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'Four Seasons' in an animal rescue centre; classical music reduces environmental stress in kennelled dogs

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HIGHLIGHTS

- Classical music increases HRV in kennelled dogs.
- Dogs display more relaxed behaviour when exposed to classical music.
- Dogs habituate to calming effects of music as soon as the second day of exposure.
- Male dogs have a more positive response to classical music than females.

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ABSTRACT

On admission to rescue and rehoming centres dogs are faced with a variety of short- and long-term stressors including novelty, spatial/social restriction and increased noise levels. Animate and inanimate environmental enrichment techniques have been employed within the kennel environment in an attempt to minimise stress experienced by dogs. Previous studies have shown the potential physiological and psychological benefits of auditory stimulation, particularly classical music, within the kennel environment. This study determined the physiological/psychological changes that occur when kennelled dogs are exposed to long-term (7 days) auditory stimulation in the form of classical music through assessment of effects on heart rate variability (HRV), salivary cortisol and behaviour. The study utilised a cross over design in which two groups were exposed to two consecutive 7 day treatments; silence (control) and classical music (test). Group A was studied under silent conditions followed by 7 days of test conditions during which a fixed classical music playlist was played from 10:00–16:30 h. Group B received treatment in the reverse order. Results showed that auditory stimulation induced changes in HRV and behavioural data indicative of reduced stress levels in dogs in both groups (salivary cortisol data did not show any consistent patterns of change throughout the study). Specifically, there was a significant increase in HRV parameters such as μRR, STDRR, RMSSD, pNN50, RRTI, SD1 and SD2 and a significant decrease in μHR and LF/HF from the first day of silence (S1) to the first day of music (M1). Similarly, examination of behavioural data showed that dogs in both groups spent significantly more time sitting/lying and silent and less time standing and barking during auditory stimulation. General Regression Analysis (GRA) of the change in HRV parameters from S1 to M1 revealed that male dogs responded better to auditory stimulation relative to female. Interestingly, HRV and behavioural data collected on the seventh day of music (M2) was similar to that collected on S1 suggesting that the calming effects of music are lost within the 7 days of exposure. A small '9-Day' study was conducted in attempt to determine the time-scale in which dogs become habituated to classical music and examination of the results suggests that this occurs within as soon as the second day of exposure. The results of this study show the potential of auditory stimulation as a highly effective environmental enrichment technique for kennelled dogs. However, the results also indicate the requirement for further investigations into the way in which auditory stimulation should be incorporated within the daily kennel management regime in order to harness the full physiological and psychological benefits of music.

1. Introduction

A recent study of pet ownership revealed that UK households were home to over 10.5 million pet dogs in 2006 [43]. Despite their position

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as one of the UK's most popular pets, welfare organisations cared for an estimated 129,743 dogs in 2009 alone [14]. The reasons for relinquishment included straying, inability of owners to care for them [18], abandonment and neglect [53]. The basis of animal welfare is contained within the concepts of the 'Five Freedoms' that is; freedom from hunger and thirst, discomfort, pain, injury and or disease, fear and distress and the freedom to express normal behaviour. Although rescue centres offer dogs a second chance through rehabilitation and rehoming, the kennel environment is inherently stressful and upon admission dogs are exposed to a variety of psychogenic stressors. For reasons often relating to practicality and expense the kennel is often spatially and socially restrictive (both intra and interspecifically), lacking in complexity, controllability and predictability [4,30,59]. In addition, isolation in a novel environment, separation from social attachment figures and regular exposure to high sound levels, all of which occur on kenneling, have been shown to elicit stress responses in dogs [51,62]. Continual exposure to such stressors results in chronic stress which is known to compromise welfare [5,6,19]. A number of studies have investigated whether there are simple, economical methods of addressing potential shortfalls in kennel design and management to minimise the stress experienced by kenneled dogs. These studies have employed a variety of forms of environmental enrichment defined as any animal husbandry principle which seeks to enhance the quality of captive care by identification and provision of environmental stimuli necessary for optimal physiological and psychological well-being [54]. Several studies have reported welfare benefits of animate (social) enrichment as a result of socialisation with conspecifics [32,41] and humans [7,16]. In addition, inanimate enrichment, that is alteration of the physical environment, can also be an effective means of alleviating kennel induced stress for example the provision of toys [52], the addition of furniture [35] and visual [26], olfactory [25] and auditory [38,68] stimulation.

The physiological [44,60] and psychological [2,57] benefits of listening to music are well documented in but not solely attributed to humans. Music has been reported to increase milk yield [2] and use of automatic milking machines in dairy cows [64], result in reduced stereotypic behaviours in captive Asian elephants [71] and have beneficial effects with regard to stress related behaviours in zoo-housed gorillas [70]. The potential use of music as an auditory environmental enrichment technique for dogs in the kennel environment has also been reported [38,68]. However, in these studies the duration of auditory stimulation and/or the trial were limited. Both studies reported changes in the activity of dogs when exposed to music; specifically, classical music increased the amount of time spent sleeping and reduced barking, while heavy metal music increased barking and body shaking. Given the intermittent nature and limited duration of exposure to music (45 min to 4 h) in these studies it is difficult to ascertain whether the observed effects of auditory stimulation, would be effective to reduce stress experienced by dogs in a working kennel environment in the long term.

In dogs, the response to stressors is multifactorial and results in both behavioural [6,9,38] and physiological changes [9,16,30]. Key elements of the mammalian stress response are the coordinated activation of the sympathetic nervous system and the hypothalamo-pituitary adrenal (HPA) axis, which drive adaptive psychological and behavioural changes. A variety of methods are available to monitor stress including quantification of circulating hormones as a measure of the physiological response and behavioural observations which provide a measure of the integrated response of an animal to its environment. The adrenal hormone cortisol is one of the main hormonal mediators of the effects of stress in mammals [29] and can be measured in several biological samples including plasma, saliva, urine and faeces. Plasma cortisol concentrations have been shown to correlate well with stress, however due to its pulsatile release and its sensitivity to acute events, including restraint and venepuncture, concentrations can be unreliable/variable. Salivary cortisol concentrations have the advantages that sample collection is relatively non-invasive [37] and that it represents cortisol secretion over a longer time period of minutes to hours rather than the single time point measurement obtained from a plasma sample. The collection technique and associated handling required to retrieve a saliva sample although usually well tolerated, can elicit a stress response. However, basal cortisol concentrations can be measured if saliva samples are collected in less than 4 min [37]. Salivary cortisol concentrations have been shown to be related to stress levels in a number of animals including sheep [23], pigs [15], cattle [47] and dogs [29,46]. The utility of cortisol concentrations as a simple measure of the activity of the HPA axis, however, is compromised by the fact that chronic stress can result in dysregulation including changes in receptor number and sensitivity [72] and changes in adrenal sensitivity [31]. In addition to activation of the HPA axis stress can also result in changes in activity within the autonomic nervous system. As the activity of the autonomic nervous system has dramatic effects on cardiac function, an alternate non-invasive means to monitor aspects of the physiological response to stress is analysis of effects on heart rate variability (HRV) [58]. Specifically, HRV is the difference in beat-to-beat intervals (R–R interval) which is derived from the non-additive input to the heart of the two branches of the autonomic nervous system. HRV analysis is based on the fundamental principle that healthy cardiac function is characterised by irregular time intervals between consecutive heart beats. Work in humans has shown that higher resting HRV is associated with the enhanced control of emotions, thoughts and behaviour [10]. HRV is also relatively easy to measure in a variety of farm animals [65], cats [1] and dogs [7]. Variation in HRV parameters has been associated with a series of factors in different species, such as genotype, behaviour, environment, temperament, performance and nutritional status in the horse [65,66] emotional state in sheep [49] and production systems in cattle [27,28]. A previous study in dogs has demonstrated that the reduction in stress, observed in response to increase human interaction, is also associated with changes in HRV parameters [7].

This study tested the hypothesis that playing classical music to dogs housed in an animal rescue and rehoming centre would reduce physiological and psychological stress. Specifically the study investigated the effects of daily exposure to classical music, for 7 days, on the HRV, salivary cortisol and behaviour of dogs living in a working rescue kennel environment and assessed whether the response to stress was influenced by factors such as sex, age, breed, gonadal status, body condition score (BCS), reason for kennelling and duration of stay.

2. Materials & methods

2.1. Subjects

This study was conducted at the Scottish SPCA Dunbartonshire and West of Scotland animal rescue and rehoming centre (ARRC) from July 2013–March 2014. This study coincided with normal husbandry and operational procedures within the centre which included visits by the general public and rehoming of animals. As the Scottish SPCA rescues and rehomes injured, neglected, abandoned or unwanted animals, dogs included in this study were from a variety of different backgrounds and varied in breed and age. In an attempt to ensure researcher safety any dogs which had displayed aggressive behaviour were not included in the study.

The subjects included in this study consisted of 50 dogs; 25 neutered (Nx) and 9 entire (E) males (n = 34) and 12 Nx and 4 E females (n = 16). The reason for being at the ARRC was n = 20 admitted as strays (S), n = 22 unwanted pets (U), n = 6 held for temporary refuge (TR) and n = 2 sequestered due to welfare issues (W). Dogs were categorised into 7 age groups (age estimated from dentition for stray dogs (n = 20)); <0.5 years (n = 6), 0.5–1.9 years (n = 8), 2–3 years (n = 17), 3.1–5 years (n = 7), 5.1–8 years (n = 10), 8.1–9.9 years (n = 1) and >10 years (n = 1). Duration of stay prior to the study was calculated as the difference between the date of arrival and the first day of data collection. This ranged from 1 to 231 days with a

mean of 31 ± 9 days. The study population included a high proportion (42%) of Staffordshire bull terriers (SBT) (n = 17) and SBT crosses (n = 4). All subjects were assigned a BCS by the researcher on the first day of data collection in accordance with the guidelines in Baldwin et al. [3].

All procedures employed throughout this study were approved by the University of Glasgow's Veterinary Ethics and Welfare Committee.

2.2. Kennel environment & general husbandry

On arrival at the centre all dogs were vaccinated (DHPPi, Leptospirosis & Canine Infectious Tracheobronchitis) and treated for parasites. Following appropriate assessment by the Scottish SPCA, S and U dogs are made available for rehoming after 7 and 3 days at the centre, respectively. Study animals remained available for rehoming throughout the trial and therefore not all dogs completed the full two weeks of study. As illustrated in Fig. 1 the study population were maintained in a kennel block which ran parallel to a central staff corridor. There were five windows along the corridor which allowed behavioural data to be collected without physically entering the kennel block. Individual kennels contained both an indoor (4.8 × 6.4 m) and outdoor (4.8 × 17.4 m) unit. The front of each kennel was lined with inch square welded mesh which incorporates a door that was bolted and padlocked in order to securely contain the dogs. The floor and all three indoor walls were tiled. The indoor unit contained food and water bowls, beds, blankets and toys. The back wall contained a 1.8 × 2.9 m 'shutter' which could be operated manually to permit/prevent the dog's access to the outdoor unit. The outdoor unit consists of a concrete runway, the front of which was lined with inch square welded mesh containing a door. A concrete runway ran perpendicular to the outdoor units allowing staff access to outdoor kennels. The outdoor runway was in turn surrounded by a cage of inch square welded mesh which allows the public to view dogs available for rehoming, but prevented them from making physical contact.

One member of staff was present on the block between 08:30 and 16:45, 7 days a week, 365 days a year. On occasion staff members were accompanied by a volunteer who assisted with husbandry duties. A standard day in the centre ran as follows: 08:30 dogs were locked outside while indoor units were deep cleaned, then they were locked inside and fed while outdoor units were deep cleaned. 10:30 onwards staff focus on general husbandry including walking, grooming and administration of medication. After 13:30 any dogs required to were taken to the on-site vet clinic for a health check and at 14:00 dogs received second feeds. At 15:30 dogs were locked outside while indoor units were spot cleaned and at 16:00 dogs were locked in for the night and outdoor runs were spot cleaned. The standard routine is supplemented with additional spot cleaning of kennels i.e., removing faeces from kennel, when required, to ensure appropriate levels of cleanliness.

2.3. Study design

Fig. 2 summarises the experimental design. The study utilised a crossover design in which dogs were exposed to two consecutive 7 day treatments; silence (control) and classical music (test). Dogs were randomly assigned to Group A or Group B. Dogs in Group A (n = 27) were exposed to 7 days of silence followed by 7 days during which classical music was played from 10.00 until 16.30. Group B (n = 23) were exposed to 7 days of classical music followed by 7 days of silence. HRV and behavioural observations were collected for 1.5 hour periods both in the morning (10:30–12:00 h) and afternoon (14:00–15:30 h) on days 1, 7, 8 and 14, subsequently referred to as S1, S2, M1 and M2 for Group A and M1, M2, S1, S2 for Group B, respectively. Saliva samples were collected at the end of each HRV/behavioural recording period.

The number of dogs studied at any one time (1–5) was dependent upon the availability of dogs being housed at the ARRC. As the study was conducted around normal operating hours, dogs were not always present for all behavioural observation/HRV recording periods. Absences only occurred, however, for veterinary checks/procedures and viewings by members of the public.

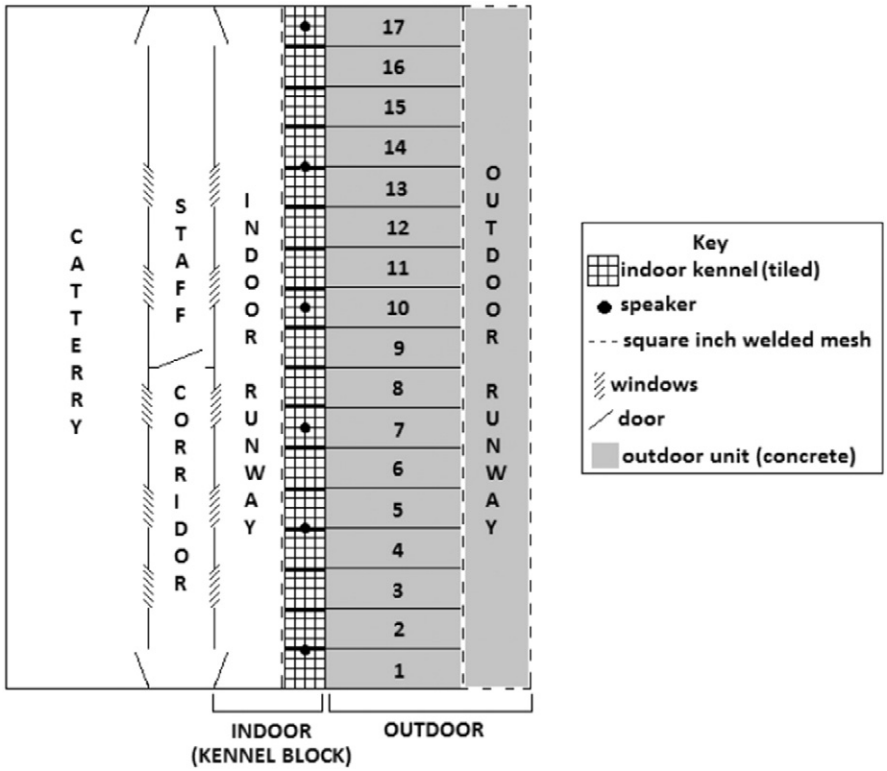

Fig. 1. This figure illustrates the general layout of the kennel block in which the study population were maintained.

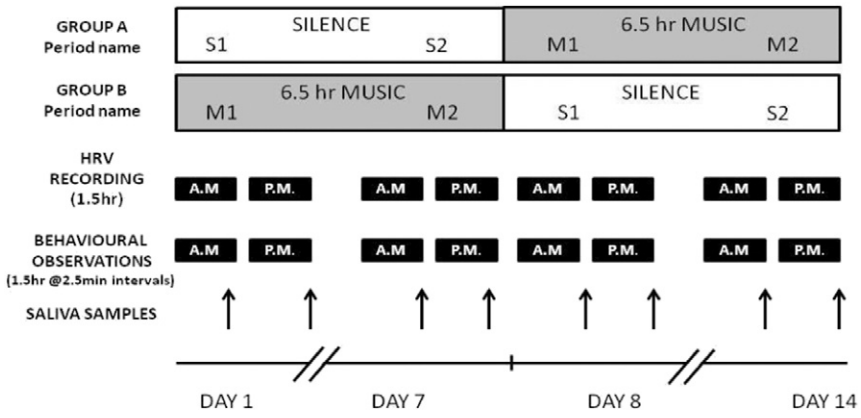

Fig. 2. Illustrates the overall study design. Group A and Group B received the same treatment in a crossover design. Each subject was studied over 14 days with HRV, behavioural and salivary cortisol data collected on days 1, 7, 8 and 14. HRV (continuous) and behavioural data (2.5 min intervals) were collected from 10:30–12:00 in the AM session and from 14:00–15:30 in the PM session. One saliva sample was collected from each subject at the end of each AM and PM session. During periods of auditory exposure (M1–M2) music was played from 10:00 till 16:30. S1 = first day of silence, S2 = seventh day of silence, M1 = first day of music and M2 = seventh day of music.

A 6.5 h playlist was generated by selecting slow tempo, low pitch tracks from the '300 Classical Favourites' CD downloaded to and played via Windows Media. The playlist was fixed and delivered through 360° Bluetooth Wireless Speakers (Veho, Hampshire, UK). The speakers were dispersed evenly throughout the block and placed on the roof of kennels 1, 4/5, 7, 10, 13/14 & 17 as illustrated in Fig. 1. The volume of the speakers was set manually and maintained at a set level throughout the study.

2.4. Data collection & analysis

2.4.1. HRV data

HRV data was collected using Polar® RS800CX human heart rate monitors (HHRM's) (Polar®, Finland). This equipment consisted of 1) Polar® wearlink strap, 2) Polar® watch-computer and 3) Polar® wireless integrated network device (W.I.N.D.). The Polar® wearlink strap was positioned around the subject's cranio-ventral thorax and the size adjusted to provide a tight yet comfortable fit. Aquasonic® Ultrasound Transmission Gel was liberally applied to the electrodes of the Polar® wearlink strap and positioned over the left, third intercostal space. The Polar® watch-computer was secured to the subjects' collar. Once the HHRM had been fitted to the dog, the same set of equipment was used to collect HRV data from that dog for the remaining experimental sessions. The Polar® watch-computers were set to record approximately 5–10 min before the start of each recording session. Two 'watch-checks', one at 30 min and one at 60 min, were incorporated into the 90 min recording session. If during the checks the watch-computers were no longer recording the watch computers were reset and more ultrasound gel applied.

After each session, HRV data was downloaded to a computer using Polar® Software and converted into an ASCII file. R–R interval data was analysed using Kubios HRV software (Version 2.0 Biosignal Analysis and Medical Imaging Group (BSAMIG), Department of Physics, University of Kuopio, Finland (http://bsamig.uku.fi)). Prior to analysis, R–R interval data was scanned manually and artefacts removed using Kubios' inbuilt 'artefact correction' feature. HRV parameters were calculated for three, 5 minute sections, selected at random, from each 90 minute recording session. The following time-domain variables were chosen for further analyses: Mean RR (μRR); Standard Deviation of R–R intervals (STDRR, ms); Mean Heart Rate (μHR); Root Mean Square of the Standard Deviation (RMSSD, ms); R–R interval Triangular Index (RRTI) and a number of successive R–R interval pairs which differ more than 50 ms (NN50) expressed as a percentage (pNN50, %). The only frequency-domain variable taken forward for further analyses was the ratio between the low frequency (LF) and high frequency (HF) band powers (LF/HF). In this study the nonlinear properties of HRV were analysed using two measures of the Standard Deviation of the Poincaré Plot (SD1 & SD2, ms). The mean values of each HRV parameter were calculated across the three 5 minute sections, from each AM and PM recording on S1, S2, M1 and M2, for each dog.

2.4.2. Behavioural data

Throughout the 90 minute recording session, behavioural data was recorded sequentially at 2.5 minute intervals (37 observations per session) using a scan sampling technique. Dogs were observed from the staff corridor outside the kennel block by the researcher. Three aspects of behaviour were recorded at each interval; position (three categories: lying, sitting, standing); location (two categories: inside, outside) and vocalisation (three categories: silent, whining, barking). At each interval the presence of staff, volunteers, visitors and other dogs was noted. The percentage of time observed performing each behavioural activity was calculated by dividing the number of observations during which that subject was performing each activity, by the total number of observations for that subject. Behavioural data was adjusted to allow for observations where the dog was recorded as out of sight i.e., in vet-clinic or out for a viewing.

2.4.3. Salivary cortisol data

A saliva sample was collected from each dog immediately after the end of each behavioural observation/HRV recording session. Saliva samples were collected using 5 × 5 tailed cotton swabs and as advised by Kobelt et al. [37] sampling time was never allowed to exceed 4 min. Gloves were worn at all times during sample collection and changed in between subjects. Dogs were lightly restrained and allowed to chew on the swab or the swab was moved around the oral cavity until it had become saturated with saliva. Immediately after collection, swabs were stored in labelled 15 ml Greiner tubes and frozen (-20°) until assay. Salivary cortisol levels were determined using a high-sensitivity competitive enzyme immunoassay kit (R&D Systems, Oxford, UK) according to the manufacturer's instructions. Salivary cortisol concentrations were measured via ELISA (R&D Systems, Minneapolis, USA). The ELISA was conducted in accordance with the manufacturer's instructions and samples were assayed at a 1:4 dilution. Assay sensitivity averaged 0.07 ng/ml and inter and intra-assay coefficients of variability were 5.4% and 4.2%, respectively.

2.5. Statistical analysis

All data sets were checked for normality (Kolmogorov–Smirnov technique). Both AM and PM data sets for μRR, μHR, the percentage time spent performing each behaviour and salivary cortisol concentrations within (M1 vs M2, S1 vs S2) and between treatment weeks (M1

vs S1, M2 vs S2), were compared using Mann Whitney tests. STDRR, RMSSD, pNN50, RRTI, LF/HF, SD1, and SD2 were compared within and between treatment weeks for both the AM and PM data sets using paired t-tests. A Kruskal–Wallis test was used to compare HRV, behavioural and salivary cortisol data between AM and PM sessions within S1, S2, M1 and M2. Given the significant effects of auditory stimulation on behaviour and HRV parameters, the effects of sex, age, breed, gonadal status, BCS, reason for kennelling and duration of stay on these response variables was investigated using General Regression Analysis (GRA). The response variable was calculated as the difference in the tested parameter between S1 and M1. With regard to reason for kennelling, welfare was not included as an explanatory variable and the age classes 8–9 and >10 years were excluded from the analysis as there was only one subject available for each classification.

2.6. '9-Day' study

A smaller study was conducted in an attempt to gain an insight into the time-scale in which kenneled dogs (n = 4) habituate to auditory stimulation during 7-day exposure to classical music. HRV, behavioural and salivary cortisol data was collected, as described in Sections 2.3–2.5 above, during all 7 days of exposure to classical music (M1–M7) and one day of silence prior to (S1) and following (S2) auditory exposure. This study was carried out after the larger study and none of the 4 dogs had previous exposure to music in the kennels.

3. Results

3.1. Mean heart rate & mean R–R interval

The mean R–R interval and heart rate observed across the groups within the study are summarised in Table 1. Within the silent week (S1 vs S2), a statistically significant difference ($P < 0.05$) was only observed in one of the four recording periods, namely Group A's, afternoon recording, where an increase was seen in the R–R interval. As might be expected, this difference was accompanied by a statistically significant ($P < 0.05$) decrease in μHR. Within a music week (M1 vs M2) there were no significant differences in μRR or μHR between M1 and M2 in either group in the morning or afternoon recording sessions.

Comparison between weeks indicated that μRR increased at the start of a music week, compared to the start of a silent week, regardless of whether the music week followed (Group A) or preceded (Group B) the silent week. The difference was statistically significant ($P < 0.05$) in all except the group A morning recording where a strong trend was noted ($P = 0.077$). This change in μRR was accompanied by a corresponding decrease in μHR, which again did not appear to be influenced by whether the music week preceded or followed the silent week. In this instance, however, statistical significance was only observed within Group A ($P = 0.0216$) in the afternoon, although a similar trend was seen in the morning in Group B ($P = 0.057$). Comparison of μRR and μHR at the end of each treatment week (M2 vs S2) did not reveal any significant differences.

Results of the GRA indicated that the changes in μRR and μHR in response to auditory stimulation were not associated with differences in age, breed, gonadal status, BCS, reason for kennelling and duration of stay. However, both values were significantly affected by sex, summarised in Table 2, specifically during the afternoon recording periods where the increase in μRR ($P < 0.001$) and decrease in μHR ($P < 0.005$), in response to auditory stimulation, was larger in males compared to females.

3.2. Heart rate variability

A full summary of the HRV parameters analysed and compared in this study is presented in Table 3. Specific variables that were found to differ within or between treatment weeks are depicted in Fig. 3. Within the silent week no consistent changes were observed in any of the assessed variables, across the two groups (A and B) over the recording day (morning and afternoon). Within the time domain variables, SSTDR and RMSDD and pNN50 differed significantly ($P < 0.05$) between S1 and S2 but only in the group that received silence first, and only in the afternoon recording sessions. In each case the values observed were higher in the S2 compared to the S1 recording session. A similar pattern of change was observed in SD1 (geometric analysis) with the value seen in S2 in the afternoon for Group A being significantly ($P < 0.05$) higher than that seen in the S1 recording session, and a similar trend being seen in SD2 ($P = 0.075$).

Within the music week, three HRV parameters differed significantly between M1 and M2. STDRR (time domain variable) was significantly lower in M2 compared to M1 in Group B (AM and PM $P < 0.05$) with a similar trend ($P = 0.077$) in Group A in the morning. RRTI was also observed to be significantly lower in M2 compared to M1, in Group A (AM $P < 0.005$, PM < 0.05) and Group B AM $P < 0.01$, PM $P < 0.05$. SD2 decreased significantly between M1 and M2 in both groups regardless of the time of day (Group A, AM and PM $P < 0.05$; Group B, AM $P < 0.01$ and PM $P < 0.05$).

More widespread significant differences were observed in the HRV parameters between the music and silence weeks, however, these differences were only observed when S1 was compared to M1. No statistically significant differences were observed between the HRV parameters when they were compared between S2 and M2.

STDRR (Group A, AM $P < 0.005$, PM $P < 0.001$; Group B, AM $P < 0.001$, PM $P < 0.001$), pNN50 (Group A, AM $P < 0.05$, PM $P < 0.005$; Group B, AM $P < 0.01$, PM $P < 0.001$) and RRTI (Group A, AM $P < 0.01$, PM $P < 0.005$; Group B, AM $P < 0.05$, PM $P < 0.01$), were significantly higher in M1 compared to S1, regardless of the time of day that the recordings were taken or whether the music week followed or preceded the silent week. RMSSD also increased in M1 relative to S1. This difference was statistically significant in Group A PM ($P < 0.001$) and Group B AM ($P < 0.01$) and PM ($P < 0.005$) with a strong trend ($P = 0.056$) being noted in the Group A morning recordings. The LF/HF ratio was significantly lower ($P < 0.005$) in the group that received the music week second,

Table 1
Mean values ± SEM for mean time duration between two consecutive R waves of the electrocardiogram (R–R Interval) μRR and mean Heart Rate μHR in Group A and Group B over different experimental sessions S1, S2, M1 and M2 for both AM and PM recordings.

Parameter	Group	Recording	S1 (mean ± SEM)	S2 (mean ± SEM)	M1 (mean ± SEM)	M2 (mean ± SEM)
μRR (ms)	A	AM	475.30 ± 23.60	507.94 ± 22.41	551.74 ± 33.99	523.97 ± 23.69
		PM	491.27 ± 13.08[a,*]	551.35 ± 22.64	568.02 ± 25.06	556.32 ± 18.77
	B	AM	485.93 ± 15.16[*]	492.02 ± 19.09	577.66 ± 35.65	524.94 ± 27.93
		PM	464.45 ± 28.63[*]	470.19 ± 40.62	560.65 ± 25.37	533.9 ± 20.32
μHR (beats/min)	A	AM	130.87 ± 4.63	134.46 ± 8.44	121.94 ± 5.34	126.72 ± 5.83
		PM	129.75 ± 2.96[a,*]	117.81 ± 3.99	116.12 ± 4.32	114.52 ± 3.65
	B	AM	132.33 ± 3.75	131.56 ± 4.69	118.21[a] ± 5.51	121.24 ± 6.46
		PM	127.55 ± 3.38	124.6 ± 5.78	118.81 ± 4.35	121.57 ± 4.83

[a] Denotes a significant difference ($P < 0.05$) within a treatment week S1 vs S2 (Group A, AM n = 24, PM n = 22; Group B, AM n = 14, PM n = 14) M1 vs M2 (Group A, AM n = 16, PM n = 15; Group B AM n = 18, PM n = 19).
[*] Indicates a significant difference ($P < 0.05$) between treatment weeks S1 vs M1 (Group A, AM n = 22, PM n = 23, Group B, AM n = 19, PM n = 18).

Table 2
Mean ± SEM for difference in μRR, μHR and HRV parameters obtained on S1 and M1 in males and females during AM and PM recordings.

HRV Parameter	Recording	Male (mean ± SEM)	Female (mean ± SEM)
ΔμRR (ms)	AM	68.95 ± 4.49	28.59 ± 5.35
	PM	118.41 ± 5.07[d]	−5.38 ± 3.48
ΔμHR (beats/min)	AM	−12.61 ± 0.78	−6.69 ± 1.56
	PM	−16.04 ± 0.55[c]	2.20 ± 1.13
ΔSTDRR (ms)	AM	36.82 ± 1.45	16.15 ± 1.86
	PM	45.86 ± 1.26[c]	8.20 ± 2.03
ΔRMSSD (ms)	AM	49.64 ± 2.33	9.14 ± 3.55
	PM	62.94 ± 2.00[c]	0.78 ± 1.93
ΔpNN50 (%)	AM	15.60 ± 0.68	2.69 ± 1.42
	PM	20.71 ± 0.62[d]	−0.12 ± 0.98
ΔRRTI	AM	3.39 ± 0.19	2.84 ± 0.34
	PM	5.51 ± 0.17[c]	0.02 ± 0.47
ΔLF/HF	AM	−0.48 ± 0.03	0.36 ± 0.15
	PM	−8.66 ± 0.59	−1.22 ± 0.5
ΔSD1 (ms)	AM	34.77 ± 1.65	6.47 ± 2.51
	PM	44.57 ± 1.42[c]	0.55 ± 1.36
ΔSD2 (ms)	AM	41.08 ± 1.45	22.00 ± 2.07
	PM	48.62 ± 1.41[b,1]	9.85 ± 3.27

Superscripts [a, b, c, d] indicate significant associations between the response variable and sex at P < 0.05, P < 0.01, P < 0.005 and P < 0.001, respectively.

specifically in the afternoon recording session for that group, although a similar trend (P = 0.051) was also seen between the afternoon sessions for Group B. Within the geometric analysis parameters, both SD1 and SD2 were higher at the start of the music week compared to the start of the silent week in all instances. The differences in SD1 were statistically significant (P < 0.001) in Group A in the afternoon with a similar trend (P = 0.056) being observed in the morning, whereas in Group B the differences were significant in both the morning (P < 0.01) and afternoon, (P < 0.005). With regard to SD2 all differences were statistically significant (Group A, AM P < 0.001, PM P < 0.001; Group B, AM P < 0.001, PM, P < 0.005).

Results of the GRA, summarised in Table 2, indicated that changes in HRV parameters in response to auditory stimulation were not significantly associated with differences in age, breed and duration of stay. Reason for kennelling was significantly associated with the mean change in RRTI (PM), specifically; the increase in RRTI in response to auditory stimulation was largest in S (5.37 ± 0.33), followed by TR (4.22 ± 1.11) and U (2.69 ± 0.30) dogs (W dogs was not included as there was only one value available for analysis). Gonadal status was also significantly associated with the response of RRTI (Entire 7.02 ± 0.55, Neutered 2.8 ± 0.17) and SD2 (Entire 59.50 ± 4.78, Neutered 29.41 ± 1.18) to auditory stimulation (PM only); in each case the value of RRTI and SD2 obtained on M1 was higher than the value obtained on S1 in entire dogs relative to neutered dogs. Sex was significantly associated with the changes in response to auditory stimulation of a larger number of the HRV parameters, particularly in the afternoon recording session. In each case, there was larger increase in STDRR, RMSSD, pNN50, RRTI, SD1 and SD2 and decrease in LF/HF in males relative to females, as shown in Table 2.

3.3. Behavioural data

The results of the behavioural observations are presented in full in Table 4. Specific variables that were found to differ significantly within or between treatment weeks are depicted in Figs. 4 and 5. Within the silent week, regardless of whether it occurred before (Group A) or after (Group B) the music week, there were no statistically significant differences in any of the behavioural measures studied.

Within the music week, however, significant changes were observed in behaviour between the M1 and M2 recording periods. Specifically, in the mornings subjects in Group A spent significantly (P < 0.05) more

Table 3
Mean values ± SEM for HRV variables in Group A and Group B over different experimental sessions S1, S2, M1 and M2 for both AM and PM recordings.

Analyses	HRV variable	Group	Recording	S1 (mean ± SEM)	S2 (mean ± SEM)	M1 (mean ± SEM)	M2 (mean ± SEM)
Time-domain	STDRR (ms)	A	AM	97.6 ± 6.01***	101.08 ± 8.3	130 ± 11.82	115 ± 8.02
			PM	91.34 ± 5.67[a,****]	120.31 ± 10.85	130 ± 8.62	112 ± 7.49
		B	AM	102.35 ± 6.95****	110.13 ± 7.26	140 ± 10.29[a]	112 ± 9.76
			PM	96.07 ± 8.02****	114.95 ± 12.39	135 ± 9.90[a]	112 ± 9.06
	RMSSD (ms)	A	AM	81.9 ± 10.54	80.54 ± 8.37	121 ± 18.83	108 ± 13.14
			PM	73.57 ± 6.66[a,****]	119.15 ± 18	124 ± 12.12	105 ± 13.76
		B	AM	88.39 ± 10.64**	97.73 ± 10.87	141 ± 16.44	103 ± 14.32
			PM	82.35 ± 9.56***	109.63 ± 16.35	129 ± 15.25	102 ± 11.33
	pNN50 (%)	A	AM	29.81 ± 3.28*	31.47 ± 3.68	42 ± 5.71	40 ± 5.01
			PM	28.32 ± 2.92[a,***]	42.51 ± 5.28	45 ± 4.3	40 ± 5.75
		B	AM	33.45 ± 4.00**	39.3 ± 5.05	49 ± 4.29	38 ± 4.67
			PM	32.82 ± 4.22****	42.83 ± 5.99	48 ± 4.1	41 ± 4.2
	RRTI	A	AM	19.54 ± 0.86**	19.51 ± 1.01	23 ± 1.17[c]	20 ± 1.1
			PM	18.03 ± 1.00***	19.15 ± 1.07	23 ± 1.53[a]	19 ± 0.79
		B	AM	20.03 ± 1.08*	21.15 ± 1.06	24 ± 1.46[b]	19 ± 1.12
			PM	18.39 ± 1.06**	19.87 ± 1.69	22 ± 1.01[a]	20 ± 1.18
f-domain	LF/HF	A	AM	1.27 ± 0.13	1.3 ± 0.13	1 ± 0.15	1.2 ± 0.18
			PM	1.35 ± 0.13***	0.97 ± 0.15	0.8 ± 0.11	1 ± 0.15
		B	AM	1.29 ± 0.19	1.16 ± 0.21	1 ± 0.19	1.1 ± 0.17
			PM	1 ± 0.12	0.86 ± 0.14	0.8 ± 0.11	0.8 ± 0.08
Geometric	SD1 (ms)	A	AM	58 ± 7.5	57 ± 5.93	86 ± 13.34	76 ± 9.31
			PM	52.06 ± 4.72[a,****]	84.35 ± 12.75	88 ± 8.58	74 ± 9.75
		B	AM	62.57 ± 7.54**	68.58 ± 7.69	99 ± 11.81	73 ± 10.13
			PM	58.29 ± 6.77***	77.59 ± 11.58	91 ± 10.8	72 ± 8.02
	SD2 (ms)	A	AM	122.6 ± 6.00****	129.92 ± 10.28	160 ± 12.15[a]	140 ± 8.56
			PM	115.92 ± 7.51****	144.33 ± 10.5	160 ± 9.49[a]	138 ± 7.05
		B	AM	128.39 ± 7.82****	137.34 ± 8.05	169 ± 10.44[b]	139 ± 10.59
			PM	121.87 ± 9.37***	140.77 ± 14.45	161 ± 10.58[a]	140 ± 10.4

Superscripts [a, b, c] indicate significant differences at P < 0.05, P < 0.01, and P < 0.005 respectively, within a treatment week S1 vs S2 (Group A, AM n = 24, PM n = 22; Group B, AM n = 14, PM n = 14), M1 vs M2 (Group A, AM n = 16, PM n = 15; Group B, AM n = 18, PM n = 19). Significant differences between treatment weeks S1 vs M1 (Group A, AM n = 22, PM n = 23; Group B, AM n = 19, PM n = 18) are indicated as follows *P < 0.05, **P < 0.01, ***P < 0.005, and ****P < 0.001. *f*: frequency; STDRR: standard deviation of the RR interval; RMSSD: the square root of the mean of the sum of the squares of differences between successive RR intervals; pNN50: number of pairs of successive RR intervals that differ by more than 50 ms (NN50) expressed as a percentage; RRTI: RR triangular index; LF/HF: low frequency/high frequency ratio: SD1; standard deviation 1 of the Poincaré Plot; SD2: standard deviation 2 of the Poincaré Plot.

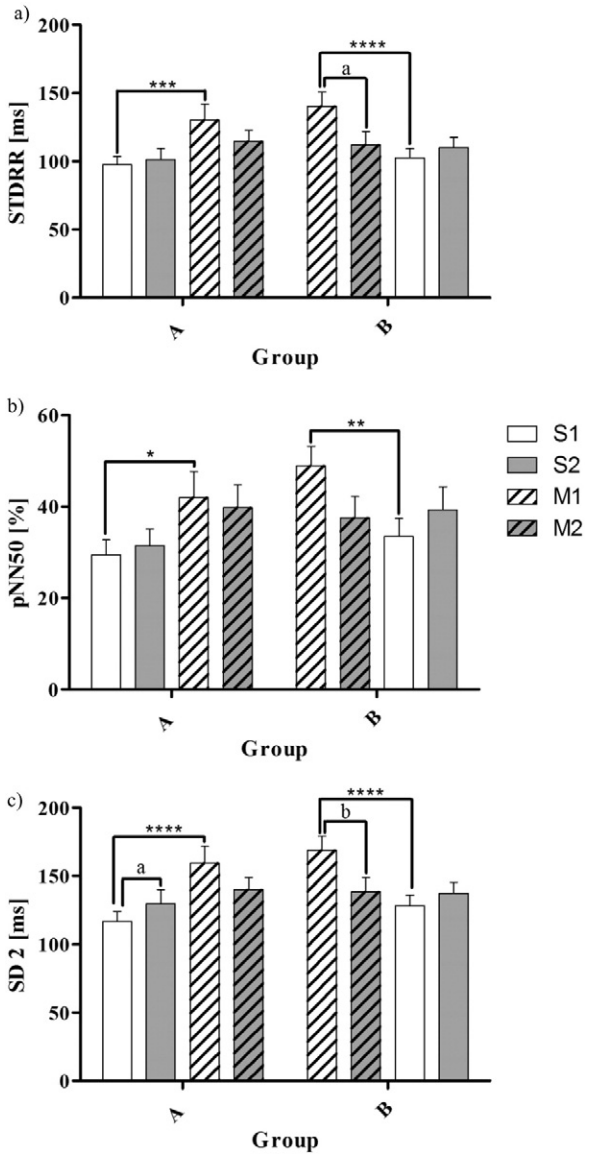

Fig. 3. Bar charts represent mean ± SEM values of a) STDRR [ms], b) pNN50 [%] and c) SD2 [ms] for Group A and Group B during the AM recording session. Superscripts [a] and [b] indicate significant differences at $P < 0.05$ and $P < 0.01$ respectively, within a treatment week (S1 vs S2 Group A, n = 24; Group B, n = 14 & M1 vs M2 Group A n = 16; Group B, n = 18). Superscripts *, **, ***, and **** indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.005$ and $P < 0.001$ respectively, between treatment weeks (S1 vs M1 Group A, n = 22; Group B, n = 19).

time sitting, with a non-significant increase in the amount of time standing and a non-significant decrease in the time lying. Subjects in Group B showed a similar pattern of behavioural changes except statistical significance was observed relative to the proportion of time standing ($P < 0.005$) and lying ($P < 0.05$). In the afternoons, Groups A and B again showed a similar pattern of behavioural changes between M1 and M2. The proportion of time spent sitting being similar, but the amount of time spent lying decreased and standing increased. Statistical significance ($P < 0.05$) was only achieved in relation to the proportion of time spent standing in Group A.

When comparing the behaviour of subjects between S1 and M1 it was found that, when music was played the dogs spent a significantly greater proportion of their time lying (Group A AM and PM $P < 0.005$; Group B AM $P < 0.001$, PM $P < 0.005$) and significantly less time standing (Groups A and B AM and PM $P < 0.001$). In both groups, in the morning and in Group A in the afternoon, these behavioural changes coincided with an increase in the proportion of time spent sitting but this was only statistically significant ($P < 0.001$) in the morning for Group A subjects.

There was no difference in the proportion of time spent indoors between the two observation periods in the silent week. In the music week dogs in Group B, spent proportionately less time ($P < 0.001$) inside during the morning observation period. Comparison of the proportion of time spent in the inside unit between S1 and M1 indicated that dogs in Group A, during the afternoon, ($P < 0.05$) and dogs in Group B during both the morning ($P < 0.001$) and afternoon ($P < 0.01$) sessions spent a greater proportion of the observation periods in the inside unit.

Comparison of vocalisations between S1 and M1 indicated that in the morning observation periods when music was being played, dogs spent a significantly greater proportion of their time silent (Groups A and B $P < 0.05$). A similar pattern was seen in the afternoon but during that observation period the difference was not found to be statistically significant. The increase in the proportion of time dogs were silent was accompanied by a non-significant decrease in the proportion of time animals were whining and barking.

In contrast to the analysis of the HRV data, no associations were found between the changes in the behavioural variables and the sex of the study animals. In response to auditory stimulation GRA demonstrated significant associations between age and the changes in the proportion of time spent sitting (AM only $P < 0.05$) and barking (AM only $P < 0.05$) in response to auditory stimulation. Dogs aged between 0.5 and 5 years of age spent approximately 21.5% (0.5–2 years 23.42 ± 3.27%, 2–3 years 21.39 ± 1.36%, 3–5 years 19.82 ± 3.34%) more time sitting, when the music was played, whereas the increase in the proportion of time spent sitting by the older and younger dogs was less (5–8 years 9.86 ± 2.99%, <0.5 years −9.86 ± 6.65%). The proportion of time dogs spent barking, was also decreased in dogs aged between 0.5 and 8 years (0.5–2 years −9.70 ± 2.45%, 2–3 years −9.77 ± 0.83%, 3–5 years −6.43 ± 1.56% and 5–8 years −7.44 ± 2.70%) but was increased in dogs aged <0.5 years (0.90 ± 0.52). A significant association was also found between the duration of stay and the changes in the proportion of time spent sitting in both AM ($P < 0.001$) and PM ($P < 0.05$) recording sessions. In both cases the increase in the proportion of time spent sitting was greatest in the dogs kennelled for more than 3 months (3–6 months AM, 50.45 ± 6.00%, PM, 36.79 ± 3.09%; 6–-9 months AM, 41.8 ± 4.29, PM, 19.43 ± 4.15%) and was significantly less or decreased for the dogs kennelled for <3 months (AM, 5.67 ± 0.61%, PM, −0.11 ± 0.62%). GRA revealed that the reason for being at the kennels was associated with significant ($P < 0.05$) changes in the proportion of time dogs spent sitting (AM), silent (PM) and barking (PM), when auditory stimulation was provided. The increase in the proportion of time spent sitting was approximately five fold larger in the TR (42.70 ± 3.00%) compared to the S (8.72 ± 1.32%) and U (7.67 ± 1.39%) dogs. With regard to vocalisation, S dogs showed the largest increase in the proportion of time spent silent, in response to classical music (6.41 ± 0.71%) and the largest decrease in the proportion of time spent barking (−5.69 ± 0.61%). U dogs showed a similar reciprocal increase in the proportion of time spent silent (3.91 ± 0.62%) and decrease in the proportion of time spent barking (−5.01 ± 0.46%). Whereas TR dogs showed an overall increase in the proportion of time spent silent (4.32 ± 2.18) with no difference in the proportion of time spent barking (0.54 ± 2.24%). Finally, an association was found between gonadal status and changes in the proportion of time spent sitting (AM) as this was increased in neutered dogs (17.24 ± 0.84%) but effectively unchanged in entire dogs (0.00 ± 1.87%) when auditory stimulation was provided.

3.4. Salivary cortisol data

The mean salivary cortisol concentrations are shown in Table 5. There was considerable variation in cortisol concentrations between subjects within each group/time and no statistically significant

Table 4
For each of the behavioural categories (Position, Location and Vocalisation) the data represents the mean values ± SEM of the percentage of time the dogs in Group A and Group B were observed performing each behavioural variable (Lying/Sitting/Standing, Inside, Silent/Whining/Barking) over different experimental sessions S1, S2, M1 and M2 for both AM and PM recordings.

Variable	Activity	Group	Recording	S1 (mean ± SEM)	S2 (mean ± SEM)	M1 (mean ± SEM)	M2 (mean ± SEM)
Position	Lying	A	AM	11.89 ± 3.11***	8.46 ± 3.72	32.00 ± 5.96	29.00 ± 7.45
			PM	14.15 ± 4.13***	18.80 ± 4.00	35.00 ± 5.50	23.00 ± 4.57
		B	AM	15.45 ± 5.44****	13.67 ± 3.51	52.00 ± 5.78[a]	31.00 ± 6.85
			PM	17.00 ± 5.44***	14.41 ± 3.51	44.00 ± 5.47	31.00 ± 6.85
	Sitting	A	AM	13.25 ± 2.61****	17.42 ± 3.80	17.00 ± 3.75[a]	31.00 ± 4.66
			PM	18.32 ± 2.88	15.29 ± 2.84	27.00 ± 3.65	25.00 ± 4.29
		B	AM	16.56 ± 4.82	19.55 ± 4.39	24.00 ± 4.68	19.00 ± 4.38
			PM	21.60 ± 4.82	12.40 ± 4.39	21.00 ± 3.67	24.00 ± 4.38
	Standing	A	AM	74.86 ± 4.63****	74.12 ± 4.80	37.00 ± 4.90	53.00 ± 7.78
			PM	67.53 ± 4.42****	65.91 ± 4.79	38.00 ± 4.15[a]	52.00 ± 4.73
		B	AM	67.99 ± 7.14****	66.77 ± 5.17	24.00 ± 3.73[c]	50.00 ± 6.12
			PM	61.39 ± 7.14****	68.32 ± 5.17	36.00 ± 4.51	46.00 ± 6.12
Location	Inside	A	AM	81.90 ± 5.86	78.04 ± 7.34	83.00 ± 6.41	79.00 ± 6.71
			PM	79.95 ± 5.20*	76.49 ± 5.86	83.00 ± 6.28	79.00 ± 6.79
		B	AM	85.93 ± 1.87****	88.82 ± 3.65	97.00 ± 1.13[b]	86.00 ± 4.51
			PM	85.98 ± 1.87**	82.51 ± 3.65	96.00 ± 1.12	88.00 ± 4.51
Vocalisation	Silent	A	AM	75.32 ± 4.38*	71.22 ± 4.32	89.00 ± 2.06	86.00 ± 7.72
			PM	80.62 ± 3.80	81.25 ± 3.84	85.00 ± 2.98	85.00 ± 6.64
		B	AM	82.72 ± 3.80*	78.27 ± 3.78	94.00 ± 1.66	91.00 ± 4.49
			PM	87.79 ± 3.80	80.27 ± 3.78	93.00 ± 1.71	91.00 ± 4.49
	Whining	A	AM	4.50 ± 2.09	2.59 ± 1.61	1.60 ± 0.81	0.90 ± 3.75
			PM	3.91 ± 1.53	1.29 ± 0.88	1.80 ± 0.92	0.60 ± 4.13
		B	AM	3.30 ± 2.10	2.23 ± 1.63	0.40 ± 0.19	0.40 ± 3.49
			PM	0.90 ± 2.10	1.27 ± 1.63	0.80 ± 0.36	1.10 ± 3.49
	Barking	A	AM	20.07 ± 20.07	26.19 ± 26.19	9.40 ± 9.35	14.00 ± 0.62
			PM	14.66 ± 14.66	16.80 ± 16.80	12.00 ± 11.82	12.00 ± 0.41
		B	AM	13.66 ± 3.55	19.50 ± 3.86	5.80 ± 1.62	8.20 ± 0.31
			PM	11.31 ± 3.55*	18.46 ± 3.86	5.60 ± 1.48	7.30 ± 0.31

Superscripts [a, b, c] indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.005$ respectively, within a treatment week S1 vs S2 (Group A, AM n = 24, PM n = 23; Group B, AM n = 17, PM n = 18), M1 vs M2 (Group A, AM n = 18, PM n = 18; Group B, AM n = 19, PM n = 20). Significant differences between treatment weeks S1 vs M1 (Group A, AM n = 24, PM n = 24; Group B, AM n = 19, PM n = 18) are indicated as follows *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, and ****$P < 0.001$.

differences were found within or between weeks. In Group A (AM and PM), the mean cortisol concentrations were on average higher in the S1 compared to the M1 sample, however, this difference was not statistically significant.

3.5. '9-Day' study

3.5.1. HRV

The mean values ± SEM for μRR and μHR and all HRV parameters studied are presented in Table 6. Fig. 6 depicts the mean values for pNN50 (AM) across all 9-days of the study. Due to the small sample size statistical analysis of the data were limited, however, the results suggest that for many of the HRV parameters, namely pNN50, μRR, STDRR, RMSSD, SD1 and SD2 values dramatically increased on M1 relative to S1 as observed in the main study. The parameters subsequently fell to a low level on M2. A gradual increase was then seen until M4 which was followed by a decrease till M7 with no further change seen between M7 and S2. The opposite pattern of change was seen in μHR and LF/HF.

3.5.2. Behaviour

For each of the behavioural variables the mean ± SEM percentage of time dogs were observed performing each behavioural activity across all 9 days of the study are presented in Table 7. As observed in the main study, from S1 to M1 there was a marked reduction in the proportion of time dogs were observed standing and an increase in the proportion of time dogs were observed lying/sitting. These observations appeared to revert by M2 where there was a marked increase in the proportion of time dogs were observed standing and reduction in the proportion of time they were observed lying/sitting. The variable position was then seen to change again on M4 whereby the proportions of time lying/sitting increased at the expense of the proportion of time spent standing. Between M4 and M7 the proportion of time the dogs are observed standing slowly increased until S2. The location of the dogs within their kennel remained unchanged before, during and after auditory stimulation. From S1 to M1 there was a marked reduction in the proportion of time dogs spent barking and a corresponding increase in the proportion of time being silent but these changes were already waning by M2. Again, on M4 there was a marked reduction in the level of barking which slowly begins to increase over the remainder of the observation period until S2.

3.5.3. Salivary cortisol

Mean values ± SEM of salivary cortisol measured during the '9-Day' study are presented in Table 8. Concentrations of salivary cortisol were very variable and no particular trend was observed.

4. Discussion

The findings of this study add to the literature relating to enrichment of the kennel environment. It is widely recognised that the kennel environment contains a series of potential stressors, including novelty, loss of an attachment figure, social isolation, etc. and that stress may have negative effects, not only on an animal's health but, due to associated changes in behaviour, its chances of being rehomed [69]. This study demonstrates that, in a working rescue kennel environment, exposure to 6.5 h of classical music results in significant changes in both physiological and behavioural markers that would suggest a reduction in the stress experienced by kennelled dogs. The response to stressors is highly complex, involving both psychological and physiological changes. In this study behaviour was used to provide an integrative measure of the complete stress response while measurement of HRV and salivary cortisol concentrations allowed non-invasive assessment of changes in activity of the autonomic nervous system and HPA axis. The results demonstrated similar effects on behaviour, HRV and cortisol, regardless of whether music was played before or after the control period and

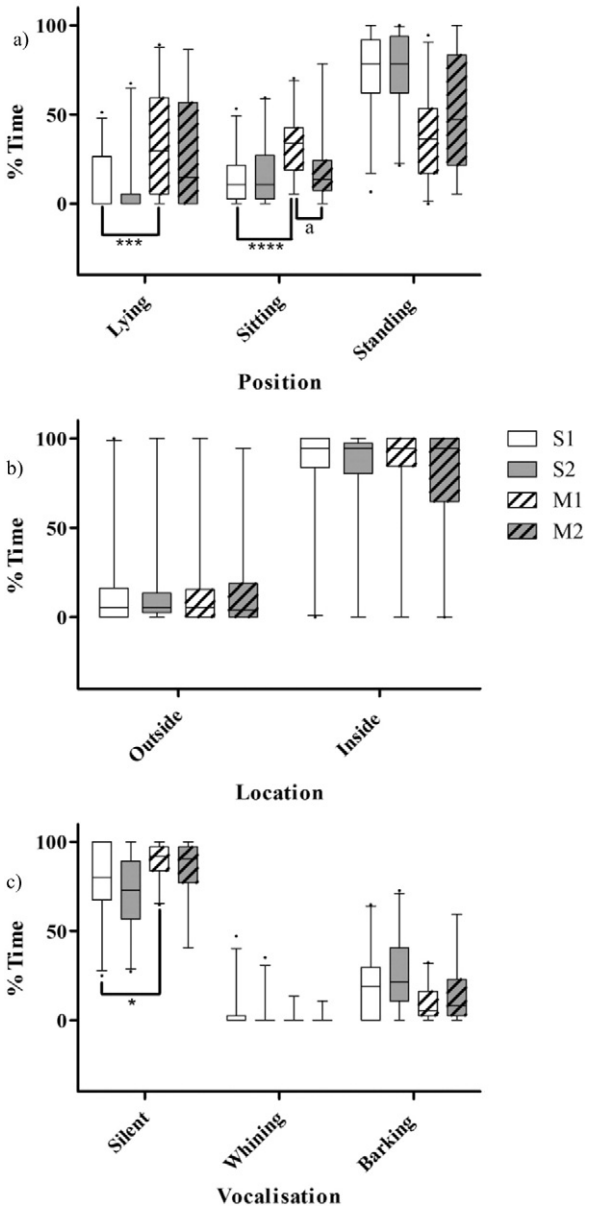

Fig. 4. Box and whisker plots (5–95 percentiles) represent the mean % of time dogs in Group A were observed performing each behavioural variable within categories a) Position, b) Location and c) Vocalisation, during the AM recording session. Superscript [a] indicates a significant difference ($P < 0.05$) within a treatment week (S1 vs S2 n = 24 & M1 vs M2 n = 18). Superscripts *, **, ***, and **** indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.005$, $P < 0.001$ respectively, between treatment weeks (S1 vs M1 n = 24).

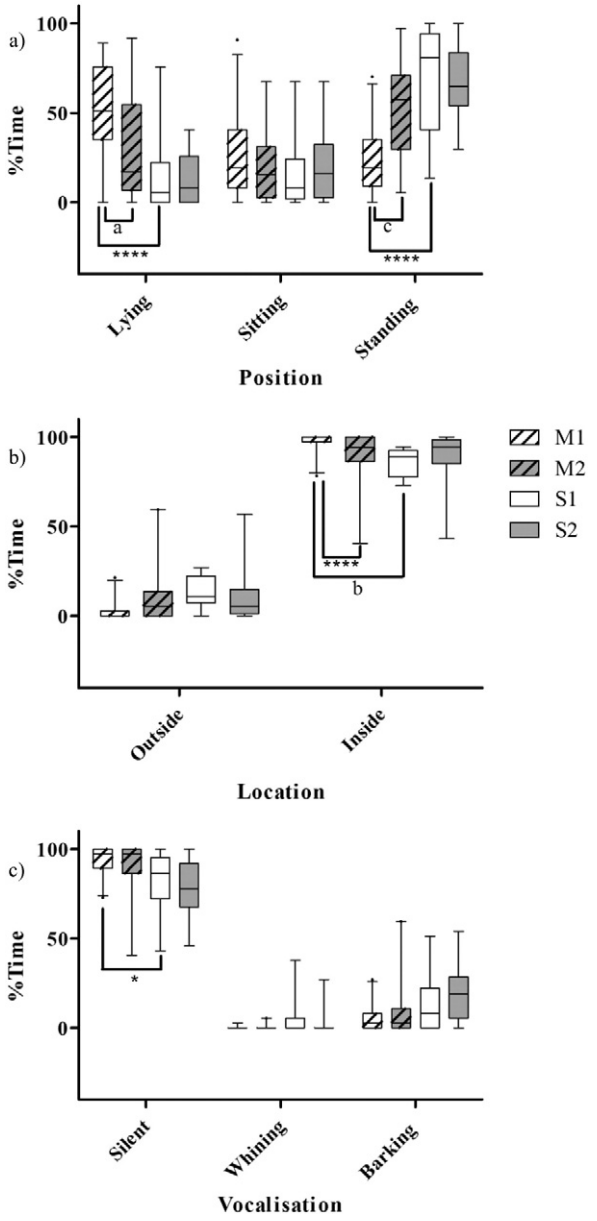

Fig. 5. Box and whisker plots (5–95 percentiles) represent the mean % time dogs in Group B were observed performing each behavioural variable within categories a) Position, b) Location and c) Vocalisation, during the AM recording session. Superscripts [a], [b], and [c] indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.005$ respectively, within a treatment week (M1 vs M2 n = 19 & S1 vs S2 n = 17). Superscripts *, **, ***, and **** indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.005$, $P < 0.001$ respectively, between treatment weeks (M1 vs S1, n = 19).

were not affected by the duration of stay in the kennels. This would suggest that the observed results are not an effect of habituation to the environment/routine within the kennel but reflect a true physiological/psychological response to the auditory stimulation. Interestingly, the effects of music on HRV and behaviour were not maintained over the 7 days during which the dogs were exposed to auditory stimulation. This result suggests that the dogs become refractory to these physiological/psychological effects of classical music when the same playlist is used repeatedly and a subsequent study suggested that this occurs in as little as one day. Finally, the physiological response to auditory stimulation was found to be significantly affected by the sex of the dogs studied. Changes in HRV parameters associated with a reduction in stress appeared to be greater in males compared to females.

HRV has been used extensively to assess autonomic nervous system function in humans and a variety of animal species. Such studies have used HRV analysis as a means to investigate responses to psychophysiological [65] and mental stress [50]. The method relies upon analysis of the variation in the R–R interval which can be recorded easily and non-invasively, using standard ECG recording apparatus or portable

Table 5
Mean values ± SEM for salivary cortisol [ng/ml] levels in Group A and Group B across different experimental sessions, S1, S2, M1 and M2 for both AM and PM recordings.

Group	Sample period	S1 (mean ± SEM)	S2 (mean ± SEM)	M1 (mean ± SEM)	M2 (mean ± SEM)
A	AM	1.4 ± 0.2	2.16 ± 0.91	1.19 ± 0.25	1.54 ± 0.43
	PM	2.08 ± 0.51	1.22 ± 0.23	1.59 ± 0.29	1.58 ± 0.45
B	AM	0.97 ± 0.51	0.96 ± 0.23	1.17 ± 0.29	1.36 ± 0.45
	PM	0.60 ± 0.13	0.86 ± 0.21	1.17 ± 0.27	1.28 ± 0.37

Table 6
Mean values ± SEM for μRR, μHR and HRV variables collected during '9-Day' study.

Parameter	Recording	S1 (mean ± SEM)	M1 (mean ± SEM)	M2 (mean ± SEM)	M3 (mean ± SEM)	M4 (mean ± SEM)	M5 (mean ± SEM)	M6 (mean ± SEM)	M7 (mean ± SEM)	S2 (mean ± SEM)
μRR	AM	454.88 ± 19.22	555.6 ± 28.43	444.07 ± 8.47	495.1 ± 33.23	541 ± 29.53	498.98 ± 22.16	464.97 ± 11.07	456.6 ± 2.69	467.47 ± 15.31
	PM	450.73 ± 33.47	519.1 ± 21.88	479.07 ± 29.35	496.9 ± 20.9	518 ± 25.63	507.95 ± 29.95	489.2 ± 25.29	482.18 ± 13.8	506.51 ± 23.12
μHR	AM	137.25 ± 5.95	117.2 ± 7.45	142.95 ± 2.29	130 ± 8.02	119 ± 6.11	128.15 ± 6.15	136.82 ± 3.81	139.07 ± 1.36	136.06 ± 5.95
	PM	138.78 ± 10.06	126.6 ± 4.93	134.28 ± 7.75	127.7 ± 5.67	124 ± 6.97	125.85 ± 7.62	128.83 ± 6.01	129.61 ± 3.85	127.93 ± 6.80
STDRR	AM	81.17 ± 6.42	148.8 ± 11.23	108.05 ± 8.89	123.4 ± 13.55	137 ± 22.18	121.17 ± 11.78	110.32 ± 13.85	103.71 ± 16.42	105.38 ± 7.53
	PM	80.95 ± 7.25	142.6 ± 14.18	122.19 ± 17.7	114.2 ± 9.11	124 ± 3.55	116.87 ± 6.93	98.03 ± 14.08	91.53 ± 6.69	131.4 ± 4.6
RMSSD	AM	70.9 ± 13.23	174.1 ± 28.04	97.07 ± 10.95	123.6 ± 9.13	146 ± 26.92	127.28 ± 15.66	112.76 ± 2.97	89.64 ± 17.58	94.77 ± 11.25
	PM	95.07 ± 13.29	165.2 ± 15.4	113.56 ± 14.89	120 ± 9.34	144 ± 20.89	127.07 ± 14.4	109.88 ± 27.42	89.33 ± 26.33	125.03 ± 7.69
pNN50	AM	24.12 ± 4.32	58.24 ± 6.04	34.64 ± 4.57	48.23 ± 4.21	51.2 ± 9.56	45.9 ± 2.93	41.64 ± 1.62	33.53 ± 7.03	35.82 ± 3.4
	PM	34.15 ± 2.84	54.61 ± 4.98	42.96 ± 5.09	43.75 ± 2.1	49.9 ± 5.7	48.01 ± 4.87	38.32 ± 7.69	31.7 ± 9.97	48.54 ± 1.58
RRTI	AM	14.56 ± 1.82	17.98 ± 1.98	19.68 ± 0.48	20.25 ± 2.66	18.7 ± 2.36	18.64 ± 1.96	18.17 ± 3.17	17.38 ± 1.78	17.66 ± 1.27
	PM	12.00 ± 0.01	18.57 ± 0.7	17.94 ± 1.97	17.17 ± 2.16	17.1 ± 2.03	17.26 ± 1.57	15.23 ± 1.79	15.51 ± 0.93	21.09 ± 1.58
LF:HF	AM	1.64 ± 0.64	0.36 ± 0.09	0.48 ± 0.02	0.54 ± 0.09	0.36 ± 0.07	0.47 ± 0.1	0.62 ± 0.18	0.83 ± 0.33	0.56 ± 0.01
	PM	0.69 ± 0.18	0.44 ± 0.01	0.36 ± 0.07	0.46 ± 0.03	0.48 ± 0.06	0.47 ± 0.12	0.73 ± 0.16	0.70 ± 0.17	0.34 ± 0.04
SD1	AM	50.17 ± 9.36	123.2 ± 19.85	68.68 ± 7.74	87.49 ± 6.46	103 ± 19.05	90.07 ± 11.08	79.8 ± 2.1	63.44 ± 12.45	67.06 ± 7.96
	PM	67.27 ± 9.4	116.9 ± 10.91	80.36 ± 10.55	84.93 ± 6.6	102 ± 14.78	89.93 ± 10.21	77.76 ± 19.42	63.22 ± 18.63	88.5 ± 5.44
SD2	AM	91.83 ± 8.14	163.5 ± 7.25	151.73 ± 10.64	135.5 ± 18.79	140 ± 27.93	137.43 ± 15.46	110.7 ± 21.35	110.3 ± 23.2	162.39 ± 11.23
	PM	7.83 ± 5.54	29.63 ± 17.11	45.78 ± 22.89	30.45 ± 15.23	11.2 ± 5.58	15.88 ± 7.94	29.86 ± 14.93	0.97 ± 0.68	16.74 ± 9.67

Fig. 6. Mean ± SEM of pNN50 [%] collected on S1, M1, M2, M3, M4, M5, M6, M7 and S2. All data displayed is that collected during the AM session.

heart rate monitors. While the majority of the published canine HRV studies have concentrated on links and associations between HRV and disease e.g., cardiovascular disease [11,13,42,45] diabetes [48], one other study has used HRV as a means to assess the effects of environmental enrichment in kennelled dogs [7]. In that study the environmental enrichment tested was human contact and, as in the current study, the results of the HRV analysis were supplemented with measurements of behaviour and salivary cortisol concentrations. While the results of that study showed that human contact had a positive effect on canine behaviour there were poor correlations between the induced behavioural changes and changes in HRV and cortisol. The changes in cortisol were, as in the current study not robust, due to high inter-subject variability. It was, however, concluded that the relationship between cortisol and the behavioural data was stronger than the correlations between the HRV and behaviour. That said changes in a number of frequency domain HRV parameters were associated with increased human contact suggesting increased vagal tone as a result of environmental enrichment. In contrast to the results of Bergamasco et al. [7] the environmental enrichment used in the current study, namely music, was associated with parallel changes in both HRV and behaviour. The initial response to music was characterised by an increased R–R interval and a consequent reduction in mean heart rate. While increased vagal tone could bring about these changes in cardiac activity, upon initial exposure to music the dogs spent a greater proportion of their time lying rather than standing and in silence rather than barking and as such the decrease in heart rate and increase in R–R interval may reflect reduced physical activity. The majority of the observed changes in the HRV parameters, induced by auditory stimulation, were in the time domain analysis parameters STDRR, RMSSD, RRTI and pNN50 and the geometric parameters SD1 and SD 2. The frequency domain parameter LH/HF ratio was only significantly affected by music in the afternoon recording sessions and in the dogs that received the music week after the silent week. A similar trend was noted in the afternoon recording session of the dogs that received the music week first. STDRR is a measure of the overall variability present within the cardiac activity, it reflects long term components of variation and as such is thought to represent different states such as sleep wake cycles and activity [55]. RMSSD and pNN50 are based on interval differences and correspond to short term variations in cardiac activity and are, as such, thought to be associated with vagal-mediated control of the heart [55,58]. In this study the initial exposure to music was associated with increased values for STDRR, while this could be a direct consequence of auditory stimulation, increased human contact has also been found to lead to increased STDRR [7] and preparing the subject for the recording session does involve close human contact, however this contact is similar for all sessions whether music is being played or not. The increase in STDRR on the first day of music could also be secondary to the increased proportion of time dogs spent lying down when the music was played [58]. However the observation of significantly increased values for both

Table 7
For each of the behavioural variables the data represents (Position; P, Location; L and Vocalisation; V) the data represents the mean values ± SEM of the percentage of time the dogs were observed performing each behavioural activity (Lying/Sitting/Standing, Inside, Silent/Whining/Barking) over the '9-Day' study.

Variable	Activity	Recording	S1 (mean ± SEM)	M1 (mean ± SEM)	M2 (mean ± SEM)	M3 (mean ± SEM)	M4 (mean ± SEM)	M5 (mean ± SEM)	M6 (mean ± SEM)	M7 (mean ± SEM)	S2 (mean ± SEM)
P	Lying	AM	24.07 ± 3.73	64.86 ± 6.34	9.46 ± 2.34	45.27 ± 6.08	39.86 ± 10.55	35.14 ± 10.64	12.16 ± 4.62	8.11 ± 1.56	13.51 ± 4.13
		PM	19.59 ± 7.35	42.23 ± 3.09	14.86 ± 7.84	34.46 ± 7.91	45.95 ± 5.73	52.03 ± 5.33	13.51 ± 9.30	15.96 ± 8.70	12.61 ± 5.91
	Sitting	AM	8.29 ± 4.81	17.57 ± 10.44	20.95 ± 13.17	14.19 ± 6.18	18.24 ± 11.33	17.57 ± 7.44	22.97 ± 13.49	24.32 ± 10.92	27.03 ± 20.29
		PM	19.59 ± 7.67	14.19 ± 11.54	25.00 ± 17.14	15.54 ± 10.25	14.86 ± 7.36	11.49 ± 4.46	20.27 ± 11.28	30.32 ± 5.91	24.32 ± 5.63
	Standing	AM	67.65 ± 8.41	17.57 ± 6.09	69.59 ± 12.45	40.54 ± 11.20	41.89 ± 13.45	47.30 ± 13.89	64.86 ± 14.89	67.57 ± 9.74	59.46 ± 19.49
		PM	60.81 ± 5.23	43.58 ± 11.09	60.14 ± 18.77	50.00 ± 11.60	39.19 ± 5.68	36.49 ± 5.46	66.22 ± 13.35	53.72 ± 11.46	57.66 ± 5.91
L	Inside	AM	95.27 ± 3.00	93.92 ± 1.29	93.24 ± 2.34	90.54 ± 3.22	90.54 ± 1.74	90.54 ± 2.81	94.59 ± 3.31	90.09 ± 2.38	93.69 ± 1.80
		PM	2.70 ± 1.91	8.86 ± 2.33	6.76 ± 5.12	13.51 ± 5.52	14.86 ± 3.58	6.08 ± 3.55	31.76 ± 7.51	5.63 ± 1.41	4.50 ± 4.50
V	Silent	AM	78.58 ± 2.40	100.00 ± 0.00	89.19 ± 2.92	95.95 ± 1.74	94.59 ± 1.91	93.24 ± 2.81	98.65 ± 0.78	95.50 ± 3.25	89.19 ± 9.49
		PM	81.76 ± 4.73	98.63 ± 0.79	95.95 ± 0.78	95.27 ± 2.31	93.92 ± 2.79	95.27 ± 2.31	91.89 ± 2.70	95.47 ± 3.24	95.35 ± 3.21
	Whining	AM	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		PM	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Barking	AM	21.42 ± 2.40	0.00 ± 0.00	10.81 ± 2.92	4.05 ± 1.74	5.41 ± 1.91	6.76 ± 2.81	1.35 ± 0.78	4.50 ± 3.25	10.81 ± 9.49
		PM	18.24 ± 4.73	1.37 ± 0.79	4.05 ± 0.78	4.73 ± 2.31	6.08 ± 2.79	4.73 ± 2.31	8.11 ± 2.70	4.53 ± 3.24	4.65 ± 3.21

RMSSD and pNN50 when music was initially played to the dogs in the kennel, would suggest that the changes in HRV are due to an increase in vagal tone and thus are as a result of the auditory stimulation. RRTI provides an additional measure of the overall change in RR interval duration as it is calculated as the total number of NN intervals divided by the number of NN intervals in the modal bin. As with the other measures of HRV, auditory stimulation was accompanied by an increase in RRTI which would again suggest increased vagal/decreased sympathetic drive to cardiac activity. The parameters SD1 and SD2 were also all higher when dogs were initially exposed to music, compared to the start of a silent week, the differences being statistically significant in seven of the eight comparisons. As an increase in SD1 is thought to indicate an increase in parasympathetic activity and an increase in SD2 a decrease in sympathetic activity [36,61,63], these changes would suggest changes in the activity of both divisions of the autonomic nervous system conducive with decreased anxiety and stress. Frequency parameter analysis of HRV describes the periodic oscillations in cardiac activity, split into bins of high, low and ultralow frequency. The interpretation of this form of analysis is controversial. It has long been proposed that the LF component reflects modulation of cardiac activity by both divisions of the autonomic nervous system while the HF component principally reflects parasympathetic activity and the LF/HF ratio the sympatho-vagal balance. However, the HF component is known to be affected by respiratory rate [58]. A recent review concluded that both LF and HF components could be affected by activity or drugs both of which affect the parasympathetic and sympathetic divisions of the autonomic nervous system. Therefore the LF/HF ratio could not simply be used as a marker of the sympatho-vagal balance [8]. In this study the LF/HF ratio was not consistently affected by auditory stimulation. A statistically significant decrease in the LF/HF ratio, in response to the classical music, was only seen in one group during one time of day. The dubiety of this result is further emphasised when compared to the study where human interaction was used as a form of environmental enrichment as in that study no consistent effect was seen on the LF/HF ratio and where a significant effect was seen, it was for the LF/HF ratio to increase following enrichment [7].

The initial effects of classical music on the behaviour of dogs within the kennel environment, in the current study, are in agreement with the results obtained by the two previous studies that looked at the effects of short term exposure to classical music, namely a reduction in barking/vocalisation and an increase in the proportion of time lying [38,68]. The observed behavioural changes are indicative of lower levels of arousal and stress and match the results obtained from human studies of the effects of music [22,39]. The observation that the initial effects of classical music on behaviour are in broad agreement with the results obtained from the HRV analysis suggesting that some of the behavioural responses of the dogs to the kennel environment may be driven by up-regulation of the parasympathetic and down-regulation of the sympathetic divisions of the autonomic nervous system. In response to auditory stimulation, a non-significant decrease in cortisol was observed. Whether this decrease drove or occurred in response to changes in behaviour could not be determined. However, the results do suggest parallel changes in the activity within a number of the body's physiological stress response systems. Previous work has reported that salivary cortisol is increased when dogs are introduced to a kennel environment but concentrations decrease after three days [16]. It is recognised, however, that cortisol concentrations are inherently variable and are affected by a host of variables such as previous housing [33] and are subject to dysregulation after chronic elevation [29], which may have affected our ability to see an effect of music on this variable.

The noted significant effects of classical music on both HRV and behaviour were the same regardless of whether the music week followed or preceded the silent week. This result demonstrates that the observed effects of music on both the physiological and behavioural responses to the kennel environment were in fact due to the auditory stimulation and not as a result of habituation to the kennel environment or the

Table 8
Mean values ± SEM for salivary cortisol data collected during the '9-Day' study.

Recording	S1 (mean ± SEM)	M1 (mean ± SEM)	M2 (mean ± SEM)	M3 (mean ± SEM)	M4 (mean ± SEM)	M5 (mean ± SEM)	M6 (mean ± SEM)	M7 (mean ± SEM)	S2 (mean ± SEM)
AM	0.80 ± 0.14	0.52 ± 0.12	0.55 ± 0.18	1.27 ± 0.46	0.50 ± 0.15	0.48 ± 0.11	0.82 ± 0.52	0.88 ± 0.30	1.35 ± 0.18
PM	0.68 ± 0.16	0.70 ± 0.25	1.34 ± 0.72	0.61 ± 0.21	0.78 ± 0.17	0.95 ± n/a	2.80 ± n/a	4.72 ± 2.39	6.96 ± 5.42

procedural aspects of the HRV recording and saliva collection both of which involved close human interactions. This conclusion is reinforced by the observation that few if any changes were seen in either HRV parameters or behaviour within the silent week.

GRA revealed several significant associations between the HRV/behavioural responses to auditory stimulation and the other variables present within the study population, namely sex, gonadal status, reason for kennelling and duration of stay within the kennel environment but no effects of BCS and breed. Of particular interest was the association seen between sex and the changes in HRV parameters in response to auditory stimulation which in general were larger in males compared to females. One possible explanation for this difference originates in sex-based genetic differences which allow males to utilise cortisol orientated stress responses in a more constructive way than females [24]. Interestingly the associations seen between the changes in HRV parameters and sex were more pronounced in the afternoon. It is likely that this reflects the fact that dogs may be less anxious/frustrated in the PM recording session as they have been fed twice, walked and socialized. The proportion of time spent sitting was significantly associated with age, reason for kenneling and duration of stay. However, as no association was found between these factors and the proportion of time spent standing or lying, we can conclude that these explanatory variables do not influence the behavioural response to auditory stimulation in kenneled dogs. A significant association was observed between barking and age that was driven by an increase in the proportion of time spent barking in the young (<6 month old) dogs, it is thought that this may reflect the lower degree of emotional regulation observed in juveniles [12] An association was seen between the changes in vocalisation (not including whining) and the reason for kenneling, however, the observed association is difficult to explain as while there was a reciprocal change in barking and silence in the stray dogs and a less well matched reciprocal change in the unwanted pets, the temporary refuge dogs exhibited an increase in the proportion of time spent in silence that was not matched by a reduction is the proportion of time spent barking. Of particular note with regard to both the HRV and behavioural results obtained in the current study was the fact that the effects of music tended to be lost over the course of the seven days during which the classical music was played within the kennel environment. This can be seen both within the M1 vs M2 comparisons and the lack of significant differences between the S2 and M2 recording periods. This striking result suggests that the dogs habituate to the auditory stimulation, when the same classical music playlist is used repeatedly. This contrasts with the finding that habituation did not occur in dogs exposed to other forms of physical enrichment including olfactory stimulation [25] and the provision of toys/chews [34]. Habituation of animals to sensory enrichment in a kennel environment has also been reported in cats in response to as little as 3 h of olfactory [21] and visual [20] stimulation. The result of the small follow on study suggests that the effects of the auditory stimulation begin to be lost as soon as the second day of exposure. The results of this follow on study also show a second decrease in physiological and psychological signs of stress over the first 4 days of auditory stimulation, however as this was again followed by an increase in our measures of physiological and behavioural stress, the results again suggest that any beneficial effects of exposure to classical music are short lived.

Within a working rescue kennel environment, the results of this study demonstrate that classical music can have a beneficial effect on dog behaviour. This is associated with changes in cardiac activity and heart rate variability and suggests that there is a change in the activity of the autonomic nervous system, away from sympathetic and towards parasympathetic dominance. The reduction in stress within this population of dogs could have both short and long term benefits with regard to health [17,19,40]. In addition through reduction of stress-related behaviours such as barking and timidity [32,56,67] music may have a beneficial effect on rehoming potential of dogs living in a kennelled environment. It was interesting to see that the effects, of auditory stimulation, were relatively short lived, when the same classical music playlist is used repeatedly. The results therefore suggest that dogs habituate when exposed to the same auditory stimulation for a seven day period and that the habituation may even occur within days. Further study is required to determine whether variation in the playlist would prevent habituation thus providing a more effective form of auditory enrichment, which would not only have beneficial effects on long term health of the animal but through effects on behaviour, may increase rehoming potential.

5. Conclusion

In this study, the findings indicate that dogs display reduced signs of physiological and psychological stress in response to auditory stimulation, relative to silent (control) conditions. Changes in HRV and behavioural data suggest that classical music is an appropriate enrichment technique with the ability to considerably reduce the stress experienced by dogs within a working rescue kennel environment. Interestingly, the results also highlights that the dogs become habituated to the calming effects of music as soon as the second day of exposure and that the effects of music as an environmental enrichment tool may be more pronounced in males compared to females. Consequently, follow on studies are underway to determine the most effective use of music as an environmental enrichment tool within a working kennel environment. The findings of this study and future work are useful to organisations such as the Scottish SPCA who constantly strive to improve kennel management and enhance the welfare of the animals in their care.

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