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The potential beneficial effect of classical music on heart rate variability in dogs used in veterinary training

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ABSTRACT

Heart rate variability (HRV), the variability between subsequent heart beats, is a measure of autonomic tone, influenced by psychophysiological factors, neurohormonal mechanisms and cardiac disease. Auditory stimulation, specifically classical music, has been documented to benefit well-being in a number of animal species. The aim of this study was to determine whether exposure to classical music improved HRV in dogs used in training during veterinary education for practical laboratories teaching canine clinical examination skills. Sixteen dogs, institutional kenneled dogs and student-owned dogs, were recruited in a cross-over study with a seven-day washout period. Dogs were fitted with a Polar® wearlink strap and HRV data were collected using a Polar® RS800CX human heart rate monitor attached to the dog's collar during the procedure. There were significant differences (P value < 0.05) in HRV indices between dogs exposed to as compared with those not exposed to classical music, specifically the mean RR interval decreased by 6% from 588 to 551 (P value $= 0.0072$). The standard deviation of RR interval (STDRR) was significantly more variable, 89 versus 109, in the dogs exposed to music (P value $=$ 0.01) and the RR triangulation index (RRTI) increased from 13 to 16 (P value $= 0.008$). One limitation of this study included small sample size. Different genres and type of music and their effect on HRV of dogs and other animals in veterinary training (and other) settings need to be explored in the future.

History

Heart rate variability (HRV) or the oscillations in interval between successive heart beats due to autonomic tone is a measure of variation of the interval between successive R waves (RR interval) recorded by an electrocardiogram (Malik et al., 1996). HRV is considered a noninvasive tool of measuring autonomic tone and influenced by behavior, cardiac and noncardiac disease (Bergamasco et al., 2010; Malik et al., 1996). Parameters include time-domain variables, which are mathematically quantified from R-R wave variability, and frequency-domain variables, which include analyses of periodicity of variation of R-R interval (Pereira et al., 2008). HRV, independent of changes in respiratory and heart rate, is influenced by stressful psychophysiological states, with emotional experiences having a dampening effect on HRV (Tiller et al., 1996). Activity diaries are important to consider during interpretation as respiratory sinus arrhythmia, most pronounced during rest, will contribute to parasympathetic dominance and more extreme HRV as seen with Poincaré analysis in normal dogs (Blake et al., 2018). Lower HRV may be associated with canine aggression and is thought to represent an inability to regulate behavioral response to stress (Häggström et al., 1996). HRV is a tool which possibly indicates not only psychophysiological state, but also cardiovascular disease. In general, a reduced HRV parameter is considered a warning sign of a potential pathological state. For example, in Cavalier King Charles Spaniels with degenerative mitral valve disease (DMVD), HRV was found to be reduced in those dogs with decompensated disease as compared with dogs with compensated mitral valve insufficiency without left atrial dilation (Häggström et al., 1996). Similar associations were found between HRV in dogs and different stages of idiopathic dilated cardiomyopathy (DCM), with lower HRV in higher classes of heart disease (Pereira et al., 2008). In noncardiovascular disease, HRV is also dampened. Dogs with poorly controlled diabetes mellitus had reduced HRV as compared with well-controlled dogs and healthy

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dogs as controls (Pirintr et al., 2012). In humans, HRV has been a well-established prognosticator in acute myocardial infarction, with reduced HRV being associated with mortality (Malik et al., 1996).

Continuous experience of elevated stress in animals and humans may lead to physiological and behavioral problems (Kogan et al., 2012; Steptoe and Kivimaki, 2012; Wolff, 1953). Dogs participating in laboratories for teaching and learning clinical skills in veterinary education demonstrate signs of chronic stress often related to the kennel environment (Bowman et al., 2015; Kogan et al., 2012). Stress in animals can compromise their life span and health (Kogan et al., 2012). In dogs, elevated stress levels are linked to increased risk of skin diseases, decreased life span, and behavioral problems (Bowman et al., 2015; Kogan et al., 2012; Wells et al., 2002). Auditory stimulation is a method of enriching the atmosphere of surrounding environments resulting in improved functioning and well-being (Kogan et al., 2012; Ullmann et al., 2008). Evidence shows that listening to classical music has positive physiological and psychological benefits for animals (Bowman et al., 2015; Kogan et al., 2012). There is opportunity to add to the evidence on the effect of music on animals and the link between music, animal stress, and behavior (Kogan et al., 2012). Current evidence shows sensory stimulation offers enrichment benefits to institutionalized captive animals (Wells, 2009). Documented benefits with auditory stimulation in animals include behavioral benefit in cows adapting to milking machines (Uetake et al., 1997), reduced stereotypic behavior in Asian elephants (Wells and Irwin, 2008), and improved HRV in kenneled dogs exposed to classical music (Bowman et al., 2015; Wells et al., 2002). HRV is improved in shelter dogs after supplementation with human interaction as compared with control dogs receiving minimal human contact (Bergamasco et al., 2010). In captive wild animals, affiliative sounds similar to the animals' conspecifics are mostly beneficial (Wells, 2009). In dogs, classical music composed by Strauss, Bach, Mozart, and Grieg, significantly reduced the amount of time barking and increased resting in shelter dogs (Bowman et al., 2015). The use of HRV monitoring using commercially available Polar® link technology has been validated for use in dogs (Bowman et al., 2015; Essner et al., 2015; Jonckheer-Sheehy et al., 2012).

During this study, first-year veterinary students practiced physical examination skills on dogs. We compared HRV indices of these dogs while undergoing a physical examination while being exposed to either classical music or regular noise laboratory environment. We hypothesized that exposure to classical music would result in improved physiological changes in dogs reflected in HRV. Our experiences can inform medical educators and university administrators in developing enriching environments and improved living conditions for animals supporting veterinary medical education.

Materials and methods

Subjects and participants

The study followed a prospective, cross-over design between the periods November 2015 and February 2016. Because of sparse literature, we were not able to obtain reliable estimates to use for the sample size calculation. For this reason, we decided to do a pilot study using 16 dogs we were able to obtain and that satisfied our inclusion criteria. Student-owned dogs and an identical number of kennel-housed dogs used for teaching purposes were recruited to meet the required numbers of dogs for a pilot study. Dogs were enrolled in this study if they fulfilled the following criteria: no history of aggression to other dogs or people, 11-30 kg in body mass, healthy, no audible heart murmur or arrhythmia, and not receiving any medication for a medical condition. Clinical examinations of each dog were performed by qualified veterinarians on a weekly basis on all kennel-housed dogs and before enrollment of all student-owned dogs. Female dogs were excluded if they were in proestrus or estrus. This study was approved by the RUSVM Institutional Animal Care and Use Committee (IACUC).

Study design and data collection

Four dogs from each group were selected randomly and exposed to classical music (the music group [MG]). Four dogs from each group were selected randomly and exposed to classical music (the music group [MG]), the remaining four were exposed to no music in an adjacent room. The owners or regular kennel companions, respectively, were not present in the same room as their companion animal; however, they participated in the examination of a dog in an adjacent room, as their presence could have a calming effect on the dog and confound the results. After a washout period of 7 days, the cross-over was performed where classical music naïve dogs were given a clinical examination in a room, where classical music was played and vice versa. Sixty minutes was allocated for each session and the physical examination was performed twice during this time; each student acted as veterinarian or veterinary technician and the roles were reversed after 30 minutes.

Dogs were fitted with a Polar® wearlink strap and contact was ensured using ultrasound coupling gel. HRV data were collected using a Polar® RS800CX human heart rate monitor that was attached to the dog's collar during the procedure and data were uploaded using an integrated network device (W.I.N.D). Watches were set to record from ten minutes before the onset of the clinical examination until ten minutes after the examination, for a total of 80 minutes. This period was allocated for each of the two students to perform a complete clinical examination and for an interval to allow the dog a short walk between examinations for toileting. A heart rate monitor visual inspection was performed approximately every 15 minutes to ensure uninterrupted recording. A complete canine physical examination, including rectal examination, was performed by one pair of veterinary students per dog. The veterinary students were volunteers but had reached a suitable level of competency in this skill in their veterinary career in that they had successfully completed the formative assessment of the canine clinical examination. HRV parameters were calculated for three randomly selected 5-minute sections, from each 80-minute recording session. Dog subject data were uploaded after each session using the Polar® software and converted into ASCII file and analyzed using the Kubios HRV software. Each patient was allocated three random periods, where a 5-minute section was analyzed.

In addition to mean heart rate (HR), the following time-domain variables were recorded: mean RR (RR), root mean square of the standard deviation expressed in milliseconds (RMSSD, ms), R-R interval triangular index (RRTI), the number of R-R intervals that differ by more than 50%, expressed as a percentage (pNN50, %), the standard deviation 1 of the Poincaré plot (SD1) for short-term HRV, and standard deviation 2 of the Poincaré plot (SD2) for long-term HRV. The following frequency-domain variables were recorded: ratio between low frequency (LF) and high frequency (HF) band powers (LF/HF) (Bowman et al., 2015). The mean value of each HRV parameter was calculated for each dog subjected to a student-performed clinical examination with and without background classical music.

Heart rate variability parameters

Mean of HRV (RR) examines the normal heart rate fluctuations using statistical techniques that include calculation of standard deviation, and the root mean square of successive differences.

RMSSD calculates the square root of the mean of the squared differences between successive normal RR intervals over 24 hours and measures short-term variation in the normal RR interval because they are entirely based on comparisons between successive beats.

RRTI examines the number of R-R intervals that differ by more than 50%, expressed as a percentage (pNN50, %). The pNN50 (percentage units) calculates the percentage of differences between successive NN intervals over 24 hours that are greater than 50 ms and measures short-term variation in the NN interval because they are entirely based on comparisons between successive beats.

Poincaré plot is a graphical method for HRV analysis based on time-domain principles. Typically, each point on a Poincaré plot corresponds to two consecutive RR intervals with standard deviation SD1 representing the short-term variability (SD1), and the standard deviation SD2 representing the long-term variability (SD2).

The ratio between low frequency (LF) and high frequency (HF) band powers (LF/HF) is a useful clinical tool that represents a measure of the sympathovagal balance.

Auditory stimulus

The auditory stimulation, “Through a Dog’s Ear music series”, is a psychoacoustic compilation designed to promote calming effects in humans and was validated to promote the same effects in dogs (Leeds et al., 2004; Wells et al., 2002). Psychoacoustic designed music allows the selection of simple and slow (50-60 beats per minute [bpm]) piano sounds that result in greater calming effects. The tempo played was considered appropriate for an average-sized dog and the expected resting heart rate (Wells et al., 2002). The loudness of the music in the laboratory room was adjusted to 55 ± 5 dB and the speakers were located within 1- to 6-meter distance from each veterinary student pair and canine subject (Figures 1 and 2).

Statistical analysis

All collected data responses were entered in Microsoft Excel® 2016 (Redmond, WA) software, and analyses were performed with R v.3.4.3 (Vienna, AT). Data were judged to be normally distributed. Means and standard deviations are reported. Student’s t-tests (paired and unpaired) were used for significance testing, as appropriate for paired and independent data. Longitudinal data analysis was used to assess heart function parameters. An *a priori* alpha level of 0.05 was specified.

Results

A total of 16 dogs met the study criteria and were enrolled in the study. Of the 16 canine subjects, 31% (n = 5) were male and most of the canine subjects were of mixed breed (81%; n = 13). The average age of the canine subjects was 3 years, weighed approximately 16 kg, and they were approximately 53 cm tall at the top of their withers.

An example of a randomly selected 5-minute recording with resultant HRV data from a dog in this study, acquired by the Polar® software and analyzed by Kubios HRVc software is represented in Figure 3.

Several HRV parameters showed significant differences between the MG and NMG (Table). The RR interval mean decreased 6% from 588 to 551 (*P* value = 0.0072). The STDRR was significantly more variable, 89 versus 109, in the dogs treated with music (*P* value = 0.01). The RRTI increased from 13 to 16 (*P* value = 0.008). The remaining HRV variables all showed increases when dogs in the MG were compared with those in the NMG.

Discussion

The aim of measuring HRV in this group of dogs was to noninvasively assess welfare of dogs used in veterinary training by using a portable, commercially available, and standardized method

Figure 1. Veterinary students and canine subject listening to music during physical examination laboratory.

Figure 2. Overview of the learning space for practice of physical examination skills.

of measuring cardiovascular autonomic tone. In addition, this information could be extrapolated and used when dogs undergo a veterinary examination and conscious diagnostic procedures. The digital Polar® RS800CX human heart rate monitor has been found to be a reliable method of measuring HRV in dogs under stationary conditions (Essner et al., 2015; Jonckheer-Sheehy et al., 2012). This technique was selected to assess the potential benefit of classical music exposure in 16 healthy dogs while being used in veterinary education, which could be perceived as potentially stressful. The aim was to demonstrate improved and therefore higher HRV in dogs exposed to classical music in contrast to dogs not exposed to classical music during the veterinary training laboratory. In this study, dogs served as their own controls. A 7-day wash-out period was selected based on the results of a previous study which demonstrated that the beneficial effect of classical music is lost after seven days of exposure (Bowman et al., 2015). This period may not have been clinically significant, as the previous study used 6.5 hours of continuous exposure per day for 14 days (Bowman et al., 2015), unlike our study, which was a once-off exposure.

Many time-domain indices were compared between the MG and NMG, and significant differences were found, specifically the mean of the RR variability, STDRR, and the RRTI. Unexpectedly, dogs exposed to classical music had a significantly lower mean RR variability ($P = 0.0072$) than those that were not exposed, which could be interpreted that exposure to classical music was a novel experience to these dogs rather than calming. The effect of auditory stimulation is not always predictable, for example, captive lowland gorillas when first exposed to rainforest noises displayed fear response (Videan et al., 2007). Discordant to this and supportive of previous studies, STDRR ($P = 0.01$) and RRTI ($P = 0.008$) were significantly higher, and the RMSSD ($P = 0.06$) and pNN50 ($P = 0.19$) were higher but the differences were not significant in dogs exposed to classical music than those that were not. These HRV time-domain benefits of classical music were similar to a previous study in shelter dogs exposed to classical music (Bowman et al, 2015). Exposure to music in that study was for 6.5 hours per day for 14 continuous days, although the dogs in that study were not used in training laboratories. Besides assessing welfare, assessment of HRV has shown to indicate presence of cardiovascular disease as in DMVD where RMSSD, a time-domain index, is decreased, indicative of parasympathetic withdrawal rather than increased sympathetic tone (Rasmussen et al., 2014). Measurement of HRV has also demonstrated benefit in examining dogs with occult DCM with respect to vasovagal tonus index (VVTI). VVTI is negatively associated with heart disease class and with survival in the more advanced stages of heart disease (Pereira et al., 2008). VVTI is a time-domain index, measuring high-frequency variation in heart rate, examined in dogs of different breeds, with brachycephalic dogs having significantly higher values due to profound respiratory sinus arrhythmia and presumed high parasympathetic tone, and dogs with congestive heart failure having a decreased index (Doxey and Boswood, 2004). Although we did not specifically calculate this index, all dogs in our study were medium to large breed non-brachycephalic dogs, and the influence of breed was not thought to account for any variability noted in our study. HRV time-domain indices have limitations, and have shown to be unable to predict the onset of congestive heart failure in dogs with cardiomyopathies. HRV time-domain indices were disappointedly unable to predict the onset of occult DCM on Doberman pinchers (Calvert and Jacobs, 2000). Similarly, time-domain variables were unable to predict sudden death in Dobermans with DCM. It is apparent that although helpful, not all time-domain variables are reliable in detecting parasympathetic withdrawal in dogs.

Poincaré plot parameters SD1 and SD2 are also helpful in assessing autonomic tone, with an increase in SD1 indicative of parasympathetic tone and an increase in SD2 indicates sympathetic tone (Blake et al., 2018). The shape of the Poincaré plot is distinctively Y-shaped in dogs, with the stalk indicative of sympathetic tone, which is longer during activity, and the arms associated with variability, becoming wider or a larger SD1, during rest

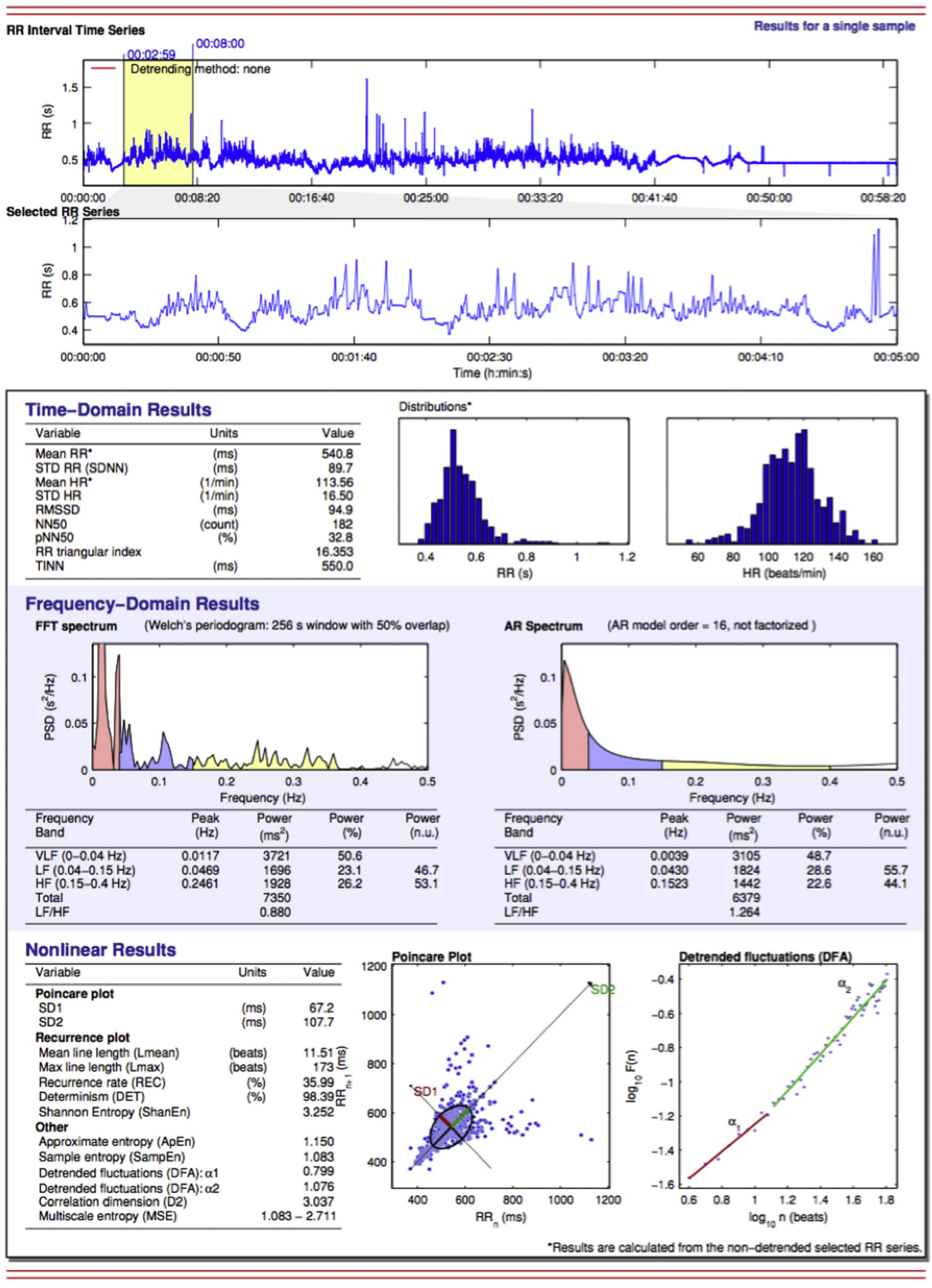

Figure 3. An example of 5-minute heart rate variability time- and frequency-domain indices collected from a dog in this study wearing a Polar® RS800CX with data imported using the Polar® software and analyzed by Kubios HRVc software.

due to respiratory sinus arrhythmia (Blake et al., 2018). All recordings of HRV in our study were collected during a laboratory and a longer stalk and narrower arms were expected. Despite lack of significance, SD1 was higher ($P = 0.06$) when dogs were exposed to music, indicative of increased parasympathetic tone and withdrawal of sympathetic tone. Discordant to this results was the SD2 parameter, a measure of sympathetic tone was significantly higher ($P = 0.008$) in dogs exposed to classical music as compared with controls. The results in our study, although only a brief period was recorded, indicate HRV of both activity and rest,

Table
Heart rate variability descriptive statistics between music group and no music group dogs and *P* values for a treatment effect adjusted for age, sex, breed, and mass of the dog

HRV parameter	NMG	MG	Treatment effect
	Mean (SD)	Mean (SD)	*P* value
μRR	588 (184)	551 (109)	**.0072**
STDRR	89 (39)	109 (92)	**.01**
RMSSD	75.1 (55.0)	94 (99)	.06
pNN50	19.8 (11.1)	23.32 (14.89)	.19
LF:HF	1.73 (1.38)	1.55 (1.19)	.80
RRTI	13.1 (5.3)	16.0 (6.2)	**.008**
HR Mean	111 (25)	117 (22)	**.02**
SD1	53 (39)	66 (70)	.06
SD2	112 (45)	137 (111)	**.008**

MG, music group; NMG, no music group; LF:HF, low frequency to high frequency ratio; pNN50, number of pairs of successive RR intervals that differ by more than 50 ms (%); RMSSD, root mean square of the standard deviation between successive RR intervals; RRTI, R-R triangulation index; SD1, standard deviation 1 of Poincaré plot; SD2, standard deviation 2 of Poincaré plot; STDRR, standard deviation of the R-R variability; μHR, mean heart rate; μRR, mean of heart rate variability (mean of 3 5-minute measurements).
Significant differences are highlighted in bold.

with increased SD1 during rest and SD2 during activity and may explain the increase in both.

Frequency-domain analyses offered more insight to the autonomic tone. High frequency is indicative of parasympathetic tone, whereas LF and LF/HF is a measure of sympathetic tone. Although not significant, LF/HF, as a marker for sympathetic tone, was higher in NMG than the MG during the training session ($P = 0.8$) and these results are similar to our interpretation of the SD1 Poincaré plots and standard deviation of HRV. The frequency domain has shown to be helpful in cardiovascular disease in dogs, with LF as a marker for sympathetic tone or parasympathetic withdrawal, and correlates with left ventricular diastolic volume and left ventricular internal diameter end-diastole diameter in dogs with DMVD before treatment with enalapril (Chompoosan et al., 2014). HRV analyses demonstrated these dogs decreased their LF in response to enalapril treatment, assumed to be due to suppression of sympathetic tone (Chompoosan et al., 2014). If classical music is beneficial to shifting tone from sympathetic to parasympathetic, it may only be a short-lived benefit, as dogs that are exposed to classical music become refractory to the benefits after 7 days (Bowman et al., 2015). The value of the apparent improved parasympathetic tone and withdrawal of sympathetic tone in the MG may be worth pursuing in a larger study.

Heart rate, unlike HRV, is dependent on vasomotor tone, exercise, thermoregulation, altered endocrine function, and cardiovascular conditioning. Owing to the nature of the study, and cross-over approach, we did not expect any differences between the two groups in terms of mean heart rate, but the MG had a significantly higher mean heart rate ($P = 0.02$), which was not considered representative of autonomic tone but more physiologic.

An important limitation in this study was the small numbers of dogs studied. We were unable to recruit additional dogs because many dogs in the kennel population of RUSVM suffered subclinical monocytic ehrlichiosis. HRV is ideally measured over continuous 24-hour Holter monitors to determine long-term trends, which was not obtained in this study, mainly due to the nature of the Polar wearlink® strap that could not be secured uninterrupted for that period.

An additional limitation was the inability to definitely exclude cardiovascular disease. Although clinical examination was performed and no audible murmur, arrhythmia, or gallop was detected in any dog, echocardiography was not performed. One dog did have a consistently low HRV. Although this dog has had normal vital parameters and laboratory testing, it is possible that this dog had occult DCM.

The last consideration is that the best way to analyze these data would be using a multivariate longitudinal model because HRV and physiologic data are correlated. This was impossible due to our small sample size ($n = 32$). Instead each of the dependent variables was modeled independently. A similar study could be undertaken with a greater number of canine subjects in the future. Any effects of the type of music, specifically instrumental versus vocal, and the tempo of the music on HRV parameters should also be explored.

Conclusions

During this AB/BA cross-over study in the canine subjects, there were significant effects from exposure to classical music. Future indications for study include implementing a study with a larger sample size and examining the short-term and long-term benefits of music therapy for canine subjects and potential benefit of auditory stimulation during veterinary examinations. Future work should examine effects of instrumental music in teaching laboratories on behavioral data and effects of different genres and type of music.

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Authors' contributions: LK, FS, and EA contributed to study conception and design. LK was responsible for conceptualizing the canine HRV hypotheses, acquisition of HRV parameters in dogs, interpretation of the results, preparation of and reviewing the article. GEG authored the analyses and results section. LK drafted the original article, and EA, FS, and GEG edited the original article making critical revisions. GEG authored the Methods section and the Results section. All authors contributed subsequent revisions of the article. All authors approved the final article.

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Supplementary Data

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Conflict of Interest

The authors declare that they have no competing interests.

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