The untrained response of pet dogs to human epileptic seizures

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Abstract

Epilepsy is a disorder of the brain and a seriously debilitating condition, which has been associated with injury, social stigmatisation and in some cases, sudden unexpected and premature death. A sense of profound isolation is felt by many individuals with epilepsy, and this community has expressed an urgent desire for an early warning system to allow them time to prepare for seizure onset. Surveys of dog owners with epilepsy have previously reported that some dogs can predict the onset of a seizure. Therefore, the current study investigated the hypothesis that if pet dogs were exposed to seizure-related odours which apparently emanated from their owners, they would respond by demonstrating attention seeking behaviours. This provides the first empirical test of this phenomenon in dogs that have not previously been trained for seizure alerting.

This study, therefore, explores the propensity of pet dogs to anticipate and respond to human epileptic seizure onset in a controlled experimental investigation. Using a repeated measures design of experiment, recordings were made of the reactions of 19 untrained pet dogs to odours from sweat samples provided by three people with epilepsy and two people without epilepsy (controls). The sweat samples were from pre-ictal, ictal and post-ictal phases. Odours from the harvested sweat samples were randomly delivered to individual dogs in a test area, using two bespoke pieces of apparatus called Remote Odour Delivery Mechanisms, (RODMs). Behavioural changes by the dogs on encountering the odour samples were recorded by video for later analysis. Consistent with our hypothesis, seizure-associated odours evoked behavioural changes in the dogs, concordant with attention seeking attempts and thereby supporting the view that seizures are accompanied by a distinctive odour.

Introduction

International surveys investigating whether dogs can predict the onset of seizures in humans with epilepsy report that dogs demonstrate attention-seeking activities directed at their owners, for example: maintaining close-proximity; intense staring; excessive panting; paw lifting; vocalisation; increased locomotor activity; licking; yawning and scratching (Dalziel et al., 2003; Kirton et al., 2004, 2008 Martinez-Caja, et al., 2019; Powell 2020). While some authors contend behaviours of that nature might be indicators of physiological canine stress (see Csoltova et al. 2017), others, such as, Lennsen et al. (2015, 2017, 2019) conjecture that stress in dogs may be demonstrated as: cringing; crouching; freezing; hiding; shaking or barking; growling; baring teeth; snapping and lunging (Serpell et al., 2006). Furthermore, in the Powell (2020) survey fight or flight behaviours were observed in less than 2% of dogs, thus reflecting the findings of previous published articles, which found that (on the contrary) seizure-associated odours effected an affiliative response in most dogs (Dalziel et al., 2003; Kirton et al., 2004; 2008; Martinez-Caja, 2019).

It is known that physiological changes and excessive electrical activity in the brain precede epileptic seizures (Ohman et al., 1976; Whealy et al., 2017; Bruno et al., 2018; Hogg et al., 2019). It is also well established that these changes are instigated by the autonomic nervous system and the hypothalamic-

pituitary-adrenal axis (HPA) and cause an increase in heart rate and respiration rate (Nater and Rohleder 2009; Linneman et al., 2016; Skoluda et al., 2017; Pereira et al., 2017). Metabolic stress and the physiological changes which accompany it, release Volatile Organic Compounds (VOCs) (Hackner and Pleil, 2017; Catala et al., 2019). These VOCs dissolve in the blood and saliva and are eventually either: exhaled as part of the respiratory process, or effused as sweat (Burda et al, 2014; Baljubasic and Buchbauer, 2015; Saima et al., 2015; Edwards et al., 2017; Reeve et al., 2019).

In addition, a range of studies have demonstrated that exhaled VOCs indicate the presence of cancers and a range of other conditions, for example: - cholera; cystic fibrosis; diabetes; gut diseases; heart allograft rejection; heart disease; liver diseases; pre-eclampsia; renal disease and TB (McCullough et al., 2006; Sonoda et al., 2011; Walczak et., 2012; Jadoon et al., 2015; Edwards et al., 2017; Reeve et al., 2019). VOCs have also emerged as a valuable diagnostic tool in identifying cases of congestive heart failure in older patients as well as people with COPD (Finamore et al., 2018). Elsewhere, it has also been documented that in the more general sense, dogs communicate not just between themselves (Sinscalchi et al., 2018), but that they can also display ‘a flexible behavioural repertoire when communicating with humans’, which include visual, tactile, auditory and olfactory signals (Sinscalchi et al., 2018, p 1). Thus, the proposal that VOCs may at some level act as a trigger mechanism for dogs which detect and respond to the onset of epileptic seizure in their owners, is plausible and warrants investigation.

Thus, it is hypothesised that epileptic seizures may be associated with some form of seizure-induced olfactory biomarkers and it is on these to which dogs respond in the pre-ictal phase. This hypothesis is further strengthened by the findings of a recently published study where it is reported that medical detection dogs had been trained to alert on seizure-specific odour(s) (Catala et al., 2019).

In the current study, our aim was to investigate at a more fundamental level if dogs with no previous training for epilepsy detection show a behavioural response when exposed to odours associated with epileptic seizures. More specifically, we hypothesized that family pet dogs owned by non-epileptic people, would show increased owner directed behaviour when exposed to odours associated with epileptic seizure, compared to control odours. We used a repeated measures experimental design in which pet dogs were exposed to odours derived from either healthy human controls or from those with epilepsy.

Methods

A repeated measures design experiment was conducted in which recruited dog-owner dyads were subjected to a series of odours taken via sweat samples from the axillae of each volunteer epileptic and non-epileptic (control) volunteers. In addition, all methods which included humans, were performed in accordance with the relevant guidelines and regulations. All experimental protocols were approved by Research Ethics Committee, School of Biological Science Queens University Belfast, PREC reference number: No 66-2015-16. The passive observation and recording of the dogs’ responses to seizure and control odours were conducted in accordance with relevant ‘Arrive’ guidelines and regulations, (https://arriveguidelines.org)

Ethical Note

Ethical approval for the study was given by the Research Ethics Committee, School of Biological Science Queens University Belfast, PREC reference number: No 66-2015-16

The seizure related samples were provided by three people who had completed Informed Consent Forms. The control samples were provided by two additional volunteers, who had completed Informed Consent Forms.

The Dogs, The Samples And Their Storage

Canine participants and their owners were recruited from a Dog Training Club in Lisburn Co. Antrim, Northern Ireland, following a routine training club night, when time was set aside for the research project to be explained. This appeal yielded a sample of 19 dogs of varying breeds and ages, and of both sexes. The following three sets of sweat samples were provided by people with epilepsy:

- pre-seizure taken when a seizure-alert dog indicated an impending seizure,
- seizure sample harvested immediately following a seizure
- post-seizure taken 6 hours after a seizure.

Sterile gauze pads were used to capture sweat samples taken from the axillae, and these were subsequently stored in pristine glass preserving jars at 4°C (Willis et al., 2004; McCulloch et al., 2006; Forbes et al., 2014; Jezierski et al., 2015; Reeve et al., 2012).

Data Collection

The dog characteristics recorded were breed, sex, age-group and years owned. Indicators of seizure odour recognition by dogs were identified by selecting three commonly observed behavioural attention-seeking activities as previously reported in the literature; *close-proximity to the owner, intense staring at the owner, and climbing up or pawing* the owner (Palestrini et al. 2005; Palmer and Custance 2008; Prato-Previde et al. 2003; Rehn et al. 2013; Topál et al. 1998; Scandurra et al. 2016 cited in, D' Aniello et al., 2018, p 70). For the purpose of this experimental design, close proximity was defined as the dogs venturing to within one metre or less from the owner. This distance was determined by the space between the seated owner and a science bench situated one metre immediately in front of him/her. Laterally, a one metre distance on each side of the owner was delineated by visual reference to a mark on the floor to the left and right of the owner.

All observations of dog responses during the experiment were conducted 'blinded', thus, neither the principal researcher nor the dog owners, had any knowledge of the sequences of odour presentation. The dogs' responses were recorded in seconds, over five trials, with each trial lasting three minutes so as to minimize the risk of the fatigue factor among dogs (Hackner and Pleil 2017). Recordings were by HP laptop installed video camera with a backup provided by an I-phone tablet camera. Analysis of the video

footage was made without knowledge of the sequence of sample odours used to prevent unconscious confirmation bias (Haidet et al., 2009; Fish et al., 2017; Holman et al. 2015; Kardish et al. 2015).

Experimental Procedure

A random number generator was used to produce groups of odour presentation which were representative of the three ictal phases and two controls. Prior to the start of each day's trial, a simple toss of the coin decided which of these groups to follow and thereafter, only the research assistant was aware of the odour deliveries during each day's trials.

Pre-test

- On arrival each dog was individually brought to the test room which measured 10.5 m X 8m X 4m (Fig. 1) and was given 3 minutes to habituate to the surroundings. This introduction was followed by the test.

Test :- Each owner was asked to sit on a chair in the centre of the test area, and to ignore their dog during testing. Two remote odour delivery mechanisms, (RODM), (Fig. 1), were used to deliver odours to the dog owner's location. The RODM is a bespoke piece of apparatus developed by the first author to deliver an odour to a specific location and has been previously validated, demonstrating that dogs respond to odours delivered by this mechanism (Powell 2020). Each RODM (Fig. 2) consisted of an aquarium pump (5W, 240/50Hz Air pump 200 delivering 200 litres/hour) and connected to a re-sealable 3.6 litre plastic watertight storage keg (UN approved). The storage keg had a resealable open top wide mouth and had inlet and outlet valves fitted at opposite sides. Pumps and kegs were connected to each other by 4mm (internal diameter) plastic aquarium hose. The outlet pipes were 15m aquarium tubing (4mm) each with a non-return valve at the end. The outlet tubes were placed, one under each thigh of the participant with the tube ends just showing at the inside of each leg. It was expected that this arrangement would increase the likelihood of the emerging scent samples remaining close to the participant's body.

During exposure to each odour, the times spent by the dogs on each of the 3 behavioural responses, detailed above, were recorded. The lengths of time for which the pumps ran while delivering scent samples and during the system flush sequences, were calculated thus:

- Pump delivers 200Lt in 60 minutes = 18 seconds/1 Litre
- Airtight keg volume = 3.6 Lt
- Time to clear 3.6 Lt = 3.6 x 18 secs = 64.8 secs = 1 min approx.
- Time to run sample scent before introducing dog = 1 min.
- Time to run sample scent, dog in room = 3 mins
- Flush time after trial (directed outside window) = 1 min.
- Total time for each scent sample = 5 mins
- Total number of samples per dog = 5 samples

- Total time needed for each dog = 5 x 5 = 25 mins

+ 3 mins initial habituation time = 28 mins/dog.

Scent Delivery Apparatus: Remote Odour Delivery Mechanism, (RODM)
Airline to scent chamber Airlines directed to participants seat

Video

Kilner preserving jar with scent sample Airtight scent chamber

Statistical analysis

All data analysis was performed using the statistical package SPSS, (version 24) IBM, Corporation New York, USA). Data from the two control odours, (C1, C2) were combined and averaged to give a mean for each of the three behavioural responses across the controls, *time near owner combined control*, *time of eye contact combined control*, and time pressing close *combined control*. The dogs' three behaviours, time near owner (TNO), time in eye contact with owner (TEC), and time pressing close to owner (TPC), were then measured across all three seizure-related odours and comparisons drawn with their responses to the combined controls.

Visual inspection of histograms and use of Kolmogorov-Smirnov tests revealed the behavioural data were not normally distributed, thus indicating the need for non-parametric statistics. Friedman tests were used to examine whether each of the three behavioural responses (TNO, TEC, TPC) differed across the four treatment conditions, (pre-ictal, ictal, post-ictal and control). Wilcoxon signed rank tests were then used to make pairwise comparisons between each of the pre-ictal, ictal, post-ictal, with the mean control where significant differences were found. The Cohen d effect size was calculated using the formula, $r = Z / \sqrt{N}$, where N = the number of observations over 4 time points which in this study is $Z/ \sqrt{76}$ (19 cases x4 time points) = Z / 8.7. According to Cohen's (1972), classification, 0.1 = small effect, 0.3 = moderate effect; 0.5 and above = large effect.

Results

4.4.1 Demographic information

As can be seen in Table 1, nineteen owners from a wide age range volunteered for this study, most of whom were female. The majority of the participating dogs were female, of pedigree status, and under 8 years of age. Most owners stated their dogs had been with them for 12 months or less and their main reason for acquiring a dog had been companionship. None of the dogs had witnessed an epileptic seizure and none of the dog owners had epilepsy.

Table 1
Owner and dog demographics

Demographic factor Number Percentage
Owner sex
Male 3 15.8%
Female 16 84.2%
Owner Age Years
18–25 1 5.3%
26–35 7 36.8%
36–45 3 15.8%
46–55 1 5.3%
56–65 6 31.6%
66 + 1 5.3%
Dog sex
Male 8 42.1%
Female 11 57.9%
Dog age months
6–35 7 36.8%
36–65 5 26.3%
66–95 3 15.8%
96–125 1 5.3%
126–155 2 10.5%
156–185 1 5.3%
Dog Breed
Pedigree 13 68.4%
Mixed 6 31.6%
Length of ownership
< 12 months 13 76.5%
1–5yrs 6 23.5%

Behavioural Responses To Seizure-related And Control Odours

TNO - **Time near owner** differed across the four treatments, pre-ictal, ictal, post-ictal and combined control, (Friedman test, X^2 (3) = 10.5, p = 0.015). The Median levels for all four odour responses were respectively, 37.0, (IQR = 43), 47.0, (IQR = 71), 53.0, (IQR = 76), and 34.5, (IQR = 27) (Fig. 3).

More specifically, Wilcoxon paired comparisons of behavioural changes to the seizure-related odours with the combined control odour revealed: TNO pre-ictal, Z = -1.53, p = 0.13; TNO ictal, Z = -3.1, p = 0.002, TNO ictal, effect size = 0.18 (small), and TNO post-ictal, Z = -2.8, p = 0.005, effect size = 0.32 (medium). Thus, dogs spent more time near their owner during the delivery of the ictal and post-ictal odours compared to the control condition, with no difference for the pre-ictal condition.

TEC- **Time in eye contact**: the dogs engaged in significantly more eye contact with their owners across the three seizure odours than they did with the combined control odours, (Friedman test, X^2 (3) = 11.8, p = 0.008); Median levels for TEC pre-ictal, ictal, post-ictal and TEC combined control were respectively, 3.0 (IQR = 7.0), 7 (IQR = 9), 8.0 (IQR = 11) and 2.5 (IQR = 5) (Fig. 4).

Wilcoxon signed rank tests comparing response to seizure-related odours and the combined control revealed, TEC pre-ictal: Z = -2.3, p = 0.028; Effect size = 0.3 (medium); TEC ictal, Z = -2.2, p = 0.022, Effect size = 0.25 (small to medium); TEC post-ictal Z = -2.9, p = 0.004 Effect size = 0.33 (medium). Thus, dogs spent more time engaging in eye contact with their owners during the delivery of each of the three seizure related odours compared to the controls.

TPC - Time pressing close

was found to differ across the four treatments (Friedman test, X^2 (3) = 8.4, p = 0.038); Median levels for TPC pre-ictal, ictal, post-ictal and Combined control were 4.0 (IQR = 7), 6.0 (IQR = 18); 1.0 (IQR = 8) and 3.0 (IQR = 5) (Fig. 5). Wilcoxon signed rank tests comparing the responses of the dogs to the seizure-related odours and the combined control revealed, TPC pre-ictal, Z = -1.17, p = 0.24; TPC ictal, Z = -2.5, p = 0.013, Effect size = 0.3 (medium), TPC post-ictal, Z = − 0.44, p = 0.66. Thus, dogs spent more time pressing close to their owner during the delivery of the ictal odour compared to control, with no difference for the other seizure related odours.

Discussion

This study hypothesised that during seizure-odour exposure untrained pet dogs would demonstrate behavioural changes concomitant with attention seeking activities, thereby implying an ability to anticipate seizure. In line with this prediction, nineteen pet dogs were found to have engaged in more attention-seeking behaviour when they detected odours from seizure associated sweat samples than when presented with control odours. This implies that epileptic seizures produced an olfactory trigger, or, volatile organic compound(s), which effected the dogs' behavioural changes. Some support for the view

that seizures are associated with VOCs comes from the findings of a previous study which recounts that trained dogs demonstrated an ability to discriminate seizure odours from controls (Catala et al., 2019).

The existence of pre-seizure odour(s) is an important finding because it offers a simple and reliable means for training dependable seizure-alert dogs to warn people of an impending epileptic event, an aim which has long been sought (Hoppe et al., 2015; Bruno et al., 2017). It would not only improve patient safety and well-being but would also, *'promote therapies aimed at rapidly treating seizures (and) be able to abort seizures through targeted therapies'* (Ramgopal et al. 2014 p 291–292).

Contrary to expectations however, not all seizure-associated odours elicited the same intensity of behavioural manifestations; for example, in the pre-ictal condition, the *time near owner* response (TNO) did not differ to that of the control sample. This outcome is somewhat contrary to previous findings (Dalziel et al, 2003; Kirton et al, 2004, 2008; Caja et al. 2019) who collectively report intense levels of pre-ictal close affiliation being demonstrated to their owners by untrained dogs. However, it has to be borne in mind that the results cited for the time near owner pre-ictal response (TNO), were participant-dependent and alerting-dog dependant for the accurate extraction of the pre-ictal sweat sample. For example, the three volunteers with epilepsy who had provided the sweat samples were entirely dependent upon their dogs to alert them at the appropriate time prior to seizure onset. That implies three possible confounding variables; firstly, the accuracy of the dogs in anticipating impending seizure episodes; secondly, the competence of the volunteers in recognizing their dogs' warning signal; and thirdly, the cognitive levels of the volunteers given that it has been reported that both seizures and the prolonged use of anti-epileptic drugs can have a negative impact on cognition (van Rijckevorsel 2006; Helmstaedter and Witt, 2016).

Yet, despite these reservations, the same criticism could not be levelled at the accuracy of sample taking in the ictal and post-ictal conditions, given the unmistakable nature of seizures and the direct involvement of a 'significant-other' who would have taken the sweat samples. In line with this, dogs spent significantly more time near the owner in both the ictal and post-ictal compared to control conditions.

Interestingly, the time near owner reaction of the dogs to the post-seizure sample, which had been taken six hours after the event, closely resembled their response to the ictal odours. This appears to suggest that 6 hours post seizure was not an adequate time interval to have allowed for the dissipation of seizure-related odours. It is suggested therefore, that this may account for the similarity of the dogs' responses to post-seizure odour and ictal odour. Future research would be advised to consider a much longer post-ictal sampling period, perhaps in the order of 12 hours, or be tested repeatedly over a number of hours.

The recorded time for the dogs making direct eye contact with their owners, (TEC), was found to be significantly greater during all three seizure conditions and although not measured, additional fleeting, direct eye contact, was observed as the dogs walked quickly past their owners. In some cases, the dogs opted to remain staring at their owners from just outside the 1meter proximity area, which precluded recording. Interestingly, the intense gaze response has been reported by others, such as Martinez-Caja et al. (2019), who cited staring as the third most frequently reported alerting behaviour shown by dogs in the

pre-ictal phase, the joint second place being described as close-proximity to owners and licking hands and face.

The third alerting behaviour tested in this study was pressing close to owners (TPC). The findings showed that this was only significant in the ictal condition, an outcome which appears to be slightly at odds with the findings cited in previous studies where dogs demonstrated pawing and jumping up alerting behaviours, across pre-ictal, ictal and post-ictal conditions (Kirton et al. 2008).

Nevertheless, in this investigation, seizure associated odours were presented to pet dogs using a novel method which was designed to deceive the dogs into believing the seizure-related odours were emanating from their owners. Thus, the current study demonstrates support for the hypothesis that a seizure-related olfactory trigger mechanism evokes spontaneous seizure alerting behaviour in pet dogs. This does not, however, exclude the possibility that other trigger stimuli may also exist and whilst beyond the scope of this study, it is acknowledged that other researchers have hypothesized the existence of a sensitivity in dogs to electro-magnetic changes (Begall et al., 2008; Burda et al., 2009; Harte et al., 2013; Martini et al., 2018). That being the case, it is conceivable that dogs may also be responding to electro-magnetic signals which are associated with pre-seizure physiological changes (Bromfield Cavazos and Sirven, 2006; Reeves and Swenson, 2008; Sano, Picard and Stickgold, 2014; Bandarabadi et al. 2015; Moshe et al. 2015; Avarez et al. 2015; Epilepsy Action 2020) and invites further investigation. Thus, it is also conceivable that dogs which anticipate seizure onset, may be responding, not only to olfactory stimulation, but also to minute variations in the body's electro-magnetic field which accompany the onset of epileptic seizure.

Conclusion

Consistent with the hypothesis identified at the beginning of this study, the data confirm that epileptic seizures, across their three phases, pre-ictal, ictal and post-ictal, are directly associated with attention-seeking behaviours in dogs. The nature of that bio-trigger remains unexplained, but the indications are that olfaction is a key component. This is a welcome outcome as it offers the opportunity for targeted training of reliable seizure prediction dogs, with a consequent reduction in accidents and injury caused by unexpected seizure occurrences and an improved quality of life for people with epilepsy. That said, several important questions have been raised by this current study and indicate the need for further research; for example, (1) why should a seizure biomarker evoke an affiliative response in dogs? (2) why are some dogs disposed to respond while others are not? (3) is the seizure trigger mechanism olfaction only, or could electro-magnetic variation also be a factor?

As has previously been reported, the nature of the owner-dog relationship may be a significant factor in the performance of dogs (Zilcha-Mano et al 2011; Horn et al 2013; Meyer et al. 2014; Dwyer et al. 2015; Jamieson et al 2017), and it is possible that this bond may explain why some dogs respond to seizures and others do not.

Declarations

Authors' Contribution Statement.

Neil Powell conducted the research outline above, as part of a PhD thesis which was conducted part-time over five and a half years at Queen University Belfast. The co-authors, Dr Alastair Ruffell and Dr Gareth Arnott, were my supervisors during this time at Queens and guided my work with great skill and dedication, in accordance with their supervisor responsibilities.

Competing interests: none

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Figures

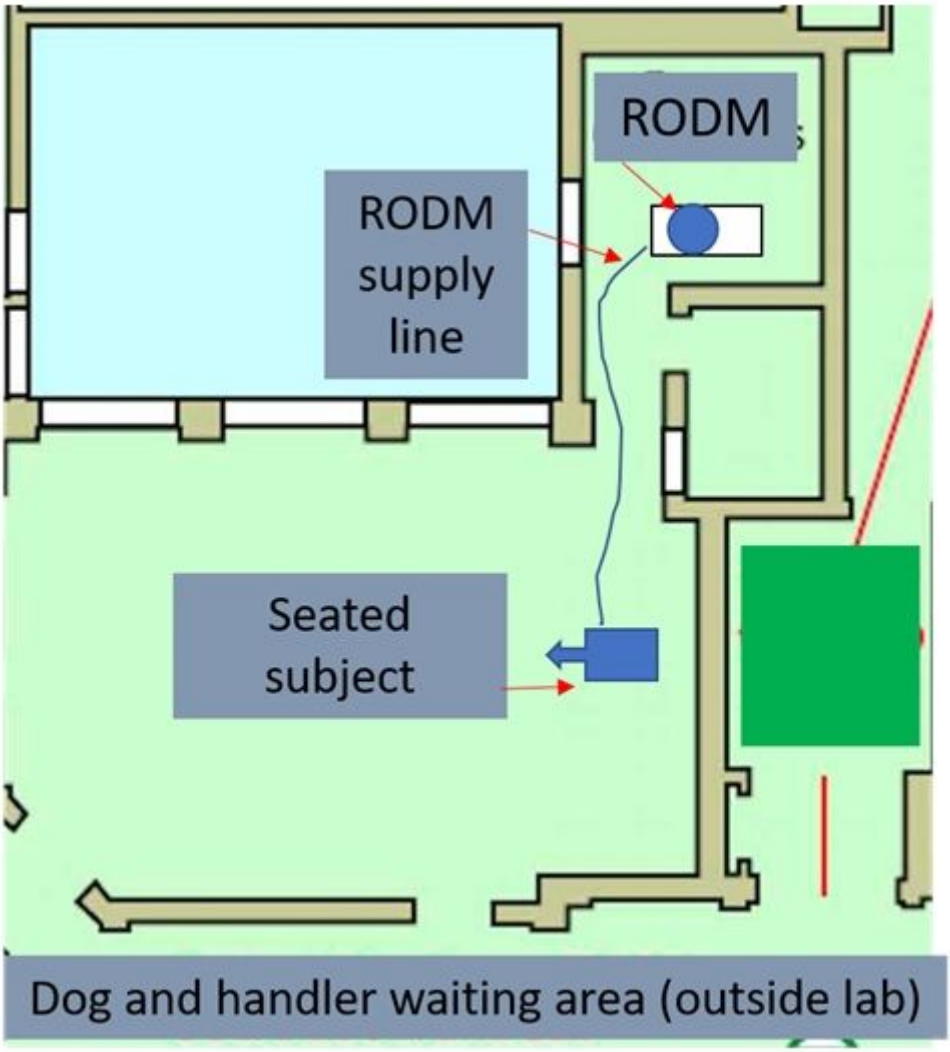

Figure 1

An overview of the test room used and arrangement for the RODM test. The test room measured 10.5 m X 8m X 4m

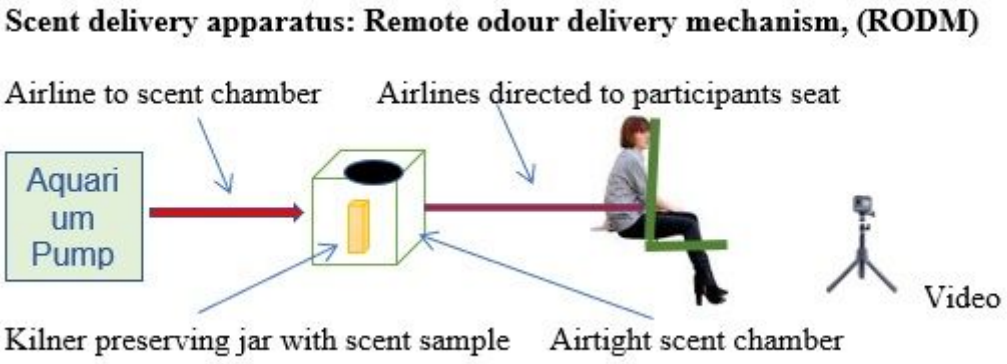

Figure 2

A schematic drawing showing how the remote odour delivery mechanism was used.

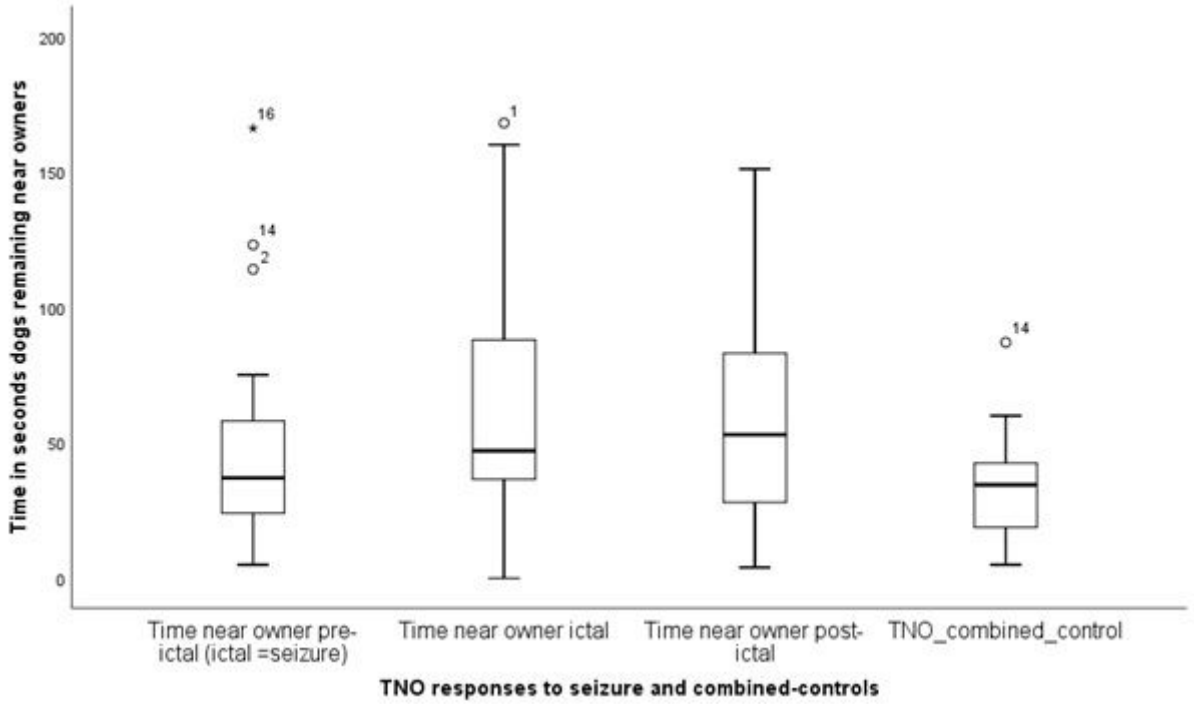

Figure 3

A box plot to show the times when the dogs remained in close proximity to their owners (one meter), measured across the four odour deliveries of seizure and combined control.

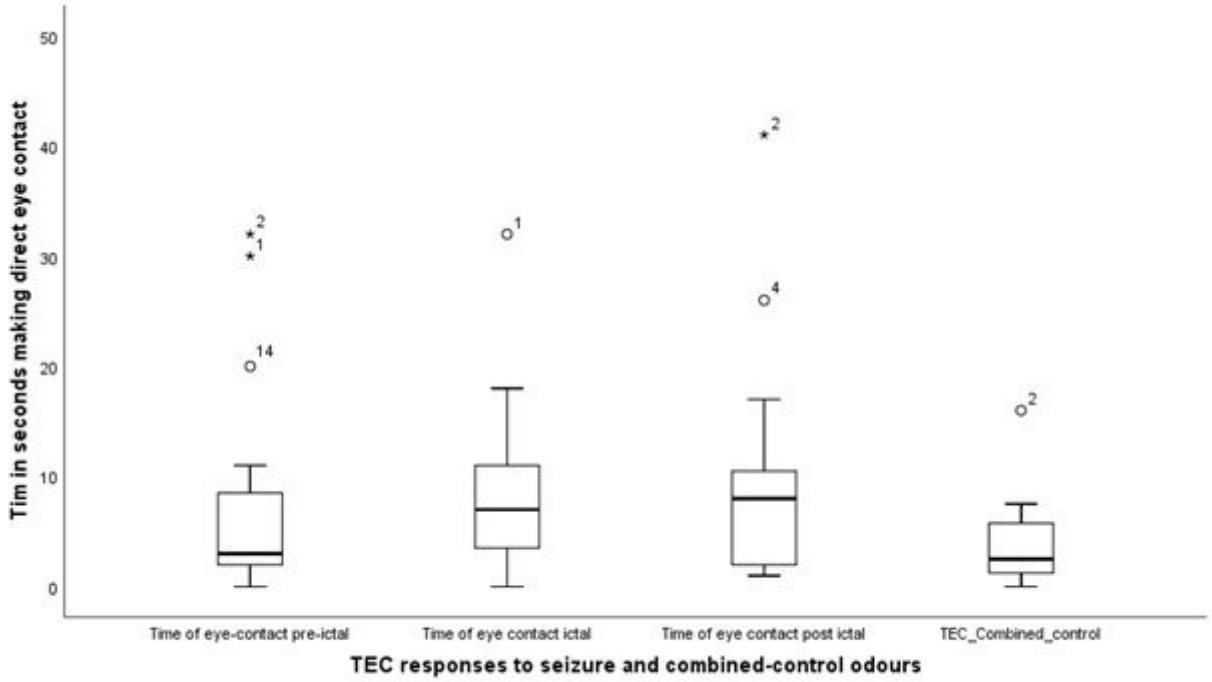

Figure 4

Showing the times when the dogs engaged their owners in a direct staring response across the 4 conditions, of seizure and combined control odours.

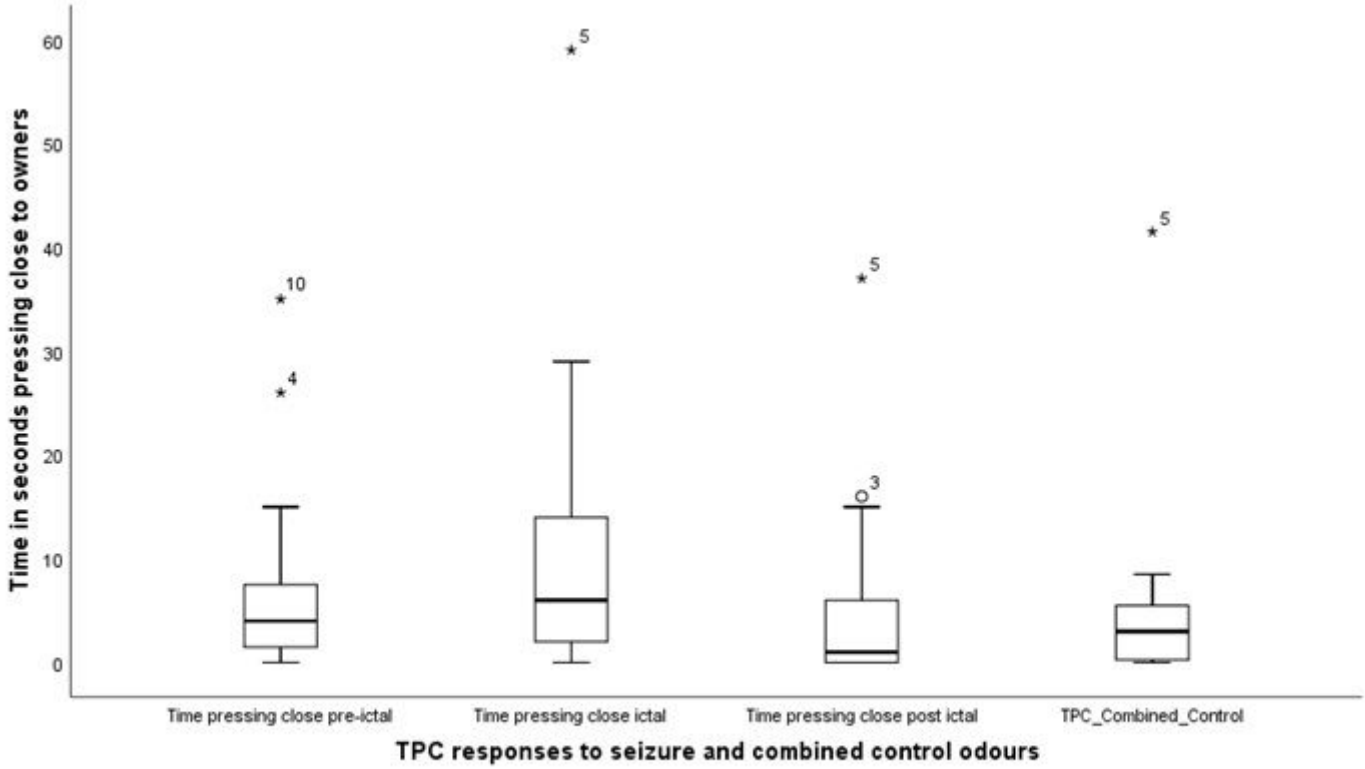

Figure 5

Box plot indicating the times spent by the dogs engaged in pressing close (TPC), to their owners by pawing or jumping up on them.