Autonomic, endocrine and behavioural responses to thunder in laboratory and companion dogs

Carla Caroline Franzini de Souza, Carolina Elisabetta Martins Maccariello, Daniel Penteado Martins Dias, Norma Aparecida dos Santos Almeida, Magda Alves de Medeiros

HIGHLIGHTS

• Thunder stimulus (103–104 dB) causes physiological and behavioural changes in dogs.
• Sound stimulus induces autonomic imbalance with sympathetic predominance in dogs.
• Companion dogs had higher cortisol levels than Beagles.
• Only companion dogs had significant increase in the cortisol.
• Laboratory dogs had more pronounced behavioural response than companion dogs.

ABSTRACT

Dogs are highly sensitive to sound stimuli, especially fireworks, firearms, and thunder, and therefore these sounds are used as models of stress reactivity in dogs. Companion and laboratory dogs may respond differently to stressful stimuli, due to differences in management and their relationship with humans. Therefore, the reactivity of beagle dogs (laboratory) and companion dogs to an acute acoustic stress model was studied by analysing the heart rate variability (HRV; cardiac interval values), serum cortisol levels and various behavioural parameters. Eight beagles and six privately owned dogs with no history of phobia to thunder were used. The sound stimulus consisted of a standardized recording of thunder for 2.5 min with a maximum intensity of 103–104 dB. To evaluate the HRV, cardiac intervals were recorded using a frequency meter (Polar RS800CX model), and later the data were analysed using CardioSeries 2.4.1 software. In both laboratory and companion dogs, thunder promoted an increase in the power of the LF band of the cardiac interval spectrum, in the LF/HF ratio and in the HR, and a decrease in the power of the HF band of the cardiac interval spectrum. Companion dogs showed higher cortisol levels than beagles, independently of the time point studied and a significant increase in the cortisol levels 15 min after acoustic stress, while beagles did not show any alterations in their cortisol levels in response to the sound. On the other hand, beagles showed higher scores in the trembling, hiding, vigilance, running, salivation, bolting and startle parameters than companion dogs. Our results showed that independently of the sound stimulus, companion dogs had higher cortisol levels than laboratory dogs. Furthermore, the sound stimulus induced a marked autonomic imbalance towards sympathetic predominance in both laboratory and companion dogs. However a significant increase in the cortisol was observed only in companion dogs. On the other hand, in general the behavioural response was more pronounced in laboratory dogs than in companion dogs.

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1. Introduction

Animals and humans are constantly subjected to stress in their daily lives. During a stressful situation two neuroendocrine axes are classically activated: the sympathetic adrenomedullary (SAM), which increases the secretion of catecholamines and hence the rapid excitation of the
cardiovascular system, contributing to increased heart rate (HR) and arterial blood pressure; and the hypothalamic-pituitary-adrenal (HPA), which leads to an increase in cortisol production, and consequent mobilization of energy [1]. Disregulation of these stress responses may affect negatively several physiological systems, including the immune and cardiovascular systems, metabolic function and behaviour [2].

Several stimuli can be considered stressors for dogs, for example: transportation [3], social and spatial restrictions [4] and loud noise, [5] among others. Dogs are highly sensitive to sound stimuli, mainly fireworks, firearms and thunder [6]. These stimuli are closely associated with anxiety disorders such as sound phobia in this species [7–9]. Thus, these sound stimuli are used as stress models for dogs [5,6,10,11] and for studies concerning animal welfare and strategies to reduce stress.

Stress levels can be evaluated by the measurement of physiological and behavioural parameters. The most commonly used parameter for stress assessment in various species is cortisol. Also, the analysis of the heart rate variability (HRV) has proved to be a reliable non-invasive tool to evaluate the cardiovascular autonomic modulation in animals undergoing several types of challenging situations [12]. In dogs, monitoring cardiac parameters and serum cortisol level assessments, as well as behavioural analysis have been used to study stress responses [4,6,13–15].

The way an individual responds to a stressful stimulus depends on several factors, including their previous experience with stimuli similar or different to the current stimulus. Thus, companion and laboratory dogs may respond differently to stressful stimuli, due to differences in their management and relationship with humans. To the best of our knowledge, no studies have been done to compare autonomic, hormonal, and behavioural responses to acute acoustic stress in companion and laboratory dogs. The purpose of the present study was to evaluate the cardiac autonomic modulation, serum cortisol levels and several behavioural parameters under sound stimuli in beagle dogs (laboratory) and companion dogs (a variety of breeds).

2. Methods

2.1. Animals

All procedures were assessed and approved by the Committee on the use of Human and Animal Subjects in Teaching and Research Ethics of the Federal Rural University of Rio de Janeiro COMEP-UFRRJ (protocol n. 23083.013953/2010-41). Two groups of dogs were studied:

2.1.1. Laboratory dogs

Eight beagles (4 males; 1 to 6 years old; weighing from 9 to 16 kg; in good health) from the Kennel of the Laboratory of Experimental Chemotherapy in Veterinary Parasitology of the Federal Rural University of Rio de Janeiro (Seropédica, Rio de Janeiro, Brazil) were used. At this kennel all dogs were kept in groups of 5 to 8 animals in semi-open kennels (total area of 30 m², with 4 m² of a sheltered area) with full access to sunlight, and water and food (commercial dog food) ad libitum. The kennels do not provide an isolated environment, so the dogs can hear thunder storms or any other natural events.

2.1.2. Companion dogs

These dogs were recruited through advertisements in the Veterinary Hospital of Small Animals and the community around the Federal Rural University of Rio de Janeiro. Inclusion criteria for the study selection were dogs of either sex, between 2 and 6 years old; weighing from 10 to 30 kg; in good health; and the exclusion criteria were signs or history of neurological or behavioural problems, primarily excessive fear of thunder or any debilitating disease. The recruitment period was from October 2013 until February 2014 and the dog owner answered a questionnaire about the general characteristics of their dogs and their behaviour as suggested by [16]. In total, 22 dog owners expressed interest in taking part in the study. Among these, based on the general physical characteristics, 4 males and 2 females were selected for the study.

None of the animals used in this study came from the same social group (same house or the same group in the kennel).

2.2. Experimental design

All individual experimental procedures started at 0900 h. Initially, with dogs at their locations (houses for companion dogs; kennels for laboratory dogs), a HR monitor designed for human beings (Polar RS800cx, Polar®, Kempele, Finland) was strapped to the chest of the dogs in order to have the HR sampled on a beat-by-beat basis. In order to maximize the contact between the electrodes and the skin, the fur of precordial region skin area was cut using a hair clipper and a conductive gel was applied. Afterwards, the dogs rested undisturbed for 10 min for baseline measurements of HR data (i.e. data classified as basal 1-house/kennel) and baseline collection of blood samples (Basal 1). Next, the dogs were taken to the test room by car (10 min) in their transit boxes (87 × 57 × 98cm).

At the test room, the dogs were left undisturbed for 30-minutes followed by another blood sample collection (Basal 2). Animals were allowed to rest quietly for 20 min after which they were placed 1 m away from the sound source. The sound stimulus was turned on for 180 s and blood samples were collected 15 (S15), 30 (S30) and 60 (S60) minutes after the end of the sound stimulus. In order to assess the behavioural responses, the dogs were videotaped before, throughout the whole sound stimulus period and for the 5 min after the sound stimulus ended. Finally, the HR monitors were removed and the dogs were returned to their houses or kennels.

The experiment room was 11m² in area, had artificial lighting and the temperature was maintained at 22 ± 1 °C. There were only a few objects in the room: two benches, a veterinarian procedure table, a bench with the sound source and a video camera. Two researchers remained in the room during the experiment but without interacting with the dogs at any time in any way. All procedures were performed individually.

2.3. Acoustic stimulus

The sound stimulus consisted of a 180-second-long recording of thunder obtained from two separate sound files (e.g. very loud thunder, a close thunder crack, and high quality stereo sound of a major thunder clap during a storm), purchased from the website http://www.sound-effect.com. The sound file format used (a wave file) has the advantage of preserving all frequency oscillations of the original sound. The sound level was adjusted to 103–104 dB and tested with a decibel meter (Sound Level Meters, Model 732A, BK Precision®, Yorba Linda, CA, USA).

2.4. Heart rate variability

HR was continuously sampled with a heart monitor (RS 800cx, Polar, Kempele, Finland). Following acquisition, the data were transmitted from the heart monitor to the custom computer software (Polar Pro Trainer v5, Polar, Kempele, Finland) through an infrared interface. The recordings were then processed by Polar Pro Trainer software, and the time series of cardiac interval values were generated and loaded into a spreadsheet software (Microsoft Excel v2003) for inspection. A line chart was built from the cardiac interval data points to perform the inspection. Then the erroneous values were visually identified and corrected by calculating the average from two points before and two points after the erroneous data point. A study from Salo et al. [17] tested the effect of correcting different amounts of beats in the cardiac interval series, ranging from 5 to 50% of correction. At 5% of correction, minor changes were seen in the results, as compared to the original non-corrected series [17]. In addition, Peltola [18] indicates that time series with >20% of correction would be rejected. In the current study, taking
into consideration an average heart rate at baseline conditions ranging from 100 to 120 bpm, a 5-minute-long time series would contain around 550 data points, and 5% would represent around 27 data points. For all the time series inspected and corrected, the number of corrections did not exceed this amount [18]. Next, the inspected time series of cardiac intervals obtained at the moments Basal 1 (i.e. at house or kennel; 300-second-long period), Basal 2 (i.e. at the test room; 300-second-long period), Sound (i.e. during the acoustic stimulus; 180-second-long period) and After-sound (i.e. 30 min after the end of the sound; 300-second-long period) were analysed.

The analysis of cardiac interval variability (CIV) was performed using custom computer software (CardioSeries v2.4 - http://www.danielpenteado.com) designed to perform time-frequency analyses of cardiovascular variability, allowing precise adjustment of the parameters related to this kind of analysis (e.g. interpolation rate, segment length, and boundaries of frequency bands). Beat-by-beat series of cardiac interval values were converted to data points every 250 ms using a cubic spline interpolation (4 Hz). The interpolated series were then divided into half-overlapping sequential sets of 512 data points, which were detrended and tested for stationarity. The existence of slow trends in time series can affect spectra calculation and the power of frequency bands [19]. Before the power spectral density (PSD) calculation, interpolated cardiac interval time series were detrended by subtracting the linear trend (obtained by linear regression calculation) from the data points [20].

In our study, a well-experienced researcher visually inspected the segments of interpolated time series searching for transients that could affect the calculation of the PSD. To confirm that the visual inspection of the time series was properly performed, a Hanning window was used to attenuate side effects and the spectrum was calculated for all segments using a direct Fast Fourier Transform (FFT) algorithm for discrete time series. All segments were visually inspected for abnormal spectra. Lastly, the results from the time series and spectra inspections were taken together for the PSD calculation; non-stationary data were not considered [21]. The spectra were integrated in the low frequency band (LF, 0.04–0.15 Hz) and high frequency band (HF, 0.15–0.4 Hz). The normalised values were found by calculating the percentage of LF and HF power with regard to the total power of the spectrum minus the very low frequency band (VLF, <0.04 Hz) power [22,23]. The LF/HF ratio was calculated in order to assess the sympathovagal balance.

2.5. Blood samples and cortisol analysis

All blood samples were collected from the jugular vein using SST Vacutainer® tubes. Following the collection, blood samples were centrifuged for 10 min at 3200 rpm. The serum was collected in plastic tubes and stored at −20 °C. Serum cortisol concentrations were determined, in duplicate, by a double antibody radioimmunoassay method using a commercial kit (RD Coated Tube Cortisol 1125 RIA, Costa Mesa, CA, USA). The sensitivity of the assay was 0.17 ng/dL and the intra assay coefficient of variation was 6.59%.

2.6. Behavioural analysis

The behavioural responses of the dogs were analysed from videotape recordings (Digital movie camera Sanyo C40, Moriguchi, Osaka, Japan) at the Basal, Sound, and After-Sound moments. The occurrence of fourteen behavioural parameters [24] in response to acoustic sound were assessed by two observers and categorized on a scale of four grades, in which some behaviours were analysed by frequency (not observed (0), observed from 0 to 25% of the time (1), observed from 25 to 70% of the time) or by intensity (no signs (0), mild signs (1), moderate signs (2) and intense signs (3)); or a mix between intensity and frequency. Table 1 details the behavioural parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
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<tbody>
<tr>
<td>Analysed by the frequency or duration of behaviours</td>
<td>Panting – An increased frequency of inhalation and exhalation often in combination with the opening of the mouth.</td>
</tr>
<tr>
<td>Analysed by the Frequency of behaviours</td>
<td>Salivation – Clearly increased salivation or increased saliva swallowing frequency.</td>
</tr>
<tr>
<td>Analysed by the Intensity of behaviours</td>
<td>Bolting – Exaggerated response of escape, running in any direction.</td>
</tr>
<tr>
<td>Analysed by the Intensity of behaviours</td>
<td>Startle – Exaggerated response of fright, jump in any direction in response to the sound.</td>
</tr>
<tr>
<td>Analysed by the frequency and Intensity of behaviours</td>
<td>Vocalising – Barks, whines, whimper.</td>
</tr>
<tr>
<td>Analysed by the frequency and Intensity of behaviours</td>
<td>Destructive activity – Tries to dig or scratch the floor or bite the room objects.</td>
</tr>
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(Adapted from [8]; Beerta et al. [14]).

2.7. Statistical analysis

The study design for the CIV and cortisol parameters allows an analysis for repeated measurements (Basal 1, Basal 2, Sound and After Sound or Basal 1, Basal 2, 15 min, 30 min and 60 min) with baseline values (Basal 1) serving as a covariate. A mixed model for the repeated-measures approach followed by Bonferroni’s multiple comparisons test was used for the analysis of the parameters. The Basal 1 was used (or not) as covariate to account for potential initial differences in CIV and cortisol levels. Behavioural data were analysed by multivariate analysis of variance and the intraclass correlation coefficient (ICC) was used to assess inter-rater reliability of the assessment between the observers. ICC is rated moderate for values >0.5, strong for values >0.7, optimal for values >0.8 and excellent for values >0.9. Statistical analyses were performed with SPSS Version 21 (IBM SPSS Inc., Chicago, IL, USA) using a two-way-random-model (confidence-interval 95%). Graphs were built using GraphPad Prism 5.0 (GraphPad Software).

3. Results

In the repeated measures ANCOVA, when the covariate basal 1 was not taken into account, in both laboratory and companion dogs, the sound of thunder promoted an increase in the power of the LF band of the cardiac interval spectrum, in the LF/HF ratio, and in the HR, and a decrease in the power of the HF band of the cardiac interval spectrum (Time factor, LF/HF: Wilks’ lambda = 0.15, F(3,39) = 16.18, p = 0.001; LF and HF: Wilks’ lambda = 0.18, F(3,39) = 13.21, p = 0.001 and HR: Wilks’ lambda = 0.291, F(3,39) = 7.309, p = 0.009). Independent of the time point (i.e. Basal 1, Basal 2, Sound, and After-Sound) companion dogs showed higher power of the LF band of the cardiac interval spectrum, higher LF/HF ratio, and lower power of the HF band of the cardiac interval spectrum (Group factor: LF/HF: F(1,11) = 11.477, p = 0.006; LF and HF: F(1,11) = 9.545, p = 0.001), when compared to laboratory dogs. Companion and laboratory dogs did not differ in the way they responded to the sound as no significance in the interaction between the time x group factors in the CIV parameters was detected. When the covariate Basal 1 was taken into account, only the influence of
time was significant in the parameters LF/HF, LF and HF (LF/HF: Wilks’ lambda = 0.16, $F_{[3,8]} = 13.875, p = 0.02$; LF and HF: Wilks’ lambda = 0.16, $F_{[3,9]} = 13.35, p = 0.02$ and HR: Wilks’ lambda = 0.291, $F_{[3,9]} = 6.40, p = 0.016$) while the differences between the two groups was not significant. These results suggested that the differences between companion and laboratory dogs for LF/HF, LF and HF are highly influenced by the basal measures of these parameters (companion dogs had higher LF/HF and LF and lower HF than laboratory dogs). Considering or not the Basal 1 as a covariate, no significant difference was detected between the companion and laboratory dogs in the HR (Fig. 1).

Repeated measures ANCOVA was also conducted to compare the effect animal group on cortisol levels at different time points after sound stimulus, using Basal 1 as covariate to account for potential initial differences. Companion dogs showed higher cortisol levels independently of the time point studied ($F_{[1,11]} = 15.25, p = 0.002$) and the cortisol levels increased in response to sound stimulus (Wilks’ lambda = 0.27, $F_{[4,8]} = 7.215, p = 0.027$). Furthermore the companion and laboratory dogs responded differently to the sound (group × time effect: Wilks’ lambda = 0.40, $F_{[4,8]} = 42.42, p < 0.001$). The cortisol levels in companion dogs assessed 15 min after acoustic stress were higher compared to Basal 1 and 60 min After Sound (Bonferroni tests: basal × 15 min $t = 3.54, p < 0.05$; 15 min × 60 min: $t = 3.22, p < 0.05$). In laboratory dogs, the cortisol had a small increase at the time point Basal 2, but with no statistical differences detected (Fig. 2).

In the analysis of behavioural data, the intraclass correlation coefficient between the two observers was 0.97, which is considered excellent. The analysis of the behavioural parameters (Fig. 3) at the Basal moment and After-Sound did not show any significant behavioural response for most of the animals. A few animals showed the try to interact, vigilance and restlessness behaviours at these moments, with no differences between beagles and companion dogs (data not shown). At the moment of the sound, taking all the behavioural parameters together, statistical analysis has shown a tendency for the companion and laboratory dogs to react differently to the sound (Wilks’ lambda = 0.01, $F_{[1,12]} = 12.72, p = 0.004$), hiding ($F_{[1,12]} = 14.02, p = 0.003$), vigilance ($F_{[1,12]} = 23.87, p < 0.001$), running ($F_{[1,12]} = 5.61, p = 0.035$), salivation ($F_{[1,12]} = 208–215

![Fig. 1. Effect of thunder sounds on the cardiac interval variability, examined through the frequency domain analysis, and heart rate. Ratio between the power of the low and high frequency bands of the cardiac interval spectrum (LF/HF, Panel A), power of the LF (Panel B) and HF (Panel C), and heart rate (HR, Panel D). Data obtained at Basal 1 (dogs at house or kennel), Basal 2 (dogs at test room), Sound (during the acoustic stimulus) and After-Sound (30 min after the end of the sound).](https://example.com/fig1.png)

![Fig. 2. Changes in serum cortisol in response to the thunder sounds in companion and laboratory dogs. Data obtained from Basal 1 (dogs at house or kennel), Basal 2 (dogs at test room), 15, 30 and 60 min after the end of the thunder sound. * $p < 0.05$ different from Basal 1 and 60 min After Sound in companion dogs. ** $p = 0.002$ difference between companion and laboratory dogs independent of the time in the repeated measures ANCOVA.](https://example.com/fig2.png)
4. Discussion

Our results show that independently of the sound stimulus, companion dogs had higher cortisol levels than laboratory dogs and, different to the laboratory dogs, companion dogs showed a significant increase in the cortisol levels in response to sound. On the other hand, although the sound stimulus induced a marked autonomic imbalance towards sympathetic predominance in both groups of dogs, the behavioural response was more pronounced in laboratory dogs than in companion dogs.

4.1. Cardiac autonomic response to stress

Following a mental stress stimulus, a combined activation of both sympathetic and parasympathetic systems is observed [25], and the CIV analysis is a non-invasive technique that can be used to investigate the cardiac modulation of the autonomic nervous system (ANS) (i.e. sympathovagal balance) [12]. The analysis of the CIV in dogs during acoustic stress is highly relevant because it allows access to the autonomic balance regardless of other factors that alter the HR. Hence, the regulatory features of ANS cannot be determined simply by measuring the average HR [12,26]. As psychological states may have an impact on the sympathovagal balance without necessarily being followed by changes in HR, the assessment of LF/HF ratio can provide an accurate appraisal of the functional regulation of ANS in response to physiological and psychological stress [27].

The present results have shown that companion dogs tend to show higher sympathetic predominance (higher LF/HF ratio and LF band and lower HF) independent of the sound stimulus; however, these results are highly influenced by the basal measures of these parameters. The basal levels are quite different between companion and laboratory dogs, and when the covariate Basal 1 was used in the covariance analysis, the difference between companion and laboratory dogs disappeared. Furthermore, both groups of dogs had similar responses to the sound, although companion dogs reached higher levels of LH/HF.

Previous studies have reported increases of the HR average HR in animals subjected to high intensity sounds [5,6,28,29]. On the other hand, other studies have shown that healthy companion dogs undergoing an acute acoustic stress protocol showed no changes in HR [14]. In our
study, HR had a significant increase in response to the sound, with no significant difference between laboratory and companion dogs.

To the best of our knowledge, no other studies in the literature have investigated the differences in CIV of companion and laboratory dogs subjected to sound stimuli. The majority of the studies have addressed the differences between shelter animals and companion animals [30], giving emphasis to the human–dog interaction as an important factor to modulate the stress response. As changes in the CIV are associated with several diseases, and such changes can be used as a risk factor after acute myocardial ischaemia, and certain breeds of dog seem to have more pronounced variations in heart rate, particularly as a result of respiratory sinus arrhythmia, a possible difference in autonomic responses between dog breeds must be considered. Dokey and Boswood [31] compared the vasovagal tonus index (VVTI), a time-domain indicator of CIV and HR of dogs of different breeds (German Shepherd, Golden retrievers, Cocker spaniels, boxers, bulldogs and King Charles spaniel) [31]. Brachycephalic dogs had a higher VVTI than other breeds of dog; however no difference was detected between individual breeds. Other studies have shown no difference in CIV parameters between breeds [30].

4.2. Cortisol

In the present study, the companion dogs subjected to an acute acoustic stress model (i.e. sounds of thunder for 180 s, at an intensity of 105–106 dB) showed higher serum cortisol levels 15 min after the acute acoustic stress. On the other hand, laboratory dogs did not only demonstrate lower basal cortisol levels than companion dogs, but they also did not show a change in cortisol after the acute acoustic stress. These data suggest a differential activation of the HPA axis between laboratory and companion dogs.

Few studies have addressed the endocrine response of dogs subjected to sound stimuli. Beerda et al. [14] used an acute acoustic stress protocol (i.e. sound intensity of 110–120 dB, for 2 s, repeated three times with 30 s intervals) and then measured the cortisol levels and HR in healthy dogs. The results showed increased salivary cortisol levels 10, 15 and 30 min after exposure to the sound [14], Ising et al. [28] observed an increase in plasma levels of epinephrine, norepinephrine, ACTH and cortisol in dogs exposed to sound stimuli (75 dB) for 3 min and also an eleven-fold increase in cortisol levels 12 min after the end of sound stimulus [28]. Firearm shots also produce a substantial increase in cortisol levels [6].

Dreschel and Granger [32], studied the interactions in HPA axis activation in response to stress, relationship quality, and behaviour in thunderstorm-anxious dogs and their owners. These authors subjected 19 highly fearful dogs and their caregivers to a 5-minute-long sound stimulus. The dogs exhibited classic signs of fear (e.g. pacing, whining, and hiding) and their cortisol levels increased by 207%. On the other hand, there were no effects of the owners’ behaviour or the quality of the dog–owner relationship on the HPA or behavioural reactivity of the dogs [32]. In this sense, our results show that companion dogs that have frequent contact with their owner exhibit a more “active” HPA axis than laboratory dogs. As will be discussed below, the owner’s presence may not always be considered a positive factor in reducing stress responses in companion animals.

4.3. Behavioural responses

In the current study, most animals showed behavioural reactions only in the presence of the sound stimulus. This suggests that the experimental environment was not stressful to the animals. In general, laboratory dogs were more responsive to the sound than companion dogs (p = 0.009), with higher scores than companion dogs in the trembling, hiding, vigilance, running salivation, bolting and startle. In general, both laboratory and companion dogs showed moderate behavioural reactions. This moderate behavioural response was expected since no phobic animals were used. Beerda et al. [13], using different acoustic stress protocols, found that the behavioural responses showed no clear relationship with increasing noise intensities or durations. Furthermore, in the present study the behavioural analysis was conducted using a semi-quantitative scale (scores 0 to 3), which should contribute to reduce the sensitivity of the analysis [13].

It is widely recognized that dog breeds have different and consistent behavioural dispositions, and therefore it is hard to completely exclude the effect of breeds in behavioural studies. In the present study we cannot associate breeds with the differential behavioural responses to acute stress between these groups of dogs. First of all, we compared beagles with a variety of dog breeds, and even in the most controlled experimental studies there are substantial differences in behaviour between individuals of the same breed [33,34]. Dogs are characterized by socialization based on family relationships, with flexible organizations and responses to a leader, besides their need for complex and varied environments [35]. Companion dogs have extensive experience to know the consequences, for example, of a slamming door, or the arrival or departure of a person. This kind of predictive behaviour is not seen in laboratory dogs. This suggests that changes in the individual reaction to stress may be strongly influenced by environmental and social conditions rather than solely genetic factors [36].

The behavioural parameters used in the present study have been previously assessed in other studies found in the literature to evaluate stress responses to sound in dogs with phobia to thunder and fireworks [8,37–39]. Despite some findings in the literature showing that phobic dogs present destructive activity, elimination and self-harm behaviours in response to sound stimuli [32,37,39], these parameters were not observed in our study.

4.4. Model of noise stimuli

Dogs are particularly sensitive to sound stimuli, mainly the sounds of thunder, fireworks, and firearms which are considered the most important sources of anxiety disorders in this species [7–9]. Therefore these stimuli are used as stress models for dogs [5,6] and can be considered a natural choice for the study of the reactivity to stress. Although there are differences between playing a recorded sound and real thunderstorms and fireworks, this technique has been used in several studies to categorize the response of dogs to these sounds [10,32,37,39].

Differently from previous studies where the main focus was behavioural analysis, in the present study the acute acoustic stress model artificially created a stress condition to measure endocrine, autonomic and behavioural responses, allowing a reliable analysis of the individual response of each dog [7,9,10,32,37–39]. The behavioural test is an important tool to analyse the effects of stress on dogs. However, when used alone it does not reflect the complex reactions of the individual to the stimulus. While recognizing the neural pathways that mediate behavioural, endocrine and autonomic responses are connected, our results show that these responses can also be dissociated.

The model used in the present study has some advantages: the sound intensity (103–104 dB) is able to induce physiological and behavioural responses without damaging the auditory system [28]; it is a simple and easy model to replicate, not requiring sophisticated technology and allowing the use of a wide variety of sounds (e.g. thunder, fireworks, etc.). On the other hand, this also represents a limitation of our model, since fear of thunderstorms and fear of loud noises are not always present together. Furthermore, the experimental method in a laboratory instead of the dog at its house allowed the observation of the behaviour of the dogs without influence of other factors, such as the owners and other animals. Data acquisition from the Polar® frequency metre was advantageous, since it was simple to use and has no effect on the behaviour of the dogs [15], allowing reliable data analysis. However, special care should be taken during transportation to the laboratory since it can lead to stressful responses in animals not accustomed to
vehicle transportation [40], such as high cortisol levels, tachycardic responses and changes in behavioural parameters [3].

4.5. Companion versus laboratory dogs

In the present study, in response to acute acoustic stress, companion dogs had a higher increase in the cortisol levels, compared to laboratory dogs, suggesting greater reactivity to acute acoustic stress. These data corroborate the concept that interaction with humans and diverse environments contribute to different stress responses. Dogs can be either more reactive or less reactive to stressful situations of everyday life depending on the proximity of their owners [7].

Companion dogs that live with humans are often subjected to a wide range of stressors. Among them, the psychological factor has shown great importance, but few studies cover the key factors of attachment and cohabitation between man and dog, and in what ways this relationship can affect the well-being of a dog [41]. However, laboratory dogs are subjected to different types of stress, since research conditions must follow rigid guidelines for animal welfare. However, correct management does not prevent the occurrence of stressful situations, on the other hand the demands of dog owners can cause conflicts and frustrations that can induce changes in the stress response and, in some animals, lead to the emergence of behavioural problems [7]. In fact, we must identify what type of relationship between dog and owner can create negative effects as well as the degree of this influence on stress response of dogs. Furthermore, we need to identify if the more active HPA axis in companion dogs is necessarily related to harmful or if it is an adaptive process.

In conclusion, the results of the current study shine a new light on how companion and laboratory dogs may react to a well-established stress model (i.e. acoustic stress) and suggest that despite the belief that companion dogs would exhibit an attenuated response to sound stimuli, intense physiological reactions are preserved. Further investigations are needed to better understand the individual and combined behavioural, autonomic, and endocrine responses in companion and laboratory dogs. Also the employment of other stress models is desirable.

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