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'The effect of different genres of music on the stress levels of kennelled dogs'

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HIGHLIGHTS

- Auditory enrichment causes positive behavioural and physiological changes in kennelled dogs.
- Different genres, particularly Soft Rock and Reggae, were associated with increased HRV and more relaxed behaviours.
- Increasing variety of music minimises the extent of habituation to auditory enrichment.

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ABSTRACT

Classical music has been shown to reduce stress in kennelled dogs; however, rapid habituation of dogs to this form of auditory enrichment has also been demonstrated. The current study investigated the physiological and behavioural response of kennelled dogs ($n = 38$) to medium-term (5 days) auditory enrichment with five different genres of music including Soft Rock, Motown, Pop, Reggae and Classical, to determine whether increasing the variety of auditory stimulation reduces the level of habituation to auditory enrichment. Dogs were found to spend significantly more time lying and significantly less time standing when music was played, regardless of genre. There was no observable effect of music on barking, however, dogs were significantly ($z = 2.2$, $P < 0.05$) more likely to bark following cessation of auditory enrichment. Heart Rate Variability (HRV) was significantly higher, indicative of decreased stress, when dogs were played Soft Rock and Reggae, with a lesser effect observed when Motown, Pop and Classical genres were played. Relative to the silent period prior to auditory enrichment, urinary cortisol:creatanine (UCCR) values were significantly higher during Soft Rock ($t = 2.781$, $P < 0.01$) and the second silent control period following auditory enrichment ($t = 2.46$, $P < 0.05$). Despite the mixed response to different genres, the physiological and behavioural changes observed remained constant over the 5d of enrichment suggesting that the effect of habituation may be reduced by increasing the variety of auditory enrichment provided.

1. Introduction

Dogs are kennelled for a variety of reasons including boarding, military, assistance training, research and rescue [44]. During kennelling, dogs are exposed to a variety of psychogenic stressors, including spatial and social restriction from humans and conspecifics, extremely high noise levels [38], lack of control and novelty. For dogs in rescue centres, the situation is further compounded by the fact that many have been separated from their owners [46] and can be in poor physical condition having been neglected and/or abused. Previous studies have shown that a physiological stress response is stimulated on arrival at a kennel and can remain activated for days [19] or even weeks [42]. Rescue dogs can remain in kennels for several months, in some cases years, and if they fail to adapt to these conditions they are at risk of becoming chronically stressed. Chronic stress can have a negative impact on the physical and mental well-being of an animal [4,13] and can induce and/or contribute to the development of undesirable behaviours [20,21,3,5]. The expression of such aberrant behaviours can reduce the rehoming potential of a dog [50,15] and is the primary reason for dogs being returned to kennels following adoption [51,33,41].

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A number of studies have explored the use of enrichment techniques, including the provision of toys [50,22,48], olfactory [17]/visual [18] stimulation and increased human [6] and conspecific interaction [28] to minimise the stress experienced by kennelled dogs. Interestingly, auditory enrichment, using music, has a positive effect on behaviour in a range of species; for example captive Asian elephants [53] and gorillas [52]. Furthermore, it has also been demonstrated that kennelled dogs spend significantly more time lying and less time barking when played Classical music [49,24,10,11]; a genre also known for its mood-enhancing [31] and relaxing effects on humans [35,45]. While many of these studies only examined short term effects, in our previous study we investigated the effects of long-term (7 days) exposure to Classical music on the stress levels of dogs housed in a Scottish SPCA animal rescue and rehoming centre. Interestingly, the markers of stress studied (heart rate variability (HRV) and behaviour) demonstrated that the positive effects of Classical music were not maintained over long periods of exposure to a Classical music playlist. Rather, the effects seen on the first day of musical enrichment were lost by day 7, and habituation to the playlist could, in fact, happen as quickly as 1 day after musical enrichment was implemented [10].

The response to stress is complex and involves the coordinated activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). A variety of techniques are available to study the physiological responses to stress including i) cortisol as a measure of HPA-axis activity ii) heart rate variability (HRV) data as a measure of autonomic function and iii) behavioural observations as a measure of the combined effects of both systems. In humans HRV has been used extensively as a marker of autonomic function to assess cardiovascular conditions [26,36], diabetes [30] and psychological disorders such as anxiety and depression [40]. The non-invasive nature of data collection, coupled with the availability of highly portable equipment, has popularised the use of HRV in veterinary research. As such, HRV has been used to evaluate cardiovascular function in disease [27,29,12], stress [23,32,1], behaviour [47], and fitness/training regimes [25] in a number of farm/companion animal species. In dogs, HRV has been compared during different physical and mental activities and in response to human interaction [6] and Classical music [10]. Essentially, HRV is a measure of the variability in the duration of RR intervals, that is, the length of time (ms) between each heartbeat i.e. how quickly the heart rate changes [14]. This variability arises due to the influence of the autonomic nervous system (ANS) [9] which achieves cardiac homeostasis by balancing sympathetic/parasympathetic input as necessary as it responds to a constant myriad of internal and external stimuli. Increased activity within the parasympathetic nervous system (PNS) i.e. vagal nerve reduces the discharge rate of the sino atrial node (SAN) and slows the conduction of impulses through the atrioventricular (AV) junction, thus producing a negative chrono- and dromotropic effect on the heart. Conversely, increased activity within the SNS increases SAN discharge rate and decreases AV conduction time, therefore producing a positive chrono- and dromotropic effect on the heart. Furthermore, differences in the conduction velocities of the two systems exist as a result of differences in the anatomical arrangement and composition of the ANS. For example, relative to the SNS, the unmyelinated post-ganglionic neurones of the PNS are much shorter and therefore transmit impulses much faster [14]. Consequently, the PNS is capable of mediating much more rapid changes to HR, relative to the SNS, thereby producing higher levels of HRV during periods of PNS dominance [14]. During periods of SNS dominance i.e. a stress response, changes to HR will occur at slower rate relative to that of the PNS, thereby producing lower levels of HRV. By exploiting differences in the conduction velocities of the two divisions of the ANS, the analysis of HRV data can indicate the relative balance between the SNS and parasympathetic nervous system (PNS) at a given time, with higher levels of HRV considered a marker of increased vagal tone. A number of HRV parameters have been developed to quantify levels of HRV from an RR interval series and they can be broadly categorised into three groups: time-domain, frequency-domain and nonlinear indices. Our previous study demonstrated that several time-domain and non-linear indices of HRV were significantly increased when dogs were played Classical music, suggesting that the PNS was more active and dogs were less stressed [10]. In addition, dogs spent significantly more time lying [10] and significantly less time exhibiting behaviours such as standing and barking which are commonly displayed during acute/chronic stress in dogs [3,4,5].

The aim of the current study therefore, was to determine whether the beneficial effects of auditory enrichment observed with Classical music could be extended if dogs were exposed to different genres of music.

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) between October 2014 and June 2015. This study coincided with normal husbandry and operational procedures within the centre, which include routine cleaning, rehoming and visits by the general public. The subjects included in this study consisted of 38 dogs; 15 neutered (Nx) and 9 entire (E) males ($n = 24$) and 7 Nx and 7 E females ($n = 14$). Dogs kennelled at the ARRC can arrive as strays (S; $n = 9$), be surrendered by owners as unwanted (U; $n = 17$), held for temporary refuge (TR; $n = 1$), sequestered from owners due to welfare concerns (W, $n = 8$) or returned following attempted rehoming (R; $n = 3$). If unknown upon arrival at the ARRC, the age of the dog was estimated by an on-site veterinarian following examination of dentition. For the purposes of this study, dogs were categorised into 5 different age groups; <1 year ($n = 6$), 1–2.9 years ($n = 10$), 3–5 years ($n = 9$), 5.1–8 years ($n = 10$) and >8 years ($n = 3$). The duration of stay at the ARRC, calculated as the difference between the date of arrival and the first day of data collection, ranged from 1 to 420 days with a mean of 46.5 ± 13.8 days. As with many rescue centres, the most common breed of dog was the Staffordshire Bull Terrier (SBT; $n = 14$). Other breeds included Crossbreeds ($n = 10$), Border Collie ($n = 5$), Lurcher ($n = 4$), Border Terrier ($n = 1$), Jack Russel Terrier ($n = 1$), Rottweiler ($n = 1$), Rottweiler X Akita ($n = 1$) and German Shepherd ($n = 1$). After undergoing appropriate assessments, all S and U dogs are made available for rehoming 7 and 3 days after arrival at the ARRC respectively, and a conscious effort was made not to interfere with the rehoming potential of any dog during the course of the study. Consequently, not all 38 subjects completed the full 11 days (14 sessions) of the study.

On arrival at the centre and then again at 8-week intervals, dogs are subject to a full clinical examination by an on-site veterinarian. Dogs were only selected if they had passed the clinical examination so that no subjects with arrhythmia were included in the study.

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

2.2. Kennel environment and general husbandry

All subjects were maintained in a kennel block that allows collection of behavioural data without physically entering the block. A detailed description of the kennel environment and the general husbandry routine are as previously published [10].

2.3. Study design

The experimental design illustrated in Fig. 1 was a modification of that used in Bowman et al. 2015 [10]. Over the course of 11 days, dogs were studied under silent (control) conditions and during exposure to five consecutive days of auditory enrichment (treatment). The study period included two silent controls; the first was conducted 2 days before

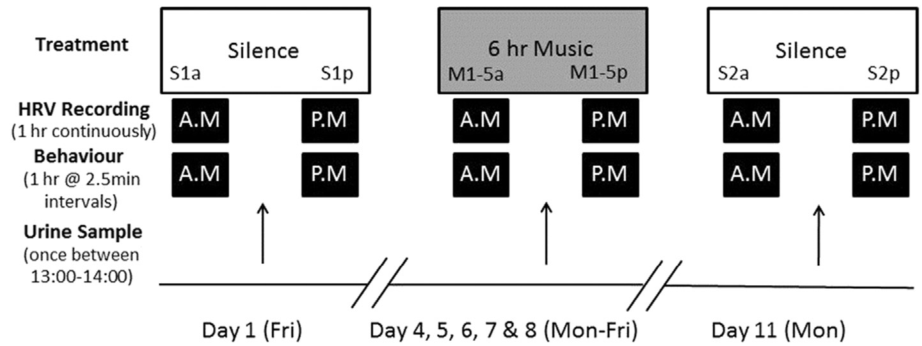

Fig. 1. Illustration of study design.

and the second 2 days after auditory enrichment and are referred to as S1 and S2, respectively. During auditory enrichment, dogs were played a different genre of music each day from the following genres; Soft Rock (M1), Motown (M2), Pop (M3), Reggae (M4) and Classical (M5). Dogs were studied in small cohorts of 2–5 at a time and, in total, 10 cohorts completed the study. In order to minimise the effect of day, the order of musical genre was randomised between each cohort. For each genre, a 6 h playlist was generated on Spotify™ and played via 360° Wireless Bluetooth Speakers (Veho, Hampshire, UK) from 10:00–16:00. In order to minimise an effect of time of day, the order of songs within each genre was randomised by shuffling the playlist. The speakers were dispersed evenly throughout the kennel block and placed on the roofs of the kennels as described previously [10]. The volume of the speakers was set manually each day and maintained at the same level throughout the study. HRV/behavioural data were collected from 10:30–11:30 (A.M. session) and 14:30–15:30 (P.M. session) and a urine sample obtained between 13:00–14:00 on day 1, 4–8 and 11 of the study.

2.4. Data collection and processing

2.4.1. HRV/behavioural data

HRV and behavioural data were collected and processed as described previously [10]. In brief, HRV data was collected continuously for 1 h in the A.M. and 1 h in the P.M. using Polar® RS800CX human heart rate monitors (Polar®, Finland). RR 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)). For each 60 min recording, three, 5 min sections were selected at random, and the mean value of each chosen parameter calculated. The HRV parameters selected for further analysis were; the standard deviation of the RR interval (STDRR), root mean square of successive differences (RMSSD), proportion of neighbouring intervals differing by >50 ms (pNN50), the ratio of low frequency (LF) to high frequency (HF) band powers (LFHF), and the standard deviation 1 and 2 (SD1 and SD2) of the Poincaré Plot.

Behavioural data was collected simultaneously and the position, location and vocalisation of each dog noted at 2.5 min intervals. At the time of each behavioural observation, the presence of anyone other than the researcher i.e. staff/staff with dogs/volunteers/visitors was recorded. For each session, an explanatory variable (presence) was generated with three levels; low (0–30%), medium (31–59%) and high (60–100%) which specifies the proportion of observations during which someone other than the researcher was present.

2.4.2. Urine

Urine samples were obtained from each subject on day 1, 4–8 and 11. Owing to the circadian manner of cortisol secretion, the samples were collected between 13:00–14:00 each day. During this time, each subject was taken on a 10 min leashed walk around the grounds of the ARRC and urine, if produced, was collected in a metal dish. The urine was transferred to a 5 ml borisilicated glass vial, labelled and frozen (−20 °C) until the time of assay.

2.4.2.1. Hormone determination. Urinary cortisol levels were determined using a high-sensitivity competitive enzyme immunoassay kit (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 0.10 ng/ml and inter and intra-assay coefficients of variability were 6.8% and 11.9%, respectively. To allow correction for urine volume, creatanine levels were determined by spectrophotometry in a commercial laboratory (Veterinary Diagnostic Services, University of Glasgow) and results expressed as a urinary cortisol:creatanine ratio (UCCR).

2.5. Statistical analysis

All statistical analyses were performed in R version 3.1.2 [37] and all graphs produced using the R package 'ggplot2' [54].

2.5.1. HRV data

All HRV parameters (apart from HR) were Log-transformed prior to statistical analysis to justify the assumption of normally distributed residuals of the linear regression model. Linear mixed effect models were used to assess the effect of treatment on HRV parameters. A change from baseline approach was employed, where the response variable of each model was the difference in the mean value of the HRV parameter obtained during S1 and treatment. The mean value of HRV parameter recorded on S1, referred to as baseline, was always the first covariate added to and retained within the model. Fixed factors sex, neuter status, size, age, breed (SBT or other), reason for kennelling and duration of stay, were added to and then removed from the model in a step-wise fashion if their contribution was not statistically significant ($P < 0.05$). In order to avoid pseudo-replication, dog identity was included in the model as a random effect. Model diagnostics included graphical inspection of residuals to ensure the assumption of homogeneity of variance and normal distribution were justified. During model construction, R automatically selects a treatment based on alphanumerical order. Therefore the effects of treatments M2-5 and control S2 are relative to the reference treatment M1 i.e. Soft Rock.

2.5.2. Behavioural data

The mean percentage of time spent performing each behavioural activity was calculated taking into account the sessions during which dogs were not present i.e. at vet clinic or viewing with potential adopter.

Across the entire data set, the percentage of time dogs spent lying or standing, was uniformly distributed. However, within each treatment, data was more normally distributed. Therefore, by application of the central limit theorem, the effect of treatment on lying and standing behaviour was assessed via mixed effect linear models.

Data for the mean percentage of time spent sitting, barking and outside were positively skewed. Therefore, binary variables were created for each behavioural activity where 1 indicated that the dog sat/barked/went outside and 0 indicated that dog did not sit/did not bark/did not go outside during the recording session. The effect of treatment on sitting, vocal and locational data was assessed using logistic regression.

In all models, fixed factors included treatment, presence and session, and dog identity was included as a random factor. The residuals for both mixed effects linear models were normally distributed. The fit of logit models was assessed by predicting the number of dogs within each category and comparing with the data set; all three models were found to make accurate predictions. In all models, the reference treatment was S1. For logit models, the odds ratio and confidence intervals were calculated by exponentiation of the estimate for each fixed factor and log confidence intervals, respectively.

2.5.3. Urinary cortisol data

Prior to statistical analysis, UCCR data was normalised using log transformation. Mixed effect linear models were used to assess the effect of treatment on UCCR. Fixed/random factors included in the model and model diagnostics were as previously described for HRV models. The reference treatment for the model was S1.

3. Results

3.1. HRV results

Mean ± SEM values of HRV parameters obtained during, silent 1 (S1, baseline) and the mean (95% C1) change in HRV parameters, relative to S1, when each musical genre was played are provided in Table 1.

3.1.1. Soft Rock (reference treatment)

Statistical analysis showed that the change in all HRV parameters in response to Soft Rock was significantly ($P < 0.001$) associated with the baseline level of that parameter. In addition, statistical analysis revealed that there was a significant increase in time-domain parameters RR ($t = 10.568$, df $= 49.61$, $P < 0.001$), STDRR ($t = 4.972$, df $= 35.92$, $P < 0.001$), RMSSD ($t = 5.847$, df $= 32.71$, $P < 0.001$) and pNN50 ($t = 8.191$, df $= 73.73$, $P < 0.001$) and non-linear indices SD1 ($t = 6.078$, df $= 33.14$, $P < 0.001$) and SD2 ($t = 4.97$, df $= 47.05$, $P < 0.001$) from S1 to Soft Rock. In addition, there was a significant decrease in HR ($t = 9.876$, df $= 62.42$, $P < 0.01$) and the frequency domain result LFHF ($t = -2.252$, df $= 17.17$, $P < 0.05$) from S1 to Soft Rock.

The changes in HRV parameters seen in response to Soft Rock were significantly associated with fixed factors age, size, breed, gonadal status and duration of stay at the centre. The decrease in RR ($t = -3.806$, df $=$ 13.72, $P < 0.01$), RMSSD ($t = 2.305$, df $= 15.97$, $P < 0.05$) and SD1 ($t = -2.92$, df $= 15.43$, $P < 0.05$) observed in older dogs (>8 years) was significantly different to the increase in these parameters observed in younger dogs (<1 year). Likewise, the increase in HR ($t = 2.663$, df $=$ 15.91, $P < 0.05$) in dogs >8 years and increase in LFHF ($t = 2.617$, df $=$ 19.3, $P < 0.05$) in dogs aged 5.1–7.9 years was significantly different to the decrease in these parameters observed for younger dogs (>1 year). In addition, the change in LFHF was significantly ($t = 2.715$, df $= 17.94$, $P < 0.05$) smaller in dogs aged 3–5 years compared with dogs <1 year. The change in RR ($t = -3.134$, df $= 22.75$, $P < 0.01$) was significantly smaller and the change in HR ($t = 2.901$, df $= 19.12$, $P < 0.01$) was significantly larger in smaller dogs compared to larger dogs. Likewise, SBT were found to have a significantly smaller increase in RR ($t = -3.312$, df $= 16.91$, $P < 0.01$) and significantly larger decrease in HR ($t = 2.901$, df $= 19.12$, $P < 0.01$) in response to Soft Rock, relative to other breeds. The decrease in HR in response to Soft Rock was found to be significantly ($t = 2.02$, df $= 19.49$, $P < 0.05$) larger in neutered dogs relative to entire dogs. Finally, the increase in LFHF was found to be significantly ($t = 2.688$, df $= 25.72$, $P < 0.05$) larger for dogs that had spent 100–201 days at the centre compared to those who had just arrived at the centre (0–1 days).

Table 1
Mean ± SEM baseline values of HRV parameters recorded during S1 and the mean difference (95%CI) in HRV parameters between S1 and following treatments; Soft Rock (A.M. $n = 23$, P.M. $n = 22$), Motown (A.M. $n = 24$, P.M. $n = 25$), Pop (A.M. $n = 23$, P.M. $n = 22$), Reggae (A.M. $n = 18$, P.M. $n = 19$), Classical (A.M. $n = 18$, P.M. $n = 20$), Silent 2 (A.M. $n = 20$, P.M. $n = 19$). Superscript ^{a} indicates that the change in HRV parameter from S1 to the reference treatment, Soft Rock, was significant ($P < 0.001$) and the magnitude of change was significantly associated with the baseline value of the HRV parameter at level $P < 0.001$. *, **, *** indicate that the difference in HRV parameters between S1 and treatments M2-M5 & S2 was significantly different from that of S1 and the reference treatment Soft Rock (M1) at level $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

HRV parameter	Baseline (S1)	Treatment											
		Soft Rock (M1)		Motown (M2)		Pop (M3)		Reggae (M4)		Classical (M5)		Silent 2 (S2)	
RR (ms)	539.09 ± 11.91	+59.62^a	(25.18–105.26)	−55.32**	(22.27–102.52)	+57.11	(22.27–102.52)	+58.95	(23.6–109.32)	+57.18	(22.85–105.93)	+57.83	(23.16–107.21)
HR (bpm)	117.71 ± 5.09	−18.22^a	(9.8–28.51)	+19.6**	(10.04–32.21)	−18.92	(10.04–32.21)	−18.33	(9.29–30.25)	−18.88	(9.7–31.22)	−19.02	(9.76–31.32)
STDRR (ms)	114.48 ± 2.55	+8.61^a	(3.43–18.85)	+7.8	(2.79–19.01)	+7.77	(2.79–19.01)	+8.32	(2.95–20.46)	+7.69	(2.73–18.95)	+8.08	(2.87–19.84)
RMSSD (ms)	105.49 ± 7.49	+9.68^a	(4.19–19.38)	+8.39	(3.13–19.45)	+8.55	(3.13–19.45)	+9.05	(3.34–21.24)	+8.04*	(2.97–18.95)	+8.68	(3.21–20.34)
pNN50 (%)	19.48 ± 0.68	+7.1^a	(4.25–12.26)	+6.73	(3.74–12.48)	−6.49*	(3.74–12.48)	+6.92	(3.83–12.9)	+6.9	(3.82–12.87)	+7.04*	(3.89–13.11)
LFHF	0.54 ± 0.08	−0.37^a	(0.18–0.73)	+0.47*	(0.19–1.19)	−0.43	(0.19–1.19)	−0.43	(0.17–1.11)	−0.44	(0.17–1.13)	+0.48*	(0.19–1.24)
SD1 (ms)	76.09 ± 5.52	+9.25^a	(4.12–17.83)	+7.96	(3.05–17.75)	+8.18	(3.05–17.75)	+8.65	(3.29–19.55)	+7.68*	(2.92–17.43)	+8.3	(3.16–18.73)
SD2 (ms)	139.87 ± 5.61	+9.56^a	(3.8–26.21)	+8.72	(3.14–26.39)	+8.65	(3.14–26.39)	+9.37	(3.35–28.55)	+8.76	(3.13–26.75)	+9.16	(3.27–27.88)

3.1.2. Motown (M2)

During Motown there was a decrease in RR which was significantly ($t = -3$, df = 222.27, $P < 0.01$) different from the increase in RR observed during Soft Rock. Likewise, the increase in both HR ($t = 2.933$, df = 224.91, $P < 0.01$) and LFHF ($t = 2.11$, df = 221.59, $P < 0.05$) was significantly different to the decrease in these parameters observed during Soft Rock. The changes in STDRR, RMSSD, pNN50, SD1 and SD2 were not significantly different to that of Soft Rock.

3.1.3. Pop (M3)

During the Pop playlist there was a decrease in pNN50 which was significantly ($t = -2.369$, df = 217.37, $P < 0.05$) different from the increase in pNN50 observed during Soft Rock. The changes in RR, STDRR SD2 HR, RMSSD and SD1 were not statistically different from those observed with Soft Rock.

3.1.4. Reggae (M4)

The change in all HRV parameters from S1 was the same for Soft Rock and Reggae.

3.1.5. Classical (M5)

From S1 to Classical, the changes in RMSSD ($t = -2.25$, df = 218.44, $P < 0.05$) and SD1 ($t = -2.25$, df = 217.15, $P < 0.05$) were significantly smaller than Soft Rock. The changes in RR, HR, STD, pNN50, LFHF and SD2 were not statistically different from Soft Rock.

3.1.6. S2

During S2 an increase in LFHF ($t = 2.164$, df = 221.19, $P < 0.05$) was detected which was significantly different to the decrease in the LFHF detected during Soft Rock. The change in RR, HR, STDRR, RMSSD, pNN50, SD1 and SD2 was the same as for Soft Rock.

3.2. Behaviour results

Fig. 3 illustrates the mean percentage time dogs were observed vocalising and in a particular location/position during silent control sessions (S1 & S2) and auditory enrichment during the a) A.M. and b) P.M. session.

3.2.1. Position

A summary of the mean percentage of time dogs spent lying and standing during control (S1 and S2) and treatment conditions is provided in Table 2.

3.2.1.1. Lying. During S1 dogs spent 28% of their time lying down. The amount of time spent lying down was significantly increased in response to Soft Rock ($t = 5.304$, df = 428.3, $P < 0.001$); Motown ($t = 3.595$, df = 427.3, $P < 0.001$); Pop ($t = 3.483$, df = 427.2, $P < 0.001$); Classical ($t = 3.321$, df = 428.8, $P < 0.001$) and a similar trend ($P = 0.1$–0.05) as noted in response to Reggae. Conversely, there was a significant ($t = -2.195$, df = 428.9, $P < 0.05$) decrease in the amount of time spent lying during the second silent control (S2). Time spent lying was associated with the time of day, specifically on average dogs spent significantly ($t = 4.414$, df = 427.6, $P < 0.001$) more time (8%) lying during P.M., relative to A.M. sessions. The presence of persons other than the researcher had no significant effect on time spent lying.

3.2.1.2. Standing. During S1 dogs spent 58% of their time standing. The amount of time spent standing was significantly decreased during all treatments; Soft Rock ($t = 8.172$, df = 428.3, $P < 0.001$); Motown ($t = -3.96$, df = 427.2, $P < 0.001$); Pop ($t = -4.283$, df = 427.1, $P < 0.001$); Reggae ($t = -2.686$, df = 428.8, $P < 0.001$); Classical ($t = -4.261$, df = 428.9, $P < 0.001$). Conversely, the amount of time spent standing was significantly ($t = 3.5$, df = 429, $P < 0.001$) increased (11%) during S2. In addition, time spent standing was associated with the time of day, specifically dogs spent significantly ($t = -2.113$, df = 427.5, $P < 0.05$) less (4%) time standing during P.M. relative to A.M. sessions. The presence of persons other than the researcher had no significant effect on time spent standing.

3.2.1.3. Sitting/location. There were no effects of music (or other fixed factors) on time spent sitting or location of dogs.

3.2.2. Vocal

A summary from the output of logistic regression on barking behaviour during control (S1 and S2) and treatment conditions is summarised in Table 3.

3.2.2.1. Barking. Relative to S1, there were no significant effects of music on barking behaviour, however, dogs were 142 times more likely to bark during S2 ($z = 2.2$, $P < 0.05$). In addition, dogs were 53 times less likely to bark during a P.M. ($z = -3.330$, $P < 0.001$) session in comparison to an A.M. session.

3.3. UCCR results

A summary of the mean ± SEM UCCR obtained during control (S1 and S2) and treatment conditions is provided in Table 4.

3.3.1. UCCR

Relative to S1, there was a significant increase in UCCR during Soft Rock ($t = 2.781$, df = 92.27, $P < 0.01$) and S2 ($t = 2.46$, df = 90.6, $P < 0.05$). Reason for arrival at the centre and the size of the dog were significantly associated with UCCR obtained during S1. The UCCR for St ($t = 2.24$, df = 19.6, $P < 0.05$), W ($t = 3.359$, df = 19.32, $P < 0.01$) and U ($t = 2.569$, df = 19.55, $P < 0.05$) dogs was significantly higher than the reference reason category which was temporary refuge. In addition, medium sized dogs had a significantly ($t = 2.415$, df = 19.73, $P < 0.05$) higher UCCR than the reference category which was larger dogs.

Table 2
The mean percentage of time (95% CI) dogs spent lying and standing during S1 and the mean change in these parameters, relative to S1, during auditory enrichment and the second silent control (S2). *, **, *** indicate that the time spent lying/standing is significantly different from S1 at level $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

	Behaviour			
Treatment	Lying		Standing	
S1	28	(15–42)	58	(45–71)
Soft Rock (M1)	$+17^{***}$	(11 – 23)	-16^{***}	(−22–(−10))
Motown (M2)	$+11^{***}$	(5–17)	-12^{***}	(−18–(−6))
Pop (M3)	$+11^{***}$	(5–16)	-13^{***}	(−19–(−7))
Reggae (M4)	+5	(0 – 12)	-8^{**}	(−14–(−2))
Classical (M5)	$+11^{***}$	(4–17)	-13^{***}	(−20–(−7))
S2	-7^{*}	(−13 – 0)	$+11^{***}$	(5–17)

Table 3
Summary of output from logistic regression to determine the effect of auditory enrichment and session on the occurrence of barking. Superscripts * and *** indicate that the estimate was significantly different to the estimate for S1 at levels $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

		Behaviour		
		Barking		
		Estimate	Odds ratio (95% CI)	
Fixed factors	S1	0.280	–	–
	Soft Rock (M1)	−0.117	0.89	(0.403–1.962)
	Motown (M2)	0.240	1.271	(0.597–2.723)
	Pop (M3)	−0.379	0.684	(0.313–1.481)
	Reggae (M4)	−0.324	0.723	(0.322–1.606)
	Classical (M5)	0.027	1.027	(0.458–2.302)
	S2	0.887^{*}	2.427	(1.104–5.44)
	Session (P.M.)	-0.745^{***}	0.475	(0.303–0.735)

Table 4
Mean ± SEM UCCR of dogs on S1 ($n = 18$) and following treatments; Soft Rock ($n = 21$), Motown ($n = 18$), Pop ($n = 19$), Reggae ($n = 20$), Classical ($n = 13$) and S2 ($n = 11$). *, **, *** indicate values of UCCR are significantly different from S1 at levels $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

			UCCR ± SEM
Fixed factors	Treatment	S1 (intercept)	3.04 ± 0.55
		Soft Rock (M1)	4.54*** ± 0.52
		Motown (M2)	3.94 ± 0.51
		Pop (M3)	3.72 ± 0.52
		Reggae (M4)	3.65 ± 0.44
		Classical (M5)	3.48 ± 0.45
		S2	4.34* ± 0.81
	Reason	Stray (St)	3.99* ± 0.43
		Unwanted (U)	3.99* ± 0.33
		Welfare (W)	4.55** ± 0.33
	Size	Size (M)	4.12* ± 0.24
		Size (Sm)	5.07 ± 1.07

4. Discussion

The results of this study are consistent with the findings of previous studies, which highlight the potential of auditory stimulation as an effective enrichment tool within a rescue kennel environment [49,24,10,11]. During exposure to different genres of music, dogs spent significantly more time lying/less time standing and had increased levels of HRV parameters, indicating increased vagal tone. Although the statistically significant differences between genres were relatively minor, Soft Rock and Reggae appeared to have the most positive effects on behaviour/HRV. Interestingly, the physiological and behavioural changes observed in this study were maintained over the 5d of auditory stimulation, suggesting that providing a variety of different genres may help minimise habituation to auditory enrichment as has been observed previously when dogs were repeatedly exposed to the same Classical music playlist over a 7 day period [10].

A number of studies have identified that during periods of acute stress dogs commonly display a number of behaviours including panting, body shaking, low body posture, vocalising and paw lifting [3,4,5]. Vocalising and paw lifting is also observed in chronically stressed dogs as well as coprophagy, autogrooming and stereotypical behaviours such as pacing and shadow/tail chasing [20,21,5]. Chronic stress negatively impacts the physical/mental well-being [5] of the animal and exacerbates the development of undesirable behaviours, which reduces the rehoming potential of dogs in kennels. It is therefore important that we try to minimise the stress experienced by dogs by identifying practical, affordable and effective methods to improve the kennel environment. A limited number of studies have been conducted to explore the effect of auditory stimulation on the behaviour of kennelled dogs [49,24,10,11]. All studies report an increase in time spent lying and reduced barking in dogs when Classical music is played. However, not all genres have the same calming properties; for example, Wells et al. [49] and Kogan et al. [25] both reported an increased incidence of body shaking when dogs were played heavy metal. Body-shaking is a fear-related behaviour which was also reportedly displayed in acoustically stressed dogs [3] suggesting that the typically loud and sudden nature of heavy metal may be unsuitable for dogs. Additionally, some genres of music, namely pop [49,24] and psychoacoustically designed Classical music for dogs [24,11], had no observable effects on behaviour. The effects of the human voice on canine behaviour are contradictory in that Wells et al. [49] reported that radio had no effect on behaviour, whereas Brayley & Montrose [11] reported that audiobooks were a more effective form of auditory stimulation compared with Classical, psychoacoustically designed Classical music for dogs and pop. In the current study, all of the genres of music tested were associated with dogs exhibiting behaviours that are indicative of reduced stress namely more time lying (except for Reggae) and less time spent standing. These observed changes in position are consistent with the findings of previous studies investigating the provision of music as a form of auditory enrichment [49,24,10,11]. Following the cessation of auditory enrichment on S2, there was a significant decrease in the proportion of time spent lying and a significant increase in the time spent standing, further reinforcing the hypothesis that exposure to music induces behavioural changes in dogs. In this study it was also noted that position was influenced by the time of day, specifically dogs spend more time lying down during the P.M. relative to the A.M. sessions. This result likely reflects the daily routine of the kennel, as by the P.M. session dogs have been exercised, received second feeds and had more human interaction relative to the A.M. session. A similar effect of time of day on vocal behaviour was also observed as dogs were 53 times less likely to bark during a P.M. session, relative to A.M., again suggesting the study population was less stressed in the afternoon.

In this study there were no significant effects of auditory stimulation on the level of barking in response to any music genre including Classical music. This is contrary to previous findings, including our own, that the provision of music, particularly Classical, is associated with reduced barking [49,24,10,11]. The lack of an effect of music on barking in this study may be a result of two factors. Firstly, the method of analysis did not discriminate between the frequency of barking i.e. dogs who barked once or for all 25 observations. Secondly, the level of barking during S1 of this study (5%) was significantly ($P < 0.001$) lower than that observed on S1 of the Classical music study (18%). In the current study however, dogs were 142 times more likely to bark following the cessation of auditory enrichment on S2, compared with S1. This result indicates that the provision of music may help reduce levels of barking in kennels, despite the lack of observable effects of auditory stimulation on vocal behaviour in this study.

Outwith the clinical setting, the collection and analysis of HRV data has been used to assess the efficacy of kennel enrichment techniques, including a programme of human interaction [6] and Classical music [10]. Therefore, one aim of this study was to determine whether exposure of kennelled dogs to different genres of music was associated with changes in HRV. The time-domain parameters analysed in this study were: the STDRR, which reflects the overall variability within the RR series arising from periodic and random sources [7]; the RMSSD, which provides an overall estimation of high-frequency beat-to-beat variations in HR that are mediated by the PNS [14] and pNN50, which is used to quantify vagal activity [9]. The only frequency domain parameter analysed was the LFHF ratio; the ratio of mainly SNS (with some PNS) mediated low frequency (LF) changes and PNS mediated high frequency (HF) changes to heart rate, respectively. This value can be used as a measure of sympathovagal balance, with lower values obtained during PNS dominance and vice versa [43,8]. Finally, non-linear indices standard deviation 1 (SD1) and 2 (SD2), provide a measure of short and long term variation and are calculated graphically by measuring the SD along the horizontal and vertical axis of a Poincaré Plot of the R to R interval, respectively. The result of the current study reinforces our previous findings, in that a significant increase in time-domain and nonlinear indices of HRV was detected during auditory enrichment. Changes in these physiological parameters indicate increased activity within the PNS (vagal tone). Relative to the first silent control (S1), dogs were found to have significantly higher values of STDRR, RMSSD, pNN50, SD1, SD2 and significantly lower values of LFHF, when played Soft Rock. Higher values of HRV are obtained as a result of more rapid changes to HR, mediated by the PNS. Considering the synergistic nature of the ANS, the observed increase in vagal tone demonstrates that the SNS is less active and reflects reduced stress levels in response to auditory stimulation. However, given that activity within the SNS is also reduced during physical inactivity, it is possible that higher levels of HRV detected in this study can be attributed to the greater proportion of time dogs spent lying down during auditory enrichment.

The largest increase in HRV parameter, from baseline (S1) to treatment, was observed during Soft Rock and Reggae. In contrast, a significant decrease in RR and increase in LFHF was detected during

Motown. This suggests that vagal tone was highest when dogs were played Soft Rock/Reggae and relative to these genres, Motown was less relaxing. Relative to Soft Rock, the pNN50 during Pop and the RMSSD/SD1 during Classical, were found to be significantly lower. The former result is in agreement with previous studies, which found Pop to have little effect on behaviour [49,24,11], whereas the latter is at odds with the results of previous studies, including our own, which demonstrate that Classical music is associated positive physiological/behavioural changes [49,24,10]. It is of interest to note that the change in HRV parameters observed in this study was much less pronounced than that of our previous study. Fig. 2 illustrates that the value of RMSSD obtained on S1 was significantly ($P = 0.0198$) higher during the current study, relative to the Classical music study [10]. This difference would suggest that on entering the present study, this specific cohort of dogs had higher vagal tone and were possibly less stressed than the group of dogs used in the previous study [10]. This would also explain the limited effects of auditory enrichment, in particular Classical music, on HRV; if this specific cohort of dogs was less stressed to begin with then there is less opportunity to improve. In addition, the range of change in HRV parameters following auditory enrichment was large. This may indicate that individual dogs are affected to a greater or lesser extent by the different genres of music and highlights the possibility that dogs may have their own preferences. An effect of personality on acoustic signalling in pigs [16] has already been demonstrated and given the highly emotive relationship humans have with music [31] it isn't unreasonable to assume that dogs could have positive/negative connotations with different genres of music based on past experiences.

The observed changes in HRV in response to auditory enrichment were also found to be affected by other aspects of the dog's history including age, breed, gonadal status and the duration of kennelling. Both RR and RMSSD were reduced in dogs aged 8–10 years and LFHF was increased in dogs aged 5–8 years. This suggests that auditory stimulation may not be as effective at reducing stress in older dogs that may perhaps, prefer a quieter environment. It is also possible that older dogs have reduced hearing and are therefore, less stimulated by auditory enrichment. Relative to other breeds, SBT were found to have a smaller increase in RR when played Soft Rock. There was also a significantly larger decrease in HR in neutered versus entire dogs when played Soft Rock.

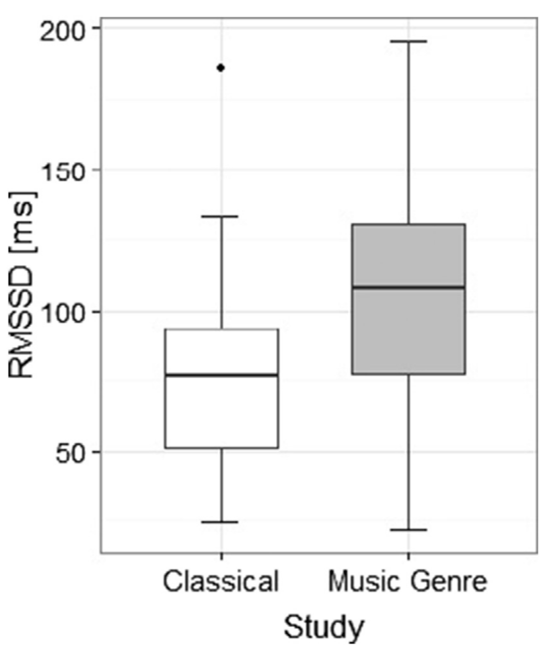

Fig. 2. Boxplot of mean RMSSD recorded on S1 of Classical Music and Music Genre Study. Baseline values of RMSSD obtained prior to auditory enrichment were significantly ($P = 0.0198$) higher in the current study, relative to the Classical music study.

Given that these fixed factors were only associated with a single parameter each, which do not directly quantify HRV, it is not possible to state this is a true effect of breed or gonadal status on the response to auditory enrichment. Statistical analysis showed that following exposure to Soft Rock, there was an increase in LFHF in dogs that had only been at the centre for 0–1 days and dogs that had spent 100–201 days at the centre. Furthermore, the increase in LFHF was larger in dogs that had spent longer at the centre, relative to those who had just arrived, suggesting that dogs that had been kennelled for prolonged periods of time had a more negative response to Soft Rock.

The levels of cortisol in plasma [19], saliva [4,10], faeces [39] and urine [2] have been used previously as a measure of HPA-activity in kennelled dogs. The advantage of urinary cortisol analysis is that sample collection is non-invasive and excretion products, including cortisol, accumulate over several hours and thereby correct for some of the natural fluctuation in cortisol [34]. The lowest UCCR was observed on S1, and UCCR was significantly ($P < 0.001$) higher when Soft Rock was played. The UCCR measured during all other genres was lower than Soft Rock but higher than S1, suggesting that auditory enrichment did not reduce HPA activity in this study. Although the UCCR was significantly higher following the cessation of auditory enrichment on S2, relative to S1, the overall results of UCCR analysis contradict HRV/behavioural parameters, which suggest the dogs were less stressed when played music. Other studies have reported poor correlation between cortisol and HRV/behavioural measures of stress as a result of high inter subject variability [6], and there are several other possible explanations for the discrepancy between parameters detected in this study. Firstly, due to failure of dogs urinating during allocated sample collection times, urine samples were only collected from half the subjects and therefore, results may not indicate the true mean UCCR for the sample population. Secondly, prolonged HPA activity during chronic stress can alter HPA activity itself meaning that some UCCR values may not reflect the true state of the animal. Finally, the accumulation of cortisol in the urine over several hours may explain difference with HRV/behavioural data which provide a more instantaneous reflection of the dogs' response to music. Interestingly, UCCR was significantly higher in stray, unwanted and welfare dogs relative to those at the centre for temporary refuge despite the fact that many of these dogs had been at the centre for a number of days or weeks prior to the start of the study. This may indicate an effect on the basal levels of cortisol secretion and HPA activity. Medium sized dogs were also found to have significantly higher UCCR than larger dogs.

5. Conclusion

The results of this study demonstrate that potentially beneficial physiological and, in particular, behavioural changes occur in response to musical auditory enrichment in kennelled dogs. The overall increase in levels of HRV during auditory stimulation reflects an increase in PNS dominance and suggests dogs were less stressed when listening to music. The magnitude of change in HRV parameters was highest for Soft Rock and Reggae, followed by Pop and Classical and lowest for Motown. However, the range of change in HRV parameters indicates that overall the response to different genres was mixed and highlights the possibility that dogs may have individual musical preferences. Despite the mixed response to different genres, the generally calming effect of music was maintained over the course of treatment, suggesting that the provision of alternative genres minimises habituation to auditory enrichment. Not only achieving, but maintaining lower levels of stress in kennelled dogs is important for their physical and mental well-being and in the display of more desirable behaviours that help facilitate rehoming. In conclusion, the results of this study reinforce those of previous studies demonstrating the potential of auditory stimulation as an effective enrichment tool but also the requirement to identify the form of audio most suited to the majority of dogs at any one time.

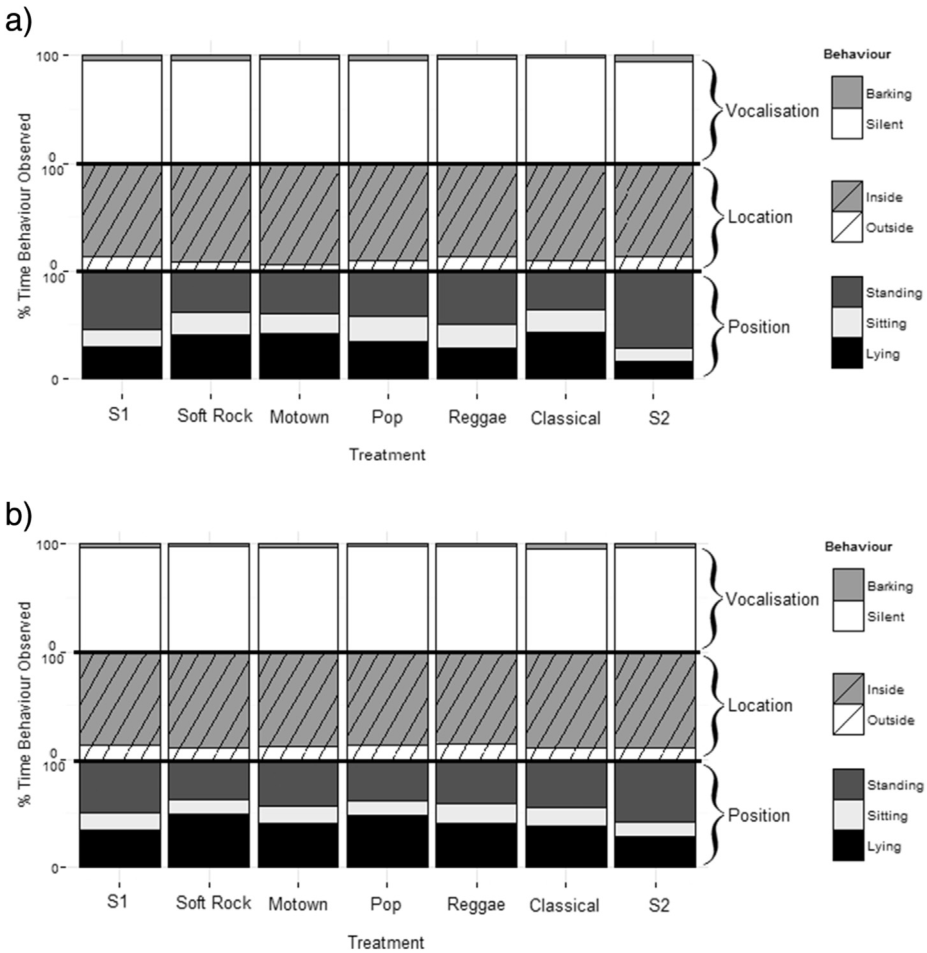

Fig. 3. Bar graph of average time dogs were observed lying, sitting or standing (position), inside or outside (location) and silent or barking (vocalisation) during silent conditions, S1 (A.M. $n = 36$, P.M. $n = 36$) & S2 (A.M. $n = 30$, P.M. $= 31$ and the following treatments; Soft Rock (A.M. & P.M. $n = 34$), Motown (A.M. $n = 37$, P.M. $n = 36$), Pop (A.M. $n = 37$, P.M. $n = 36$), Reggae (A.M. & P.M. $n = 32$), Classical (A.M. & P.M. $n = 31$), Silent 2 (A.M. $n = 30$, P.M. $n = 31$).

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