated here, as individuals with elevated cortisol levels that do not return quickly to baseline are thought to be at an elevated risk of negative effects on their immune, cardiovascular and neuroendocrine systems [50]. Studying carer cortisol profiles throughout the day may provide further insights; chronic stressors that threaten physical integrity, are uncontrollable, or involve trauma tend to result in a high flat diurnal profile of cortisol release, with lower than normal levels in the morning and higher than normal levels in the evening [51].

Comparing the cortisol responses of dogs with epilepsy against healthy dogs may provide more insights. Previous studies have shown that dogs living in chronically stressful environments (e.g. dog shelters) exhibit lower levels of cortisol [29], thought to be due to 'vital exhaustion', where chronic stress leads to exhaustion dysregulation of the HPA axis, resulting in depressed cortisol output [52]. This phenomena is seen in chronically stressed humans when they no longer feel able to cope with chronic life stressors, and is reportedly accompanied by feelings of increased irritability and demoralization [52].

The study took place in the home, a naturalistic setting for the dog and carer that is beneficial for the collection of saliva samples as it avoids the potentially stress-inducing effects of travel and the novel experimental environment for data collection. Requesting carers to collect saliva samples avoided the potential interference of a stranger's presence that would be introduced by the researcher collecting samples. In the same meta-analysis of salivary cortisol in dogs, dogs whose carers or regular handlers were present at the time of testing had significantly lower salivary cortisol concentrations than those that did not [29]. This emphasises the importance of using a person with the same role in the dog's life to collect samples in study populations (e.g. the primary caregiver) to avoid introducing this

additional source of variation to the data. There was potential bias introduced to the study during the recruitment of primary caregivers, as 15/16 carers included in this study were female and there are known differences in cortisol reactivity of males and females in response to psychological stress [53]. There are a number of reasons why this bias may have occurred, including the finding that the presence of an adult female in the household is associated with dog ownership [54]. In addition, previous research has identified that a female response bias to surveys is relatively common [55].

The use of carers to collect samples carries the risk of insufficient sample volume being collected. In this study, 16/64 (25%) of samples taken from the dogs were of insufficient volume to analyse, which reduced the power of the study. In contrast, only 3/64 (4.7%) carer samples were of insufficient volume to analyse. Sample volume might vary greatly between and even within individuals, and in future studies, it is recommended that multiple samples should be collected at the same time point to avoid this. Exploring alternative saliva collection techniques that are reliable and do not interfere with the cortisol assay may be beneficial e.g. using the smell of food to stimulate saliva production.

The samples were collected following spontaneous rather than induced seizure activity, which has the benefit of studying the natural disease process, but introduces limitations due to the lack of control of the event. In contrast to a study of cortisol responses to a simulated thunderstorm in thunderstorm-phobic dogs [21], no baseline samples prior to the seizure could be collected here, with comparisons instead made at time-matched points on a 'non-seizure day'. We were unable to control for the time of day the seizure took place due to the spontaneity and inherent unpredictability of seizure activity, features which may also contribute to chronic stress for carers. This may have introduced circadian rhythm effects on cortisol levels; however, these effects may be minimal as time of day was not found to be associated with cortisol levels in dogs or carers. The timing of the sample collection was controlled for by including timing instructions in the sampling protocol for carers, and asking carers to record on a sheet the times their samples were taken, to verify whether they corresponded with the protocol as described. None of the submitted samples collected were outside the protocol as described.

We expected that there would be a relationship between the strength of the dog-carer bond (as measured by the MDORS) and the degree of HPA reactivity, with more closely bonded carers hypothesized to exhibit a more pronounced HPA response to witnessing their dog having a seizure. Questions within the MDORS subscales have previously been correlated with carer cortisol levels, with lower carer cortisol levels associated with a perception of being less bothered about the dog stopping them from doing things, a greater frequency in bringing their dogs when visiting people, and a perception that the dog's death would be traumatic [46]. In the present study, none of the three MDORS subscales appeared to play a role in the HPA response of their carers, which we speculate may be due to the consistently high levels of all three MDORS subscales observed in this study. MDORS scores in comparable studies were lower than that seen in the current study, with the exception of perceived costs (Table 1) [56, 57]. MDORS is based upon social exchange theory, specifying that benefits and perceived costs of a relationship need to be balanced for the relationship to be successful. The high perceived cost score (which indicates a more positive dog-care relationship i.e. that the carer perceives lower costs associated with their dog) is somewhat surprising, as it is recognized that carers of dogs with epilepsy face the burden of long-term management of their dogs, which may inhibit their independence and have an impact on their personal, social and working lives [13]. In contrast, it is perhaps unsurprising that the dog-owner interaction subscale was higher than in previous studies, as a high level of interaction is required as part of the routine management of a dog with epilepsy e.g. medicating their dog several times per day, monitoring for signs indicating a seizure may be about to occur, monitoring for drug side effects, regular trips to the vets for blood testing.

Carers who scored more highly for the personality trait neuroticism exhibited higher levels of cortisol at both 20 and 40 minutes post-seizure. Neuroticism is one of the five basic dimensions of the five-factor model of personality, with this dimension sometimes termed 'emotional stability' or 'negative emotionality' [37]. It was first delineated as a personality trait seventy years ago as part of the original "Big Two" personality traits [58]. Neuroticism is a dimensional measure of an individual's tendency to experience negative emotions that are manifested at one extreme as anxiety, depression, and

moodiness and at the other as emotional stability [59]. The results here are in line with those of previous studies, that have demonstrated that people with high neuroticism show significantly greater levels of salivary cortisol throughout the day (as measured in 3 hour intervals) [60], at 30-60 minutes after waking [59], and have a greater cortisol response to acute psychological stress [61]. It is possible that carers with higher neuroticism experience greater anxiety when witnessing their dog have a seizure, and catastrophize the event more than a carer with low neuroticism.

## 5. Conclusions

In conclusion, seizures act as a significant stressor for both dogs with epilepsy and their carers. As there was a gender bias in the carer sample (15/16 were female), and there are known sex differences in cortisol reactivity in response to psychological stress, the conclusions of this study may be limited to female carers. Future efforts to mitigate the tractable aspects of seizure-related stress, including the reduction of post-ictal anxiety in dogs (through owner support, pharmacological or behavioural means), and implementation of better support for carers managing seizures may improve the quality of life of both members of this relationship.

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Highlights

- Seizure activity is potentially stress-inducing for dogs with epilepsy and their carers

- This study examined HPA reactivity following spontaneous seizure activity in dogs

- Salivary cortisol levels increased in dogs and carers following seizure activity

- Carers with higher neuroticism scores exhibited higher cortisol levels

- No associations were found between cortisol reactivity and the dog-carer bond