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*Article*

**Rabbit pheromone acting as an interomone, dog appeasing pheromone, and Sergeant’s pheromone change heart rate and behavior of anxious dogs**

**W. Garrett Thompson1 and John J. McGlone 1\***

1. Laboratory of Animal Behavior, Physiology and Welfare, Texas Tech University, Lubbock TX;

E-Mail: garrett.thompson@ttu.edu

2 E-Mail: john.mcglone@ttu.edu

**\*** John J McGlone; john.mcglone@ttu.edu;
Tel.: 806-742-2805, ext 246; Fax: 806-742-4003

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**Simple Summary:** Two studiesexamined the effects of ***trans*-**2-methy-2-butenal (RP, a rabbit maternal-neonatal pheromone) acting as an Interomone in dogs. The studies were summarized together using two different groups of anxious dogs. In conclusion, RP acts as an interomone in adult dogs. RP has shown that it changes heart rate (HR) and behavior of more dogs compared to other treatments. In most cases dogs become less active and HR is decreased when treated with RP.

**Abstract:** All pheromones that were tested in this study had at least one effect on HR and behavior. The objective of this study was to assess efficacy of pheromones/interomones to modulate HR and behavior in adult anxious dogs. The dogs (n=8 were estimated 5 to 8 years of age) were obtained from a local research facility. Each dog received each treatment in a Latin square design with repeated measure over time. This model allowed evaluation of effects of treatment, dog, treatment by dog, time, and treatment by time. Treatments were given in collar forms as supplied from their manufactures.

 The study was analyzed in two different phases: phase 1 that consisted of the first 24 hours after the collar was placed around the dog’s neck. During phase 2 dogs were startled then HR and behavior were recorded for 2h after. The treatment by dog interactions were significant for HR, standing, sitting, lying, active, active2 behaviors during both phase of the study. Dogs HR was reduced with the DAP and RP treatments (62.5%) during the phase 1. The significant treatment by dog interactions for the behaviors indicates that dogs react differently when treated with different pheromone collars.

**Keywords:** dog; pheromone; heart rate; behavior

1. **Introduction**

Fear and anxiety-related behaviors are becoming more common in today’s domestic dog and these behaviors can lead to physiological stress and health problems. A physiological stress response occurs following exposure to a fear or anxiety provoking stimulus [1]. This type of stress can have both short- and long-term effects on health of the individual [2]. It was shown that rats with neophobia characterized by a larger HPA response than their non-fearful littermates die sooner compared to their cohorts [3]. Dreschel [1] found dogs that were “well-behaved” lived longer than dogs that were considered stressed and had high levels of anxiety.

There are many daily activities such as leaving for work, changes in weather, and routine veterinary examinations that can be considered stressful for dogs. Dogs experiencing stress are more inclined to change their “normal behavior” to behaviors that are unwanted by pet owners. Vacalopoulos and Anderson [4] estimated that up to 90% of dogs may exhibit behaviors that their owners find unacceptable. Some of these behaviors include: separation anxiety, fearfulness, excessive vocalization, destructiveness, and hyperactivity [4,5]. Wells [6] found that over 30% of dogs are relinquished by their owners to rescue shelters are abandoned and not directly handed over to an animal care service.

Pheromones are semiochemicals that provide communication between animals. Pheromones were originally defined as “substances secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction” [7]. Pheromones change the behavior and/or physiology of the animal that perceives the chemical signal. Some pheromones have unexpected effects. For example, the reproductive pheromone androstenone reduced prepubertal swine agonistic behaviors [8]. Some chemical signals operate across species and can either benefit or harm either the sender or the receiver. Allomones are chemicals produced that benefit one species (ex., plants) compared with another species (ex., certain insects;) [9]. A kairomone is a chemical produced and released by one species, benefiting another species, and often harming the emitter [10]. For example, a cat kairomone is detected by a rodent and used to avoid the cat (usually benefiting the rodent). The term interomone is defined as a chemical that operates in a given species but will have a very different effect, often unpredictable, on receiver of another species [11]. An interomone does not have to benefit or negatively impact the sending or receiving species. Because some pheromones such as DAP operate as related molecules across species (ex., the dog, cat and pig DAPs are similar structurally), the interomone concept holds that a pheromone in one species may have a related or different effects in another species.

The need for alternative therapies is important because of the large number of animals taken to shelters because, in part, pet owners are unable to control unwanted behavior. Salman et al. [12] found that that between 1995 and 1996 1,984 dogs were relinquished to animal shelters for behaviors such as biting, aggressiveness, escaping, destructive behaviors, and disobedience. Training tools such as electric collars have been shown to be stressful in dogs when they are not able to associate their action with the electric shock [13].

1. **Materials and Methods**

*2.1 General*

The study was performed in a laboratory setting at Texas Tech University. The institution is AAALAC International accredited and the work was approved by the Institutional Animal Care and Use Committee prior to its conduct. Dogs were obtained from a research facility where they had been used as test subjects primarily in flea and tick research. In that facility, the animals were kept in concrete-floored kennels with chain link fencing. Anxious dogs were selected from that pool animals and then transported to the Texas Tech University facility, bathed, given a physical exam by a veterinarian and rested for more than a week before testing. Dogs were initially selected because the investigators considered the subjects anxious. Each dog was fearful of people and/or anxious. All dogs displayed hyper-vigilance, avoided novel people and showed minimal interest in socializing with any but a few very well-known individuals (their primary caretakers). They startled easily when presented with novel stimuli and demonstrated avoidance behaviors, including shaking and cowering with tails tucked and ears lowered. Eight anxious dogs were mixed breeds and estimated to be 5 to 8 yr of age. Dogs were housed in separate rooms. Each room had 100% fresh air intake and exhaust. Dogs were fed twice (am and pm) per day and water was available ad libitum. Rooms provided a minimum of 12 m² of floor space (well in excess of the space required by USDA). The rooms were cleaned while the dogs were exercised daily.

The work was performed in phases or studies. Baseline dog behavior and HR were first quantified. In phase 1, dogs with different collars containing control or pheromone treatments were evaluated for potential behavioral and HR effects. In phase 2, each dog was startled and the resulting behavior and HR were quantified.

2.2 Treatments

Placebo, Rabbit pheromone and Sergeant’s pheromone collars were manufactured by Sergeant’s (Ohmaha, NE). The dog appeasing pheromone (DAP) collars were purchased commercially. The placebo collar was the same material (Co-Polymer) as the other collars, but with no active ingredient. The Sergeant’s (formula H or FH) collar contained the 6 % of Sergeant’s pheromone that is currently marketed (its contents are proprietary; but described in their patent (Sergeatn, 2011). The Rabbit Pheromone (RP) collar contained 0.02 % 2-methylbut-2-enal embedded in the plastic. The Dog Appeasing Pheromone (DAP) collar contained 5% of the DAP formula (DAP—Ceva Santé Animale).

Heart rate was measured using two different systems. A telemetry system (Data Science International, St. Paul, MN USA) was used on dogs A-D. Leads were placed on the dogs’ shaved skin while sensors measured heart rate. A specially-fit jacket held the transmitter. A computer collected HR data continuously by telemetry with data points recorded each 5 seconds. HR data were averaged each hour. The lab technician was blind to treatment days and scanned and eliminated any misleading entry points (as happens when the leads become loose). The second system was the Polar Heart Rate System (Polar Heart Rate RS800CX, Warminster, PA USA) was used on dogs E-H. Heart rate was taken every 5 seconds once all the heart rate belts were placed on the animals.

The switch between systems was made because the studies were conducted at different times of the year. By switching to the Polar HR system there were less instances of lead failure (leads becoming loose or chewed) that was occurring with the DSI telemetry system. The Polar HR system was better able to stay in contact with the shaved hair. Also the amount of time needed to reapply the HR gel could be done quickly and more regularly.

Video cameras were mounted overhead in the rooms. The dogs’ behaviors were captured on media at a sampling rate of 30 frames/second. Data were played back while observers, blind to treatments, recorded behaviors. Behaviors that were recorded are defined in Table 1. A scan sampling method ([Altman, 1974] change to numbers) was used to record dogs’ behaviors every 5 minutes. Data were summarized by each hour. Behaviors were mutually exclusive in the ethogram (Table 1). Observers were trained to evaluate the dogs overall behavior at the 5 minute scan sample data point.

**Table 1. Definitions of dog behaviors used in this study.**

|  |  |
| --- | --- |
| Behavior | Definition |
| Pace/walk | Locomotion in a repeated pattern or in any non-repeating pattern |
| Stand | Supported by limbs, not moving/walking/pacing |
| Sit | Posterior on ground while front feet support the animal |
| Digging | The act of scratching the bottom of the kennel with the two front paws |
| Lick self | Tongue touching any self-body part |
| Self-Grooming | Cleaning of the body parts by the individual (Tongue to body or limbs) |
| Jumping | Front or all four feet off the ground |
| Lying | Dog’s body not supported by any limb.  |
| Active or activity | All behaviors other than lying down |
| Active2 or activity2 | All behaviors other than lying down and sitting |

2.3 Experimental Design and Statistics

This study was a replicated Latin Square design. Four dogs represented one square and the four by four Lain Square was replicated. Within the Latin Square design measures were repeated over time (24 h per day). This model allowed evaluation of effects of treatment, dog, dog by treatment, time, treatment by time and dog by time. The treatment and dog effects were tested using the dog by treatment effects. The remaining independent variables were tested using the residual error term. Data are presented using Least Squares means generated from the General Linear Models procedure within the SAS software (Statistical Analysis Systems, 2009). Standard errors were produced by SAS using the appropriate error term. Percentage data were transformed (because percentage data are not normally distributed) by square root arcsin transformation before analysis to approximate normality. Least Squares means are presented as percentage.

The study was conducted in three phases: Baseline, Pheromone/Placebo application, and Startle Test. Baseline can be described as the period of time when no collar was applied. After the baseline period, treatments were administered in random order. Pheromone/Placebo application is the period of 24 h when the dogs received each treatment via a collar. A lab technician would enter the rooms in the same order and place the collar on the dog. HR data was then analyzed from the point each collar was placed on each individual dog. Lastly, startle refers to the 2-hour time period after the 24 h of collar application when dogs were startled with an air horn. The air horn produced a noise at 110 db (measured by a sound level meter from Radio Shack) and was placed by a technician approximately 17-35 cm away from the dogs head. Each dog received a blast from the startle horn in the same order that the collars were placed on the dogs. The startle period was measured 2 h after the startle, then all collars where removed. Behavior and HR data were measured for all the phases previously mentioned. Dogs were allowed at least a 24 h recovery period in which no pheromone or data measurements took place between treatments. Heart rate data were collected each 5 s for 24 h.

Additional data was collected on HR and behavior at times when the dogs would come into contact with the technician collecting HR data (to fix a malfunction or check the contact of leads on the skin) and the interaction with facility cleaning members. HR data was taken out for these periods of time and behavior data was omitted. Cleaning personnel would take the dogs (out of view) outside the rooms while the rooms were cleaned daily. This amount of time was approximately around 5 minutes.

In addition to the General Linear Models analysis as described above, regression equations were calculated to describe HR over time after startle. We wished to document if HR increased, did not change, or decreased over time after startle with use of pheromones/interomones by calculation of the regression equation and R2 for each treatment group. These regression lines provided are descriptive. Due to the variable nature of HR and behaviors, Chi-square analyses were used to assess the direction of the response of each treatment relative to the placebo collar effect.

**3. Results and Discussion**

*3.1 Phase 1 – Collars over 24 h*

Dogs responded differentially to the pheromone collars. The dog by treatment effect was significant for this study as it was in past work (Table 2) for HR and most behaviors. This means that some dogs responded to a given pheromone while other dogs responded to other pheromones.

The DAP and RP collars lowered anxious dogs’ heart rates of 62.5% of these anxious dogs. FH decreased HR among only 37.5% of these anxious dogs. Chi-square analysis showed that DAP and RP decreased HR and FH increased HR of anxious dogs relative to dogs with the placebo collars (Chi-square value = 12.5, P < 0.001).

The pattern of how pheromones changed HR differed among treatments. Data in Figure 1 are sorted from least to highest placebo HR. Table 3 presents each dog’s change in HR relative to its HR under the placebo collar in a non-parametric ranking of the data. The data were divided into low, medium and high HR dogs. The middle category had insufficient data and variation to analyze. The Chi-square values showed that low-HR dogs increased HR more than dogs with DAP or FH collars. Also, high-HR dogs had a significant Chi-square value with RP causing a decrease in dog HR and the DAP and FH having a more variable effect. The RP collar caused a distinctive distribution. RP tended to raise HR of dogs with low HR and lower HR of dogs with a high basal HR. This could be a normalizing effect. Future research ought to clarify this physiological observation of RP on anxious dogs.

**Table 2. Basal HR and behavior P-values. Note significant dog\*treatment effects.**

|  |
| --- |
| 24 h Treatment P-values and Behavior Means |
| Model effect | HR | Pace | Stand | Sit | Lying | Active | Active2 |
| Treatment | 0.75 | 0.95 | 0.95 | 0.99 | 0.68 | 0.68 | 0.58 |
| Dog | 0.67 | 0.0001 | 0.001 | 0.0001 | 0.0008 | 0.0008 | 0.0001 |
| Treatment\*dog | 0.0001 | 0.45 | 0.01 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Time | 0.03 | 0.02 | 0.0001 | 0.0001 | 0.41 | 0.41 | 0.63 |
| Time\*Treatment | 0.06 | 0.39 | 0.89 | 0.69 | 0.67 | 0.67 | 0.79 |

**Figure 1: Basal Heart Rate Dog by Treatment Interaction.**

**\*Indicates that treatment Least Squares means differ (P<0.05) compared to placebo.**

**Table 3. Dog numbers ranked from lowest (dog C) to highest (dog H) HR. Note the pattern that RP increased dogs with low HR and decreased dogs with high HR – a normalizing effect. DAP and FH had effects that were variable in relation to placebo level of dog HR. Within the table, an “I” indicates an increase, a “–“ indicates nearly identical HR as control and a “d” indicates a decrease in HR relative to the placebo HR of each dog.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dog | HR, bpm | RP | DAP | FH | χ2 | P-value |
| C | 89.1 | i | d | i | 101.01 | < 0.0001 |
| A | 98.7 | i | d | d |
| F | 99.9 | i | i | i |
| E | 110.3 | - | - | i |  |  |
| G | 135.2 | d | i | - |  |  |
| B | 142.1 | d | d | i | 39.76 | < 0.01 |
| D | 117.5 | d | i | d |
| H | 209.2 | d | d | d |

*3.1.2 Phase 1: 24h Behavior*

Behavior was changed in some dogs by some pheromone collars (note significant dog by treatment interactions, Table 2). Dog behavior responded differently when treated with different pheromones. Percent time spent being active (P < 0.0001), standing (P < 0.01), sitting (P < 0.0001), and lying (P < 0.0001) had significant dog by treatment interactions (Table 2).

**Table 4: Phase 1 (before startle) behavior treatment Least Squares Means.**

|  |
| --- |
| 24h Treatment Means |
|   | Means |
| Treatments | HR | Pacing | Standing | Sitting | Lying | Active | Active2 |
| Placebo | 133.4 | 2.84 | 4.34 | 2.86 | 65.4 | 34.59 | 25.95 |
| DAP | 115.4 | 3.24 | 3.83 | 3.24 | 74.4 | 25.59 | 17.07 |
| Formula H | 128.7 | 3.08 | 4.21 | 2.86 | 70.1 | 29.81 | 21.09 |
| Rabbit Pheromone | 133.5 | 3.16 | 4.42 | 3.12 | 68.2 | 31.77 | 22.35 |
| SE | 14.86 | 0.50 | 0.79 | 2.42 | 5.36 | 5.36 | 4.52 |

All pheromone collars changed the level of activity in at least one dog (Figure 2). DAP pheromone collars changed the totally activity of two dogs. Dogs E and G became less active when treated with the DAP collar compared to placebo. Dog F tended to increase activity with a DAP collar compared with a placebo collar.

Dogs A and G became significantly less active when treated with the FH collar compared to placebo (Figure 2). Dog B became more active when treated with the FH collar compared with the placebo collar. Dogs B and E became more active when treated with the RP collar, while dog G became less active when treated with RP compared to placebo.

Dog C increased standing behaviors when it wore a FH or RP collar compared with the same dog wearing a placebo collar (Figure 3). Dog E tended to stand more with DAP or RP collars compared with placebo collar.

Sitting is a behavior that represents being alert or awake but not active. In Figure 4, one can see that dog A reduced its sitting behavior with DAP, FH or RP collars. Dog B had the reciprocal effect in that it increased its sitting behavior when it wore DAP, FH or RP collars compared with when the same dog wore a placebo collar. Dog G’s behavior changed with all pheromone collars – DAP tending to increase sitting and FH tended to decrease sitting while RP significantly decreased its sitting behavior.

While the behavioral effects are difficult to interpret, it is clear that all collars caused some change in the behavior of anxious dogs. This sort of quantification of dog behavior in a controlled setting is one of the first documented effects of pheromone collars on the behavior of anxious dogs.

**Figure 2: Basal Period Percent Active Dog by Treatment Interaction**

**\* Indicates that treatment Least Squares means differ (P<0.05) compared to placebo. # Indicates a trend (P < 0.10) in treatment Least Square means compared to placebo.**

**Figure 3: Basal Period Percent Standing Dog by Treatment Interaction**

\*

 **\*Indicates that treatment Least Squares means differ (P<0.05) compared to placebo. #Indicates a trend in treatment Least Squares means (P < 0.01 to P > 0.05) compared to placebo.**

**Figure 4: Basal Period Percent Sitting Dog by Treatment Interaction**

**\*Indicates that treatment Least Squares means differ (P<0.05) compared to placebo. #Indicates a trend in treatment Least Square means differ (P<0.01-P.>0.05) compared to placebo.**

*3.2.1 Phase 2 – HR After Startle*

Pheromone collars did not cause a significant treatment effects (Table 5) on post-startle dog HR mean values (Table 6). However, the dog by treatment effect was highly significant which indicated that some dogs responded differently than other dogs. Shown in Figure 5 are the individual HR values after startle for each treatment and each dog.

The HR data after startle were summarized in a non-parametric ranking in Table 7. Note that the same general pattern was observed after startle as was observed during the 24 h behavior collection period. RP tended to increase dog’s HR after startle that had low HR, but it decreased HR among dogs with a high base HR. Both FH and DAP had a more variable response for dogs of varying basal HR (Table 7). Again, conducting a non-parametric analysis, the general pheromone responses of anxious dogs after startle was similar to their responses before startle. While RP caused an increase in HR after startle and a decrease in HR after startle for dogs with low and high HRs respectively, the DAP and FH collars did not cause any discernable pattern in HR after startle.

**Table 5. Phase 2 -- Startle HR and behavior P-values. Note significant dog\*treatment effects. Dogs did not pace during the startle period.**

|  |
| --- |
| Startle Period Treatment P-values |
| Model effect | HR | Stand | Sit | Walk | Lying | Active | Active2 |
| Treatment | 0.97 | 0.41 | 0.97 | 0.47 | 0.64 | 0.64 | 0.37 |
| Dog | 0.53 | 0.18 | 0.01 | 0.16 | 0.13 | 0.13 | 0.80 |
| Treatment\*dog | 0.0001 | 0.002 | 0.0001 | 0.34 | 0.002 | 0.002 | 0.21 |
| Time | 0.74 | 0.22 | 0.31 | 0.69 | 0.04 | 0.04 | 0.14 |
| Time\*Treatment | 0.66 | 0.43 | 0.73 | 0.91 | 0.02 | 0.02 | 0.01 |

**Table 6. Startle Behavior Treatment Means.**

|  |
| --- |
| Startle Treatment Means |
|   | Means |
| Treatments | HR | Pacing | Standing | Sitting | Lying | Active | Active2 |
| Placebo | 139.0 | 2.7 | 3.4 | 15.3 | 72.2 | 27.8 | 23.96 |
| DAP | 121.3 | 6.3 | 6.3 | 12.5 | 63.8 | 36.2 | 15.27 |
| Formula H | 126.1 | 5.9 | 10.9 | 11.9 | 60.7 | 39.3 | 24.22 |
| Rabbit Pheromone | 124.3 | 3.7 | 6.2 | 13.0 | 71.8 | 28.2 | 15.17 |
| SE | 13.4 | 1.08 | 2.07 | 1.89 | 8.13 | 8.13 | 7.16 |

**Figure 5: Startle Heart Rate Dog by Treatment Interaction.**

**Table 7. Dog numbers ranked from lowest (dog #C) to highest (dog #H) HR. Note the pattern that RP increased dogs with low HR and decreased dogs with high HR – a normalizing effect. DAP and FH had effects that were variable in relation to placebo level of dog HR. Within the table, an “i” indicates an increase, a “–“ indicates nearly identical HR as control and a “d” indicates a decrease in HR relative to the placebo HR of each dog.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dog | HR, bpm | RP | DAP | FH | χ2 | P-value |
| C | 88.6 | i | d | - | 117.1 | P < 0.0001 |
| A | 130.4 | i | d | d |
| F | 106.4 | i | i | i |
| E | 1051 | i | i | i |
| G | 134.0 | d | i  | - | 62.5 | P < 0.001 |
| B | 156.1 | d | d | i |
| D | 181.2 | d | i | d |
| H | 209.3 | d | d | d |

*3.2.2 Phase 2 – Behavior after startle*

The overall treatment effects were not significant for post-startle behaviors (Table 6). However, several behaviors were changed in some dogs as indicated by a significant dog by treatment interaction. And some behaviors changed over time after startle.

Four dogs (A, B, C and E) showed changes in overall activity with certain collars (Figure 6). RP significantly decreased activity of three dogs (A, B and E). DAP reduced activity of one dog (dog number A). FH decreased activity of dog A but increased activity of dogs B and C (Figure 6). Most of the changes in activity after startle were dogs standing (Figure 7) and sitting (Figure 8) and some dogs laid down after startle.

A key questions is did the dogs have decreased activity after startle, under the hypothesis that more inactivity would represent a more calm outcome after startle. Using a simple non-parametric examination of the data, DAP reduced post-startle activity in 75% of the dogs; FH reduced activity in 55.6% of dogs and the RP decreased activity in 87.5% of these anxious dogs (χ2 = 11.3, P < 0.01).

Presented in Figures 7 and 8 are the data representing the dog by treatment effects for standing (P < 0.0001) and sitting (P < 0.0001). At the initial startle point dogs treated with FH were more active (lower lying down) compared to the same dogs with the placebo collar. Some dogs decreased standing and lying down, but the effects were quite variable among dogs. Dogs more often laid down 1 to 2 hours after startle. Shown in Figure 9 are the data for each treatment over time after startle. Note that by 2 hours after startle, all treatment collars had increased lying down compared with dogs with the placebo collar.

**Figure 6: Startle Period Percent Active Dog by Treatment Interaction**

**Figure 7: Startle Period Percent Standing Dog by Treatment Interaction**

**\* Indicates that treatment Least Squares means differ (P<0.05) compared to placebo.
# Indicates a trend in treatment Least Square means differ (P<0.01-P.>0.05) compared to placebo.**

**Figure 8: Post-startle sitting behavior showing lease squares means for the dog by treatment interaction. An \* indicates that mean differed from placebo.**

**\*Indicates that treatment Least Squares means differ (P<0.05) compared to placebo. #Indicates a trend in treatment Least Square means differ (P<0.01-P.>0.05) compared to placebo.**

**Figure 9: Post-startle lying behavior showing lease squares means for the treatment by time interaction. An \* indicates that mean differed from placebo.**

**\* Indicates that treatment Least Squares means differ (P<0.05) compared to placebo.
# Indicates a trend in treatment Least Square means differ (P<0.01-P.>0.05) compared to placebo.**

1. **Conclusion**

This experimental is valuable and sensitive for three primary reasons. First, the study was performed in a controlled laboratory setting. Second, test subjects were uniformly anxious. Third, the replicated Latin Square design with data collected over time provides a powerful statistical method to reduce variation among subjects. Because behavior and HR are quite variable for anxious dogs, these controls make the studies less variable and more sensitive.

Collection of highly-controlled data on dog behavior in a laboratory setting should complement field studies and individual experiences with animals. Pheromone therapy is viewed by some as a therapy that is difficult to develop clinical data. What happens in the laboratory may reflect what happens in dogs kept in homes. However, our recurring observation that there is a significant dog by treatment interaction indicates that all dogs are not affected in the same manner by pheromone therapy. Some dogs will clearly respond to pheromones differently.

The DAP has been used on dogs for some time in homes and a commercial setting using a variety of models. If dogs are selected from families, one can expect the subjects to be variable. For instance Pageat and Gaultier [14] suggest that DAP may have calming effects on dogs that have separation anxiety from their owners. While other studies look at stress induced from transportation [15]. These studies were looking at different types of beahvior issues and therefore the subjects enrolled in these studies were subjective based on the owner as well as unblinded researchers.

The rabbit pheromone is clearly a maternal-neonatal pheromone produced in the mother’s milk. The DAP is reported to be produced in the mammary skin [16]. The composition of DAP is reported to vary slightly among species of mammals (ref patent). It is possible that the dog appeasing pheromone and cat appeasing pheromone are structurally similar enough to work across species. The behavioral effect of the rabbit pheromone is well described in the literature [17,18,19]. Variation in 2-methylbut-2-enal has not been reported. Still, we were operating under the hypothesis that the RP may have effects in other mammalian species. Because there is not a term for substances that are a pheromone in one species but that change the behavior of other species in non-evolutionary ways, the term Interomone was applied. The RP, acting as an Interomone, had more powerful effects on the behavior and physiology than did DAP.

We know that for rabbit pups, RP has different behavioral results at different concentrations [18]. Still, even very low concentrations of RP have effects on dog behavior and physiology. We observed a stronger behavioral response in the adult anxious dog with RP compared to DAP.

Due to the recent questions the efficacy of pheromones and their use in dogs [20], we used a highly-controlled experimental model. We demonstrated here that pheromones/interomones can change the behavior of anxious dogs. By using this scientific model, we can conclude that Rabbit Pheromone collars had the highest rate of success at either changing the heart rate or the behavior of anxious dogs but the SERG and DAP collars also changed the physiology or behavior of some dogs. Futhermore, this is the first study that shows how pheromone collars can change a dogs’ heart rate and behavior before and after startle.

We confirmed here that significant variation in response among dogs is found in pheromone/interomone efficacy in a controlled setting. In our highly-controlled setting, our data support the hypothesis that the pheromones/interomones tested will change behavior and heart rates of some anxious dogs.

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**Conflict of Interest**

The corresponding author declares a perceived conflict of interest in that he was (but is not now) a consultant to the research sponsor during the conduct of this work. He does receive royalties from sale of pheromones/interomones.

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