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# Dog-appeasing pheromone collars reduce sound-induced fear and anxiety in beagle dogs: a placebo-controlled study

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**The objective of the study was to assess the effects of a dog-appeasing pheromone (DAP) collar in reducing sound-induced fear and anxiety in a laboratory model of thunderstorm simulation. Twenty-four beagle dogs naïve to the current test were divided into two treatment groups (DAP and placebo) balanced on their fear score in response to a thunderstorm recording. Each group was then exposed to two additional thunderstorm simulation tests on consecutive days. Dogs were video-assessed by a trained observer on a 6-point scale for active, passive and global fear and anxiety (combined). Both global and active fear and anxiety scores were significantly improved during and following thunder compared with placebo on both test days. DAP significantly decreased global fear and anxiety across 'during' and 'post' thunder times when compared with baseline. There was no significant improvement in the placebo group from baseline on the test days. In addition, the DAP group showed significantly greater use of the hide box at any time with increased exposure compared with the placebo group. The DAP collar reduced the scores of fear and anxiety, and increased hide use in response to a thunder recording, possibly by counteracting noise-related increased reactivity.**

Noise-induced fear and anxiety is a significant behaviour concern of dog owners. Treatment is often delayed until responses are extreme (Sherman and Mills 2008). In a survey of 383 dog owners (Blackwell and others 2013), 49 per cent of the dogs showed at least one sign of fear when exposed to noises, such as fireworks, thunder and gunshots. In a US survey of 337 noise-phobic dogs, 86 per cent, 74 per cent and 41 per cent were fearful of thunderstorms, fireworks and vacuum cleaners, respectively (Denenberg and others, 2013), with most showing sensitivity to multiple noise sources.

A range of interventions of varying efficacy have been used primarily focused on desensitisation and counterconditioning combined with one or both of psychotropic drugs and natural products, such as pheromones (Crowell-Davis and others 2003, Mills and others 2003, Sheppard and Mills 2003, Levine and others

2007, Levine and Mills 2008, Sherman and Mills 2008). Several non-pharmacological treatments, such as the Storm Defender Cape (Cottam and Dodman, 2009), Anxiety Wrap (Cottam and others 2013) and HarmonEase (DePorter and others 2012), have been studied for the management of storm-related disorders in dogs. Importantly, many of the studies using drugs or natural therapeutics in veterinary behavioural medicine do not include placebo control.

The dog-appeasing pheromone (DAP) (Adaptil, Ceva Santé Animale) is a synthetic analogue of the pheromone secreted after parturition by the intermammary sebaceous glands of the lactating bitch (Pageat and Gaultier 2003). This pheromone is responsible for the sense of wellbeing experienced by puppies when with their mother. It has demonstrated calming properties in many clinically tested stressful situations such as kennelling (Tod and others 2005), veterinary visits (Mills and others 2006, Siracusa and others 2010), car travel (Estelles and Mills 2006), human separation (Gaultier and others 2005), firework exposure (Mills and others 2003, Sheppard and Mills 2003, Levine and others, 2007, Levine and Mills 2008) and introduction of puppies into a home (Gaultier and others 2008, Gaultier and others 2009). Additionally, improved socialisation of puppies attending socialisation classes has also been reported (Denenberg and Landsberg 2008).

DAP has only been studied for its effect on noise sensitivity during firework exposure (Mills and others 2003, Sheppard and Mills 2003, Levine and others 2007, Levine and Mills 2008). The present study examined noise-induced fear to a thunder recording. Fireworks differ from thunder in the duration of 'cracks and booms', distance from the source, variability of weather events and other associated stimuli that might cause fear or distress. For example, of 936 recorded storm events (Crowell-Davis and

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others 2003), 35 per cent consisted of rain only; 25 per cent of rain, lightning and thunder; 17 per cent of rain and thunder; 12 per cent of rain, strong wind, thunder and lightning and the remaining 11 per cent of other combinations. Additionally, the stimuli can have wide variability in type, intensity, frequency, duration and proximity of sound and lightning, as well as a lowering of barometric pressure and static electricity. Thus, when compared with fireworks, the perception of thunderstorms is much more complex. Moreover, dogs enrolled in a clinical trial will experience varied weather events, and are further influenced by owner and environmental differences between households. Consequently, a laboratory model using thunderstorm recordings, which has been used in behavioural modification for noise-related fears and phobias (Levine and others 2007, Levine and Mills 2008) and which induces measurable fear response in both clinical and laboratory environments (Dreschel and Granger 2005, Araujo and others 2013), offers a non-specific, but standardised, model of noise-induced fear and anxiety for the assessment of interventions such as pheromones.

To assess the effectiveness of a DAP collar in reducing sound-induced fear and anxiety in dogs, the thunderstorm simulation model was used (Araujo and others 2013), with two critical revisions: first, an observational assessment that distinguished between behavioural responses; and second, a hide box to provide a possible 'retreat' site. The authors hypothesised that the DAP collar would be more effective than placebo in reducing measures of fear and anxiety in response to and following the sound recording (Araujo and others 2013). As fear is a state of alarm or agitation in response to a stimulus while anxiety is a response in anticipation of prospective danger or memory of past danger (Sherman and Mills 2008, Radosta 2011), signs exhibited in response to the thunder sounds are due to fear while signs observed between sounds and after the thunder recording (anticipation of danger) represent anxiety. Thus, fear and anxiety both describe the dog's response in this model.

## Material and methods

### Study population

Twenty-seven healthy beagle dogs of both sexes, more than seven and less than 12 years old, naïve to thunderstorm test and with no history of noise phobia were included. Subjects were housed in the facility for at least three months, and were rehoused by treatment group at least five days before any testing began (see Table 1).

Dogs were housed in groups of four, based on interdog compatibility, in pens measuring 5.2 x 1.5 m. Dogs were provided free access to water, and were fed a standard commercial dry diet once daily. Environmental management included regulation of temperature, ventilation, humidity and lighting. During the study, each dog was provided with a food-dispensing toy, music, raised platforms and a shelter in their home pen. Dogs were provided with daily human interaction on all non-test days in the

form of cleaning, observations and grooming. They were also inspected daily in regard to general health and behaviour by the research technicians.

The study facility is registered by the provincial regulatory authorities, and the study was approved by the local animal care and use committee as per the guidelines of the Canadian Council on Animal Care. All dogs were observed regularly by the facility veterinarian and animal care technicians, and no effects of the testing were seen on general health, behaviour or feeding nor were there any residual effects subsequent to the study.

### Study design

The study was conducted as a blinded parallel-group placebo-controlled design, including three open field tests per treatment group. The first test was a baseline thunderstorm session, which was used for initial subject selection. On the following day, dogs were scored and allocated to one of the two treatment groups as described below. Four days later, the first group was fitted with their collars and then exposed to two additional thunderstorm tests on the following two days (tests 1 and 2). Test 2 was conducted to establish the effect of the treatment on the production of conditioned fear and anxiety, the intensity of the response to thunder when repeatedly tested and the response to two days of treatment. The second group was then tested in an identical manner following cleaning and aeration of the test room (Table 1).

All dogs were individually tested, by group, with no overlapping testing and with the last two tests occurring at approximately the same time of day for each subject. Before each dog entered the open field arena, it was disinfected with a multi-purpose industrial cleaner (Dynamite Big Job Cleaner, CP Industries) and vented by opening the windows for 10 minutes, thus ensuring that adequate air exchanges would occur between subjects. Moreover, the temporal separation of the groups was set up to further ensure no treatment contamination between groups.

The collars (DAP or placebo) were fitted to the dogs' necks according to the manufacturer's instructions. Collars were regularly checked to ensure they were snug enough to the skin to be warmed by the body heat for proper diffusion of the active ingredient. In total, the collars remained on the dogs from approximately 24 hours before the thunderstorm test 1 until after test 2.

All personnel involved in the study were blinded to treatment conditions, with the exception of the person responsible for performing allocation. Analysis of the dogs' behaviours (fear/anxiety scores and time spent in hide, as described below) was conducted by a trained observer and a technician, respectively, both blinded to the treatment provided.

### Treatment allocation

Initially, 27 dogs were recruited to select 24 for the test phase, based on their responsiveness to exposure to the thunderstorm simulation during the baseline session. The 24 subjects showing the greatest global fear score (compiled from both active and passive scores as explained below) were selected for the treatment phase. Dogs were then ranked based on decreasing scores and alternatively allocated into the two groups of 12 animals each: DAP collar and placebo collar. The placebo group had three neutered male dogs and nine female dogs (one entire), with a mean age of 9.2 years (7.4–10.5 years) and mean global score at baseline, during the thunderstorm phase, of 4.2 (3.5–5.2). The pheromone treatment group consisted of four neutered male dogs and eight spayed female dogs with a mean age of 9.3 years (7.5–10.6 years) and mean global fear/anxiety score at baseline, during the thunderstorm phase of 4.2 (3.5–5.0). Once selected, the dogs were rehoused in pens of four by treatment group, with a minimum of five days to adapt to housing changes. All dogs with the same treatment were housed in contiguous pens. Conversely, each treatment group was separated by at least one empty pen to limit cross-diffusion of pheromone to the placebo dogs.

TABLE 1: Schedule of testing procedure

Study day	Placebo group	DAP group
0	Baseline thunderstorm (test 0)	
1	Analysis of baseline thunder test and group assignment	
5	Collar placement	
6	Thunderstorm test 1	
7	Thunderstorm test 2	
8	Full cleaning and eight-hour aeration	
8		Collar placement
9		Thunderstorm test 1
10		Thunderstorm test 2
DAP, dog-appeasing pheromone		

## Testing procedure

The thunderstorm simulation test consisted of an open field testing room measuring 2.74 x 3.66 m, and an audio-recording of thunderstorm sounds (Araujo and others 2013). Dogs were placed individually into the room, and their behaviour observed and objectively scored. The two windows in the room were covered with a solid lightproof window covering.

Fear and anxiety were analysed using an observational assessment scale, developed to distinguish active signs (increased activity) and passive signs (decreased activity), as well as a global score.

In this trial, a box 58.4 cm long x 61 cm wide x 58.4 cm high constructed of high-density polyethylene was provided in the corner of the open field room to allow the dog to enter during the test, as a possible coping strategy. Therefore, a second camera was added, directed towards the entrance of the box to record the dog's behaviour while inside the box for observational analysis (Fig 1). The other camera was fixed to the ceiling of the room, both to capture the objective measures and to record the dog's behaviour for observational analysis over the entire room (Fig 2).

Each testing session lasted nine minutes. The first three minutes provided baseline data. During minutes 3–6, the dogs were subjected to a taped presentation of thunder (the 'during' thunder exposure). No audible stimulus was provided during the final three minutes ('following'), which served as the post-thunder or recovery interval (Fig 3).

The thunderstorm track consisted of recorded segments from the Sounds Scary! Thunder Therapy CD ([www.soundtherapy4pets.co.uk](http://www.soundtherapy4pets.co.uk)), which has been developed as an aid to behaviour therapy for desensitisation and counterconditioning of dogs to thunderstorms (Levine and others 2007, Levine and Mills, 2008). This track was played over a stereo system during the middle phase of the test. The sound produced was on average 83.9 dB, which is similar to a vacuum cleaner, a shouted conversation or heavy city traffic, but less than actual thunderstorms or shotgun firing, which can reach up to 130 dB (National Institute of Health 2013).

## Assessment of efficacy

In this study, the authors looked at two measures of fear and anxiety. The first was an observational scale in which the dogs were ranked from 1 (no fear) to 6 (highest fear) (Gruen and others 2015, Landsberg and others 2015). The second was time spent in the hide box.

Dogs were assessed during each of the three-minute intervals, before, during and after thunder exposure. Fear and anxiety observational scoring was developed by GML, based on observation of canine facial signalling and body posturing, and a review and

compilation of the signs described in the veterinary literature for assessment of dogs with fear of noises (Sheppard and Mills 2003, Dreschel and Granger 2005, Levine and others 2007, Cracknell and Mills 2011). Behavioural signs were evaluated over each three-minute interval as either active when associated with increased activity and reactivity, or passive when associated with decreased activity and autonomic signs. This is consistent with the recently described classification of dog behaviour of 611 dogs with fear of thunder in which principal component analysis identified two behaviour types, extrovert and introvert (Mariti and others 2013). Each dog was also given a global fear/anxiety score for each three-minute interval based on the frequency and intensity of all signs (described below). Box 1 summarises the behaviours used in the assessment of the different presentations of fear and anxiety. The global score was used for subject selection and served as the primary variable for assessing efficacy.

With this study design, the 'pre'-thunder phase from the first open field test assessed the initial fear and anxiety during the first three minutes due to entry into the room and before exposure to the thunder track. The 'during' phase assessed the fear and anxiety related to the sound simulation while the 'following' phase corresponded to the refractory period, after the thunder track stopped. In addition, the potential development of conditioned fear and anxiety and the effect of the treatment on it could be explored through the 'pre' time period, when analysed as a repeated measure, from thunder test to thunder test (Fig 3).

With this fear and anxiety scale, each score consisted of a mark ranging from 1 to 6, combining the assessment of intensity and frequency of the behaviours. For the global score, a score of 1 was given if there was an absence of any signs of fear and anxiety. A score of 6 represented marked signs, most of the time (approximately 80 per cent or more); a score of 4 represented moderate signs, some of the time (approximately 50 per cent) and a score of 2 represented mild signs that were occasional (approximately 20 per cent or less). An active score based on running, scanning, startling, digging and jumping was given based on the intensity and frequency of these signs during the time they were observed over the three-minute interval and a passive score based on freezing, cowering, lip licking and trembling was assigned based on the intensity and frequency of these signs during the time they were observed over the three-minute interval (Box 1). For active or passive fear and anxiety, a score of 6 represented marked and multiple signs and high duration (most of the time and high intensity) while a score of 2 represented mild signs seen infrequently over the three-minute session. Scores were to be given to the nearest 0.5 on a linear scale. With a 6-point scale (1–6), even an improvement of 0.5



FIG 1: Camera view of dog in hide

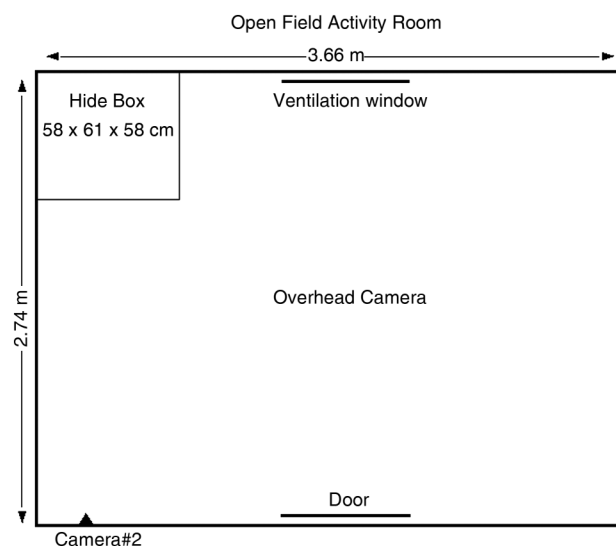


FIG 2: Open field activity room





TABLE 3: Evolution of pre-stimulus measures ('before'), according to the repetition of the tests

Test	N	Variable	Mean	Sd	Median	Minimum	Maximum
Group: placebo							
Test 0	12	Passive score before	1.67	0.49	1.75	1.00	2.50
		Active score before	2.04	1.10	1.75	1.00	5.00
		Global score before	1.88	0.69	2.00	1.00	3.50
Test 1	12	Passive score before	2.79	0.72	3.00	2.00	4.00
		Active score before	2.08	0.95	1.75	1.00	4.00
		Global score before	2.38	0.80	2.25	1.50	4.00
Test 2	12	Passive score before	2.58	0.87	2.50	1.50	5.00
		Active score before	2.33	1.27	2.25	1.00	5.00
		Global score before	2.46	0.66	2.25	1.50	3.50
Group: DAP							
Test 0	12	Passive score before	1.54	0.66	1.25	1.00	3.00
		Active score before	1.50	0.64	1.25	1.00	3.00
		Global score before	1.56	0.48	1.50	1.00	2.50
Test 1	12	Passive score before	2.58	1.02	2.25	1.00	4.00
		Active score before	3.04	1.48	3.00	1.00	6.00
		Global score before	2.79	0.84	3.00	1.50	4.00
Test 2	12	Passive score before	3.04	0.89	3.00	1.50	4.00
		Active score before	2.38	1.51	1.75	1.00	5.00
		Global before	2.71	0.84	2.50	2.00	4.50

DAP, dog-appeasing pheromone

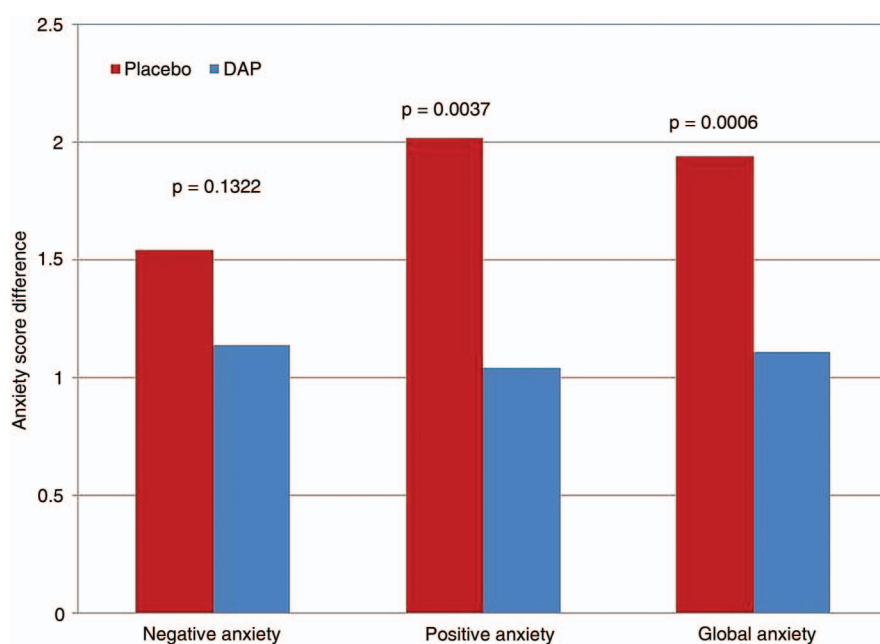
with 'following' thunder ( $P < 0.0001$ ), and both were consistently higher than 'before' thunder, confirming the model's validity.

On both test 1 and test 2 combined, a significant difference was observed between the two groups on the active and global scores. Relative to the before thunder exposure scores, active and global fear and anxiety during ( $P = 0.0037$  and  $P = 0.0006$ , respectively) and following ( $P = 0.0015$  and  $P = 0.0010$ , respectively) the thunderstorm exposure were attenuated significantly in the DAP group compared with placebo (Figs 4 and 5). Specifically, for active and global scores least squares means (LS-means) increases from before to during thunderstorm exposure were 1.94 and 1.75 times larger, respectively, in placebo-treated dogs compared with dogs exposed to treatment. Moreover, the placebo dogs continued to demonstrate increased fear/anxiety following the thunder compared with before, whereas in the DAP group, the fear/anxiety level following thunder was lower than before thunder exposure on average (Fig 5).

Although significant group differences were not found on passive score, the DAP-treated animals demonstrated lower mean increases from before thunder both during and after thunderstorm exposure compared with the placebo group (Figs 4 and 5), when combined across tests 1 and 2. Notably, the relative increases in fear and anxiety from before to during and after thunder in the DAP group compared with placebo were marginally lower ( $P = 0.0606$  and  $P = 0.1119$ , respectively) at test 2, which suggests that the anxiolytic effect of DAP on passive fear/anxiety measures is less robust than on active and global fear/anxiety measures.

The hide box analysis revealed a statistically significant treatment difference regardless of time (i.e. before, during or following thunder). DAP-treated dogs used the hide box more frequently than placebo-treated dogs (Table 4).

At baseline, no difference in the number of dogs using the hide box was found; however, there was a statistically significant



\* Anxiety "before" corresponds to the value before each thunder

Note: LS-MEANS and p-values are issued from a generalized mixed model for repeated measurements, including treatment effect and test effect as fixed factors and the corresponding baseline measurement

FIG 4: Change in anxiety scores during to before. DAP, dog-appeasing pheromone; LSMEANS, least squares means

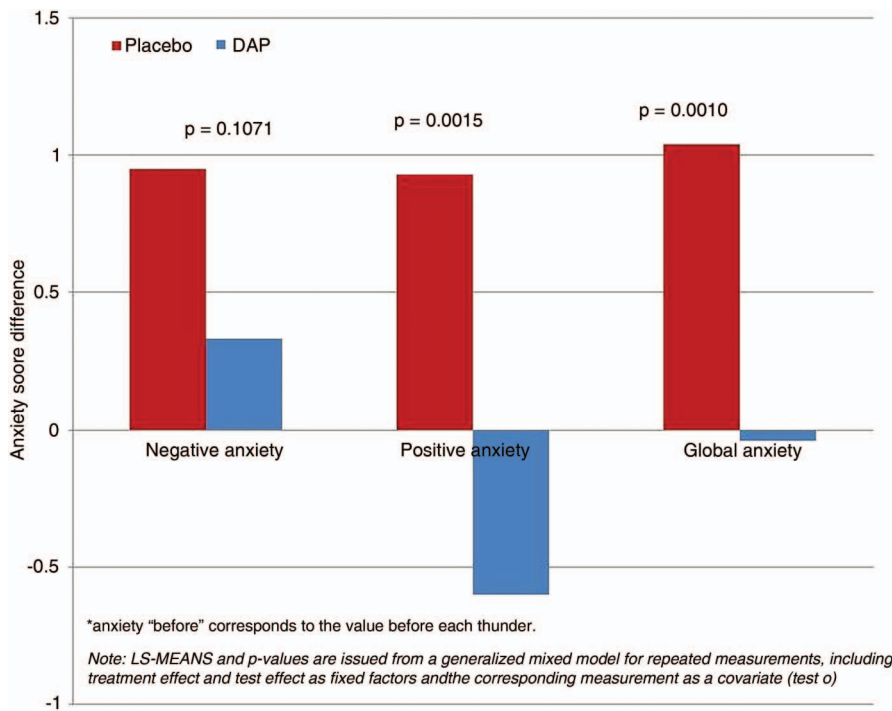


FIG 5: Changes in anxiety score following to before. DAP, dog-appeasing pheromone; LSMEANS, least squares means

difference at test 2 (Chi squared, P=0.0013), with three times more dogs in the DAP group using the hide cage during the test compared with placebo (Fig 6).

**Discussion**

The purpose of this trial was to provide standardised conditions for the evaluation of treatment with a DAP collar compared with a placebo collar in a controlled laboratory model of noise-induced fear and anxiety.

In clinical settings, dogs with thunderstorm aversion display considerable variability in their fear and anxiety to stimuli associated with thunder, including wind, barometric pressure changes, darkening skies and lightening. Moreover, owner responses to the pet's behaviour and the home environment play a role in the development and progress of the problem, as well as in introducing confounding variables when evaluating treatments. For these reasons, a placebo-controlled trial assessing storm aversion poses significant challenges in standardising groups at baseline, controlling for owner responses and in the variability in frequency, intensity and quality of each storm event. Therefore, standardised laboratory-based trials limit the confounds related to housing, intensity and duration of stimulus exposure, scoring measures, methodologies and pet owner influences in evaluating therapeutic effects.

In an initial laboratory study using the thunderstorm model, dogs were assessed in an open field for two minutes before exposure to thunder, three minutes during exposure and five minutes after a thunderstorm recording (Araujo and others

2013). In that study, when compared with behaviour during a control open field test, inactivity duration and frequency increased both during and after exposure to the thunderstorm. However, these observations are true in only a subset of dogs; other dogs showed a hyperactive response when exposed to thunder recordings. In fact, during the course of the thunder exposure, dogs may shift between the two strategies. These behaviours are consistent with avoidance and freezing signs displayed by pet dogs when exposed to fear-evoking sounds (Cottam and Dodman 2009, Cracknell and Mills 2011, Blackwell and others 2013). Variability in response may reflect individual differences related to personality, previous experience and age, with young dogs, especially those that are naïve to the thunder model, being more likely to respond with increased activity than older dogs. Therefore, the current study used observational scoring of active and passive signs of fear and anxiety, as well as a global fear and anxiety score rather than the previous measures.

Previous studies demonstrate an improvement using a DAP diffuser in dogs with fear of fireworks in combination with desensitisation and counterconditioning using a CD recording (Mills and others 2003, Levine and others 2007, Levine and Mills 2008). In these trials, there was no placebo group for comparative purposes. In the present study, the pheromone therapy was compared with placebo using a standardised methodology in the

TABLE 4: Comparative repeated measures analysis of the use of the hide cage

	DAP vs placebo OR (95% CI)*	p Value
Before	32.4 (4.4 to 239)	0.0016
During	16.6 (1.9 to 144.9)	0.0135
Following	10.0 (1.5 to 65.7)	0.0187

\*ORs from a generalised mixed model for repeated measurements, including treatment effect and test effect as fixed factors and the corresponding baseline measurement as a covariate (test 0).  
 DAP, dog-appeasing pheromone

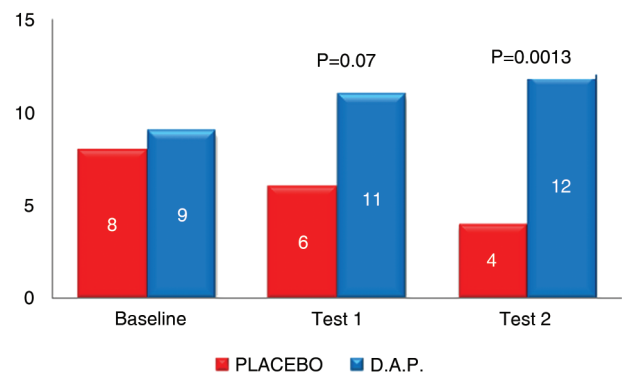


FIG 6: Number of dogs using hide cage. DAP, dog-appeasing pheromone

absence of concurrent behaviour modification, to establish the efficacy of the DAP collar alone.

The results of this study demonstrate a significant reduction in both global and active fear and anxiety in dogs wearing a DAP collar both during and following exposure to a thunder recording. This improvement was demonstrated on both the first (test 1) and second (test 2) thunderstorm test days. Passive fear/anxiety showed a trend towards a reduction at test 1 and a non-significant decrease at test 2. In addition, there was a significantly greater use of the hide box in the DAP group compared with the placebo group with repeated thunderstorm exposure. Collectively, these behavioural findings suggest an adaptive 'settling' response to the fear-evoking thunderstorm stimulus in DAP-treated dogs, although further studies are warranted to confirm this. The absence of a significant reduction in the passive score suggests that the primary effect of the pheromone therapy is calming of the active signs of fear and anxiety. However, it is possible that the current recording system is less able to detect the subtle signs associated with fear and anxiety such as tenseness and salivation that comprise the passive score. Therefore, this study supports the use of DAP as an adjunct to a behaviour management programme for dogs with fear of noises. The findings suggest a potential benefit for the use of DAP in the home environment in conjunction with a hide or den for dogs that have demonstrated a fear response to noise on initial exposure.

## Conclusion

The DAP collar reduces global and active fear and anxiety to a thunder recording and increases hide use, possibly by counteracting noise-related increased reactivity. These findings support a possible use for DAP in the prevention and management of noise-related fear and anxiety.

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