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Auditory enrichment for arousal reduction in non-vocal learning species

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Abstract

Passively listening to music and other auditory enrichments has been repeatedly demonstrated to be effective at reducing physiological arousal in a wide range of non-human species. Although statistically significant arousal reduction has been demonstrated, the size of this effect in most studies is generally small. To strengthen the previously demonstrated arousal-reducing effects of auditory enrichment, the aims of this thesis are:

> To understand what specific aspects of auditory enrichment have the greatest influence on arousal in dogs and horses.
>
> To determine if dogs have the auditory perceptive abilities that justify any assumptions of musical appreciation.
>
> To establish if a positive association with specific music can influence how that music can manipulate arousal in dogs.

Classical music with the pitch and tempo altered; music based on the owner's voice; and a range of metronome beats where trialled. Heartrate variability was the primary measure of effect and methods of measurement were validated in both dogs and horses prior to these studies. To test perception, a two-choice go/go selection paradigm was used.

Changing the pitch or tempo of music made no difference to the arousal of dogs or horses. Bespoke music based on the owners' voices had an equivalent effect on arousal in dogs as classical music and white noise. Limited testing of perception in dogs failed to demonstrate any ability to discriminate between different tempos, but the results of forming a positive association were suggestive of an increase in effect. An incidental finding was that auditory enrichment was more effective at reducing arousal when used in a noisy environment than when used in a quiet environment.

Auditory enrichment has been demonstrated to have an arousal-reducing effect, however, the arousal-reducing effects of auditory enrichment in non-vocal learners may be stemming from simple mechanisms such as acoustic masking and/or the formation of positive associations.

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Preface

This PhD is part-funded by the Scottish SPCA, and it was intended that the majority of the data collection would be conducted on dogs in their care within the Animal Rescue and Rehoming Centres (ARRCs) close to Glasgow. However, due to COVID-19 restrictions, from mid-March 2020, all access was lost to the ARRCs, and was never fully re-established. As a result, this project was extended to also encompass horses, as there was ready access to a population of horses at a time when dog availability was greatly reduced. Much of the dog work after the first year had to be conducted outside of the ARRCs and was moved to the University of Glasgow's School of Biodiversity One Health and Veterinary Medicine, using staff and student owned dogs. This has impacted on the composition of the study populations used in some of the experimental chapters and introduced differences in study conditions. These adjustments mean that it is hard to draw direct comparisons between early work conducted in a noisy, uncontrolled environment and the remaining work that was conducted in a quiet, controlled environment. Using different populations of dogs in different environments at different times over four years was not a methodology of choice but has, however, generated some very interesting incidental findings relating to HRV parameters, discussed in Chapter 2, and how music may be having an effect, discussed in Chapters 3-7.

Acknowledgements

Firstly, and most obviously, I need to thank my supervisory team, Neil Evans and Fiona Dowell. I have learnt so much from them both over the past four years, particularly the value of positive and supportive mentorship. I hope that if I am ever privileged enough to be entrusted with the nurturing and development of an early career researcher, my experiences as a PhD student will have moulded me into a far better supervisor than I would otherwise have been.

Less obvious is the huge amount of positive support that I have received from staff at the University of Glasgow that have absolutely no vested interest in either my success or development. Dorothy McKeegan, Mark McLaughlin, Maria Afonso, Jason Matthiopoulos, Andrew Brownlow, Lance Voute, Mariel Tendoeschate, Lorenzo Viora, George King, Nick Jonsson, Ilyena Hirskyj-Douglas, Praille Kriengwatana, Kathryn Elmer, Kate Griffiths, Catherine Stalin, Jasmine Clarkson, Joel Hotchkiss, Zamantha Marshall, Lissann Wolf, Tyler Stevenson, Julien Le Kernec, Guillaume Rousselet, Pablo Murcia, Paul Johnson, Angie Rupp, Anna McGregor – just some of the names. Some, I've bounced ideas off that have informed my research, some have helped me develop technical skills and knowledge that I would otherwise never have had, some have introduced me to collaborations I would never have found, and some have just made me feel that I belong. No one should underestimate the impact a five-minute chat in the corridor can have on a person's sense of direction and self-worth. I know they will never read this, but it just seems wrong not to acknowledge the vital role that the wider family of the University of Glasgow has played in getting me this far and in shaping my future. In addition, no organisation would function without its technical and administration staff, and although I have spent precious little time in the laboratory, Ana Monteiro and Lynne Fleming have been vital in helping me find my way around the procurement system and are always good for a chat. The technical staff at the Clinical Skills department have also been of huge support, particularly Susan Glattbach who gave very helpful advice and the loan of equipment when I was first setting up the Animal Behaviour Centre. Mark Paterson also deserves an honourable mention, if only for having the patience to repeatedly sort out my travel claims!

The bulk of this project should have been conducted at the Scottish SPCA Animal Rescue and Rehoming Centres. It is a matter of deep regret that COVID-19 curtailed my access to the centres, as all the staff I had dealings with from the centre managers to the volunteer dog-walks were so enthusiastic about the potential applications of this work and were so invested in the improvement of animal welfare. I really wish I could have spent more of the last four years in the company of such passionate and enthusiastic people. By necessity, much of the data used throughout this thesis was collected from privately owned dogs and horses, and I am hugely grateful to those owners for entrusting me with their beloved animals.

Finally, and most importantly, I am deeply indebted to my wife, Susan Hawthorne, without who’s unwavering support I would never made it this far. I do not underestimate the unique privilege I have in having such an experienced academic in my corner, convincing me I am good enough to be a successful PhD candidate. Her Northern Irish upbringing has also given her a unique set of motivational skills, and the threat of “a good hiding” if I was to quit has got me through some of the lower points of the last four years.

Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this thesis is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Richard Mott

Outputs from this Thesis

Publications

Mott, R., Dowell, F. and Evans, N., 2021. Use of the Polar V800 and Actiheart 5 heart rate monitors for the assessment of heart rate variability (HRV) in horses. *Applied Animal Behaviour Science*, *241*, p.105401.

Kriengwatana, B.P., Mott, R. and ten Cate, C., 2022. Music for animal welfare: A critical review & conceptual framework. *Applied Animal Behaviour Science*, p.105641.

Keynote Address

Music for Captive Animals. Association for the Study of Animal Behaviour auditory enrichment workshop 2021

Oral Presentations

The use of positive associations to strengthen the effects of auditory enrichment. Proceedings of the 55th Congress of the International Society for Applied Ethology (ISAE) 2022

Enhancing auditory enrichment for domestic dogs using the owner’s voice. The Association for the Study of Animal Behaviour (ASAB), Easter Meeting 2022

Use of the Polar V800 and Actiheart 5 heart rate monitors for the assessment of heart rate variability (HRV) in horses. Proceedings of the 54th Congress of the International Society for Applied Ethology (ISAE) 2021

Sex differentiated neural processing predicts response to auditory enrichment in domestic dogs. International Society for Applied Ethology (ISAE) UK/Ireland Regional Meeting 2020

List of Abbreviations

ABC	Animal Behaviour Centre (University of Glasgow)
ANS	Autonomic Nervous System
ARRC	Animal Rescue and Rehoming Centre
BPM	Beats Per Minute
dB	Decibels
ECG	Electrocardiogram
EDR	ECG Derived Respiration
EEG	Electroencephalogram
FFT	Fast Fourier transform
GABA	Gamma-Aminobutyric Acid
GSR	Galvanic Skin Response
HF Power	High Frequency Power (absolute value)
HPA	Hypothalamic Pituitary Adrenal
HR	Heartrate
HRM	Heartrate Monitor
HRV	Heartrate Variability
IBI	Inter Beat Interval
K448	Mozart's piano sonata for 2 pianos in D major

PNS	Parasympathetic Nervous System
PTSD	Post Traumatic Stress Disorder
RMSSD	Route Mean Square of Successive Differences
RSA	Respiratory Sinus Arrhythmia
sAA	salivary Alpha Amylase
SAM	Sympathetic Adrenal Medullary
SBOHVM	School of Biodiversity, One Health and Veterinary Medicine
SBR	Spontaneous Blink Rate
SDNN	Standard Deviation of the IBIs
SNS	Sympathetic Nervous System
SSPCA	Scottish Society for the Prevention of Cruelty to Animals
SSRI	Selective Serotonin Re-uptake Inhibitors
TCA	Tricyclic Antidepressants
WN	White Noise

Chapter 1 Introduction

1.1 Overview

In 2019, there were around 250,000 domestic animals in UK Animal Rescue and Rehoming Centres (ARRCs) (Hansard, 2019) and this number has increased due to the current cost of living crisis, with the RSPCA reporting a 25% increase in abandonments (Vet.Times, 2023). There is an average stay for dogs at an ARRC of five weeks before being re-homed (Battersea, 2017), although for some dogs, this can be considerably longer. Even though most centres have excellent facilities, many dogs still find it to be a highly stressful environment, particularly in the first four weeks (Righi et al., 2019), with stressors including separation, isolation, confinement, noise and odours. The chances of being rehomed quickly would be greatly improved if anxiety-related unwanted behaviours such as constant barking and excessive activity could be reduced (Wells & Hepper, 1992). Dogs that have been in an animal shelter for over five weeks are characteristically highly reactive to stressors (Raudies et al., 2021) which can be both the reason for not being rehomed and the response to a protracted stay in the shelter.

Although there is a clear imperative to enrich the ARRC environment to improve welfare and increase rehoming rates, for some domestic dogs the home environment can also be a source of stress. The RSPCA (2022) estimate that 45% of dogs in the UK show signs of fear when exposed to fireworks and according to the Blue Cross (2022), fear of thunderstorms is also very common. The unique circumstances of the 2020/2021 COVID lockdowns and the prevalence of working-from-home led to many dogs never learning that it is acceptable to be left alone, and the Dogs Trust (2022) cite this as one of the causes of the increase in separation anxiety. With behavioural problems being one of the main reasons to surrender a dog to an animal shelter (Fuh et al., 2012) it is feasible that cheap and effective methods of stress reduction developed for the ARRCs may have further utility in the wider domestic dog population in reducing the numbers of dogs surrendered. Finally, as Cobb et al. (2022) highlighted, it is vital that effective outreach is conducted to bridge the gap between scientific research and the key industry stakeholders to ensure evidence-based practice is utilised within ARRCs.

Passive listening to music and other auditory enrichments can reduce anxiety in humans (Graff et al., 2019) as well as behaviours and other markers of stress and anxiety in a range of non-human species (Barcellos et al., 2018; Bowman et al., 2015; Crouch et al., 2019; Hampton et al., 2019; Kühlmann et al., 2018; Williams et al., 2017; Zapata Cardona et al., 2022). However, the size of the arousal-reducing effect can often be small. This thesis therefore sets out to take this inexpensive and proven method of stress reduction and improve its effectiveness. This first chapter provides the necessary background, firstly by outlining the stress response, what the welfare implications are, how these responses can be mitigated and how stress responses can be measured. Section 1.3 then looks at what is already known about auditory enrichment for both humans and non-humans, then goes on to describe factors specifically affecting dogs. The final section then assesses the best way to quantify the effect of enrichment studies. This important question is so often overlooked, but it is vital to define at the outset of any enrichment study how any improvement in welfare is to be determined. This review chapter will therefore identify important knowledge gaps and justify main aims of the thesis as detailed below.

1.2 Responses to Stressors

1.2.1 The mechanism of stress responses

There are two main distinct pathways for a physiological response to a stressor. These are the autonomic nervous system and the hypothalamic pituitary axis. Striatal dopamine is also released in response to stressors, but this is probably linked to the activation of the HPA axis. Although these systems are functionally separate, they do not work in isolation.

1.2.1.1 The Autonomic Nervous System (ANS)

The ANS is part of the peripheral nervous system that automatically regulates many of the body's involuntary functions, such as heart rate, breathing rate, blood pressure, digestion, and temperature control. The ANS is divided into two pathways that mainly work in opposition to maintain balance: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) (Figure 1-1). The efferent neurones of the PNS pathway are both cholinergic and use acetylcholine as their main neurotransmitter while the SNS pathway uses cholinergic presynaptic and adrenergic post synaptic neurones and noradrenaline as the main neurotransmitter (Squires, 2010). The efferent of the SNS is the spinal cord via the chain of sympathetic ganglia, whereas the PNS bypasses the spinal cord via the vagal nerve (Cranial Nerve X). Increase in PNS activity is therefore also referred to as an increase in vagal tone. The PNS is dominant in the relaxed state (rest and digest) driving, amongst other functions, a reduction in heart rate, and the SNS increases in dominance when under threat, triggering the release of adrenaline and noradrenaline from the adrenal medulla (Bitsika et al., 2014), the two components of the response driving the immediate "fight or flight" responses such as increased cardiac output and reduced visceral circulation (Squires, 2010). The nucleus of the solitary tract of the brain stem is vital to the SNS and is the 'hub' for sensory afferents triggered by physical stress (Gidron, 2019) and innervates the paraventricular nucleus of the hypothalamus with noradrenergic neurones. Under conditions of purely physical stress, the dominance of the SNS increases due to a decrease in PNS activity (i.e., reduced vagal tone) whereas during psychological stress, the dominance of the SNS increases due to reduced vagal tone and an increase in SNS activity (Fu & Levine, 2012). Although the end result is the same, this phenomenon may be useful in determining whether an increase in heart rate is due to physical activity or psychological stress (Von Borell et al., 2007) and will be discussed in Section 1.2.4 – Measuring Physiological Responses. However, it is hard to precisely partition physical and psychological stress. Social stress (as normally tested as a measure of psychological stress (Woody et al., 2018)) may be different to the psychological stress of a physical threat, and it is plausible that the fear of a physical threat will bring about the same response as the actual physical threat i.e., preparation for fight or flight.

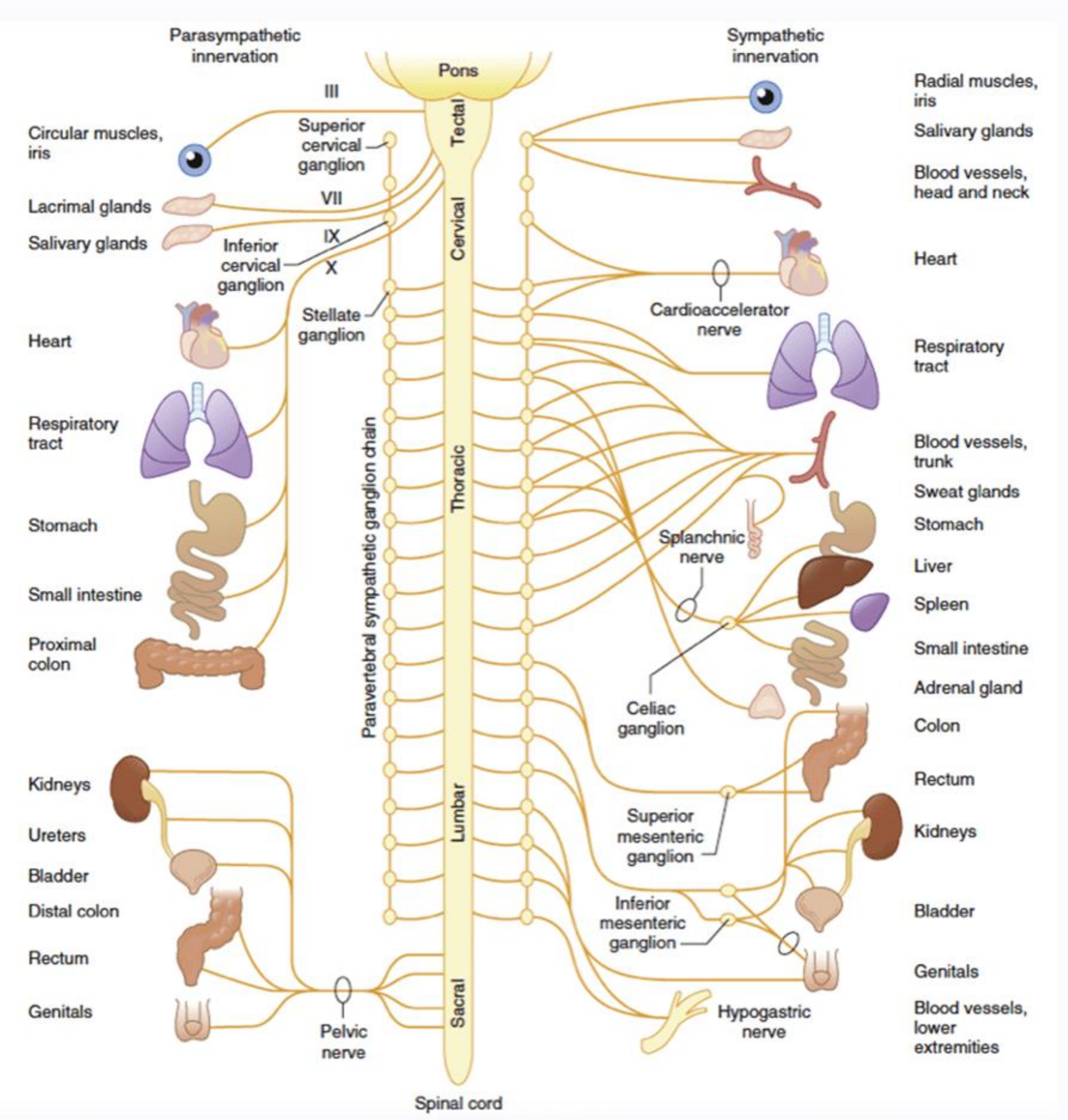


Figure 1-1 Schematic representation of the ANS, with the SNS depicted on the right and the PNS depicted on the left. Note the importance of the Xth Cranial Nerve (vagal nerve) on the functioning of the PNS (reproduced from Stein & Glick, 2017)

1.2.1.2 Hypothalamic Pituitary Adrenal (HPA) axis

When an organism is presented with a psychological stressor such as the social-evaluative threat test used in humans (Woody et al., 2018), the HPA axis is triggered leading to a cortisol response, the prominent neuroendocrine response to a stressor (Ranabir & Reetu, 2011). Fundamental to the psychological stress response is the limbic system, a collection of brain regions (hippocampus, amygdala, thalamus, hypothalamus and basal ganglia) that regulate behavioural and emotional responses. The initial response to a psychological stressor comes from the prefrontal cortex being stimulated by dopaminergic projections from both the ventral tegmental area and the *nucleus accumbens* (Gidron, 2019). The prefrontal cortex then projects back to the amygdala, entering the basolateral amygdala via the lateral amygdala and then exits the medial nucleus of the amygdala to the paraventricular nucleus (Godoy et al., 2018) (Figure 1-2). Corticotropin-releasing hormone and arginine-vasopressin are released from the paraventricular nucleus into the hypothalamic pituitary portal blood vessels, leading to a rise in the levels of pro-opiomelanocortins including ACTH, β-endorphin, and α-melanocyte-stimulating hormone (α-MSH) (Gidron, 2019). ACTH in turn increases the output of glucocorticoids from the adrenal cortex, the most important of which in most mammals is cortisol (Squires, 2010). Afferents from the hippocampus also take the same path through the lateral / basolateral amygdala in response to psychological stressors. (Godoy et al., 2018). Therefore, the brain regions involved in the initial stages of a response to a psychological stressor are also the ones involved in cognition, emotions and memory as opposed to the brain stem mediated ANS.

The HPA axis is stimulated at the same time as the ANS (i.e., on exposure to a stressor) but the HPA response occurs more slowly (minutes to hours) (Squires, 2010) and has a more generalised effect than the ANS. In long-term stress, one of these generalised effects is the negative feedback of glucocorticoids on ACTH may lead to normal basal cortisol levels and HPA suppression (Nicolaides et al., 2018). In the context of an ARRC, where long-term residents may feasibly be displaying a chronic stress response, baseline cortisol measures may not reflect the animal’s current state.

1.2.1.3 Dopamine Response

Dopamine is involved in attention and motivational processes, and the regulation of the responses to stimuli, either rewarding or aversive (Belujon & Grace, 2015). Striatal dopamine release increases in response to both acute and chronic stressors (Belujon & Grace, 2015; Finlay et al., 1995; Pruessner et al., 2004; Puglisi-Allegra et al., 1991), but due to the demonstrated link between dopamine release and cortisol levels (Oswald et al., 2005; Pruessner et al., 2004), it is likely that dopamine levels reflect HPA axis activation rather than being a separate system. The relevance to this thesis is that Ferreri et al. (2019) demonstrated that, in humans, dopamine modulates musical responses in both positive and negative directions, with artificially increased dopamine levels leading to increased “pleasurable” feelings when listening to music. Depending on the extent to which dogs have a subjective experience of musical pleasure, their stress (therefore dopamine) levels prior to a musical intervention may influence how effective that intervention is. Indeed, King et al. (2018) suggested that the processing of auditory stimuli is mediated by the individual’s internal state, as well as the context in which the sounds are detected, and this may be as important as the sounds themselves.

1.2.1.4 Systems Working in Concert

In discussing how the various stress pathways work together, it is important to consider whether the stressor is physical or psychological. Both can manifest in similar physiological responses (i.e., increased heart rate), but the mechanisms of response of both are very different. In the context of an ARRC, the stressor is likely to be psychological but within this division there are various subdivisions. For example, both anxiety and depression can be symptoms of psychological stress, although there is a complex bi-directional relationship with anxiety often being the cause of stress (Phillips et al., 2015) but both have different mechanisms and can be managed by different pharmacological agents (Rang et al., 2015). One of these mechanisms was demonstrated by Wohleb et al. (2013) who showed that that the psychological stress caused by repeated social defeat induced the development of anxiety and proposed that this was caused by the recruitment of inflammatory monocytes to the brain, the mechanism for which was further elucidated by McKim et al. (2018). The terms ‘stress’ and ‘anxiety’ are therefore not interchangeable although measuring a physiological stress response may give an implied measure of anxiety. However, there is no single non-specific response that is generated by all stressors (Squires, 2010). Skoluda et al. (2015) showed that under conditions of either physical or psychological stress, both the HPA and ANS were activated as part of the acute phase response but that the ANS was dominant at times of physical stress and the HPA axis was dominant at times of psychological stress, and Schwabe et al. (2008) demonstrated the importance of a psychological stressor to engage the HPA axis.

When differentiating between physical and psychological stressors, a useful consideration is whether conscious thought mediates the response or whether the response is entirely automatic. This indicates which brain regions and structures are involved in each circumstance. Physical stressors such as haemorrhage, pain or exertion are brainstem-mediated and stimulate the ANS (Potts et al., 2019). The response to a psychological stressor is predominantly initiated by the forebrain regions (Godoy et al., 2018; Potts et al., 2019), and will stimulate both the HPA axis and, to a lesser extent than a physical stress, the ANS. Potts et al. (2019) stated that it is almost impossible to disassociate the two systems and Skoluda et al. (2015) suggested that being placed in a condition of physical stress may well result in a psychological stress condition which is hard to quantify. It is, therefore, overly simplistic to consider the HPA and ANS as independent systems as there is a bidirectional relationship between the two. ACTH can cause an increase in noradrenaline, so the HPA axis stimulates the ANS, and stimulation of the *locus coeruleus* (part of the ANS) can lead to an increase in ACTH secretion (Gidron, 2019). Due to the linkage between the ANS and HPA axis, Chrousos (2009) referred to them together as the ‘stress system’ with both stimulating each other in a mutual positive feedback manner.

When considering the brain regions involved in invoking a stress response, a key neural “hub” for the integration of external stimuli and initiation of either an HPA or ANS response is the amygdala (LeDoux & Damasio, 2013) The amygdala is divided into various regions which each have discrete functions, and there are different pathways to stimulate the amygdala dependant on the nature of the stressor which in turn dictates its output, as outlined in the schematic at Figure 1-2. The lateral amygdala (LA) receives inputs from both the brain stem (physical stress) and from the limbic system (psychological stress) (LeDoux & Damasio, 2013). The basolateral amygdala (BLA) receives inputs from the anterior cingulate cortex (ACC) which contributes to the innate fear response (Jhang et al., 2018) and there is also top-down control from the medial prefrontal cortex (mPFC) to the basomedial amygdala (BMA) managing anxiety and freezing (Adhikari et al., 2015), with sub-divisions of the mPFC responsible for fear expression and fear suppression (Giustino & Maren, 2015). There are also projections from the auditory cortex (AC) to the lateral amygdala (LA) that mediate fear and aversive behaviour in response to auditory stimuli

(Bertero et al., 2019). The output from the amygdala is then either via the central nucleus of the amygdala (CeA) to the *locus coeruleus* (LC) to trigger the SNS (Gidron, 2019) or from the basolateral nucleus of the amygdala (BLA) to the paraventricular nucleus of the hypothalamus (PVN) thus stimulating the HPA axis (Godoy et al., 2018). Neuroimaging has identified increased activity in the BLA in humans when presented with fearful images (Hyman & Cohen, 2013), providing further evidence that the HPA axis is the primary responder to psychological stress. The paraventricular nucleus has a regulatory role in the HPA axis, particularly in an excitatory role (Herman et al., 2003) and thus forms a link between the SNS and HPA, but as yet there is no evidence of the SNS down-regulating dopamine release in the *nucleus accumbens,* an important part of the stress response.

For the recipient of a stressor, an important distinction is whether the stressor is acute or chronic. Acute stress can be defined as a short-lived response to a negative situation that allows a quick and complete recovery of the physiological balance (adaptation), while chronic stress is a long-lasting condition (which could be sequential acute stressors) from which the subject either cannot fully recover or becomes maladapted (Squires, 2010; Trevisi & Bertoni, 2009), and this applies regardless of the nature of the stressor. Kim and Diamond (2002) highlighted the significance of the lack of control over a stressor which leads to maladaptation, and this may be an import factor in ARRCs where animals have very limited control over their environment. It is important also to note that the acute phase stress response will involve both the ANS and the HPA axis – the degree to which each is involved is governed by the physical or psychological nature of the stressor (Skoluda et al., 2015) and it is therefore overly simplistic to suggest that the ANS is the acute response and the HPA axis is the chronic response. In an ARRC context, it is likely that animals are confronted by both acute and chronic stressors. The maladaptation caused by chronic stress is a key welfare concern as the long-term changes in endocrine, physiological and immune functions can manifest in pathological consequences (Trevisi & Bertoni, 2009). However, reduction and mitigation of acute stressors may also improve a dog's chances of being rehomed and may be a more pertinent aim when seeking to have a positive impact both in ARRCs and in domestic homes, and this will be discussed further in the section on defining success.

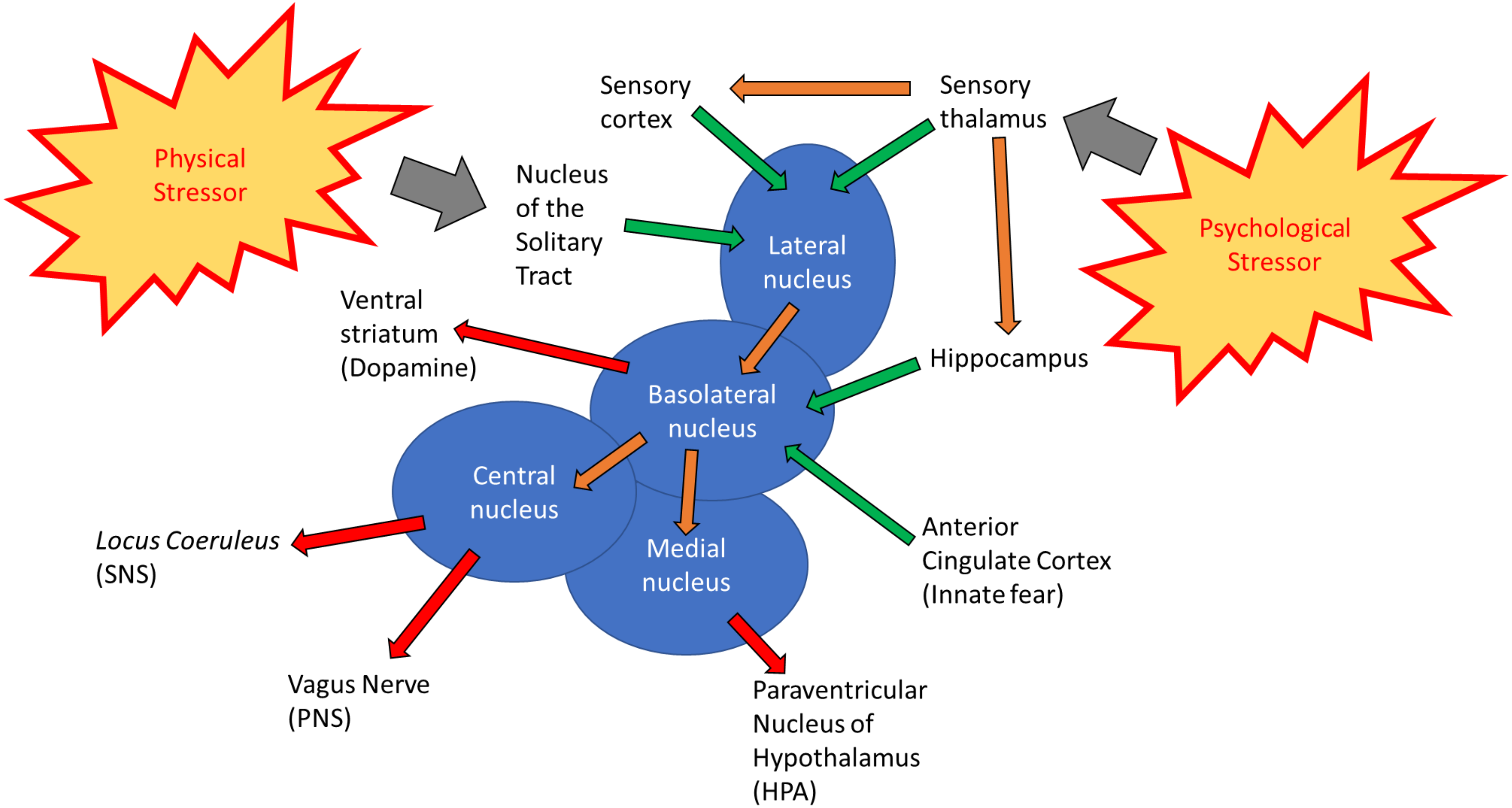


Figure 1-2 **Schematic of the role of four of the nuclei of the amygdala in the response to physical and psychological stressors, demonstrating the interplay between the ANS, HPA axis and Dopamine release, and the difficulty of defining a stress response as coming from purely a physical or psychological stressor. The green arrows indicate inputs to the amygdala, the red arrows indicate outputs from the amygdala (and the systems they influence) and the orange arrows indicate transmission between amygdala nuclei or between other neural structures.**

1.2.2 Impact of Stressors on Welfare

Although there are demonstrated developmental advantages to being exposed to both physical and psychological stressors, there are profound implications for both health and welfare if those stressors overwhelm the body's ability to adapt.

1.2.2.1 Early Life Stressors

Exposure to mild early life stressors is an important way of building resilience in adult life (Fontana et al., 2020), but chronic stress, particularly during developmentally critical periods, can have negative effects that may last the lifetime of the individual (Chrousos, 2009). An example of this is the exaggerated ventral striatal dopamine release in response to a psychosocial stressor seen by Pruessner et al. (2004) in adult humans who had experienced poor early life maternal care. This indicates that early life chronic stress can have long-term effects on the mesolimbic dopamine system, leading to up-regulated stress responses in later life. The implications for dogs that have been exposed to sub-optimal welfare early in life is that they may become irreparably affected, causing them to be labelled as 'difficult' or 'problem' dogs, and therefore there is a particular imperative for ARRCs to minimise exposure to stressors for young (< 6 months old (Dietz et al., 2018)) dogs in their care.

1.2.2.2 Impact of Stressors on Physical and Psychological Health

The dysregulation of glucocorticoids caused by chronic stress has a profound impact for physical health and welfare, including dysregulation of proliferation, differentiation and programmed cell death (apoptosis) as well as important epigenetic changes (Zannas & Chrousos, 2017), immune deficiency (Pruett, 2003), reproductive abnormalities (Kaplan & Manuck, 2004) and altered metabolism (Xu et al., 2020). Stereotypical behaviours may also be triggered by environmental stressors (Vaz et al., 2017), and these behaviours can impact on physical health (Shih et al., 2016). There is also a profound impact on psychological health, with the activation of the HPA axis by the amygdala being fundamental to the pathology of post-traumatic stress disorder (PTSD) in humans (Gidron, 2019). PTSD may not be unique to humans, and Bolman (2019) suggests that African grey parrots *(Psittacus erithacus)* can manifest signs that may be analogous to human PTSD, however limited attention has been given as to whether non-humans remember traumatic effects to the extent that they suffer from those memories. Wild birds have been demonstrated to show both behavioural and neurobiological responses analogous to PTSD (Zanette et al., 2019) and in chimpanzees *(Pan troglodytes)*, compromises in functionality consistent with PTSD have been seen (Bradshaw et al., 2008), but most of the work in animals has been to establish a model of human PTSD (Richter-Levin et al., 2019) rather than studying animal PTSD *per se*.

1.2.3 Interventions

An overwhelming and unrelenting stress response that impacts negatively on either physical or psychological heath needs to be mitigated. There are various options available from pharmacological interventions to environmental enrichment, and this section discusses some of those in relation to their suitability and appropriateness for use in an ARRC.

1.2.3.1 Pharmacological

As outlined previously, a psychological response to a stressor may manifest as either anxiety or depression and these two conditions can be managed with different pharmacological interventions, indicating that the biology of both conditions is different. By way of example, benzodiazepines are the main class of anxiolytics and work by the facilitation of the binding of gamma-aminobutyric acid (GABA) to $GABA_A$ receptors.   They also act as an anticonvulsant to counter blockage of $GABA_A$ receptors, which is an interesting observation as music has been shown to have anxiolytic (Graff et al., 2019) and anticonvulsant (Coppola et al., 2015) effects. The main class of antidepressants are monoamine uptake inhibitors which include tricyclic antidepressants (TCA) and selective serotonin re-uptake inhibitors (SSRI). These act by inhibiting the uptake of noradrenaline and/or 5-hydroxytryptamine (5-HT) and although many also have an anxiolytic effect, it can take days or weeks to produce an effect (Rang et al., 2015). In contrast to the use in humans, SSRIs and TCAs are routinely used to manage various anxiety disorders in dogs (Bazin & Desmarchelier, 2022). Although benzodiazepines would have a faster onset of action, they are not licensed for use in dogs in the EU. The prescription cascade regulations state that a drug licenced for a different condition in the same species (i.e. SSRI) should be given in preference to a human drug used ‘off-licence’ (McPeake & Mills, 2017).

1.2.3.2 Psychobiotics

The gut-brain axis, a vagal-mediated interaction between the gut microbiome and psychological functions (Fülling et al., 2019), has proved to be a promising approach for managing both anxiety and depression through the manipulation of the gut microbiome. Bravo et al. (2011) demonstrated the beneficial effects of *Lactobacillus rhamnosus* on anxiety-related and depressive-like behaviours in mice *(Mus musculus albula)* and showed that these effects where blocked following vagotomy. *Lactobacillus plantarum* supplementation altered GABAergic and serotonergic signalling in the brain of zebra fish (*Danio rerio*) (Davis et al., 2016) and *Lactobacillus helveticus* supplementation given to gestating Sprague Dawley rats *(Rattus norvegicus)* led to offspring with reduced anxiety-like behaviours (Niu et al., 2020). Prebiotic treatment with fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) for stress-related behaviours has also proved successful in mice (Burokas et al., 2017).

1.2.3.3 Placebo Effect

The placebo effect is a psychobiological phenomenon (Benedetti et al., 2005) leading to a physiological response due to different mechanisms, such as expectation of benefit and Pavlovian conditioning (Benedetti et al., 2005; Hall et al., 2015). The evidence is clear that in humans, the placebo effect can have meaningful therapeutic benefits (Finniss et al., 2010) or, as outlined by Lilienfeld et al. (2014), be a confounder to accurately evaluating improvement in psychotherapy. Where music has been used as an anxiolytic intervention, a greater effects size has been seen in studies where patients could select their own relaxing music (Binns-Turner et al., 2008; Gillen et al., 2008; Krout, 2007) as opposed to studies where the relaxing music was specified by the researcher (Graff et al., 2019). One conclusion from this is that, when a patient has selected their own music, they know it is music they find relaxing and therefore there is already an expectation of benefit, meaning that at least some of the effect may be placebo. This is a plausible hypothesis for human studies, but it is not clear if a non-human can have an expectation of benefit, although the other mechanism suggested by Benedetti et al. (2005) is Pavlovian conditioning, and this is a basic form of learning that most species are capable of.

1.2.3.4 Prophylaxis versus Treatment

Much of the focus in the proceeding sections has been on the treatment of anxiety and other stress-related responses, however from an animal welfare perspective, perhaps a more important objective is preventing the stress response from occurring. Although this could be managed pharmacologically by the prophylactic administration of drugs, for most domestic and captive animals a more appropriate approach is improvement of their environment to make it as optimally suited to good animal welfare as possible.

1.2.3.5 Environmental Enrichments

In their book, Shepherdson et al. (1999) discussed the importance of the general enrichment of the kennels' environment for enhancing the welfare of kennelled dogs. Bloomsmith et al. (1991) identified physical, occupational, nutritional, social and sensory as five broad categories of enrichment for non-human primates, and these are areas that are routinely considered when environmental enrichment for captive species, including kennelled dogs, is being undertaken.

Physical enrichment may involve enhancing the environment to prevent chronic inescapable boredom and the related frustration behaviours (Burn, 2017), and it can also involve the removal of stressors such as flickering lights (Morgan & Tromborg, 2007) and triggering sounds (Castelhano-Carlos & Baumans, 2009). Increasing the complexity of the kennel, such as providing raised platforms and access to outdoor areas can help improve the welfare of dogs (Hubrecht, 1993) and providing hiding places for cats (Vinke et al., 2014) has been demonstrated to have a positive welfare benefit. Considerations such as these are important when designing a kennel or rescue centre, but as most ARRCs already have fixed infrastructure, major changes to size or design may not be feasible.

The lack of novel stimulation and physical activity in kennelled dogs can lead to boredom, inflexible behaviour, reduced reactivity, and the development of stereotypic behaviour (Hubrecht, 1993; Wells, 2004). While the necessity of supplemental exercise is a controversial issue, studies have shown that exercise may not only provide welfare benefits through a reduction in boredom, but also increase rehoming potential of the dog (Menor-Campos et al., 2011; Wells & Hepper, 1992). Moreover, positive experiences such as pleasure or play are indicative of good welfare (Boissy et al., 2007). However, toys provided as enrichment tools may not be the most effective means of reducing boredom as, while dogs may show interest in toys, they may not prevent the development of repetitive behaviour, particularly circling (Hubrecht, 1993). The presence of a toy in the kennel may indirectly benefit the dogs through effects on rehoming and it has been shown that potential buyers are more attracted to dogs housed in kennels with toys, helping them to visualise the dog as a desirable pet (Wells & Hepper, 2000). Providing toys filled with food have been shown to increase appetitive behaviours, activity, and reduce barking (Schipper et al., 2008).

Feeding certain diets, such as low protein supplemented with tryptophan, has been found to reduce aggressive behaviour in dogs (DeNapoli et al., 2000). Additionally, feeding dogs a higher quality diet can lead to reduced plasma ACTH concentrations, which may indicate lower stress levels (Hennessy, 2013). Psychobiotics can alter the gut microbiome and bring about changes in stress reactivity, anxiety-related, depressive-like, and social behaviours, working through a vagal-mediated mechanism (Fülling et al., 2019). Chronic stress has also been shown to influence the gut-microbiome composition in dogs (Mondo et al., 2020) and Yeh et al. (2022) showed that oral administration of *Lactiplantibacillus plantarum* PS128 to dogs reduced behavioural problem severity. Although now approved as a method to treat patients with refractory depression in humans, it’s utility in an ARRC may be limited, primarily from a cost perspective.

As dogs are highly social animals with a need for conspecific contact and human attention, with single-housing and the prevention of social interaction can lead to the development of maladaptive behaviours and stress-related problems, (Hetts et al., 1992; Mertens & Unshelm, 1996). Group-housing may prevent such problems (Grigg et al., 2017) but can also present challenges such as kennel mate compatibility.

The kennel environment for dogs is sensory-deprived compared to their natural environment in the wild, so sensory enrichment methods such as visual, olfactory and auditory stimulation have been explored to alleviate the boredom and stress that dogs experience in kennels (Wells, 2009). Graham et al. (2005) investigated the use of moving visual images for the stimulation of dogs and although they found that the behaviour of kennelled dogs was influenced by visual stimulation, they suggested that dogs may not benefit from this type of enrichment to the same extent as species with more well-developed visual systems such as primates. Olfactory stimulation using scents, such as vanilla, valerian, coconut and ginger has been used to greater effect and has been demonstrated to reduce both activity and vocalisations in kennelled dogs (Binks et al., 2018). Auditory enrichment has been trialled extensively with a range of species and will be discussed in detail in section 1.3.

1.2.3.6 Interventions within an ARRC

In the context of an ARRC, both anxiety and depression may need to be addressed, not only as a welfare issue, but because dogs that are depressed or anxious are less likely to be rehomed (Wells & Hepper, 1992). As the pharmacological mechanisms for managing these two conditions are different, it is perhaps optimistic to expect any single non-pharmacological intervention (such as music) to effectively manage both conditions. Although pharmacological interventions are effective, their blanket use to manage behaviour in the ARRCs may be both inappropriate and undesirable. Although clearly an area of research worth pursuing, implementing specialist pre/probiotic diets within the ARRCs would require very careful consideration of the cost versus benefit. One of the drives for any animal charity is to have maximum impact for minimum cost, so psychobiotics may not be suitable for widespread use. Enhancing or modifying the acoustic environment is a potential candidate as part of a general enrichment programme, particularly for ARRCs, as its application can be both cheap and simple.

1.2.4 Measuring Physiological Responses

Having discussed potential interventions, it is important to consider exactly how the effect of those interventions will be measured. Options for quantifying stress responses are discussed in this section in relation to the mechanisms of stress responses and their suitability for use in an ARRC.

1.2.4.1 Heart Rate Variability (HRV)

When conducting cardiac auscultation, a heart in normal sinus rhythm will appear to have a regular, consistent beat pattern. However, when the electrical activity of the heart is measured with a device such as an electrocardiogram (ECG), it can be seen, as in the example in Figure 1-1-3, that the time between every beat varies by a few milliseconds. Heart rate variability (HRV) is the measure of this variance and is controlled by the ANS. Therefore, the degree of HRV gives an insight to the functioning of the ANS at that time.

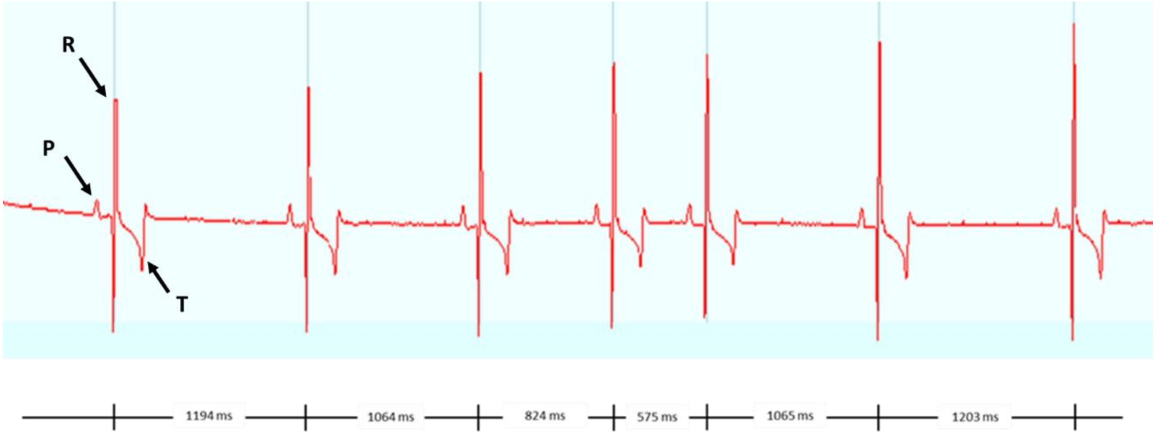


Figure 1-1-3 - Example of an ECG trace (canine) showing the normal variance in timing between each beat. The P, R and T waves are annotated on the first beat.

The R wave on an ECG (electrocardiogram) is the first positive deflection seen after the P wave. It represents the depolarization of the ventricles of the heart, leading to the contraction of the heart muscle. In humans, the R wave is usually the largest wave in a normal ECG tracing, but there are species-specific variations, discussed in Chapter 2. The inter-beat variability is usually measured between R peaks and so is often referred to as the R-R interval. A major contribution to this variance in R-R interval is from Respiratory Sinus Arrhythmia (RSA) (Phillips and Donofrio, in Bloom et al. (2009)) and reflects the activity of the PNS branch of the ANS. The principle behind RSA is that, on breathing in, the inter-thoracic pressure reduces which reduces blood pressure. Baroreceptors activate to suppress vagal tone, raising heart rate to counter the lowered blood pressure. The reverse happens during expiration so there is a continual rhythmic increase and decrease in heart rate from which the respiration rate (ECG derived respiration (EDR)) can be estimated. RSA is therefore always going to be present, but what is important is the amount of change each time the heart rate rises and falls. High variability is indicative of healthy ANS function and good physiological recovery, but from an animal welfare perspective, high variance is also indicative of high vagal tone / dominance of the PNS which suggests the animal is in a state of ‘rest and digest’ rather than ‘fight or flight’.

In humans, HRV has a wide range of applications such as a marker of heart disease and stroke (Jarczok et al., 2019), evaluating post MI prognosis (Huikuri & Stein, 2012), as a measure of physical training adaptation (DeBlauw et al., 2021) and in the evaluation of major depressive disorder (Caldwell & Steffen, 2018). In the fields of animal welfare and behavioural sciences, HRV is primarily used as a non-invasive measure of stress responses and has been used in a range of species including dogs (Bowman et al., 2017; Katayama et al., 2016), production animals (Erdmann et al., 2018) and horses (Squibb et al., 2018). Whereas clinical evaluation is conducted against reference ranges and fitness evaluation is conducted using repeat measures over a protracted period of time, animal studies generally measure the changes in HRV over the course of an experimental paradigm and can provide a resolution down to 1 min epochs (Camm et al., 1996). There is debate about whether HRV can elucidate anything about the functioning of the sympathetic nervous system, but it is universally accepted that increased variance equates to an increase in vagal tone / PNS activity and a decreased variance equates to a withdrawal of vagal tone. Therefore, although it is common to interpret reduced variance as “more stressed” it is technically more correct to interpret this as “less relaxed”. Although it may seem intuitive that these two terms could be used interchangeably, it must be remembered that the SNS/PNS balance is not directly reciprocal thus a reduction in one does not necessarily lead to a proportional increase in the other.

HRV can be quantified with regard to i) time-domain parameters such as the Route Mean Square of the Successive Differences (RMSSD) or the Standard Deviation of the IBIs (SDNN) (Allen et al., 2007). ii) frequency-domain parameters that quantify what proportion of the signal can be accounted for by pure sine waves from very low, low and high frequency (VLF/LF/HF) bands (Kuss et al., 2008) and iii) Non-linear parameters (SD1 and SD2), derived from Poincare plots, which give a measure of the complexity and unpredictability of the signal (Shaffer & Ginsberg, 2017). RMSSD and SDNN are both surrogate indexes of PNS activation, but SDNN is a more suitable metric for long-term variability (minimum of 5 min required) (Teckenberg-Jansson et al., 2019). SD1 is identical to RMSSD (Ciccone et al., 2017), although some authors still report these as separate measures. SD2 closely correlates to SDNN and is therefore only suitable for assessing long-term (>5min) variance in IBIs. The LF/HF ratio is commonly reported, but this (and LF power) are now seen as unreliable measures of SNS activity (in humans) (Thomas et al., 2019) as LF Power is representative of changes in both SNS and PNS. HF Power has previously been demonstrated to be the measure most closely aligned to PNS activity in horses (Stucke et al., 2015). The metric used to analyse the IBI data needs to be selected taking into account the length of sample and the quality of the data. Both RMSSD and HF Power can be reliably assessed using 1 min recordings whereas SDNN and any parameter using LF Power requires at least 5 min (Seppälä et al., 2014). RMSSD is not adversely affected by missing data (e.g., missed beats or sections of date corrupted due to artefact) but HF Power appears to be more sensitive (Sheridan et al., 2020).

For reliable frequency domain analysis, species-specific frequency bands must be selected (Joachim A. Behar et al., 2018) as the respiration rate must be within the HF band. Power (area under the curve) reflects the amount of variance in each band. Higher HF power indicates greater RSA, but may be at the same peak (i.e., same respiration rate) as a sample with lower total power. HF Power peaks at respiratory frequency, hence the ability to estimate the EDR rate. Figure 1-1-4 and Figure 1-1-5 are from 5 min sections of HRV analysis from a horse, taken approx. 20 min apart. It can be seen that the respiration rate is approx. the same (indicated by the Power Spectral Density (s^2/Hz) peaking at 0.24 Hz in both cases), however, the total HF power (area under the curve) in Figure 1-1-5 is nearly eight times that in Figure 1-1-4 (13,090 ms^2 vs. 1,719 ms^2), this would indicate a higher vagal tone in Figure 1-1-5.

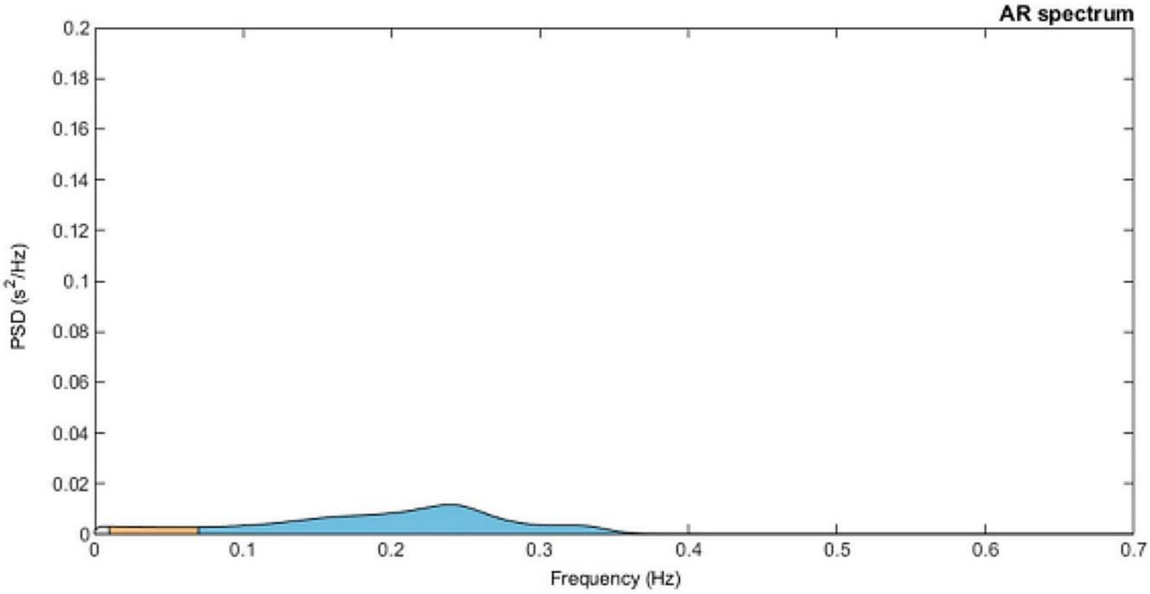


Figure 1-1-4 - Auto-regressive HRV spectral analysis - low vagal tone (equine). HF spectrum in blue, LF spectrum in orange. HF peak 0.24 Hz (approx. 15 breaths per min.), HF Power 1,719 ms^2, HR 38 bpm, RMSSD 83 ms

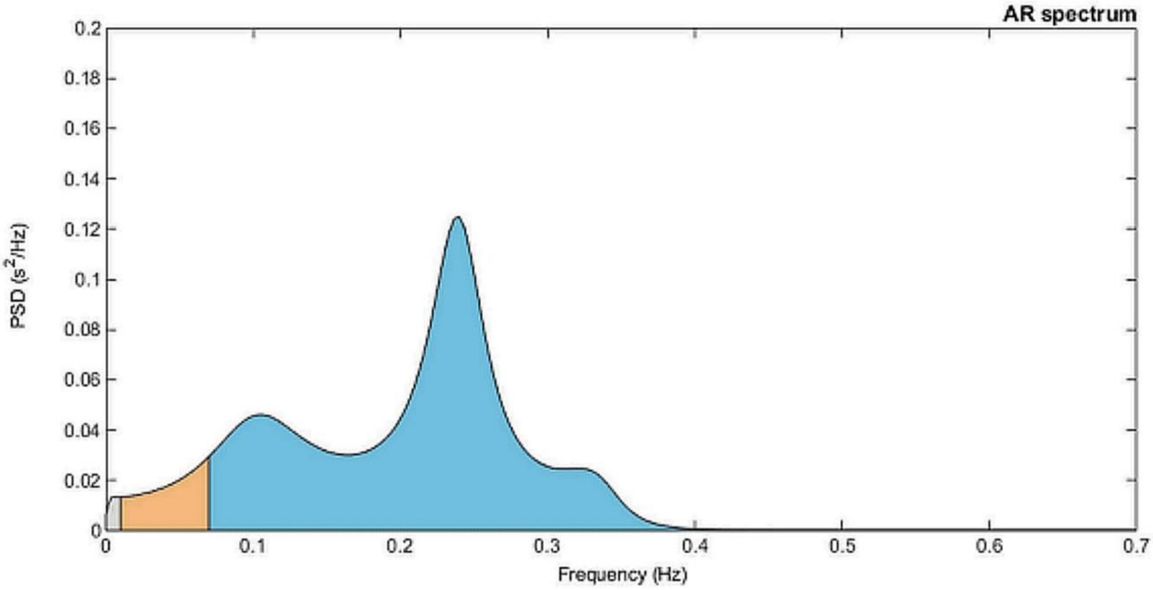


Figure 1-1-5 - Auto-regressive HRV spectral analysis – high vagal tone (equine). HF spectrum in blue, LF spectrum in orange. HF Peak 0.24 Hz, HF Power 13,090 ms^2, HR 39 bpm, RMSSD 234 ms

It is also of note that the average HR for each of these samples was the same and that although RMSSD was greater in the sample in Figure 1-1-5 (234 ms versus 83 ms; also indicative of high vagal tone) the magnitude of change was not as large as for HF Power. This exemplifies how HRV analysis can provide more information about the functioning of the ANS than either heart rate or respiration rate alone.

1.2.4.2 Cortisol

The levels of circulating cortisol within an animal are a reliable indicator of HPA axis functioning and stress reactivity (Gotlib et al., 2008) although it can only ever be an indirect measure of the triggers of the HPA axis (Hellhammer et al., 2009). It has been shown to be a quantitative and proportionate measure of induced acute stress (Kidd et al., 2014) and has been used in numerous animal studies (Ellis et al., 2014; Peeters et al., 2013; Scheidegger et al., 2016; Schmidt et al., 2010).

Cortisol levels can be measured in plasma (Bohák et al., 2013), faeces (Palme et al., 2005), saliva (Ellis et al., 2014; Peeters et al., 2013), hair (van Eerdenburg et al., 2021) and urine (Blackwell et al., 2010). Faecal sampling is the least invasive method of measuring cortisol, but the time lag between a stressor and a rise in cortisol being seen is approximately 24 hours (Palme et al., 2005) depending on the species, and is therefore only suitable for monitoring long-term chronic stress or acute stressors that occurred 24 hours earlier. The time lag for cortisol to appear in hair is considerably longer (Bryan et al., 2013) and cortisol levels in hair are less sensitive to acute stress (Ashley et al., 2011). There is less of a delay in reaching peak cortisol in urine than in either hair or faeces, but it is still about 3 hours (Rooney et al., 2007). It is also important to account for fluctuations in urine volume as this will influence concentration, but this can easily be done by calculating the urinary cortisol/creatine ratio (Behrend et al., 2013). It is also difficult to ensure dogs pass urine at specific times, thus making the time from stressor to sampling hard to replicate for each sample. Taking blood samples for plasma analysis is invasive (therefore potentially stressful), and in the UK requires licencing under the Animals (Scientific Procedures) Act (ASPA). Taking saliva samples does not require an ASPA licence, however, some animals may find the necessary restraint stressful and as pointed out by Skoluda et al. (2015), may be a confounding influence on the results. When using cortisol concentrations in plasma, saliva or urine, it must be remembered that cortisol is secreted in an ultradian and circadian related fashion (Nicolaides et al., 2017) and this impacts on the timing of sampling and the interpretation of the results to be confident that the changes seen are stress related.

Narayan et al. (2019) proposed that animals in a high stress environment would show a blunted cortisol response to a stressor and a slower recovery than animals in a low stress environment. This could potentially confound measurements made in an environment known to produces a prolonged activation of the HPA axis, such as an ARRC (Hennessy, 2013; Hennessy et al., 1997). An alternative approach was proposed by Trevisi and Bertoni (2009) who demonstrated an exaggerated cortisol response from chronically stressed cows. The response to a challenge may therefore be a more valid assessment of animals exposed to chronic stressors than the baseline levels, but intentionally distressing animals raises ethical concerns.

Cortisol would initially appear to be an ideal measure of effect for the current study as it reflects the HPA axis and thus psychological stress, which is likely to be the cause of poor welfare in ARRCs, and cortisol has also previously been used to demonstrate the medium to long-term effects of music (Bowman et al., 2017). However, regardless of sampling method employed, there is always going to be a lag (30 mins to 24 hours) between a change in physiological arousal and peak cortisol levels, and if the animals are showing a chronic stress response, the absolute cortisol concentration may be misleading. Despite the utility of cortisol in quantifying responses to psychological stress, cortisol concentrations are not able to reflect the nuances of minute-by-minute responses to a changing environment.

1.2.4.3 Spontaneous Blink Rate (SBR)

Spontaneous Blink Rate (SBR) is widely used to study stress and anxiety in humans. Giannakakis et al. (2017) found that SBR significantly increased during specific stressful and anxious situations when compared to the person-specific control situation and correlated this with an increase in heartrate. When studying concentration, Berguer et al. (2001) and Joiner et al. (2016), found that SBR significantly reduced when concentrating on reading a text and in human studies, SBR is now routinely used as a measure of cognitive effort (Kwee-Meier et al., 2017). Although these studies have not been widely replicated with animals, Mott et al. (2020) demonstrated that SBR was a reliable measure of both increased arousal (increased SBR) and increased vigilance (reduced SBR) in horses and postulated that it may be a reflection of striatal dopamine levels. SBR has also been shown to increase in dogs in response to an acute stressor (fireworks) (Gähwiler et al., 2020). Although a promising and completely non-invasive measure of acute stress, accurate measurement does require a constant view of one or both eyes and this can make assessment difficult. This can be overcome with restraint but as with the collection of invasive samples, this could be a confounding factor during stress assessment. Measuring SBR is almost impossible on free-roaming animals, and so is probably not suitable for use in a ARRC setting where dogs are free in individual kennels/runs.

1.2.4.4 Other Physiological Measures

As detailed above, an increase in cortisol does not reflect SNS activity, however, another salivary biomarker, salivary alpha amylase (sAA), has been used as a marker of the SNS response (Bitsika et al., 2014; Vogel et al., 2019). In some circumstances, taking a saliva sample may be more appropriate than constant monitoring using a heart rate monitor but taking a saliva sample require the animal to be restrained and so may add a confounding stressor. Skin conductance, also known as Electro Dermal Response (EDR) or Galvanic Skin Response (GSR), is a reliable measure of a response to a stressor (Liapis et al., 2015), specifically the activity of the SNS (Nepal et al., 2018). Since HRV can only give a reliable measure of PNS activity, coupling EDR with HRV has the potential to give the full picture of ANS activity.

As outlined in paragraph 1.2.1.3, there is a dopamine response to stressors. Dopamine levels can be measured in peripheral blood using techniques such as HPLC (Low & Mathias, 2005). However, as dopamine cannot cross the blood-brain barrier, the levels of dopamine in the blood are not a reliable indicator of the levels of dopamine in the brain, and therefore not a reliable measure of arousal.

Electroencephalograms (EEG) have been widely used to study human emotional responses (Hamada et al., 2018) and require the placement of electrodes on the scalp to record electrical activity from the brain. In relation to their brain size, dogs have very thick skulls and highly muscular heads, which leads to issues gaining reliable data from the surface electrodes (Bunford et al., 2017). Needle electrodes give better resolution, but this is generally only performed on anaesthetised dogs. (Cauduro et al., 2017). Ambulatory devices are available for veterinary use and have been used by James et al. (2017) for epilepsy screening in dogs and by Stomp et al. (2021) for quantifying stress responses in horses. Whether the resolution in dogs would be good enough to quantify an emotional state may be questionable, but there may be utility for perception testing by using an EEG for measuring Brainstem Auditory-Evoked Responses (BAER) in dogs (Ter Haar et al., 2002).

1.2.4.5 Behavioural Measures

Behavioural ethograms are routinely used in animal studies to quantify responses to stressors (Dunbar et al., 2016; Hall & Heleski, 2017; Rose & Riley, 2021; Stanton et al., 2015) in place of self-reports used for the same purpose in human studies (Baker et al., 2002). When studying the effect of musical mode and tempo on human subjects, Trochidis and Bigand (2013) recorded physiological responses (EEG) and found that the physiological responses were able to capture the subtle effects and interactions that were not obvious from similar previous studies relying on self-reports. Whilst recording behavioural responses clearly has utility (non-invasive, no lab work, potentially integrative of the ANS and HPA axis), particularly as the aim is to affect a behavioural change, behavioural measures may be more susceptible to confounding factors such as learned behavioural inhibition (Foraita et al., 2021) which might a particular issue for dogs in a ARRC that may have a background of sub-optimal welfare. In this context, physiological measures may give a more objective measure of effect.

1.2.4.6 Summary of Response to Stressors

A range of physiological parameters reflecting the responses to stressors can be measured, with some measurements being more invasive than others, and this may conflate results. Some, such as cortisol, can show a change in state over hours or days, whereas EEG will instantaneously show changes, and HRV can show changes minute-by-minute. It is worth reiterating that all these measures are only recording a physiological response and leaving the researcher to make assumptions about internal states. On balance, HRV is the most suitable metric for use in this study and although it does not reflect the HPA axis (psychological stress) it does give the better temporal resolution required for this study.

1.3 Auditory Enrichment

As discussed in section 1.2.3 above, enhancing or modifying the acoustic environment is a potential candidate to reduce stress responses, particularly for ARRCs, as its application can be both inexpensive and simple. This section discusses the extensive evidence from human and animal studies and explores how this work fits with dogs' perceptive abilities.

1.3.1 Musical Interventions

1.3.1.1 Human Studies

The field of music therapy first evolved from the treatment of PTSD in veterans returning from World War II (Collins & Fleming, 2017). Interest in therapeutic applications for music has grown since then, and there is a body of evidence to support the use of music to improve outcomes in a disparate range of conditions in humans such as autism (Whipple, 2004), Alzheimer's disease (Fang et al., 2017), epilepsy (Coppola et al., 2015; Lin et al., 2012), stroke (Särkämö et al., 2008), acute pain (Lee, 2016), chronic pain (Bradt et al., 2016) and Parkinson's disease (Ghai et al., 2018). Some of these studies involve music therapy in its true sense (i.e. the patient being actively involved in the production of music) and this has shown good effect in the management of neurodegenerative conditions such as Alzheimer's disease (Palisson et al., 2015; Simmons-Stern et al., 2010), however many studies involve passive listening, more formally known as music medicine interventions (Bradt & Teague, 2018).

In humans, the calming effect of passively listening to music is widely reported in both clinical and non-clinical settings (Finn & Fancourt, 2018) and has been used as a non-pharmaceutical method of reducing pain and stress during prostate biopsies (Chang et al., 2015), childbirth (Wulff et al., 2017) and dental treatments (Bradt & Teague, 2018). Kühlmann et al. (2018) reported that anxiety was reduced when music was played prior to surgery and that the perception of pain was reduced if music was played after surgery. No significant effect was found of intraoperative music, which supports the earlier work of Migneault et al. (2004) who reported no effect of music on the hormonal stress response of surgical patients under general anaesthesia. Some of this anxiolytic and analgesic effect of passively listening to music may be explained by the work of Moraes et al. (2018) who demonstrated that classical music increased striatal dopamine activity in Wistar rats and by Chen et al. (2017) who, demonstrated up-regulation of the striatal D2 receptors of young adults when exposed to the popular music ‘Gangnam Style’. The music/dopamine link has also been demonstrated in humans by Salimpoor et al. (2011), who showed that listening to music increased striatal dopamine release and Chanda and Levitin (2013) who, in their review, proposed that musical reward may be driven by dopamine release associated with expectation and predictions.

One of the most recent studies to investigate the anxiolytic effect of music (Graff et al., 2019) concluded that music offered an effective alternative to intravenous midazolam (benzodiazepine) but cautioned that the results be interpreted with care due to a number of limitations in the study including a small sample size and the self-reporting subjective nature of the data. Unusually, Graff et al. (2019) did not allow the patients to select their own music when previous recommendations (Binns-Turner et al., 2008; Gillen et al., 2008; Krout, 2007) has been that the greatest effect can come when patients select their own music, particularly when trying to reduce anxiety. The music selected by Graff et al. (2019) was ‘Weightless’ by Marconi Union, a piece of electronic music specifically designed to aid relaxation by the use of carefully arranged harmonies, rhythms, and bass lines. Another piece of music favoured by researchers is Mozart’s piano sonata for 2 pianos in D major (K448) which has been made popular in music research by the reported “Mozart Effect” (Rauscher, 1994), which although was investigating improvements in IQ from listening to K448, has been used repeatedly in studies on the management of anxiety and pain (Conrad et al., 2007; Coppola et al., 2015; Lin et al., 2012). It appears in humans that the anxiolytic and analgesic effects are dependent on personal preference, with Wulff et al. (2017) showing that preferred musical choice has a more positive effect. This aspect of personal choice has also been demonstrated with Turkish (Yüksel et al., 2015) and Iranian (Abedi et al., 2017) subjects listening to nation-specific traditional music. Education and socio-economic status may also influence music choice, as shown by Račevska and Tadinac (2018) who demonstrated a positive correlation between general intelligence and a preference for instrumental music. Therefore, in human subjects, no one musical selection will have the desired effect on every individual, and musical preferences need to be taken into consideration (Krout, 2007), although 'classical' music is often demonstrated to have a relaxing effect. The effect of personal choice may be explained by Bradt et al. (2013) who, when reviewing the evidence for an anxiolytic effect of music in humans, highlighted that none of the participants could be blinded to the study. Faria et al. (2012) discussed the widely reported issue of the placebo effect in pharmacological anxiolytic human studies - the regular finding of therapeutic effect of inert substances. The placebo effect is dependent on the subject's expectation of benefit (Robson, 2022) but can still generate a powerful physiological response. In non-blinded studies such as those used to investigate the therapeutic use of music, the placebo effect cannot be discounted, with individuals believing they should be more relaxed after listening to the music.

The recent systematic reviews of the clinical effects of music in humans (Bradt et al., 2013; Brady et al., 2018; Finn & Fancourt, 2018; Guerra et al., 2019; Wilson, 2018) have generally been measured in their interpretation of the evidence. Both Bradt et al. (2013) and Finn and Fancourt (2018) highlighted the issue of the high risk of bias in all the reviewed studies and methodological weaknesses were identified in the majority of studies reviewed by both Bradt et al. (2013) and Wilson (2018). Guerra et al. (2019) found limited evidence to either support or refute the use of music as an analgesic or sedative although Finn and Fancourt (2018) determined 11 out of 20 clinical trials they reviewed demonstrated some stress reducing effect. A review produced 10 years earlier (Gillen et al., 2008) was similarly non-committal but as there was enough evidence to suggest that patients may derive benefit from an intervention that was both safe and cost effective, they recommended that it was worth investigating further. Likewise, Bradt et al. (2013) concluded that, despite the many weaknesses, the body of evidence was that music may be a viable alternative to anxiolytic drugs for pre-operative anxiety, however they later determined that the evidence of efficacy for anxiety during dental treatment was less conclusive (Bradt & Teague, 2018). One of the key recommendations from Bradt and Teague (2018) was that a coordinated programme of music therapy may be more powerful than simple passive listening.

1.3.1.2 Animal Studies

Some of the earliest work testing the effect of music on dogs in ARRCs was conducted by Wells et al. (2002). They found an arousal-reducing effect of classical music but that, as later also demonstrated by Kogan et al. (2012), heavy metal led to increased signs of agitation. Bowman et al. (2015) demonstrated a short-term effect of classical music on the HRV of shelter dogs and suggested a rapid habituation occurred. Previous work in humans as outlined above has focused on an immediate reduction of the acute stress response for the duration of the auditory intervention, typically during medical procedures and for shelter animals, this clearly has utility in alleviating stress in the first few days in a novel environment. However, as many animals will spend a protracted period of time in this environment, it is important to identify how to counteract the chronic stress response. Bowman et al. (2017) found that the urinary cortisol results contradicted the HRV and behavioural results, possibly suggesting that music (in this case soft rock) has an effect on the SAM axis (and therefore physical stress response) rather than the HPA axis (and therefore the psychological stress response) and Conrad et al. (2007) also suggested that in humans music had an effect on the SAM axis, having found a decrease in adrenaline concentrations but no significant alterations of ACTH and cortisol concentrations whilst listening to music. However, with the previously demonstrated effect of music on striatal dopamine (Chanda & Levitin, 2013; Chen et al., 2017; Moraes et al., 2018; Salimpoor et al., 2011) it is possible that the HPA axis is the target, although both pathways do work in concert. However, identifying the pathway activated doesn’t explain the habituation seen by Bowman et al. (2015). A potential solution to the habituation seen is that, when reinforcement is used as part of learning acquisition, a partial reinforcement schedule is far less prone to extinction than a continuous one (Sangha et al., 2002). The positive effect of music may therefore be less prone to extinction if presented at variable intervals rather than continuously.

In an attempt to reduce the long-term stress of zoo-housed monkeys, Wark (2015) trialled a number of different sounds including recordings of the natural habitat and the sound of a waterfall but found the most restful environment was one of complete silence. This is in contrast with the meta-analysis by Buxton et al. (2021) who found, in humans, large effects sizes from listening to ‘natural’ sounds. This indicates that rather than investigate sounds that humans would intuitively consider to be restful, it is important to investigate the effect of particular sounds at a neuro-physiological level. An issue that is often overlooked is that, although a recording of a waterfall may sound just like a waterfall to human ears, animals with different auditory ranges may not hear a recording as a true representation (Kriengwatana et al., 2022), and may be hearing something aversive in the recording that humans would be unaware of. In agreement with this, Lange et al. (2020) demonstrated that cows preferred “live” spoken word over a recording of the same, even when a human was present in both conditions.

As with humans, in animals there may be different responses to the same piece of music depending on a range of factors including previous associations. As neuro-physiological responses have been identified in rats (Moraes et al., 2018) and humans (Chen et al., 2017) and a behavioural response has been observed in dogs (Bowman et al., 2017) to various genres of music, it is plausible to suggest that the same mechanisms exist in other species. Indeed, auditory enrichment with music has been seen to have an effect in horses (Hartman & Greening, 2019), rats (Kühlmann et al., 2018), cats (Hampton et al., 2019), pigs (Zapata Cardona et al., 2022), cattle (Crouch et al., 2019) and birds (Williams et al., 2017).

1.3.1.3 Mechanism of Action

As shown above, there has been at least a modest anxiolytic effect of music demonstrated in both human and animal studies. One of the aspects that needs to be considered is whether this effect is a response to the specific piece of music selected. In a review, Krout (2007) summarised the key musical elements which improved relaxation in humans include a slow and stable tempo, low volume, absence of percussive rhythms, connected melodies, and simple harmonic or chord progressions. The Sonata for Two Pianos in D major, K. 448, composed by Wolfgang Amadeus Mozart in 1781, has been used by a number of researchers to manage pain (Kishida et al., 2019), anxiety (Conrad et al., 2007) and seizures (Coppola et al., 2015). The supposed ability of this particular piece of music to improve concentration and IQ led to the popularised term “Mozart Effect” (Rauscher et al., 1993). Although this particular piece may conform to the tempo, structure, melodic and harmonic consonance and predictability at outlined by Krout (2007), it is a completely different musical experience from the carefully arranged harmonies, rhythms, and bass lines of modern music specifically designed for relaxation in humans such as "Weightless", Marconi Union, which was demonstrated to have an anxiolytic effect comparable to midazolam by Graff et al. (2019).

For the human brain, listening to music is an extremely complex process as it involves the connection of a range of cognitive and emotional processes. Imaging studies have shown increased activity well beyond the auditory cortex including frontal, temporal, parietal and subcortical areas (Bhattacharya et al., 2001; Janata et al., 2002; Koelsch et al., 2004; Popescu et al., 2004), as well as limbic and paralimbic regions (Blood & Zatorre, 2001; Blood et al., 1999; Brown et al., 2004; Koelsch et al., 2006; Menon & Levitin, 2005) so for humans, the perception of music is an holistic process occurring throughout the brain (Edwards & Hodges, 2007) and the positive effects may derive from a cognitive component associated with anticipation, prediction, and working memory (Colver & El-Alayli, 2016). The wide-spread involvement of these brain regions related to attention, memory, semantic processing, motor functions and emotions may be explained by the global workspace theory of consciousness where stimuli activate multiple regions of the brain, to generate a “conscious” experience (Baars, 2005). In humans, difference in responses to motivational and calming music may be explained by the involvement of different regions of the prefrontal cortex (Bigliassi et al., 2015). This suggests that in humans, at least some of the effect comes from an understanding of what is being heard, with linkages back to previous experiences, and there may be emotional ties to music that are uniquely human (McDermott & Hauser, 2007). For animals with very limited prefrontal cortices, this may not be the case, and it is currently impossible to quantify the richness of the inner world experienced by animals. Physiological responses from listening to music being the result of a conscious experience may explain the results seen by Binns-Turner et al. (2008), who (in humans) despite seeing a reduction of pre-operative anxiety and post-operative pain, saw no physiological response to music being played whilst the patient was anaesthetised.

Särkämö et al. (2008) postulated that the improvement in cognitive recovery seen in their study of stroke patients may have been due to music inducing a positive mood and hence an activation of the dopaminergic mesocorticolimbic system, particularly the pathway from the ventral tegmental area to the *nucleus accumbens*, an inhibitory pathway that has been implicated in the suppression of pain and other aversive stimuli (Menon & Levitin, 2005). It has also been proposed that music has a suppressive action on the sympathetic nervous system, (Gillen et al., 2008) which is also a plausible explanation for the anxiolytic effects seen. However, when studying the beneficial effect of music for epilepsy patients, Lin et al. (2012) suggested that musical enhancement of parasympathetic tone may be the key mechanism.

An altogether simpler mechanism has been proposed by Krout (2007) and Scharine et al. (2009), that music may act as a masker and/or distractor, covering-up other unwanted sounds and thus aiding the listener's relaxation process. It is important to tease-out whether the relaxing effects seen are related to specific musical elements acting on the SNS/PNS or if acoustic masking is the key mechanism. As discussed by Kriengwatana et al. (2022), this can be achieved by the inclusion of a non-music sound control (such as white noise) rather than simply comparing music to silence.

There is likely also to be something innate about the response of all animals to particular sounds. Arnal et al. (2019) showed in humans the rough sounds in the 30-80 Hz range (such as a typical fire alarm) "hijack" brain regions involved in auditory processing and suggested a link to natural alarm calls, adding salience above what would be expected from that volume of sound. In rats, it has been seen that pro-social frequencies (50 kHz) cause an increase in striatal dopamine whereas alarm calls (22 kHz) did not (Willuhn et al., 2014). Using the same frequencies, Sadananda et al. (2008) demonstrated that pro-social and alarm frequencies stimulated completely different brain regions. For humans, the tempo and pitch of the music are important, and a study by Husain et al. (2002) showed that when changing the tempo of Mozart's K.448, performance on a special task improved with faster tempos and changing the key from minor to major improved mood. There might therefore be a particular pitch and / or tempo that is most relaxing, and this may well be species (and possibly individual) specific. When considering auditory enrichment, it is also necessary to consider pitches and tempos that animals may find to be particularly aversive, and these might be outside of the human hearing range and therefore not given due consideration. It is important, therefore, not to be anthropocentric when considering a welfare-friendly acoustic environment, and a greater understanding of how animals perceive the world needs to be developed.

1.3.2 What dogs hear

An important question to address when designing auditory enrichment of any kind, is to determine what the target species can hear. As outlined above, it is too easy to be anthropocentric and potentially use enrichments that the animal cannot hear, and not take account of stimuli that they can. A useful starting point is to develop a frequency / intensity (FI) curve for dogs and compare that to one representing human hearing, an example of which is Figure 1-6 below.

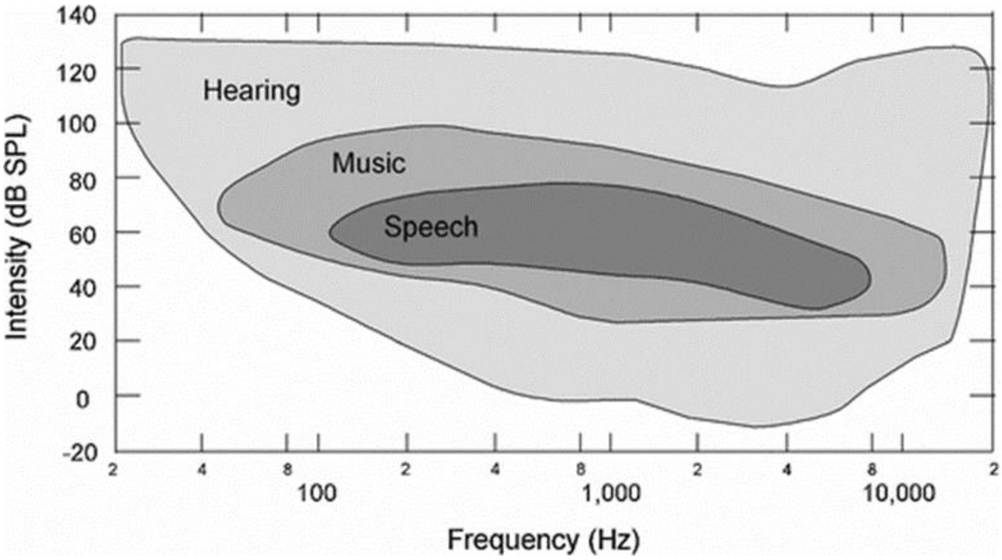


Figure 1-6 - Human FI curve (Herman, 2016)

It is of note that, although the minimum and maximum hearing ranges are often referred to, it can be seen from Figure 1-6 that at the extreme limits of hearing (approximately 20 Hz to 20 kHz), the volume must be very high to be heard, and this general pattern is repeated in all species. Of greater practical relevance is what is referred to as the sensitive range i.e., the range of frequencies that can be heard at about 40-60 dB, and in Figure 1-6, this overlaps with the area covered by music and speech, approximately 50 Hz to 10 kHz.

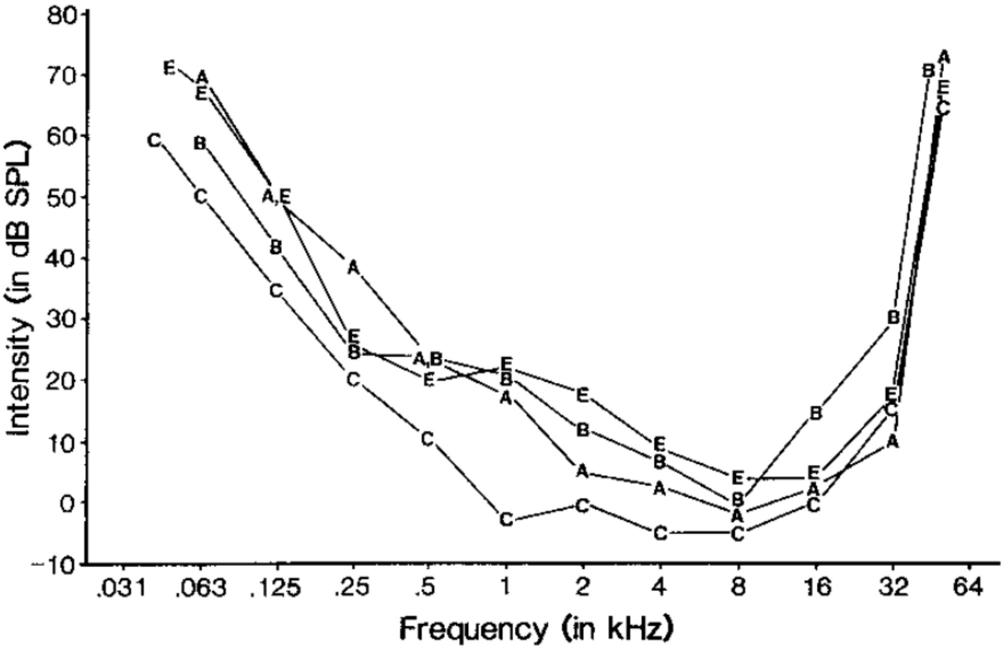


Figure 1-7 – Audiograms of four dogs (A, Chihuahua; B, Dachshund; C, Poodle; E, St Bernard) (Heffner, 1983)

The frequency / intensity curves of four different dog breeds are shown in Figure 1-7, and demonstrates firstly, that there are relatively small inter breed differences in hearing, and secondly, that dogs have a far wider hearing range than humans, approximately 40 Hz to 50 kHz (Heffner, 1983; Sales et al., 1997), which is why ultrasonic dog whistles work. This is important from a welfare perspective, as there may be aversive ultrasonic sound in the environment e.g., from electrical equipment, that humans are completely unaware of (Kruger et al., 2021). Although the hearing range of dogs is much wider than humans, the sensitive range of healthy dogs is around 1-16 kHz (Heffner, 1983; Ter Haar et al., 2010), similar to humans. Human music falls within this range, so can at least be heard by dogs at modest volumes. It is of note, however, that within this sensitive range, they will be able to hear sounds 24 dB lower than that of humans (Sales et al., 1997), a consideration in the larger ARRCs where the background sound levels can routinely be over 80 dB.

As in humans, dogs' hearing deteriorates with age, occurring from a mean age of 8–10 years, with the greatest loss being in the 8-32 kHz range (Ter Haar et al., 2008). Poncelet et al. (2000) highlighted that in very young dogs (1.5 to 2 months) there may be breed differences in the development rate of auditory ability, but for adult dogs (over 1 year) there are only marginal breed differences (Heffner, 1983), although some breeds, notable Dalmatians, are prone to heritable hearing loss

(Cargill et al., 2004). Taking this information, any study of the effect of auditory enrichment on dogs should only use adult dogs and factor in age and breed to identify any confounding effects.

1.3.3 What dogs perceive

Having addressed what dogs can hear by way of frequency and volume governed by the physical properties of their ears, it is equally important to address what dogs perceive, and this is a more complex question of neural processing and how dogs make sense of auditory stimuli.

1.3.3.1 Comparative Neuroanatomy

In a human study, Hoefle et al. (2018) found that listening to music caused activation of both the anterior and posterior regions of Heschl's gyrus, areas previous been shown to be involved in the auditory processing of speech (Scott et al., 2000). Further evidence of the link between music and speech comes from (Sammler et al., 2011) who determined that the left inferior frontal gyrus (IFG) (the location of Broca's area), plays a functional role in the processing of musical syntax and as well as language. Music and language share several cognitive processing mechanisms (Patel, 2017; ten Cate & Honing, 2022), including a common dependence on declarative memory (Miles et al., 2016) suggesting a common origin of both (Koelsch et al., 2003). Because of this, development of musicality is seen by many as an evolutionary precursor to the development of a vocal language. Therefore, language ability may be a useful proxy for musicality (there cannot be a vocal language without a sense of rhythm and pitch). The first step to developing a vocal language is vocal learning, which is the ability to learn new vocalizations through imitation and is different from auditory learning, which is the ability to learn associations with sounds (Jarvis, 2006). Dogs are not vocal learners - this is a trait restricted to humans, cetaceans, bats, elephants and some birds (Hoeschele et al., 2015) and even our closest primate relatives do not have this ability (Petkov & Jarvis, 2012). Vocal learning species have numerous high-level auditory regions involved in the processing and recognition of complex vocalization and without this, they cannot be a vocal learner although can use set calls to communicate. Dogs therefore cannot learn a new type of bark, but they can learn the salience of discreetly different sounds such as different vowel sounds (Root-Gutteridge et al., 2019). It is therefore worth considering the neuro-physiological barriers to vocal learning and consider to what extent this debars dogs from having any musicality. It is also worth considering what regions of the human brain are activated whilst listening to music and considering how well these regions are represented in dogs.

In humans, brain activity in response to musical stimuli has been widely studied, particularly using functional magnetic resonance imagery (fMRI). Although fMRI doesn’t give the temporal resolution that an EEG will, it will give far better spatial resolution and so has great utility in showing activity in specific brain regions (Newman, 2019). Auditory signals feed from the auditory cortex back to sub-cortical regions, particularly the thalamus. Sub-cortical circuits are important for rapid, fear related responses via the thalamus to amygdala, more considered cortical responses take longer but are more discriminating (Oertel & Doupe, 2013). Section 1.3.1.3 above has already outlined the interconnected network of subcortical and cortical brain regions engaged when listening to music, with the dopaminergic mesocorticolimbic system proposed as area of importance. The sub-cortical regions involved in the emotional response to music in humans (ventral striatum, *nucleus accumbens*, amygdala, insula, hippocampus, hypothalamus, ventral tegmental area, anterior cingulate) are conserved in dogs (Uemura, 2015). Within the prefrontal cortex, the orbitofrontal cortex is a key part of the reward system, involved in representing stimulus-reward value (O'Doherty, 2007) and, in humans, is engaged when listening to music (Särkämö et al., 2008). As with the sub-cortical regions, the orbitofrontal cortex is also present in dogs (Uemura, 2015), although as the relative size of the overall prefrontal cortex is much smaller than in humans, the interpretation of emotional stimuli outlined as by Bigliassi et al. (2015) may well be less developed in dogs.

When comparing the neuro anatomy of comparable brains of a vocal learning songbird and a non-vocal learning chicken, the chicken lacks the inter-connected neural nodes in the thalamus, striatum and pallium that the songbird has, and this cortico-striatal-thalamic loop also appears in humans (Petkov & Jarvis, 2012). Therefore, rather than specific brain regions, the key to musical perception is almost certainly the strength of the connections between them. Patel and Iversen (2014) have hypothesised that entrainment (the ability to move in time with a beat) relies on the strength of the connections between the auditory and motor cortices, and not the areas themselves, and Chapin et al. (2010) demonstrated the fundamental importance of an extended motor network in beat perception. Dogs' brains have areas that respond to human voices (Gábor et al., 2020), and these areas show a similar pattern of fMRI response to those in humans (Andics et al., 2014; Boros et al., 2021; Gábor et al., 2020), but dogs still lack the capacity for vocal learning. Therefore, the field of connectomics may ultimately elucidate comparative cognitive abilities better than simply identifying similarities in brain anatomy.

1.3.3.2 Musical Perception

The question of whether animal can perceive and enjoy human music was first pondered by Darwin (1871). Since then, musical discrimination has been widely studied in an attempt to answer this question. Following the early work demonstrating an arousal-reducing effect of playing music to dogs in a rescue centre (Wells et al., 2002), much of the subsequent research has focused on trying to identify which genre of music produces the largest arousal-reducing effect in dogs (Bowman et al., 2017; Brayley & Montrose, 2016; Kogan et al., 2012). However, there can be the erroneous assumption that all music of a particular genre is similar enough to have the same effect, with the classical music genre being given particular attention (see review by Lindig et al. (2020)). This anthropocentric view assumes that dogs tell different genres apart, and although musical discrimination has been widely studied in non-human species, including fish (Chase, 2001), rats (Celma-Miralles & Toro, 2020), non-human primates (McDermott & Hauser, 2007) and birds (Dooling et al., 2002; Porter & Neuringer, 1984), there is no published work specifically related to dogs. While much of the published work has focused on elucidation of the origins of musicality (Honing et al., 2015), a characteristic defined as "*the capacity that underlies the human ability to perceive, appreciate, and produce music*" (Hoeschele et al., 2015), many of the abilities that generate a perception of music are not uniquely human (Hoeschele et al., 2015). As an example, carp are able to distinguish between classical and blues music (Chase, 2001) and different classical composers (Shinozuka et al., 2013). There is mounting evidence, however, that musicality, at least in non-vocal learners, may, if present at all, be restricted to the most basic element; the discrimination of rhythmic regularity (Celma-Miralles & Toro, 2020). When making cross-species comparisons of auditory perception, birds have far better resolution of temporal fine structure in acoustic stimuli (periods as short as 1 ms) than humans (3-4 ms) (Dooling & Prior, 2017) and this ability may be explained in-part by differences in the VIIIth cranial nerve compound action potentials that are unique to birds (Dooling et al., 2002). The greater perception seen in birds may therefore be due to differences in the peripheral nerves rather than in cortical or sub-cortical regions. Therefore birds, with comparable cortical regions to humans, do not perceive the auditory environment the same way humans do, and it is likely the other species have different neural sampling rates, and therefore different perceptive abilities.

It has been demonstrated that dogs can discriminate between different speakers and vowel sounds (Root-Gutteridge et al., 2019), and between English and Spanish (Mallikarjun et al., 2022). Dogs have also been shown to be able to discriminate between the distress vocalisations of human infants and puppies (Root-Gutteridge et al., 2021). Dogs clearly have some auditory discriminatory abilities, but with regard to languages, it is unclear what specific cues dogs are using to tell them apart (Mallikarjun et al., 2022) but it has not been determined whether dogs' express aspects of musical discrimination including perception of tempo and pitch, which may underpin any language perception they have. When training dogs, fast short auditory cues such as whistle blasts innately produce physical activity whereas slow long auditory cues reduce physical activity (McConnell, 1991) which indicates at least some temporal perception (length and/or timing of cues), although, as with language, it is impossible to identify to what specific aspect of the cue the dogs are attending.

Limited evidence of musical discrimination is provided by the observation that the response of dogs can be influenced by certain aspects of the music to which they are exposed. For example, it has been reported in dogs that some forms of music (specifically heavy metal) can result in increased expression of anxiety-related behaviours (Kogan et al., 2012). Given that beat perception is a fundamental element of musicality (Wittmann & Pöppel, 1999), determination of whether dogs have this basic ability may allow greater understanding of what is perceived by dogs and maximise the effectiveness of auditory enrichment paradigms through selection of appropriate stimuli.

1.3.4 Comparison of Dogs and Horses

Due to the logistical constraints imposed by the COVID-19 protection measures, horses are also included in this study. Domestic horses have similarities with ARRC dogs in so much as they are group housed, are in regular contact with humans and undergo regular training, sometimes using vocal cues. They are highly reactive, and like dogs, can become rapidly distressed by aversive noise such as fireworks (Gronqvist et al., 2016) and thunder (Riva et al., 2022). Auditory enrichment has been demonstrated to have a positive effect on arousal levels (Stachurska et al., 2015; Wiśniewska et al., 2019), although there are fewer studies than in dogs. In many stables, it is common for a radio to be playing (Rørvang et al., 2020), but with limited acoustic studies involving horses, the effects are not well understood.

Horses have better high frequency hearing than humans, extending up to 33.5 kHz (Heffner & Heffner, 1983) and this may in-part be to compensate for relatively poor vision (Saslow, 2002). Their sensitive range is comparable to dogs (1 – 16 kHz) (Heffner & Heffner, 1983), therefore any auditory enrichment suitable for dogs will also be heard by horses at a modest volume (approximately 60 dB). As with dogs, horses are also prone to age-related hearing loss, although with horses, this is particularly apparent in the 14 - 25 kHz range (Wilson et al., 2011), higher than human music or speech. It is therefore probably not necessary to exclude older horses from this study.

Regarding perception, horses, like dogs, are non-vocal learners. As such, according to the “vocal learning and rhythmic synchronization hypothesis” (Patel, 2006), they should not be able to synchronise movement with a beat (thus demonstrating beat perception). However, unlike in dogs, with horses there is anecdotal evidence of horses synchronising their trotting gait with a musical beat, and Bregman, Iversen, et al. (2012) proposed a method to scientifically test this phenomenon, but to date, there is no published literature using this method to demonstrate rhythmic entrainment in horses.

Compared to dogs, there is far less published work on auditory discrimination, and this may be due to less reliance on vocal cues during training. In one study that did investigate vocal discrimination, Heleski et al. (2015) found horses could not differentiate between harsh voice tones and soothing voice tones used during a learning task. There is also less reliance on conspecific vocal communication in horses than dogs, although they do communicate emotional states using whinny, nicker, squeal, and snort (Yeon, 2012).

1.3.5 Sex Differences

Having considered in section 1.3.2 the potential confounding effects of both age and breed on what dogs hear, and having discussed the neurophysiology of what they perceive in section 1.3.3, it is finally worth considering if there may be any sexual dimorphism in the way dogs perceive sounds. This is an important consideration, as it may be that male and female dogs will benefit for subtly different auditory enrichment.

Although Eliot et al. (2021) saw no sexual dimorphism in any human brain structure once size is accounted for, structural sexual dimorphisms have been seen in other species, specifically mice and rats (Tobet et al., 2009). Maybe more important than any structural differences (or lack thereof) is the cross-species evidence for sexually dimorphic function. In response to stressors, Rhodes and Rubin (1999) found a significant difference in the HPA response in male and female rats and McEwen (2017) proposed brain-system differences mediating the way stressful stimuli are interpreted by males and female rats, such as impairment of hippocampal dependant memory in males but not females. Stress induced sex-specific alterations within cortical and midbrain structures seen in rats may explain the greater prevalence in human females of depression and anxiety (Rincón-Cortés et al., 2019).

When considering sexually dimorphic responses to music, much of the work has been conducted with humans. A meta-analysis conducted by Wilson (2018) showed a stronger effects size in women than men, although (Bigliassi et al., 2015) saw stronger activation of the PFC in men than in women, with effects persisting longer in men. However, Guétin et al. (2016) found an equal reduction in self-reported anxiety by both men and women after listening to music. Men and boys have been seen to process negative sounds in either the left (Koelsch et al., 2003) or right (Flores-Gutiérrez et al., 2009) hemispheres, but both these studies found that this processing was bilateral in both women and girls. Despite the lack of consensus, it does seem that men and women recruit different networks for processing emotionally valanced audio stimuli (Goshvarpour & Goshvarpour, 2018) and it has been suggested that the confounding effects of these sexual dimorphisms have to be considered if music is being used to modify emotional states (Nater et al., 2006). However, it is interesting that many of the studies that have reported a sexually dimorphic responses to music have been imaging or EEG studies, with studies not reporting a difference using either self-report anxiety levels (Guétin et al., 2016; Nilsson et al., 2009) or HRV (Dureau, 2005; Nozaki et al., 2015). Therefore, although sex biases still need to be considered, differences in HRV might not be expected between different sexes.

1.4 Defining Success in Enrichment Studies

To quantify the success of auditory enrichment, what is trying to be achieved in any given situation needs to be clearly defined. For example, in a zoo setting, it is common practice to measure welfare by the amount of normal, natural behaviours that are being exhibited (Wolfensohn et al., 2018). For domestic dogs, the aim in most settings (rescue centres or within the home) is a “calm” or “relaxed” dog which, in quantitative terms, is reduced physiological arousal, although it is debateable whether reduced physiological arousal is always a good proxy for high welfare. Reduced arousal (within reason) is likely to increase the chances of dogs in ARRCs being re-homed and reduces the chances of them being returned and therefore, for the purposes of this thesis, reduced physiological arousal in response to auditory enrichment will be considered “success”. A full discussion of subjective experience, emotional consciousness and narrative construction (or lack of) and how emotional valence rather than just physiological arousal is measured in animals, is beyond the scope of this thesis. As the autonomic nervous system is fast-responding and equally sensitive to psychological and physiological stressors (Skoluda et al., 2015) it is logical to use this to quantify physiological arousal. As the aim is to increase relaxation, quantifying PNS activity by measuring heart rate variability is the obvious candidate, with the desired outcome being an increase in PNS activity / vagal tone.

1.5 Conclusion

Animal Rescue and Rehoming Centres can be highly stressful environments, and dogs manifesting a stress response whilst in an ARRC are less likely to be rehomed quickly, and if they are rehomed, are more likely to be returned. Exposure to a chronically stressful environment can also be both physically and psychologically damaging to dogs, particularly puppies at sensitive neuro-developmental stages, and this is a welfare concern. Reducing the triggers to a stress response is a fundamental welfare consideration, and auditory enrichment is an obvious candidate as it is low cost, easy to implement and does not pose any welfare risks. There is a considerable body of evidence of the arousal and anxiety reducing effects of the passive listening to music in both humans and a range of non-human species, although the exact mechanism by which music is having this effect is unclear. In humans, there is likely to be some element of placebo effect, but in non-humans, investigation of the expectation of benefit has not been reported in the literature. Quantifying the benefit of any enrichment in the absence of a reliable way to establish emotional valence is a challenge, however, when aiming to improve rehoming rates from an ARRC, success of auditory enrichment can be defined as a reduction in physiological arousal, specifically quantified by an increase in vagal tone / PNS dominance, as measured by HRV.

Auditory enrichment is effective at reducing arousal, but the effects sizes can be small. To strengthen the previously demonstrated arousal-reducing effects of auditory enrichment, the aims of this thesis are:

Aim 1 – To validate the HRV data collection and processing used to quantify effect in the subsequent experimental chapters.

Objectives:

To validate the Polar V800 and Actiheart 5 heart rate monitors against a clinical ECG in dogs.

To validate the Polar V800 and Actiheart 5 heart rate monitors against a clinical ECG in horses.

To determine the resting HRV reference ranges in healthy horses and dogs.

Aim 2 - To understand what specific aspects of auditory enrichment have the greatest influence on arousal in dogs and horses (Chapters 3 and 4).

Objectives:

> Compare the effect on arousal of different tempos of the same music.
>
> Compare the effect on arousal of different pitches of the same music.
>
> Investigate the effect of play-back of the owner's voice within music on the arousal of dogs.

Aim 3 - To determine if dogs have the auditory perceptive abilities that justify any assumptions of musical appreciation (Chapter 5).

Objectives:

> Develop a two-choice go/go selection paradigm to test dogs' ability to discriminate between two different auditory conditions.
>
> Determine the extent of dogs' categorical perception of tempo, examined with metronome beats and complex music.
>
> Determine the extent of dogs' categorical perception of pitch, examined with pure tones and complex music.

Aim 4 - To establish if a positive association with specific music can influence how that music can manipulate arousal in dogs (Chapter 6).

Objectives:

> Determine if the arousal-reducing effect of auditory enrichment for dogs can be enhanced by the formation of a positive association with it in an Animal Rescue and Rehoming Centre setting.
>
> Test the ability of previously conditioned auditory enrichment to aid the transition to a new environment for dogs on being rehomed.

It was intended that the majority of the data collection would be conducted on dogs in the care of the Scottish SPCA. However, due to COVID-19 restrictions, from mid-March 2020, all access was lost to the ARRCs, and was never fully re-established. As a result, this project was extended to also encompass horses, as there was ready access to a population of horses at a time when dog availability was greatly reduced. The addition of horses to the study was initially due to the logistical constraints imposed by COVID-19, but they do provide an interesting comparative model as they have a similar auditory sensitive range (1 – 16 kHz) to dogs (Heffner & Heffner, 1983), and like dogs, are a non-vocal learning species.

Chapter 2 Use of the Polar V800 and Actiheart 5 heart rate monitors for the assessment of heart rate variability (HRV) in dogs and horses

2.1 Introduction

2.1.1 IBI Data Collection and Processing in Non-humans

As detailed in Chapter 1, HRV, derived from inter-beat interval (IBI) data, is routinely used by animal behaviour and welfare scientists in a range of species as a non-invasive method to quantify vagal tone as a proxy of arousal / response to a stressor. The ‘Gold Standard’ for recording IBI data in any species is a 4, 6 or 12 lead electrocardiogram (ECG), however, for reasons of practicality, such equipment is rarely used outside of a clinical setting. The advent of inexpensive wearable heart rate monitors (HRM) such as the Polar V800, designed for human athletic training, has provided a more practical option for the capture of IBI data from unrestrained animals. However, as they only provide the IBIs and not a full ECG trace, there is no way of confirming the accuracy of what is reported. While HRMs have been validated against ECGs for the collection of IBI data in humans (Giles et al., 2016), in animals, data errors are identifiable in HRM-generated IBI data (Marchant-Forde et al., 2004). These errors may be attributable to interspecies differences in ECG profiles, body shape, movement artefact or species-specific cardiac arrhythmia (Durham, 2017; Quintana et al., 2016). Due to this, the cardiac parameters of both dogs and horses differ from those of humans (detailed in the relevant sections below) and it must be remembered that both the hardware and software being used for data collection and processing have been specifically designed for use with human subjects. If valid conclusions are to be drawn from IBI data collected from non-human subjects, both the equipment and the processing of the raw IBI data needs to be validated for each species, rather than relying on assumptions taken from human physiology. For frequency domain analysis to be accurate, the frequency bands (i.e., the ranges which reflect very low, low and high frequency) need to be specified. These are different for each species (Joachim A. Behar et al., 2018) as they are highly dependent on respiration rate (see Chapter 1 for an explanation of respiratory sinus arrhythmia). Based on previous validation studies, the required bands for horse data are: VLF 0 – 0.01 Hz, LF 0.01 – 0.07 Hz and HF 0.07 – 0.6 Hz (Stucke et al., 2015) and for dog data are: VLF 0.0033 – 0.067 Hz; LF 0.067 – 0.235 Hz; HF 0.235 – 0.877 Hz (Joachim A. Behar et al., 2018). These frequency bands were used for all IBI data processing throughout this thesis.

2.1.2 HRV Reference Ranges

HRV reference ranges are well established for humans (Acharya et al., 2006), but are less well defined for other animals. Much of the previous work assessing HRV in dogs has been carried out in situations that may generate at least a mild stress response such as veterinary practises (Bogucki & Noszczyk-Nowak, 2015), light restraint (Wormald et al., 2017) and in rescue and rehoming centres (Bowman et al., 2017). Matsunaga et al. (2001) established resting HRV parameters, but this was conducted with "dogs placed in a sling and restrained in the hanging position" and determined 'normal values' of HR 98 ± 14 bpm and HF Power 5,868 ± 1,714 ms^2. These figures are in agreement with Bogucki and Noszczyk-Nowak (2015), but it is likely that neither environment was entirely 'stress free' for the dogs. Breed differences in HRV have also been seen in dogs (Doxey & Boswood, 2004), although the only significant differences in healthy dogs were seen between brachycephalic and non-brachycephalic breeds. The authors of that study suggested that this was due to respiratory sinus arrhythmia being affected by the greater upper airway resistance to breathing in brachycephalic breeds.

The HRV literature relating to horses is more extensive than that relating to dogs, and this may be driven by the use of HRV to assess athletic performance (or lack of) in horses (Cottin et al., 2005; Evans, 1991; Evans & Polglaze, 1994; Frick et al., 2019; Verheyen, 2012; Younes et al., 2016). Reference HRV ranges for performance are therefore well established in horses and resting reference ranges have also been established (Eggensperger & Schwarzwald, 2017). It is also possible that baseline HRV measurements from horses better reflect a 'relaxed' state than those taken in dogs as 1) measurements of HRV are often taken whilst the horse in is its own stable, and 2) working horses are conditioned to having to having various equipment (saddles, girths etc.) attached to them, so may find the attachment of ECG equipment less intrinsically stressful than may be the case in dogs.

Enrichment interventions are generally evaluated as pre and post intervention states, with a reduction of arousal seen as a success. This is both a valid and useful measure of effect but understanding the HRV values of truly ‘relaxed’ animals will give an arousal-reduction target to aim for, and an understanding of what effect size may be required. Establishing reference ranges for ‘relaxed’ animals will also assist welfare assessment, as relative arousal can be established rather than just the absolute values.

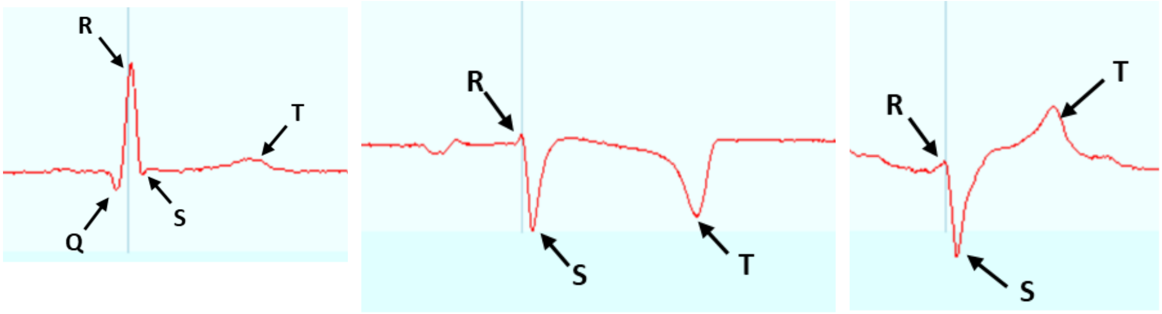
2.1.3 Aim

The aim of the work included in this chapter was the validation of the equipment and methods to be used to collect and process IBI data from two important domestic species (2.2 horses and 2.3 dogs). For each species, the objectives were to: validate the Polar V800 HRM and Actiheart 5 ECG against a 4 or 5-lead ECG including validation of species-specific artefact correction levels used for IBI processing in the Kubios HRV analysis software; and determination of HRV reference ranges for animals at rest.

2.2 Horses

2.2.1 Uniqueness of Horses’ HRV

Horses present particular issues for IBI recording using HRMs that are designed primarily for human use. As can be seen in Figure 2-1, compared to a human ECG trace, the ECG of a horse exhibits a very small R, a very large S and a T that can be positive or negative and can be as large as the S. In addition, the T-wave can be particularly labile, there can be periodic changes in P-wave configuration, sinoatrial block, and non-respiratory sinus arrhythmia (Durham, 2017).



Human QRS complex c. 60 bpm. The blue vertical line indicates the beat detection in the Actiheart 5 software.

Equine ECG c. 30 bpm. Note the lack of identifiable “Q”, the small “R” and pronounced “S” when compared to a human ECG. Also note the size and direction of the “T”.

Equine ECG c. 80 bpm. This is from the same horse. Note the change in polarisation of the “T” at the higher HR.

Figure 2-1 ECGs recorded using the Actiheart 5 heart rate monitor from a human and a horse (at 30 bpm and 80 bpm) showing intra and interspecies variation. Identifiable QRST components are labelled on each trace

The situation is further complicated by the prevalence of second-degree AV block in a significant number of clinically normal horses at rest (Figure 2-2) (Eggensperger & Schwarzwald, 2017). Second-degree AV block is generally physiological and associated with high vagal (parasympathetic) tone and will be removed as HR increases (Figure 2-3) (Reef & Marr, 2010). During second-degree AV block, the SA node fires, and a normal P-wave can be observed, but the electrical impulse does not pass beyond the AV node, therefore there is no corresponding R-wave. As cardiac monitoring with HRMs typically only records the R-R interval, these missed beats effectively double the IBI where the block occurred, and data must be corrected for missed beats if the R-R interval is to be used in HRV analysis as a metric of PNS activity.

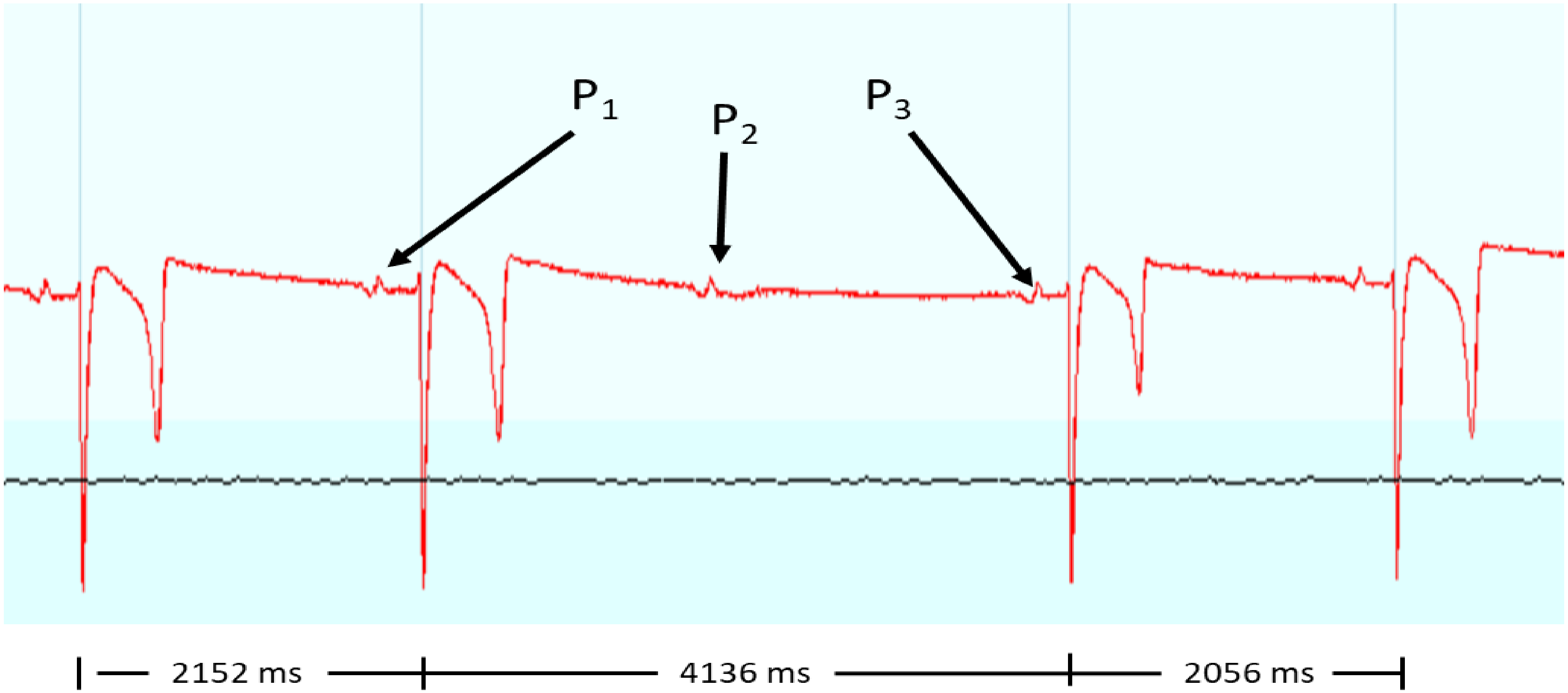
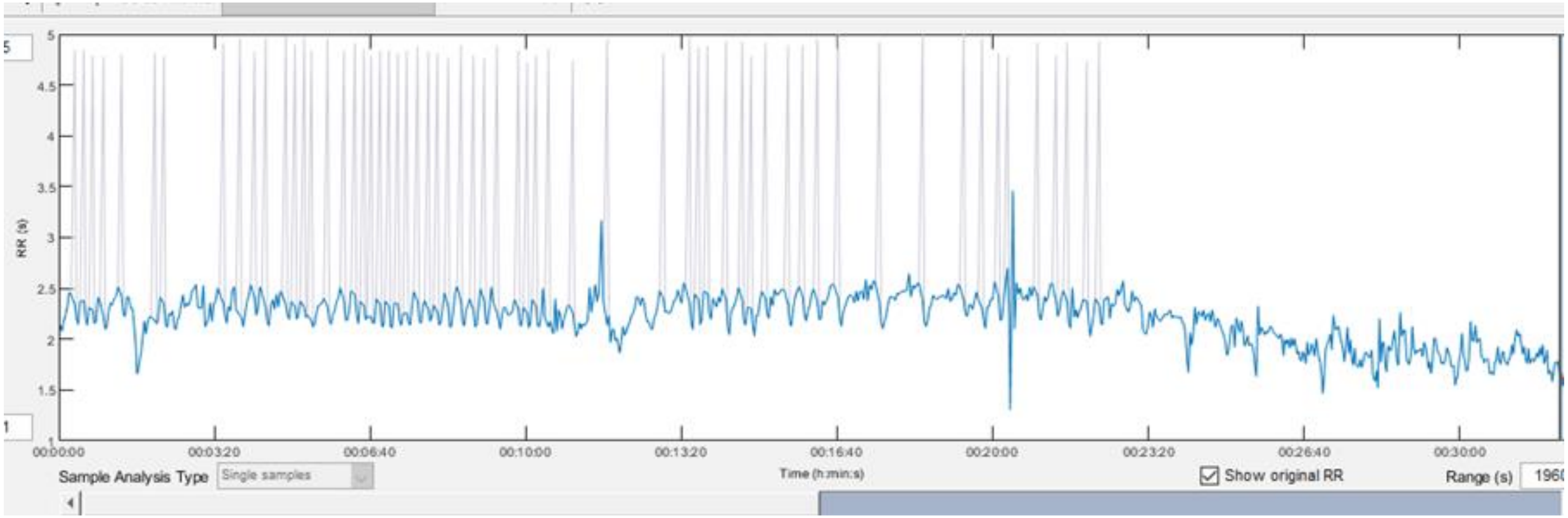


Figure 2-2 Equine ECG c. 28 bpm showing second degree AV block. P_1 and P_3 are in the correct location with respect to the subsequent QRS complexes. P_2 is in the correct position for where the missed beat should be but is not follow by a QRS complex – the SA node has fired and the atria have contracted correctly, but the electrical signal has not progressed past the AV node. This example is physiological rather than pathological and disappeared with a modest increase in HR (Figure 2-3). Inclusion of these double IBIs causes problems with the interpretation of HRV and recording the P-P intervals rather than R-R is one potential solution.



Sample Analysis Type Single samples

☑ Show original RR

Range (s) 196

Figure 2-3 R-R data output from Kubios where IBI data was collected using a Televet and a 0.9 s artifact correction applied. This horse displayed many AV blocks which are seen as a spike in the R-R to more than double the average R-R but in this trace, these have been corrected and appear as the grey lines. The increase in HR in the final quarter of the recording was associated with a loss of the AV block confirming that it is a physiological rather than a pathological dysrhythmia.

The Polar S810 HRM (Polar Electro Oy, Kempele, Finland) has been validated for use in static horses (Parker et al., 2009; Ille et al., 2014) but discrepancies within IBI data have been reported when used with moving animals (Parker et al., 2009). While recent technological improvements (i.e., the newer Polar V800) could improve IBI data reliability (Randle et al., 2017) as demonstrated in humans (Giles et al., 2016), the V800 has not been validated for use with horses. An alternative HRM is the Actiheart 5 monitor (CamNtech Ltd., Fenstanton, UK). This 2- lead system generates a full ECG trace, has been validated for use in humans (Barreira et al., 2009) and has been used in some animal studies (Bouthegourd et al., 2009; Sun et al., 2017), but has not been reported to have been validated for, or used with, horses.

2.2.2 Equine Validation Study

2.2.2.1 Subjects

Test subjects were 17 horses (8 geldings, 9 mares) age (mean ± SD) 16±1.7 years, height 164±1.9 cm. Breed composition of the sample was 5 Irish Sport Horses, 3 Dutch Warmbloods, 4 Cobs, 4 Thoroughbreds and 1 Irish Draft. All were in light to moderate work and stabled at 1 of 4 yards in Northern Ireland or were teaching horses at the Weipers Centre Equine Hospital, University of Glasgow. The IBI recordings were all made in the horses' own stable, with horses at rest.

Following initial HRV data analysis, the sample was sub-divided into those that displayed normal sinus rhythm (2 or less second degree AV blocks over 30 min, Group S) (n=13, 6 geldings, 7 mares, mean age 15±2.0 years, mean height 163±2.3 cm) and those that displayed >2% cardiac dysrhythmias (Group D) (n=4, 2 geldings, 2 mares, mean age 20±2.9 years, mean height 167±2.4 cm).

The sample size was calculated using the R package "pwr" to perform an *a priori* power analysis for an inter-class correlation comparing the output of the two HRMs with the ECG in terms of HR, RMSSD and HF Power. The effect size for the current analysis was estimated as very high (0.7) based on a previous study using an earlier model of the Polar device in horses (Ille et al., 2014). For either of the devices to produce valid data, there needs to be a very strong correlation with a clinical ECG, therefore there is limited value in being able to identify an effect size smaller than 0.7. This effect size estimate was entered into the power analysis with the following parameters: alpha (2-sided) = 0.05, power = 0.80, allocation ratio 1:1. The power analysis results suggest that a sample of n=13 was required to detect a correlation of >0.7 with an 80% probability.

2.2.2.2 Data Collection

Each horse was fitted with a telemetric ECG (Tevelvet 100, Jørgen Kruuse, Marslev, Denmark) and two HRMs (Polar V800 with H7 sensor, Polar Electro Öy, Kempele, Finland and Actiheart 5, CamNtec, Fenstanton, UK). The Actiheart 5 (Figure 2-4) is a two-lead ECG designed for human athletic training, but unlike the Polar V800, the Actiheart 5 provides a full ECG trace and incorporates motion sensors.

Figure 2-4 The Actiheart 5 two lead ECG (Bluetooth version)

The four leads of the Televet 100 were attached to Kruuse aqua-wet Ag/AgCl ECG electrodes and configured as per the Dubois method (da Costa et al., 2017) (selected to keep the electrodes away from the Polar belts) (Figure 2-5). Two

Polar equine science belts were fitted next to each other, with the cranial belt located as close to the elbow as possible (Figure 2-5). ECG conductive gel (Henry Schein Medical, Gillingham, UK) was liberally applied to the electrodes of the Polar belts to ensure uniform contact. One belt had a Polar H7 sensor fitted, the other the Actiheart 5. As there can be a slight delay from starting the Polar V800 and signal recording, the Televet and Actiheart were set to begin recording synchronously a few minutes after the Polar monitor. The subsequent time delay was removed *post hoc* from the Polar raw data to provide a synchronous start time for all three devices. Recordings were made for 30 min whilst the horse remained at rest in their stable. Date from all three devices were up-loaded to a laptop for later analysis.

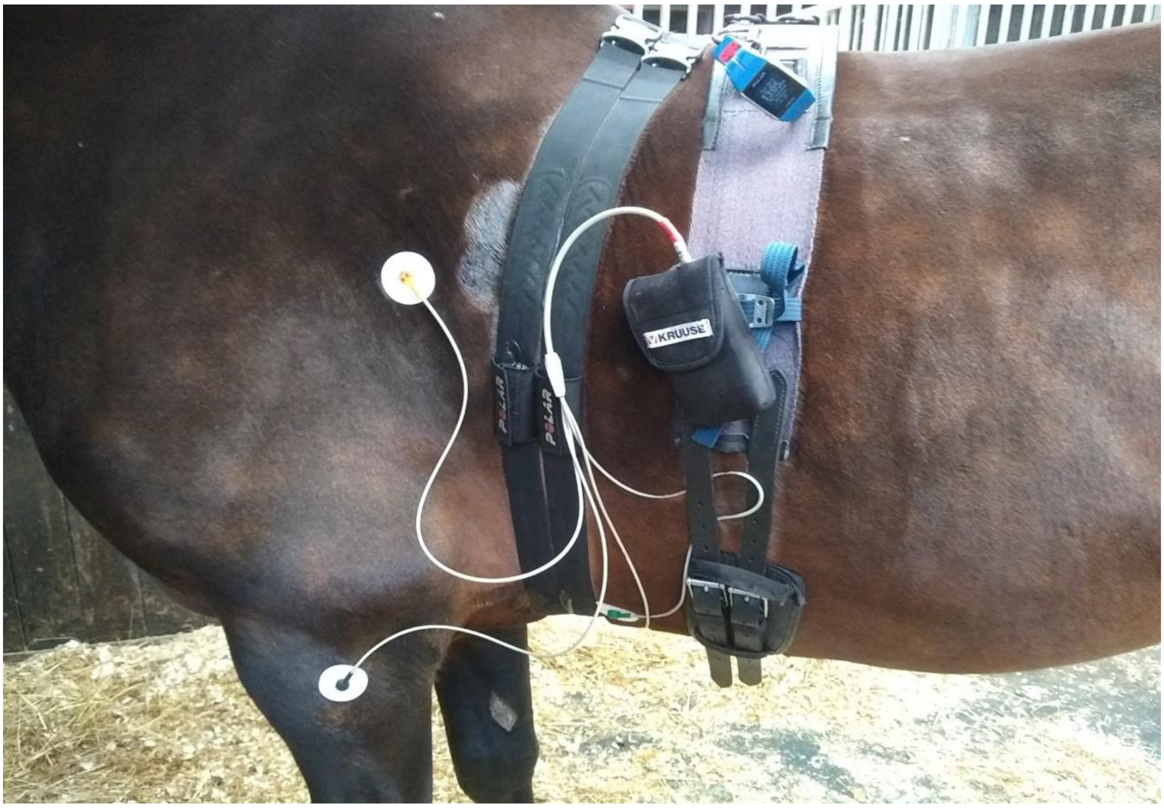


Figure 2-5 Electrode Configuration. The Televet 100 is attached to the lunging roller and the electrodes are fitted in the Dubois method (red electrode is in the same position as the yellow on the opposite side of the horse). Two Polar equine science belts are fitted, the most cranial fitted with the Actiheart 5 sensor, the most caudal fitted with the Polar H7 sensor. The Polar V800 watch is seen attached to the top ring of the roller.

2.2.2.3 Data Analysis

Televet data were processed using the Televet 100 5.1 software with the beat identification function enabled. Mis-identified beats (i.e., where the software marked a beat not on a R-wave) were identified visually and manually corrected before the IBI data were exported as a .txt file. Actiheart data were processed using the Actiheart 5 software. Where an AV block occurs and the IBI > 2800 ms the software does not recognise this as correct data (as it is set for human parameters), and ‘NA’ is reported. Where this occurred, the NA was manually replaced by insertion of an IBI that was twice the mean of the IBI on either side of the reported NA. The resultant data file was then exported as a .txt file. The Polar data was uploaded into the Polar Flow software and exported as a .txt file. The three .txt files associated with each period of data recording were compared and the start points were manually matched.

HRV analysis was then conducted on the data from each device using Kubios software (Kubios HRV Standard 3.4.1, Kubios Oy, Finland). The Kubios software allows the user to correct for artefacts such as missing, extra or misaligned beats, or dysrhythmias such as AV block or premature ventricular/atrial complexes (PVC/PAC) within the data. This is achieved using a threshold-based artefact correction algorithm which compares every IBI value against the local median interval calculated from 30 successive beats. Where an IBI differs from the local average by more than the threshold value specified by the user (between 0.01 sec and 0.99 sec) it is replaced with an interpolated value using a cubic spline interpolation (Tarvainen et al., 2021). For human studies, a 0.25 s difference between the IBI and the local median is specified by the software developers (Tarvainen et al., 2021) as a ‘medium’ threshold at 60 bpm, and 0.3 sec has previously been used as a threshold in equine studies (Ille et al., 2014; Squibb et al., 2018). As AV block gives an IBI of double the local average interval (c.4 sec verses c.2 sec @ 30 bpm), a correction of 0.9 sec is sufficient to pick up and correct for missed beats but not impact on the rest of the data. To determine the best agreement with Televet data to which a 0.9 sec correction had been applied (and second-degree AV blocks removed), the Actiheart and Polar data had zero, 0.9 sec, 0.6 sec and 0.3 sec corrections applied, and the results compared. Additional corrections of 0.45 sec and 0.4 sec were subsequently applied to the Polar data to achieve the smallest possible bias. Frequency bands of VLF 0 – 0.01 Hz, LF 0.01 – 0.07 Hz and HF 0.07 – 0.6 Hz were specified for equine-specific frequency domain analysis (Stucke et al., 2015) and the fast Fourier transform (FFT) output was used. Each 30 min recording was analysed in 1 min epochs, and the results exported to an Excel spreadsheet and saved as a .csv file for statistical analysis in R Studio (running R version 4.0.2).

2.2.2.4 Statistics

The data for HR, RMSSD (time domain analysis) and HF Power (frequency domain analysis) were compared for each device and each correction level in R Studio, initially producing Pearson correlation tables and then performing inter class correlations using the “psych” package (Revelle, 2023), in which each device/correction level was treated as a single fixed rater (ICC3). Based on the estimated relationships between the three devices obtained using Pearson’s and ICC, an agreement interval was determined using the Bland Altman test of agreement (Giavarina, 2015). Acceptable limits were defined *a priori* as a bias within 5% of the sample median for that parameter (Lenoir et al., 2017) and with the 95% CI of that bias encompassing 0. HR acceptable bias < ±2 bpm, RMSSD acceptable bias < ±4 ms, HF Power acceptable bias < ±128 ms^2.

2.2.3 Determination of Equine HRV Reference Ranges

Reference ranges for all the HRV parameters generated by the Kubios software were calculated. Values were derived from a log transform of the 30 min 0.9 sec corrected Televet values for 13 horses showing 2 or less second-degree AV blocks over 30 min (Group S). The reference range was then calculated as the mean ±1.96 x SD.

2.2.4 Results

2.2.4.1 Automated Artefact Correction

Table 2-1 shows the percentage and number of “beats” identified as erroneous for each horse, from all three devices, when a 0.9 sec artefact correction threshold was applied in Kubios i.e., the IBI is >0.9 sec longer than the local median interval. There was parity between the devices, as to the number of beats identified as artefact and corrected, but there was wide variance between horses. A high percentage of erroneous beats as seen in Horses 1, 2, 3 and 15 is indicative of dysrhythmia. Examination of individual ECG traces (data not shown) for horses 1, 2 and 3 indicated that the majority of artefacts were due to second-degree AV block, whereas horse 15 had previously been diagnosed with atrial fibrillation (AF) and displayed the characteristic irregularly irregular HR (Ludhwani & Wieters, 2018). The studied horses were therefore subdivided into groups S (normal sinus rhythm) and D (>2% cardiac dysrhythmias) for subsequent analysis.

Table 2-1 The number of "beats" identified as erroneous and the percent of data that those beats represented for each horse from the S and D groups and for each device for the entire 30 min recording period when a 0.9 sec artefact correction is applied in Kubios.

Horses in Group S	ECG		AH		Polar	
	%	Beats	%	Beats	%	Beats
4	0.11	1	0.11	1	0.23	2
5	0.07	1	0.07	1	0.08	1
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0.09	1
9	0	0	0	0	0.50	6
10	0.06	1	0.06	1	0	0
11	0	0	0	0	0.67	8
12	0.07	1	0.07	1	0.08	1
13	0	0	0	0	0.06	1
14	0.18	2	0.18	2	1.56	17
16	0.17	2	0.08	1	0	0
17	0.18	2	0	0	0	0
Horses in Group D						
1	2.14	17	2.16	18	2.05	17
2	8.19	61	8.17	61	9.80	74
3	17.21	126	17.19	129	18.36	121
15	7.88	106	7.60	103	7.68	104

2.2.4.2 Determination of Reference Ranges

Reference ranges were derived for all HRV parameters from the Kubios analysis of the Televet IBI data (with 0.9 sec correction threshold), of the horses in Group S (Table 2-2).

Table 2-2 HRV Reference Ranges for 13 resting healthy adult horses showing 2 or less second-degree AV blocks over 30 min (Group S). The range is the mean ± 1.96 x SD and uses equine-specific frequency bands for the analysis.

HRV Parameter	Reference range	Units
Mean RR	1,115 – 1,998	ms
Mean HR	30 – 54	beats/min
RMSSD	31 – 216	ms
NN50	165 – 1,244	beats
pNN50	13 – 100	%
SDANN	12 – 257	ms
SDNN index	73 – 222	ms
RR tri index	9 – 28	
TINN	227 – 1,095	ms
LF Power	604 – 3,529	ms^2
HF Power	547 – 12,191	ms^2
LF/HF Ratio	0.1 – 2.5	
SD1	22 – 152	ms
SD2	47 – 123	ms
SD2/SD1 ratio	0.6 – 2.7	
Approximate entropy	1.3 – 1.7	
Sample entropy	1.4 – 2.2	
Detrended fluctuations α1	0.5 – 1.6	
Detrended fluctuations α2	0.1 – 0.6	

When Kubios HRV analysis was applied to the uncorrected IBI data obtained from the horses in Group D (Table 2-3), both RMSSD and HF power estimates consistently fell outside the range seen in horses from Group S. Application of a 0.9 sec correction threshold resulted in both the mean RMSSD and HF Power being brought within the reference range for the three horses with clinically normal dysrhythmias, but not for the horse with the severe pathological dysrhythmia.

Table 2-3 Mean RMSSD and HF power, for the horses in Group D (n=4) when the Televet IBI data were analysed with no artefact correction or following a 0.9 sec threshold correction

Horse	RMSSD (ms) Normal range 31 – 216 ms		HF Power (ms^2) Normal range 547 – 12,191 ms^2	
	No Correction	0.9 sec Correction	No Correction	0.9 sec Correction
1	355	108	25612	3081
2	1024	162	269101	6691
3	1200	85	338023	1844
15	770	393	212620	60917

2.2.4.3 Inter-device Correlation and Agreement

The results of the interclass correlations for RMSSD and HF Power for data from each device and at each correction level tested are shown in Table 2-4. For the Actiheart (AH) data, the best correlation was seen for both parameters when a 0.9 sec threshold for correction was applied. For the Polar data, the best correlation, was obtained when a correction of 0.4 sec was applied.

Table 2-4 ICC3 for data from Group S (n=13) relative to the 0.9 sec corrected ECG (Televet) data.

Device/Correction	RMSSD	HF Power
AH 0	0.83	0.59
AH 0.9	0.97	0.99
AH0.6	0.94	0.8
AH 0.3	0.84	0.52
Polar.0	0.24	0.02
Polar.0.9	0.76	0.52
Polar.0.6	0.87	0.56
Polar 0.45	0.87	0.69
Polar 0.4	0.87	0.73
Polar.0.3	0.82	0.48

The results of the Bland-Altman tests of agreement for RMSSD and HF Power for Group S are shown in Table 2-5. The full agreement tables for HR, RMSSD and HF Power can be found in Appendix A. Only the Actiheart data with a 0.9 sec correction and the Polar data with a 0.4 sec correction gave an acceptable level of agreement with the 0.9 sec corrected Televet data for both parameters, having a bias of < 5% (< 4 ms and < 128 ms^2) and having the 95% CI encompassing 0.

Table 2-5 Bland-Altman test of agreement on RMSSD and HF Power data against 0.9 sec corrected ECG, Group S (n=13). Acceptable limits were defined as a bias within 5% of the sample median for that parameter and with the 95% CI of that bias encompassing 0. RMSSD acceptable bias < ±4 ms, HF Power acceptable bias < ±128 ms^2.

	RMSSD (ms)			HF Power (ms^2)		
Device / Correction	**Bias (< ±4 ms)**	**95% CI (Lower)**	**95% CI (Upper)**	**Bias (< ±128 ms^2)**	**95% CI (Lower)**	**95% CI (Upper)**
AH.0.9	0	-2	1	45	-51	141
Polar 0.4	0	-2	3	-90	-505	325
AH.0.6	1	-1	3	209	-123	542
AH.0	-4	-7	0	-619	-1349	111
AH.0.3	6	3	9	683	232	1133
Polar 0.45	-1	-4	1	-371	-855	112
Polar.0.3	5	2	8	471	-32	975
Polar.0.6	-4	-7	-1	-525	-1137	87
Polar.0.9	-11	-15	-7	-1681	-2642	-721
Polar.0	-31	-47	-14	-11096	-20151	-2042

The output from the Actiheart closely mirrored the Televet in that the closest agreement in time domain and frequency domain came when a 0.9 sec artefact correction was applied. For the Polar data, a 0.9 sec correction gave an underestimation well outside the acceptable levels of agreement for both time and frequency domain parameters. A stronger correction of 0.6 sec also gave an underestimation in both parameters and a 0.3 sec correction gave an over estimation. Further corrections of 0.45 sec and 0.4 sec where trialled, with 0.4 sec providing an acceptable level of agreement in both parameters. This agrees with the ICC results in Table 2-4. HR data for both HRMs, regardless of the correction factor used, exhibited good agreement with the Televet data (ICC >0.99, bias < 0.2 bpm) in every case.

Figure 2-6 shows the Bland-Altman plots for the cardiac data from Group S derived from the Polar V800 (figures a-c) with a 0.4 sec correction applied and the Actiheart (figures d-f) with a 0.9 sec correction applied and show that, although both devices have a small bias when compared with corrected (0.9 sec) Televet data, the Actiheart provides narrower limits of agreement in all 3 parameters.

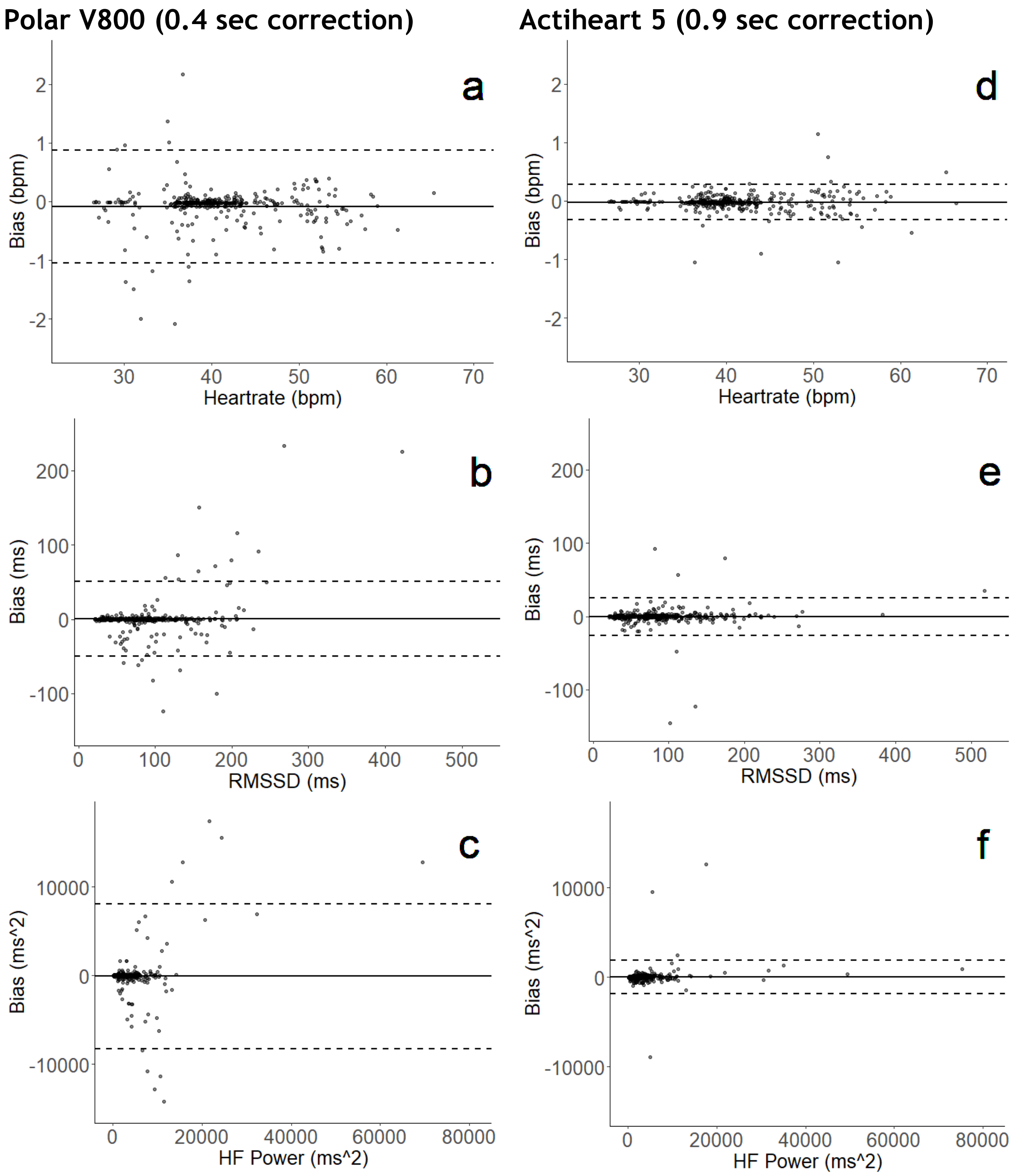


Figure 2-6 Bland-Altman plots for cardiac data derived from the Polar V800 with a 0.4 sec correction applied (figures a-c) and the Actiheart 5 (figures d-f) with a 0.9 sec correction applied. The bias from corrected (0.9 sec) Televet data is indicated by the solid line and the upper and lower levels of agreement are shown by the dashed lines.

2.2.5 Discussion of Horse HRV

The results of this validation indicated that the output from the Actiheart 5 was almost interchangeable with that of the Televet 100, and the best agreement was obtained when, during analysis, the same artefact correction was applied to the data from both devices in Kubios. The data derived from the Polar V800 had a strong correlation and acceptable agreement with the Televet 100 output when a 0.4 sec artefact correction was applied to the Polar data in kubios.

An issue with HRV analysis in horses is the prevalence of second-degree AV block in a significant number of clinically normal horses at rest (Eggensperger & Schwarzwald, 2017), and appears as intermittent double (or greater) IBIs. Second degree AV block in horses is generally physiological and associated with high vagal (parasympathetic) tone and so will be removed as HR increases (Reef & Marr, 2010) but for horses being monitored in an inactive, resting state, these ‘missed beats’ add variance to the inter-beat data that is not actually there, which becomes problematic when attempting to quantify HRV.

Applying a 0.9 sec artefact correction in Kubios is sufficient to correct for the extreme values generated by second-degree AV block without affecting the rest of the data. The correction effectively adds in the missing beat, so would give approximately the same result as reporting the P-P intervals as suggested by Eggensperger and Schwarzwald (2017). Kubios software reports how many beats and what percentage of the data have been corrected in this fashion, as reported in Table 2-1. A key consideration is that, although these ‘missing beats’ have been added in using a cubic spline interpolation and thus are representative of the surrounding beats, they are still artificially created data points. As can been seen in Table 2-1, for the data for one of the horses, nearly 18% of it had been artificially created in this manner.

It is common practice to discard data corrected by more than 5% (Schöberl et al., 2015; Von Borell et al., 2007). From Table 2-1, it can be seen that 4 of the 17 horses had more than 2 beats identified as errors/artefact when a 0.9 sec correction was applied and 3 of these horses had over 5% errors as defined by Kubios. As the ECG and Actiheart data had been manually corrected there can be a high degree of confidence that these ‘errors’ are missing beats from second-degree AV block that have been added in. Although it is not possible to view the ECG trace when data is collected using Polar HRMs, as almost an identical percentage of Polar IBI data were identified as artefact as that seen when data was collected with an ECG, it is likely that a 0.9 sec correction applied to the Polar data also identifies and corrects for second-degree AV block. Indeed, when this correction was applied to the IBI data from horses 1, 2 and 3 their HRV parameters were brought within the range of the horses without dysrhythmia (Table 2-3), however, the validity of results from data, in this case up to 18% of which has been artificially created, must be questioned. Horse 15 had previously been diagnosed with atrial fibrillation, and it was notable that a 0.9 sec correction was not able to bring the HRV results within the reference range. A 5% rejection threshold remains prudent even if the artificially created beats are mathematically correct.

When determining the correction thresholds to use to assess the agreement between the HRMs and the ECG, 0.9 sec was selected as it would remove second-degree AV block without affecting the rest of the data and 0.3 sec was selected as it has previously been used in equine studies (Ille et al., 2014; Squibb et al., 2018). Although this has sometimes erroneously been considered to represent a 30% threshold – 0.3 sec is only 30% when the IBI is exactly 1000 ms (i.e., HR is 60 bpm). For horses with a resting HR of c. 30 bpm, a 30% threshold loosely equates to a 0.6 sec correction, so this was selected as the third option. These were compared with the Televet data with a 0.9 sec correction applied.

The output from the Actiheart 5 closely mirrors the Televet in that the closest agreement in time domain and frequency domain comes when a 0.9 sec artefact correction is applied. However, for the Polar data, a 0.9 sec correction gives an underestimation well outside the acceptable levels of agreement for both time and frequency domain. A stronger correction of 0.6 sec still gives an underestimation in both parameters and a 0.3 sec correction gives a marked over estimation. Further corrections of 0.45 sec and 0.4 sec were trialled, with 0.4 sec providing an acceptable level of agreement in all 3 parameters. It is likely that this stronger level of correction is required to remove the artefact from the Polar data that had already been manually removed from both the Actiheart and Televet data.

It is of note that the level of agreement between the devices for heartrate is very good regardless of the device or correction applied. In contrast, there is a poorer (although still acceptable) agreement with HF Power, and this is to be expected, as frequency domain data are highly sensitive to missing or misclassified beats, so even small inconsistencies will be magnified (Parker et al., 2009).

The current study was only conducted on static horses at rest. For exercise assessment, the electrodes would require more robust attachment and it has been hypothesised that movement of the Polar belt would produce additional artefact and the reduced agreement as seen by both Parker et al. (2009) and Lenoir et al. (2017). This has also seen in human studies (see Georgiou et al. (2018) for review). Both Parker et al. (2009) and Ille et al. (2014) found better agreement between the Polar and ECG when the horses were static compared to when exercising. The Actiheart 5 is designed to be attached to conventional ECG electrodes and further work is required to validate this method of attachment for exercise assessment in horses.

2.3 Dogs

2.3.1 Uniqueness of Dogs' HRV

Unlike the cardiac parameters of horses outlined above, the HR and QRS complexes of dogs are similar to those of humans. It might therefore be assumed that equipment and software designed for humans will work well with dogs, without modification. However, dogs have naturally very high IBI variance compared to other species (Minors & O'Grady, 1997) and this can lead to errors in interpretation of data if anthropocentric assumptions are applied to the analysis and can confound analysis algorithms that are designed to work with human parameters.

2.3.2 Canine Validation Studies

Due to size limitations, unlike in the horse it was not possible to compare both Actiheart and Polar HRMs with a 4/5 lead ECG on the same dog. As such a pilot study compared the Polar V800 to the NovacoR ECG using two animals and in a full validation study using nine different dogs, the Actiheart 5 was compared with a Televet 100. Canine reference values were determined in a separate study population.

2.3.3 Polar V800 versus NovacoR ECG

2.3.3.1 Data Collection

In a pilot study, IBI data was simultaneously collected using a Polar V800 and a NovacoR Vista Access 5 lead Holter ECG from two dogs (female, 2.3±0.75 years, 18.5±2.5 kg) at the University of Glasgow's School of Biodiversity, One Health and Veterinary Medicine (SBOHVM). The pre-gelled ECG pads and leads were fixed to the dogs using the standard configuration shown in Figure 2-7. The elasticated Polar chest strap with H7 sensor (Polar Electro Oy, Kempele, Finland), was placed so that the electrodes sat against the chest wall, close behind the elbows of each dog. To ensure uniform electrical contact, ECG conductive gel (Henry Schein Medical, Gillingham, UK) was liberally applied to the electrodes and the dogs' chests. To ensure consistent contact and reduce movement artefacts, the recording apparatus was secured in position with veterinary cohesive bandage (AniWrap, J.A.K Marketing Limited, York, UK). Using this method, good electrical contact was achieved without the need to clip the dogs' fur.

Veterinary cohesive bandage was then applied to hold the ECG pads and the Polar elastic strap in position. The NovacoR Vista Access ECG was connected to the 5 ECG leads and secured to the dorsal aspect of the dog, also using the veterinary cohesive bandage. The complete set-up is shown in Figure 2-8.

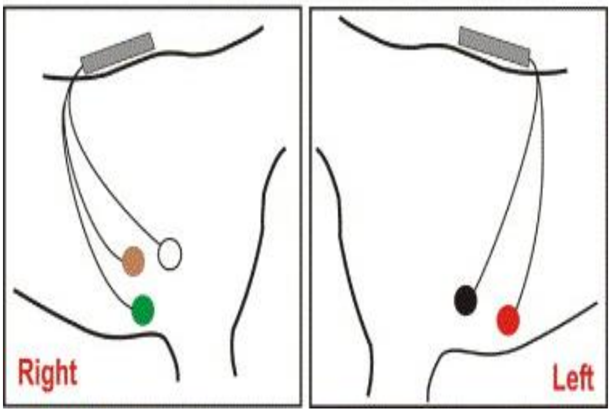


Figure 2-7 Correct precordial positioning of leads for the NovacoR Vista Access 5 lead Holter ECG (from Petrie (2005))

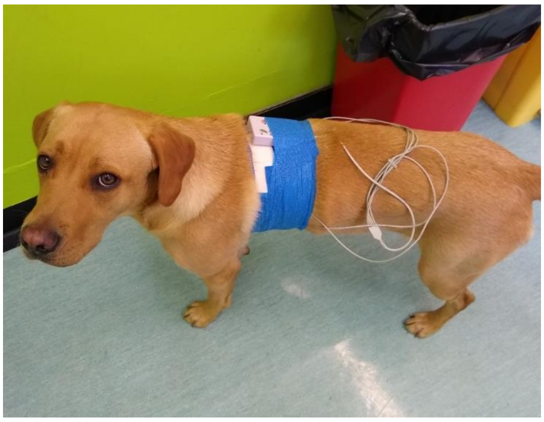


Figure 2-8 Dog fitted with NovacoR Vista Access 5 lead Holter ECG and Polar V800

Recordings were made for 30 min whilst the dogs were in a 5 m x 3 m empty room. The dogs were not restrained and were free to move around the room. On completion of recording, the ECG and HRM were removed. The ECG data were transferred on a memory card to a bespoke stand-alone PC and analysed using the Holtersoft Ultima Software. The QRS complexes were visually inspected, and any mis-identified beats (i.e., where the software identified a beat anywhere other than on the R-wave) were manually corrected (moved to the nearest R-wave or deleted). Following manual correction, the IBI were exported to a text file for HRV analysis with Kubios 3.3.1 software. No automated artefact correction was applied to these data in Kubios. Data from the Polar V800 were downloaded using the Polar Flow software and then the IBIs were exported as a text file for HRV analysis with Kubios 3.3.1 software. To examine how the different levels of the inbuilt artefact correction within Kubios affected the correlation of HRV data with that obtained by ECG, the Polar data were reanalysed with 0.3 sec, 0.6 sec, 0.9 sec and no artefact correction. Canine specific frequency bands of VLF 0 – 0.07 Hz, LF 0.07 – 0.24 Hz and HF 0.24 – 088 Hz were used for the frequency domain analysis (Joachim A Behar et al., 2018) and the fast Fourier transform (FFT) output was used.

2.3.3.2 Statistics

Agreement between the IBI output, the NovacoR ECG and the uncorrected Polar V800 was first determined using a Pearson's correlation. The data for RMSSD (time domain analysis) and HF Power (frequency domain analysis) were compared for each device and each correction level in R Studio, performing inter class correlations using the "psych" package (Revelle, 2023), in which each device/correction level was treated as a single fixed rater (ICC3). Based on the estimated relationships between the two devices obtained using Pearson's and ICC, an agreement interval was determined using the Bland Altman test of agreement (Giavarina, 2015) for the RMSSD and HF Power data. Acceptable limits were defined *a priori* as a bias within 5% of the sample median for that parameter (Lenoir et al., 2017) and with the 95% CI of that bias encompassing 0. Acceptable biases determined as: HR < ±2 bpm; RMSSD < ±5 ms; HF Power < ±666 ms^2 for the Polar dataset.

2.3.3.3 Results

When comparing the IBI data between the Polar V800 and the NovacoR ECG, there was a highly significant strong correlation (r= 0.99, p<0.001 (Figure 2-9)) when the Polar data had no artefact correction applied.

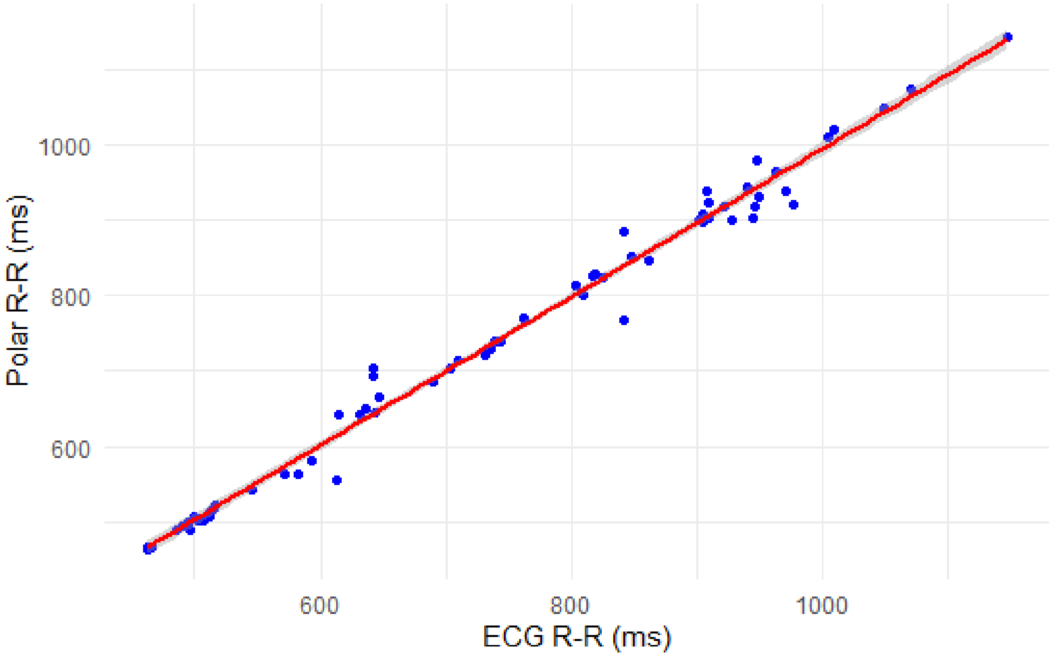


Figure 2-9 Correlation (r= 0.99, p<0.001) between the R-R output of a Polar V800 and a NovacoR Vista Access 5 lead Holter ECG, sampled every min for 30 min, n=2

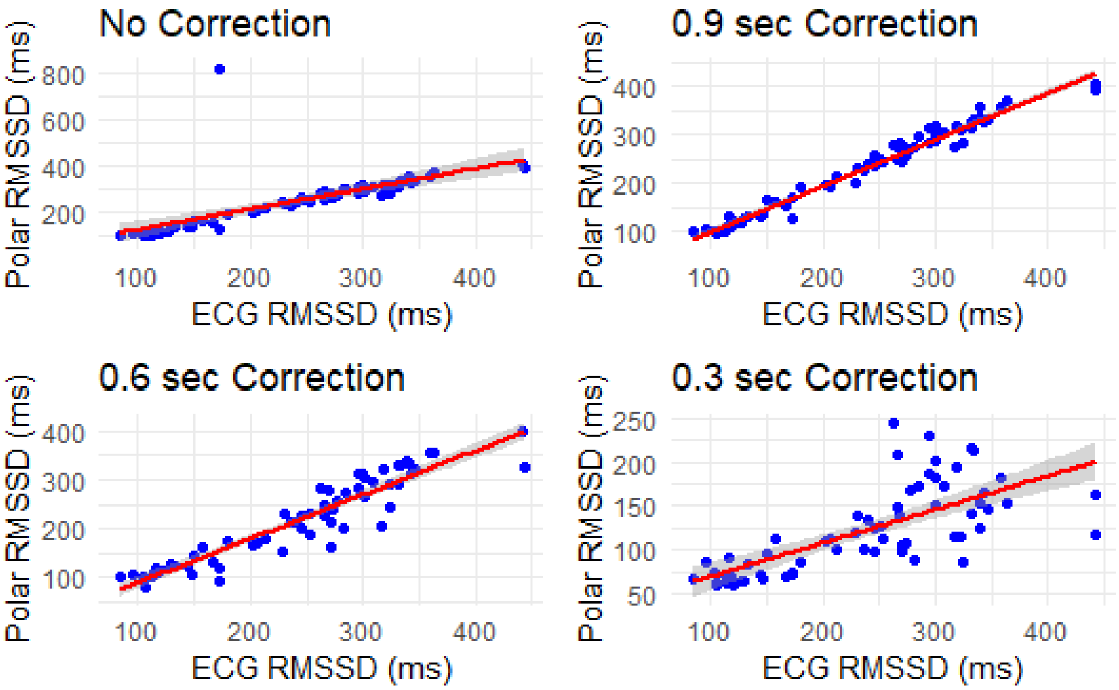


Figure 2-10 Inter-class correlations plots comparing the RMSSD output of a Polar V800 and a NovacoR Vista Access 5 lead Holter ECG with 4 different levels of artefact correction applied to the Polar data (no correction ICC=0.68, 0.9 sec correction ICC=0.98, 0.6 sec correction ICC=0.94 and 0.3 sec correction ICC=0.59 (p<0.001))

Figure 2-10 illustrates the Inter-Class Correlation (ICC3) between RMSSD data collected using the Polar V800 and NovacoR Vista ECG devices when four different levels of artefact correction were applied to the Polar data. The correlation was significantly affected by the different corrections applied ($F_{4, 314} = 25$, $p < 0.001$), with the best correlation (ICC=0.98) when a 0.9 sec correction was applied. Table 2-6 displays the full results for the ICCs between the IBI and two HRV parameters (RMSSD and HF Power) obtained from the Polar V800 and NovacoR ECG when four artefact correction levels were applied to the Polar V800 data.

Table 2-6 Inter Class Correlations between output of a Polar V800 and a NovacoR Vista Access 5 lead Holter ECG for Inter Beat Intervals (IBI), Route Means Square of Successive Differences (RMSSD) and the High Frequency (HF) Power. Four different levels of artefact correction were applied to the Polar data.

Correction	IBI (ms)	RMSSD (ms)	HF Power (ms^2)
No Correction	0.99	0.68	0.41
0.9 sec	0.99	0.98	0.87
0.6 sec	0.98	0.94	0.75
0.3 sec	0.95	0.59	0.18

To further investigate the relationship between the data from the two devices, Bland-Altman tests of agreement were conducted for RMSSD and HF Power for all four artefact correction levels (Table 2-7). The correction level offering the closest agreement were then plotted (Figure 2-11).

Table 2-7 Bland-Altman test of agreement on RMSSD and HF Power data against un-corrected ECG.

	RMSSD (ms)			HF Power (ms^2)		
Device / Correction	**Bias (< ±5 ms)**	**95% CI (Lower)**	**95% CI (Upper)**	**Bias (< ±666 ms^2)**	**95% CI (Lower)**	**95% CI (Upper)**
No Correction	-8	-28	13	-974	-4315	2368
0.9 sec	5	1	9	613	-526	1751
0.6 sec	24	17	32	1982	439	3525
0.3 sec	116	99	132	8567	6276	10858

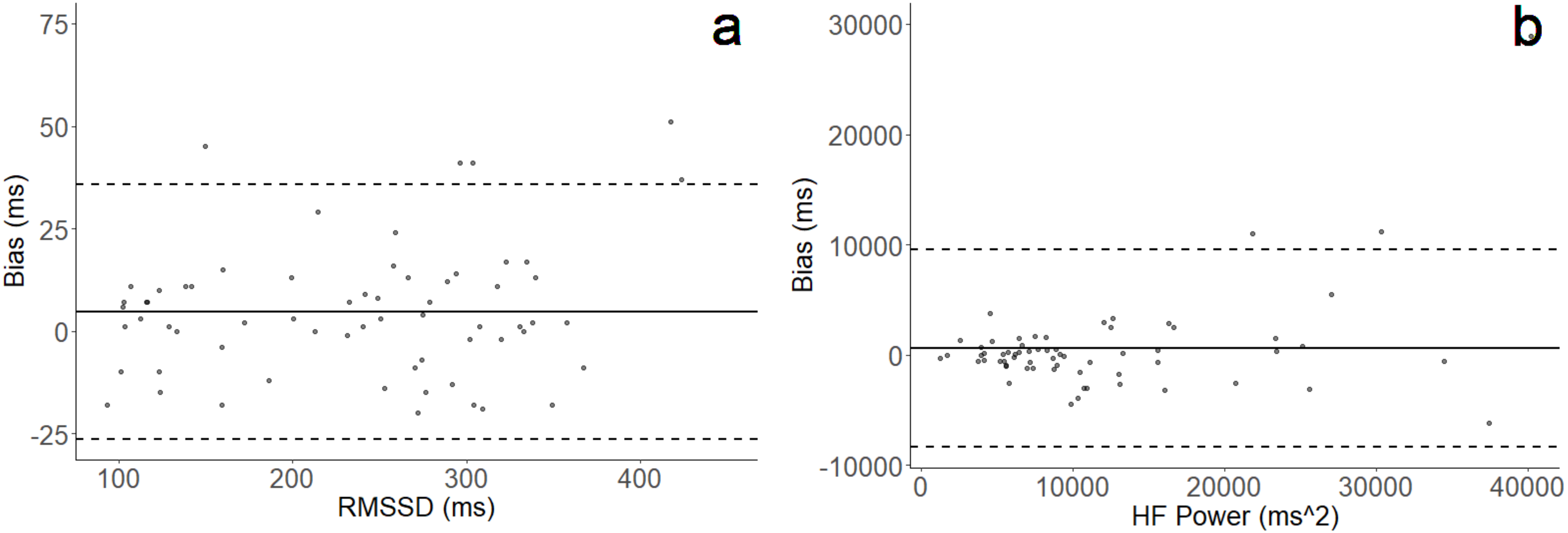
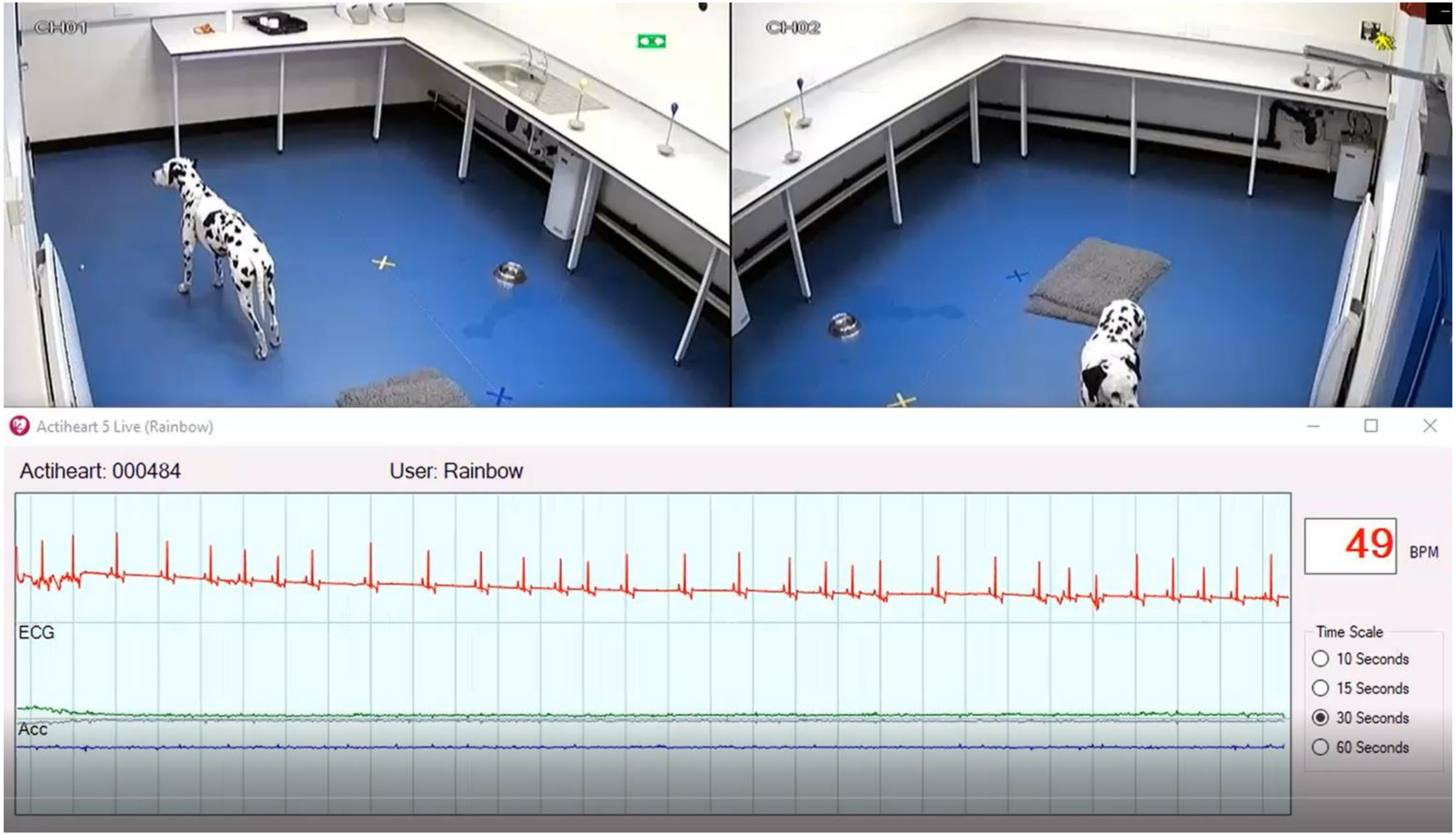


Figure 2-11 Bland-Altman plots for a) RMSSD and b) HF Power data derived from the Polar V800 with a 0.9 sec correction applied. The bias from uncorrected NovacoR data is indicated by the solid line and the upper and lower levels of agreement are shown by the dashed lines.

2.3.4 Actiheart 5 versus Televet ECG

2.3.4.1 Data collection

The validation study was conducted on 9 dogs (3 male, 6 female) of mixed breeds, mean age 4 ± 2.2 years, mean weight 21 ± 7.2 kg, to compare the Actiheart 5 with a Televet 100 four-lead veterinary ECG. The sample size was calculated using the R package "pwr" to perform an *a priori* power analysis for an ICC comparing the output of the Actiheart 5 with the Televet 4-lead ECG in terms of HR, RMSSD and HF Power. The effect size for the current analysis was estimated as very high (0.9) based on our previous work with the Actiheart 5 in horses (Mott et al., 2021). This effect size estimate was entered into the power analysis with the following parameters: alpha (2-sided) = 0.05, power = 0.95, allocation ratio 1:1. The power analysis results suggest that a sample of n=9 was required to detect a correlation of >0.9 with a 95% probability. The entire method used was the same as used for the Polar/NovacoR validation outlined above, with simultaneous recordings with both devices being conducted for 30 min whilst the dog was unrestrained at rest. This was conducted in the University of Glasgow's Animal Behaviour Centre, where there was provision for constant remote monitoring of the dogs, an example of which is shown in Figure 2-12. Live monitoring ensured that any issues with the equipment could be identified in real-time, and not at the end of the recording period.



Actiheart: 000484

User: Rainbow

49 BPM

Time Scale
○ 10 Seconds
○ 15 Seconds
● 30 Seconds
○ 60 Seconds

Figure 2-12 A screen shot of one of the video recordings from the Animal Behaviour Centre showing the two video feeds and the live ECG recording from the Actiheart 5. The lower three lines on the trace are the output from the three accelerometers and show that the dog is static.

2.3.4.2 Statistics

An agreement interval was determined using the Bland Altman test of agreement with acceptable limits defined *a priori* as a bias within 5% of the sample median for that parameter and with the 95% CI of that bias encompassing 0. Acceptable biases determined as: HR < ±3 bpm; RMSSD < ±12 ms; HF Power < ±1103 ms^2, for the Actiheart dataset.

2.3.4.3 Results

For the Actiheart 5 and Televet 100 data, the beat detection was manually corrected from the ECG traces so, unlike the Polar V800 derived data, there was no requirement to apply any automated artefact correction in the Kubios software. Table 2-8 shows the ICC3 between the output of a Actiheart 5 and a Televet 100 4 lead Holter ECG.

Table 2-8 Inter Class Correlations between output of a Actiheart 5 and a Televet 100 4 lead Holter ECG for IBI, RMSSD and the HF Power

Device	IBI (ms)	RMSSD (ms)	HF Power (ms^2)
Actiheart 5	1	0.99	1

Table 2-9 displays the results of the Bland-Altman test of agreement and shows the bias of the Actiheart 5 derived data from that of a Televet 100, as well as the upper and lower limits of the 95% confidence interval of that bias. Acceptable bias was specified *a priori* as RMSSD ± 12 ms and HF Power ± 1103 ms^2.

Table 2-9 Bland-Altman test of agreement between a Actiheart 5 and a Televet 100 on RMSSD and HF Power data

	RMSSD (ms)			HF Power (ms^2)		
Device	Bias (±12 ms)	95% CI (Lower)	95% CI (Upper)	Bias (±1103 ms^2)	95% CI (Lower)	95% CI (Upper)
Actiheart 5	0	-3	3	72	-255	399

Figure 2-13 shows all the data-points from the Actiheart 5 for RMSSD and HF Power plotted against the Televet 100 output.

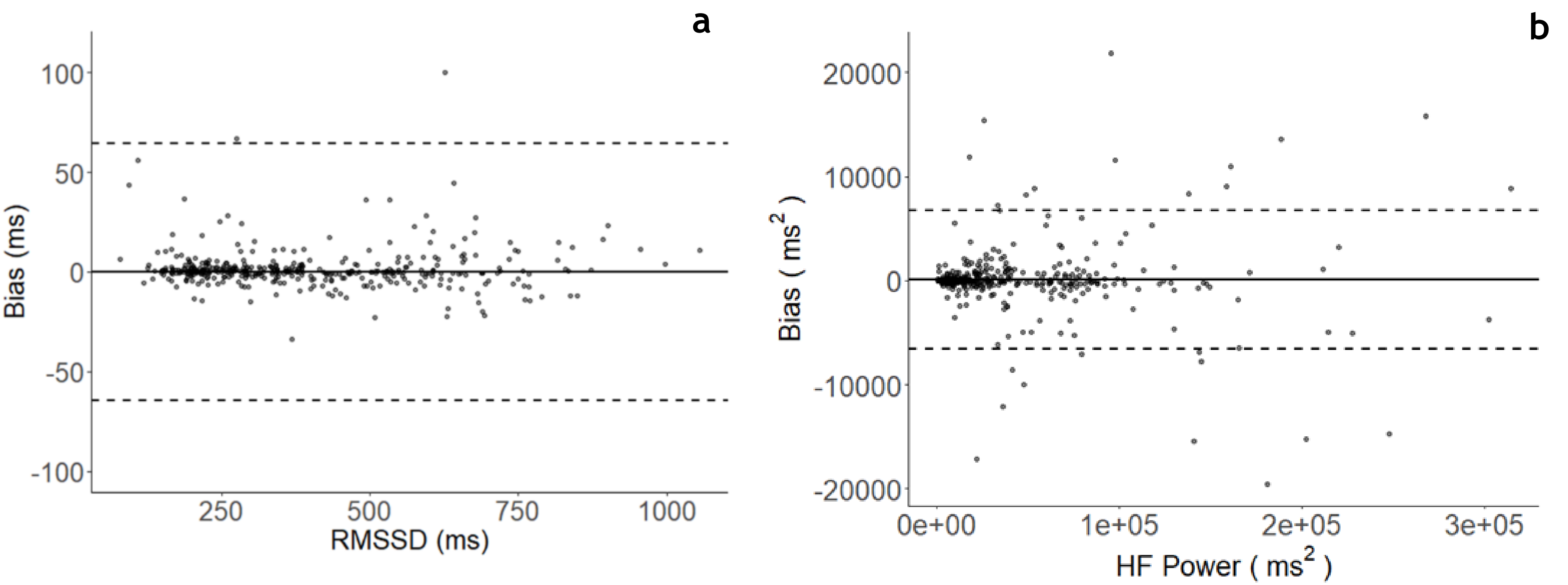


Figure 2-13 Bland-Altman plots for RMSSD (2-8a) and HF Power (2-8b) data derived from the Actiheart 5. The bias from the Televet 100 data is indicated by the solid line and the upper and lower levels of agreement are shown by the dashed lines.

2.3.5 Canine HRV Reference Ranges

2.3.5.1 Data Collection

Baseline HRV measurements were recorded from 22 dogs (8 male, 14 female, 8.7 ± 6.1 years) at four different locations, to investigate if the location and environment would influence baseline HRV. The locations selected where:

1. The University of Glasgow kennels located within the School of Biodiversity, One Health and Veterinary Medicine (SBOHVM), where staff could leave their pet dogs during the day. Dogs were kept in individual 2 m x 1.5 m pens with mesh sides, with up to 8 dogs accommodated at any one time.

2. The clinical skills examination room at SBOHVM. This is a 5 m x 3 m room used by veterinary students to practise their clinical skills. The dogs that were recorded in this room were all familiar with it, as they were used for clinical skills teaching. Only one dog was in the room at any time.

3. Dunbartonshire and West of Scotland Scottish SPCA ARRC – short-stay dogs ready for rehoming. Indoor pens were 2 m x 1.5 m with solid walls and a mesh front, facing into a corridor and solid wall. Up to 17 dogs were housed in the study block.

4. Bothwell Scottish SPCA ARRC – long-stay dogs not ready for rehoming. Indoor pens were 2 m x 1.5 m with solid walls and a mesh front facing another row of pens. Up to 30 dogs were housed in the study block.

During the period of the recording, dogs remained undisturbed and free to move around either their kennel or the examination room, depending on where the recording was taking place. For all the recordings, the location was familiar to that dog. The measurements were made using Polar V800s, fitted in the method described above, and up to five dogs were recorded at one time (other than in the clinical skills examination room, where dogs were recorded singularly). Recordings of 45 min were made, with the first 15 min of data being discarded to ensure the results were not influenced by any arousal caused by the handling of the dogs and the attachment of the equipment. A dog became unintentionally distressed during one of the recording sessions, the data from this dog gave an incidental opportunity to question the ability of HRV to determine the emotional valence of arousal.

For 5 of the 10 dogs accessed in the SBOHVM kennels / examination room, the recording period coincided with the dog displaying both increased and decreased arousal, based on HR / HRV. The recordings were repeated to get 30 min of baseline data whilst the dog was relaxed, and comparison of the two data sets from these 5 dogs also allowed examination of the range of HRV in these individual dogs. One of these 5 dogs was in a position of mild distress (desperate to go to the toilet, which was identified too late). To examine if HRV could determine valence, the same dog was presented with the same experimental set-up the following day, but this time after an initial baseline recording, the dog was presented with toys and treats in an attempt to increase arousal with a positive valence. The other 4 dogs were mildly aroused (as demonstrated by dramatically reduced HRV (Figure 2-14)) by the process of the experimental set-up, so their initial 15 mins of recording was used as the “aroused” baseline. For all remaining dogs, IBI recording commenced at least 30 min after the equipment was set-up.

2.3.5.2 Statistics

To compare the effect of location on RMSSD and HF Power, data were analysed in R 4.1.2, using generalised linear mixed effect models (GLMM) (with individual ID included as a random effect) and location modelled as a fixed effect, and the difference across the locations determined with the ANOVA function.

2.3.5.3 Results

The mean baseline HRV parameters for dogs in each of the 4 locations are shown in Table 2-10. RMSSD ($F_{3,18} = 11.6$, $p<0.001$) and HF Power ($F_{3,18} = 2.8$, $p=0.02$) were significantly different across the 4 locations.

Table 2-10 **Baseline pre-intervention mean HRV parameters for 22 dogs at 4 locations – School of Biodiversity, One Health and Veterinary Medicine Kennels/Examination room; SSPCA Dunbarton/Bothwell.**

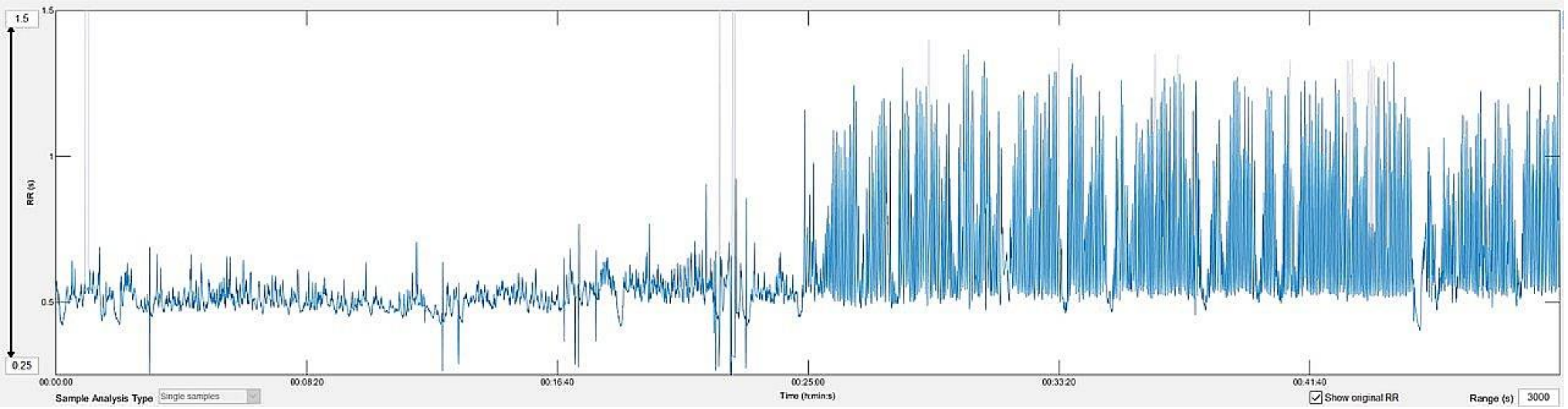
Location	*RR (ms)*	*SDNN (ms)*	*HR (bpm)*	*RMSSD (ms)*	*pNN50 (%)*	*LF Power*	*HF Power*	*LF/ HF*	*SD1*	*SD2*	*N*
SBOHVM K	791	218	82	296	69	27083	26432	2.6	211	217	6
SBOHVM E	766	160	83	201	57	10159	13573	1.8	143	170	4
SSPCA D	575	118	107	135	49	6324	9884	1.4	95	135	7
SSPCA B	445	90	136	86	29	5549	3414	1.8	61	112	5

Data from 5 dogs at the SBOHVM that displayed heightened arousal in response to the experimental set-up were partitioned *post hoc* into the first 15 min following the experimental set-up and minutes 30-45 (Table 2-11). RMSSD ($T_{1,4}$ = -10.3, p< 0.001) and HF Power ($T_{1,4}$ = -3.3, p 0.03) were significantly different between the 2 conditions.

Table 2-11 **HRV parameters from the same 5 dogs from the SBOHVM taken from the first 15 min following the experimental set-up and minutes 30-45 following the set-up**

Time (min)	*RR (ms)*	*SDNN (ms)*	*HR (bpm)*	*RMSSD (ms)*	*pNN50 (%)*	*LF Power*	*HF Power*	*LF/HF*	*SD1*	*SD2*	*N*
0 - 15	540	79	112	92	36	3629	3634	1.2	65	91	5
30 - 45	856	230	71	304	80	25146	24219	2.0	215	241	5

Figure 2-14 shows an example of the R-R interval output over 50 min of a control condition when no enrichment was provided, and shows a greatly reduced HRV for the first 25 min following the setup of the equipment, and gives an indication of how long this particular dog took to acclimatise to the experimental set-up.



Sample Analysis Type Single samples

☑ Show original RR

Range (s) 3000

Figure 2-14 RR interval trace exemplifying the ease of identifying a period of reduced HRV indicating mild stress following the initial experimental set-up (Polar data, 0.9 sec correction applied)

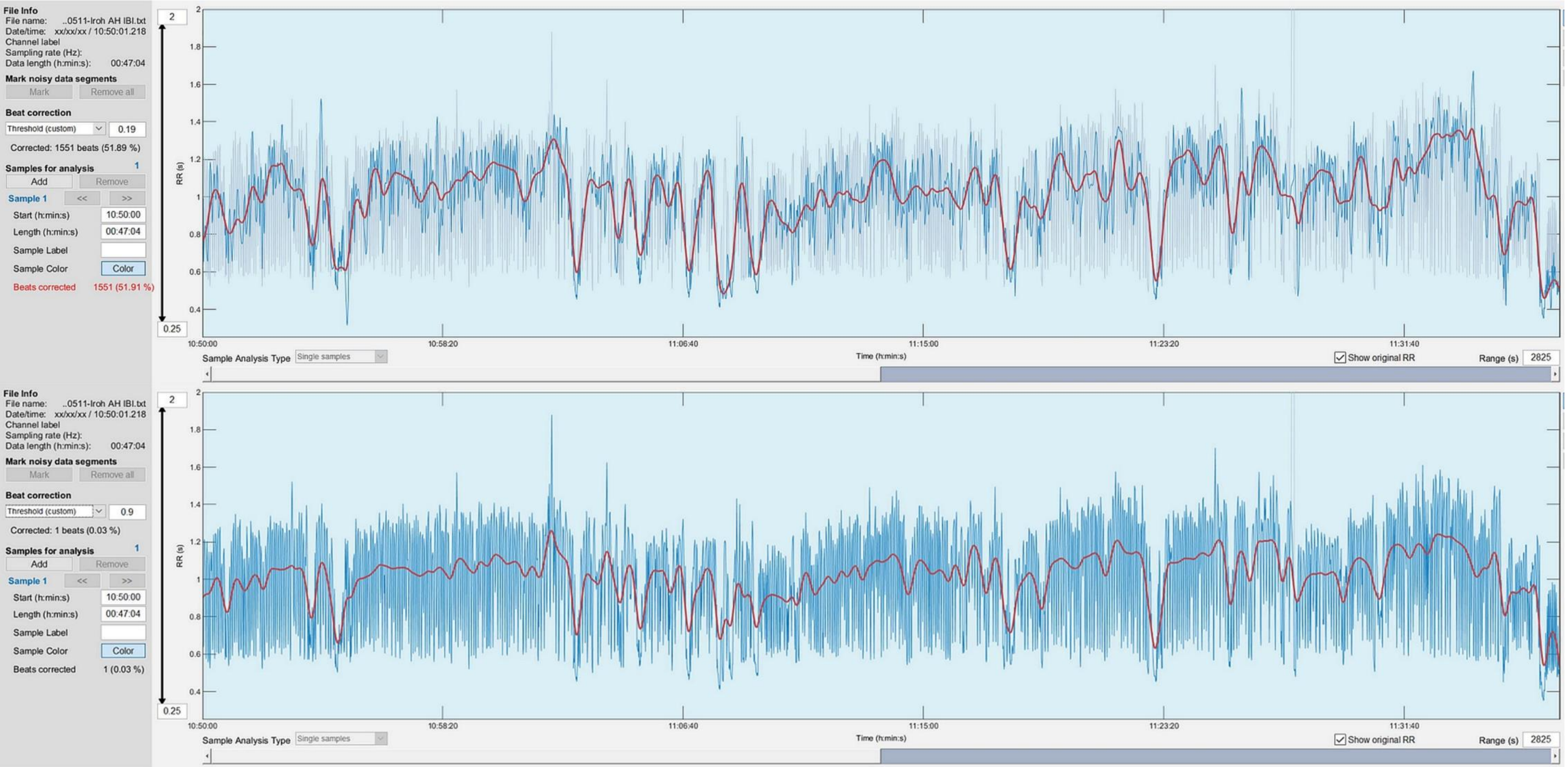
Table 2-12 shows data from the single dog that had inadvertently become distressed (negative valence) and on a separate day when at rest (neutral valance) and when treats and toys were presented (positive valence).

Table 2-12 HRV parameters from the same single dog at the SVM showing relaxation/neutral valence, excitement/positive valence and distress/negative valence

Valence	*RR (ms)*	*SDNN (ms)*	*HR (bpm)*	*RMSSD (ms)*	*pNN50 (%)*	*LF Power*	*HF Power*	*LF/HF*	*SD1*	*SD2*	*N*
Neutral	928	236	65	304	87	24838	22265	1.1	215	256	1
Positive	521	76	115	94	38	2343	4385	0.5	66	84	1
Negative	505	102	119	124	46	2694	6300	0.4	88	114	1

2.3.6 Discussion of Dog HRV

From Table 2-6 it can be seen that, relative to the ECG data, the Polar V800 derived data, exhibited an excellent ICC with all 4 artefact correction levels for IBIs. When looking at the output that is routinely used for statistical analysis (RMSSD and HF Power), the 0.9 s artefact correction gave the best correlation with the ECG output. This is a lower level of correction than is typically applied during HRV analysis in other species where standard artefact correction is 20% (Joachim A Behar et al., 2018) (i.e., identifying beats as artefact where the IBI differs by more than 20% from the previous IBI). For the dog data shown at Figure 2-15 with an average IBI of 966 ms, this would equate to a 0.19 s correction using the Kubios software which, for this dog, would identify and remove as artefact over 50% of the normal variability, as illustrated in Figure 2-15, which is a screenshot of Kubios analysis of Polar derived canine IBI data.



File Info
File name: ..0511-Iroh AH IBI.txt
Date/time: xx/xx/xx / 10:50:01.218
Channel label
Sampling rate (Hz):
Data length (h:min:s): 00:47:04

Beat correction
Threshold (custom) 0.19
Corrected: 1551 beats (51.89 %)

Samples for analysis 1
Sample 1
Start (h:min:s) 10:50:00
Length (h:min:s) 00:47:04
Sample Label
Beats corrected 1551 (51.91 %)

Sample Analysis Type Single samples
☑ Show original RR
Range (s) 2825

File Info
File name: ..0511-Iroh AH IBI.txt
Date/time: xx/xx/xx / 10:50:01.218
Channel label
Sampling rate (Hz):
Data length (h:min:s): 00:47:04

Beat correction
Threshold (custom) 0.9
Corrected: 1 beats (0.03 %)

Samples for analysis 1
Sample 1
Start (h:min:s) 10:50:00
Length (h:min:s) 00:47:04
Sample Label
Beats corrected 1 (0.03 %)

Sample Analysis Type Single samples
☑ Show original RR
Range (s) 2825

Figure 2-15 **A section of normal ‘relaxed’ dog data showing that when a 20% (0.19 s) correction is applied (upper panel), 51.91% of the data has been ‘corrected’ (greyed-out). Appling a 0.9 s correction to the same section of data (lower panel) corrected just 0.03% of the data, which equated to a single erroneous beat, leaving the ‘good’ data intact.**

The use of a 0.9 s artefact correction with Polar derived IBI data is further validated by the results (Table 2-7 and Figure 2-11) of the Bland-Altman test. Bland-Altman is a robust measure of agreement (Giavarina, 2015) and provides a more useful output than an ICC as it provides better insight into the relationship between the data from the two devices. Data derived from the Actiheart 5 more closely resembles that observed by ECG than Polar derived data, following manually correction of the Actiheart and ECG output. Table 2-8 and Table 2-9 show that the Actiheart 5 data has a near-perfect correlation with the Televet 100 data and an acceptable level of agreement.

The mean HRV measurements from dogs at four different locations shown in Table 2-10 demonstrate a significant difference in HRV between locations. This reinforces the importance of exercising caution when comparing between studies conducted in different environments. Figures previously reported from animal shelters (Bowman et al., 2015) or from dogs that are being ‘lightly restrained’ (Matsunaga et al., 2001) may well not reflect true relaxation. This possibility is supported by the data in the current study which shows that dogs at Table 2-11 ARRC had lower HRV, suggesting higher levels of arousal, compared to the dogs at the SBOHVM.

There have been attempts to use HRV to establish emotional valence in dogs (Katayama et al., 2016), horses (Kowalik et al., 2017), sheep (Reefmann et al., 2009) and goats (Briefer et al., 2015) Fundamentally it is not possible from a single form of data pertaining to the ANS to determine if the animal is in a positive or negative state of arousal as the autonomic response to stressors is independent of the nature of the stressor and can also be activated by non-stressors. Although only taken from a single dog (as no dogs were going to be intentionally distressed), Table 2-12 illustrates the difficulty of establishing valence from HRV alone.

Although the collection of IBI data was non-invasive, the handling of the dogs by a non-familiar person and the attachment of the equipment to them was a potential stressor. Figure 2-14 is a very good visual representation of how much HRV can change in dogs, and how abruptly that change can happen, in this case with no external intervention, and further supports the findings articulated in Table 2-11. Although this is a particularly extreme example, it also demonstrates the risk of using the first 30 min of the experimental protocol as the control condition, as with this particular dog, this would have led to skewed results. For this reason, HRV data collection for subsequent chapters started at least 30 min after the equipment was fitted.

2.4 Conclusion

Throughout this thesis, the physiological effects of auditory enrichment are primarily quantified using HRV. To support the validity of that data, this chapter has demonstrated that: the hardware being used (Polar V800 and Actiheart 5) can provide reliable beat detection in the target species; the correct *post hoc* processing of raw data can remove artefact and correct anomalies without damaging good data; and has established species-specific normal ranges for healthy, relaxed horses and dogs.

For dogs, the Polar V800 gives results comparable to an ECG providing a 0.9 sec artefact correction is applied to the Polar data when analysed with the Kubios software. Due to the unusually high HRV of dogs, the stronger artefact corrections commonly used in other species (including humans) risks over-correcting the data and should be avoided. The Actiheart 5 provided a better correlation and closer agreement with the reference ECG and has the advantage that the QRS complexes can be inspected and manually corrected if required, so for dog Actiheart data there was no need to apply any automated artefact corrections. The disadvantage of data collection using the Actiheart 5 system is that manual correction of an ECG trace, beat by beat, can be very time-consuming depending on the amount of misidentification of beats by the software, which in some individual horses and dogs, was extensive. There is therefore a balance between the practicality of manually processing large amounts of ECG data verses the rapid but less accurate automated processing with the Polar V800 derived data.

For static horses, due to the prevalence of second-degree AV block in relaxed animals, manually corrected ECG data requires a 0.9 sec artefact correction applied to correct for these ‘missed beats’, as it is the firing of the SA node that is of interest for HRV analysis, rather than the contraction of the ventricle. In comparing 0.9 sec corrected ECG data with data derived from the Polar V800 and the Actiheart 5, an acceptable agreement was found for both time and frequency domain HRV parameters, when a 0.9 sec correction was applied to the Actiheart data, and a stronger 0.4 sec correction was applied to the Polar data. In horses, as with dogs, the Actiheart 5 provided a closer agreement with the reference ECG than the Polar V800, mainly because the QRS complexes generated by the Actiheart could be inspected and manually corrected prior to analysis.

Non-aroused HRV values were established for horses and dogs, which differ from those previously published. Investigating the methods previously used, it is likely that previously published figures have come from animals displaying at least a mild stress response (due to either the environment or unfamiliar handling) and this point is further emphasised by the significant difference in baseline HRVs shown by dogs in the four different environments tested. The data collected from a dog that is used to being handled by a wide variety of unfamiliar people on a daily basis showed that even in dogs that are very familiar with being handled, there can be an elevated stress response for up to 30 min following the fitting of heart rate monitoring equipment. This must be accounted for when baseline measurements are taken.

Whilst this work validates equipment and methods for the assessment of HRV in two commonly studied species, it was all conducted on relatively static animals. For exercising animals, the elastic Polar straps used here may be prone to slipping, producing skin movement artefacts. Conventional ECG pads attached to shaved, prepared areas of skin may be a more suitable method of non-invasive attachment when monitoring exercising animals, and this warrants further investigation.

The hardware and software routinely used for HRV analysis in non-humans is specifically designed for use in humans, which is maybe due to the economy of scale meaning that the production of animal-specific equipment is not financially viable. This chapter has highlighted the differences in cardiac parameters between humans, horses and dogs and has shown that, despite differences in QRS complexes, beat detection is reliable with both the Polar V800 and the Actiheart 5. For the analysis of IBI data, species-specific frequency bands have been previously reported (Joachim A Behar et al., 2018), and here the appropriate automated artefact correction levels have also been determined, taking into account the high prevalence of physiological second degree AV block in horses

(Reef & Marr, 2010) (0.9 sec ECG derived data / 0.4 sec Polar derived data) and the unusually high variance of IBIs in dogs (Minors & O'Grady, 1997) (no correction for ECG derived data / 0.9 sec Polar derived data). Appropriate artefact correction ensures that the HRV analysis conducted on IBI data derived from both the Polar V800 and Actiheart 5 throughout this thesis in both horses and dogs, can be used as a measure of effect with a high degree of confidence.

Chapter 3 The effect of changing the tempo and pitch of auditory enrichment on the arousal of dogs and horses

3.1 Introduction

Human studies have identified the tempo of passively listened to music as having an effect on neurological (Trochidis & Bigand, 2013) and cardiovascular (Bernardi et al., 2006) markers of emotional valence and physiological arousal. EEG analysis has shown, in humans, slow tempos lead to decreased left frontal lobe activity, (an area of the brain demonstrated by Packheiser et al. (2021) to be associated with the processing of positively valenced emotions such as excitement), when compared to fast tempos, which increased activity in the same region (Trochidis & Bigand, 2013). HR, as a measure of arousal, can also be manipulated by listening to different tempos, with a gradual reduction in tempo leading to a proportional reduction in HR (Nomura et al., 2013; Van Dyck et al., 2017). Cardiac entrainment has been seen in human athletes whereby HR increases in participants listening to fast tempo music (Karageorghis & Priest, 2008). Fast tempo music can increase intensity of athletic activity, whilst slow tempo music has been shown to speed the recovery to resting HR (Lee & Kimmerly, 2016). The tune ‘Weightless’ by Marconi Union, a piece of electronic music specifically designed to induce relaxation, is written to be played at 71 bpm (Song, 2022) (normal human adult resting HR is between 60 and 100 bpm). If such a relationship was to be applicable to other species, it could be hypothesised that species-specific relaxing music should be based around a tempo close to that species’ resting HR. However, there is currently limited evidence to support this in mammals, fish or birds when arousal is assessed by behavioural, hormonal or cardio-vascular parameters (Snowdon, 2021). There is, however, a generally accepted principle across species that short rapid calls generally have an arousal effect, and long tonal calls have a calming effect (Snowdon, 2021), so tempo may be key to the effects on arousal in animals in the same way it is in humans. There is, however, a potential conflict that slow tempos may produce lower arousal but at the cost of a more negatively valenced emotional state.

The impact of the pitch of auditory stimuli on arousal is also a consideration. A pure tone will have a frequency, which is a measure of how often a periodic wave repeats itself. Frequency and pitch are related (higher frequency = higher pitch), but they are not interchangeable terms. Pitch is a musical rather than scientific construct and is subjective. For example, a survey of European church bells made in 1862 reported frequencies of the note ‘A’ ranging from 370 Hz to 567.3 Hz (Ballora, 2003). A pure tone will be a single frequency, but a musical note will have a main (fundamental) frequency and higher frequency harmonic components (partials) of the fundamental (Stainsby & Cross, 2009). The frequency of most of the partials are related to the fundamental by a small integer ratio (Gerhard, 2003). The fundamental frequency can be removed (referred to as the ‘missing fundamental’), leaving just the higher frequency harmonics, and the pitch will still be perceived (by humans) as the same note (Bendor & Wang, 2005). This perceptive ability may have developed in humans to derive an overall unitary precept from a mixture of frequencies, vital for both vocal language comprehension and musical appreciation (Stainsby & Cross, 2009), but is not necessarily present in other species.

Changing the key that a piece of music is played in will change the pitch of each note by one semitone for each key change (Krumhansl & Kessler, 1982). Changing from major to minor modes is more complex and depends on where the harmonic centre of the music is i.e., music in a major mode/key will revolve around the chords and notes of the major scale. The notes in every major scale are arranged in the same scale pattern and so has a similar quality (bright, cheerful, uplifting). Minor scales by contrast, use a different scale pattern and have a very different quality to major scales, sometimes described as sounding more solemn, sad, mysterious, or ominous than music that is in a major key (EarMaster, 2022). In human studies, changing between major and minor modes has been shown to influence the emotional response to music, with major keys being associated with self-reported feelings of happiness and serenity when compared to minor keys (Trochidis & Bigand, 2013). When changing between major and minor modes, pitch is changed, but so is a great deal more about the music, so it is impossible to conclude if pitch is the causal factor of the different effects of the two modes. However, lower pitch levels have both been associated with negative valence and high arousal compared to higher pitches, which reported as more pleasant, happier and brighter (Collier & Hubbard, 1998; Jaquet et al., 2014). Similar findings have been reported in dogs, with low pitched music being linked to greater arousal and alertness behaviour (Amaya et al., 2020).

A factor that may impact on musicality or musical perception is whether that species is a vocal learner or not as, according to the "vocal learning and rhythmic synchronization hypothesis" (Patel, 2006), non-vocal learners should not be able to synchronise movement with a beat. Vocal learning is defined here as animals that are capable of vocal production learning (Vernes et al., 2021) i.e., where an animal can learn a new vocalisation outside of its normal repertoire. Animals (including dogs and horses) may learn to use a normal vocalisation in a novel context, referred to as vocal usage learning (Vernes et al., 2021), but the vocal learning referred to by Patel (2006) means only vocal production learning.

The investigation of tempo and pitch detailed in this chapter was planned to be conducted using dogs in the Scottish SPCA ARRCs, but due to COVID-19 restrictions, access was lost to all the ARRCs from March 2020, and was never fully re-established. Horses were used for some of the studies because there was access to them at a time when there was no access to dogs. Although a departure from the original study species, the arousal-reducing effects of music have also been observed in horses (Stachurska et al., 2015; Wiśniewska et al., 2019), although with no reports specifically relating to the effects of pitch or tempo. As a non-vocal learning species, the mechanisms involved may be similar to those in dogs, thus supporting a theory that all non-vocal learning species react similarly to aspects of music. The dog pitch and pure beat studies were conducted in the University of Glasgow's Animal Behaviour Centre as there was ready access to a study population of dogs belonging to staff and students whilst access to the Scottish SPCA ARRCs was not available. By necessity, the studies detailed in this chapter were conducted at different times, using different study populations at different locations over a three-year period.

The hypotheses tested in this chapter are:

1. Cardiac entrainment with tempo is present in both horses and dogs, as measured by a correlation between tempo and heart rate.

2. Increasing pitch will reduce arousal both horses and dogs, as measured by changes in HRV.

3.2 Materials and Methods

3.2.1 Subjects

Dogs The dogs used to investigate the effects of pitch and tempo on arousal were all owned by staff and students from the University of Glasgow or were dogs in the care of the Scottish SPCA housed at the Dunbartonshire ARRC. Not every dog took part in every study, and some dogs only took part in one study.

> **Tempo Study** Seventeen dogs of mixed breeds in the care of the Scottish SPCA (5 female (entire), 2 male (entire), mean age 4 ± 1.9 SD years) and at the University of Glasgow SBOHVM kennels (6 female (spayed), 4 male (neutered), mean age 6 ± 5.1 SD years)
>
> **Metronome Study** Ten dogs of mixed breeds (6 female (spayed), 4 male (neutered), mean age 3 ± 1.9 SD years) (University of Glasgow Animal Behaviour Centre)
>
> **Pitch Study** Eleven dogs of mixed breeds (6 female (spayed), 5 male (neutered), 7.6 ± 4.2 SD years) (University of Glasgow Animal Behaviour Centre)

Horses (Pitch and Tempo) The seven horses used in the studies (4 geldings, 3 mares, mean age 20 ± 5.3 SD years) were either owned by the University of Glasgow and maintained at the university's Weipers Equine Centre (n=3) or privately owned horses at a yard in Northern Ireland (n=4).

These studies were approved by the University of Glasgow School of Veterinary Medicine Ethics and Welfare Committee and were conducted in line with the International Society for Applied Ethology's ethical guidelines (Sherwin *et al.*, 2017). Informed consent was obtained from all owners.

3.2.2 Auditory Enrichment

The music used throughout was Mozart's piano sonata for 2 pianos in D Major (K448), as this has been previously shown to have an arousal-reducing effect when played at normal speed (92 bpm) and pitch (D major) (see Kriengwatana et al. (2022) for review). The pitch and tempo of this track were modified in Audacity recording and editing software (V 2.3.0, Pittsburgh, USA) using the ‘effect’ function as detailed by Amaya et al. (2020). Pitch changes were made without changing tempo and *vice versa*. For the tempo studies, a 3 min excerpt was modified so that the tempo was 40, 80, 120, 160 or 200 bpm and trimmed to make a 1 min recording. These tempos were selected so that, if cardiac entrainment was evident, there was scope to move dogs’ average resting HR of 100 bpm up and down. For the pitch studies, a 1 min excerpt was produced in keys A, B, C, D and G (same excerpt for each key).

3.2.3 Data Collection

Tempo Studies (Dogs) To investigate the effect of tempo on canine HR, two complementary studies were conducted. In one, the tempo of a piece of Mozart K448 was changed while in the other, simple tempo was tested using a metronome. The two studies were conducted at different times in association with other studies, hence they were conducted in different environments.

To examine the effect of musical tempo, IBI data was collected from seventeen dogs whilst in a familiar environment (kennels at the University of Glasgow’s School of Biodiversity, One Health and Veterinary Medicine (n=10) or Dunbartonshire Scottish SPCA ARRC (n=7) when no auditory enrichment was provided (control, day 1) and when auditory enrichment (70 dB) was present (day 2). During the control condition, background noise levels from the dogs barking peaked at around 80 dB for both locations. The auditory enrichment comprised a 35 min playlist made up of extracts of K448, modified to 40, 80, 120, 160 or 200 bpm, presented in a randomised order, interspersed with 1 min sections of silence and 1 min sections of white noise, with each 1 min condition repeated 5 times. The order of presentation was the same for each participant. IBI data was collected at the same time of day on both occasions to take account of circadian and ultradian rhythms of HRV (Stein et al., 2006). IBI data were collected for HRV analysis using Polar V800 heartrate monitors using the methods described in Chapter 2. Arousal levels were assessed during each auditory condition and the control condition in 1 min epochs using HRV, specifically, RMSSD and HF Power.

To examine the effect of simple metronome tempo, a study with a different ten dogs was conducted at the University of Glasgow’s Animal Behaviour Centre. The method was similar to the previous study with the exception that instead of changing the tempo of K448, a metronome beat of 40, 80, 120, 160 and 200 bpm was used. The randomised order and sequences of white noise and silence remained the same as that used in the Mozart tempo study, with each 1 min condition repeated 5 times. For this study, the control used was the IBI data recorded from a 15 min period prior to the period of auditory enrichment and 10 min after it finished, making the total length of the IBI data collection 60 min. In the Animal Behaviour Centre, a Bluetooth speaker system (JBL Flip 3) was suspended above the centre of the arena at a height of 2.2 m. Auditory enrichment was played through this at a sound pressure level of 65 dB when measured 1 metre above floor height, i.e., at approximately the height of the ears of a large dog. This is quieter than played in the kennel environment as there was less background noise (generally around 30 dB). As with the above study, IBI data were collected for HRV analysis using Actiheart 5 ECGs, using the methods described in Chapter 2. Arousal levels were assessed during each auditory condition and the control condition in 1 min epochs using HRV, specifically, RMSSD and HF power.

Pitch Studies (Dogs) The effect of changing pitch was investigated with eleven dogs of mixed breeds and took place at the University of Glasgow's Animal Behaviour Centre. Using the same method to that employed for the metronome tempo study above, the 1 min sections of pitch modified Mozart K448 were presented to the dogs in a randomised order, interspersed with 1 min intervals of silence and 1 min periods of white noise, played at 65 dB. As with the above study, IBI data were collected for HRV analysis using Actiheart 5 ECGs, using the methods described in Chapter 2. Arousal levels were assessed during each auditory condition and the control condition in 1 min epochs using HRV, specifically, RMSSD and HF power.

Horses (Tempo and Pitch) The effect of music played at different tempos and pitches was investigated with seven horses using a 3-day protocol: Day 1 - control, Day 2 – Tempo Test, Day 3 - Pitch Test. All data collection was conducted at the same time each day, and IBI data were recorded using Actiheart 5 ECGs using the methods outlined in Chapter 2. Auditory enrichments were played through a Bluetooth speaker system (JBL Flip 3), suspended at a mid-point between the horses, at a sound pressure level of 65 dB when measured 2 metres above floor height (horse ear height).

Using a similar method to that employed for the dog tempo study, the tempo modified K448 was presented in a randomised order with 1 min sections of silence and 1 min sections of white noise. The order of presentation was the same for each participant. As with the metronome study in dogs, each 1 min condition was repeated 5 times and with 15 min of silence at the start and 10 min silence at the end, the total test phase was 60 min. The effect of changing pitch was investigated on Day 3, using the same 60 min playlist as the dog pitch study. As with the previous tempo and pitch studies above, IBI data were collected for HRV analysis using Actiheart 5 ECGs, using the methods described in Chapter 2. Arousal levels were assessed during each auditory condition and the control condition in 1 min epochs using HRV, specifically, RMSSD and HF power.

3.2.4 Statistics

Tempo and Pitch Studies Data from all studies were analysed in R 4.1.2, using general linear and mixed effect models using nlme (Pinheiro & Bates, 2023). Musical condition, age, sex and location (for split studies) were modelled as fixed effects, and model was selection conducted using AIC using AICcmodavg (Mazerolle, 2023). The various model options for the dog tempo study are shown in Table 3-1, along with their AICs. Plotting a histogram of the residuals confirmed the assumptions of normality were met.

Table 3-1 AIC table for the range of models that could have been applied to the dog tempo data. The full model has the lowest AIC and therefore was the model selected. It is of note that the musical condition being tested has the highest AIC therefore the least impact on the RMSSD of HRV

Name	Model	AIC
Combination	lm(RMSSD ~ Condition + Sex + Age + Location)	8509
Interactions	lm(RMSSD ~ Condition * Sex * Age * Location)	8540
Location	lm(RMSSD ~ Location)	8551
Age	lm(RMSSD ~ Age)	8633
Sex	lm(RMSSD ~ Sex)	8642
Condition	lm(RMSSD ~ Condition)	8660

When individual ID was added as a random effect to the combination model above, the model performance was assessed using the “performance” package (Ludecke et al., 2021), the AIC reduced to 8017 and the ICC was 0.619, indicating that approximately 62% of the variance in RMSSD was accounted for by differences in individual dogs.

For the dog music tempo study and the horse tempo/pitch studies, overall effect of enrichment was compared to the unenriched control condition recorded the previous day. Variance in effect was determined using the ANOVA function. For the dog metronome and pitch studies, and the horse tempo and pitch studies, variance in effect across the enrichment conditions was determined using the ANOVA function, and the ‘Before’ and ‘After’ conditions were compared using a paired t-test.

3.3 Results

Investigation of the effects of music tempo (dogs) RMSSD ($F_{1, 1129}$ = 122, p<0.0001) and HF Power (F1, 1129 = 8, p=0.0042) significantly increased when auditory enrichment (music/silence/white noise) was provided in a noisy (kennels) environment, when compared to the control condition of no enrichment recorded the day before (Figure 3-1 a and b). Playing Mozart at different tempos or periods of white noise and silence had no significant effect on RMSSD ($F_{6, 640}$ = 0.3, p=0.9513) or HF Power ($F_{6, 640}$ = 0.6, p=0.7421) across the period of enrichment (Figure 3-2 a and b). Age and sex had no significant influence on either the general effect of enrichment or the specific effect of individual tempos. Location had a significant impact on RMSSD ($F_{1,13}$ = 6.9, p=0.02) but not on HF Power ($F_{1,13}$ = 3.3, p=0.09). When filtered by location, the effect on both HRV parameters of changing tempo was still non-significant.

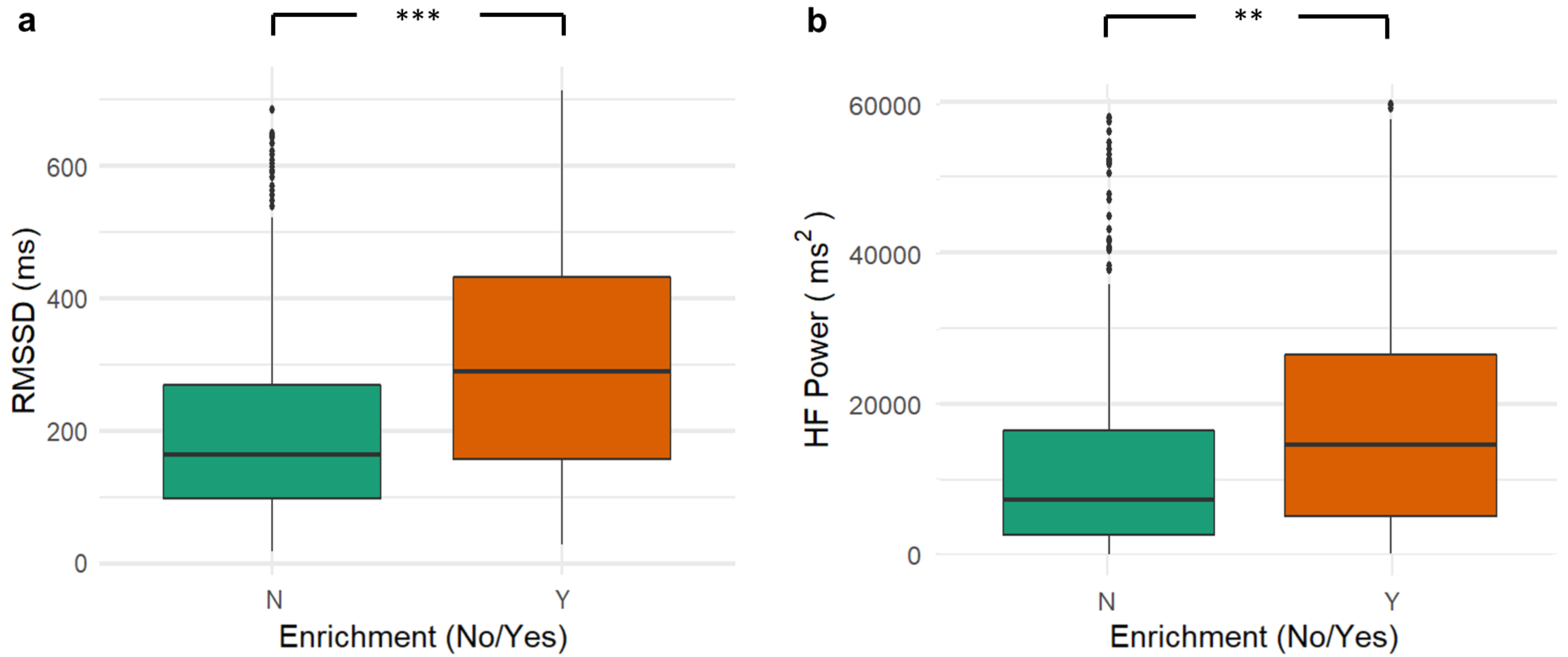


Figure 3-1– Boxplots (dog Tempo) representing the median and inter-quartile range, showing that RMSSD (a) ($F_{1, 1129}$ = 122, p<0.0001) and HF Power (b) ($F_{1, 1129}$ = 8, p=0.0042) were both significantly increased (RMSSD increased from 202 ± 138 ms to 300 ± 164 ms and HF Power increased from 15,776 ± 19,849 ms^2 to 22,248 ± 22,083 ms^2 (mean ± SD)) when auditory enrichment was provided (P<0.05 (*), P<0.01 (**), P<0.001 (***))

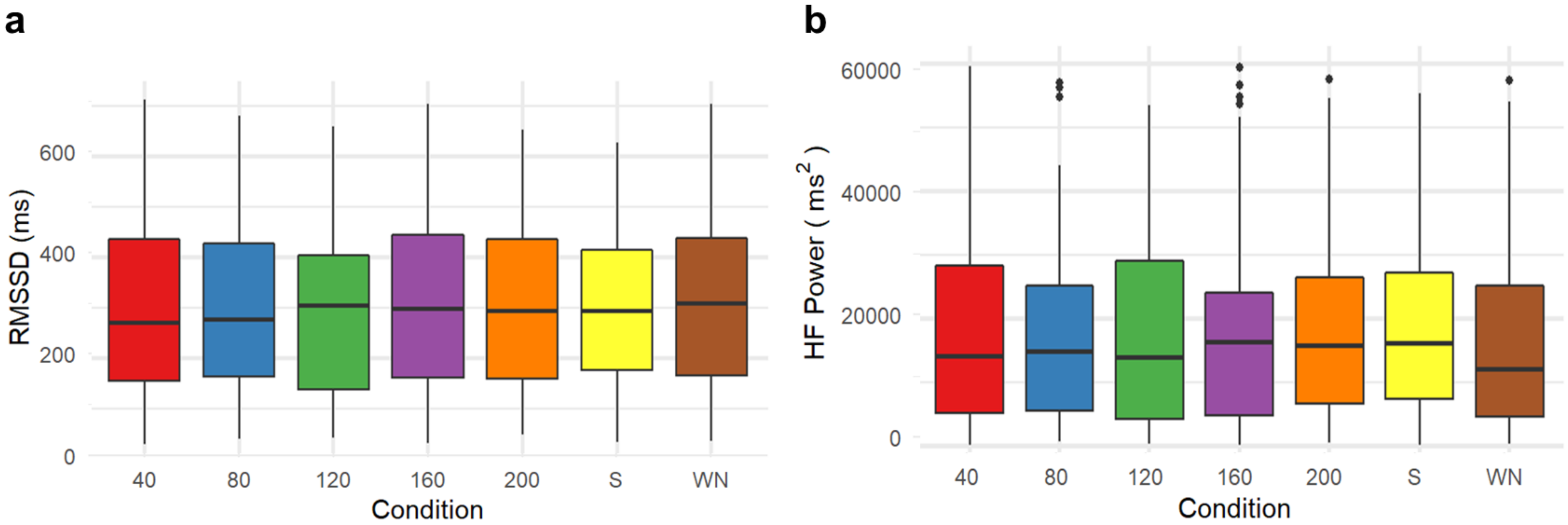


Figure 3-2– Boxplots (dog tempo) showing that RMSSD (a) and HF Power (b) did not significantly differ when dogs were exposed to each of the randomly ordered enrichment conditions (silence (S), white noise (WN) and Mozart played at 5 different tempos 40-200 bpm)

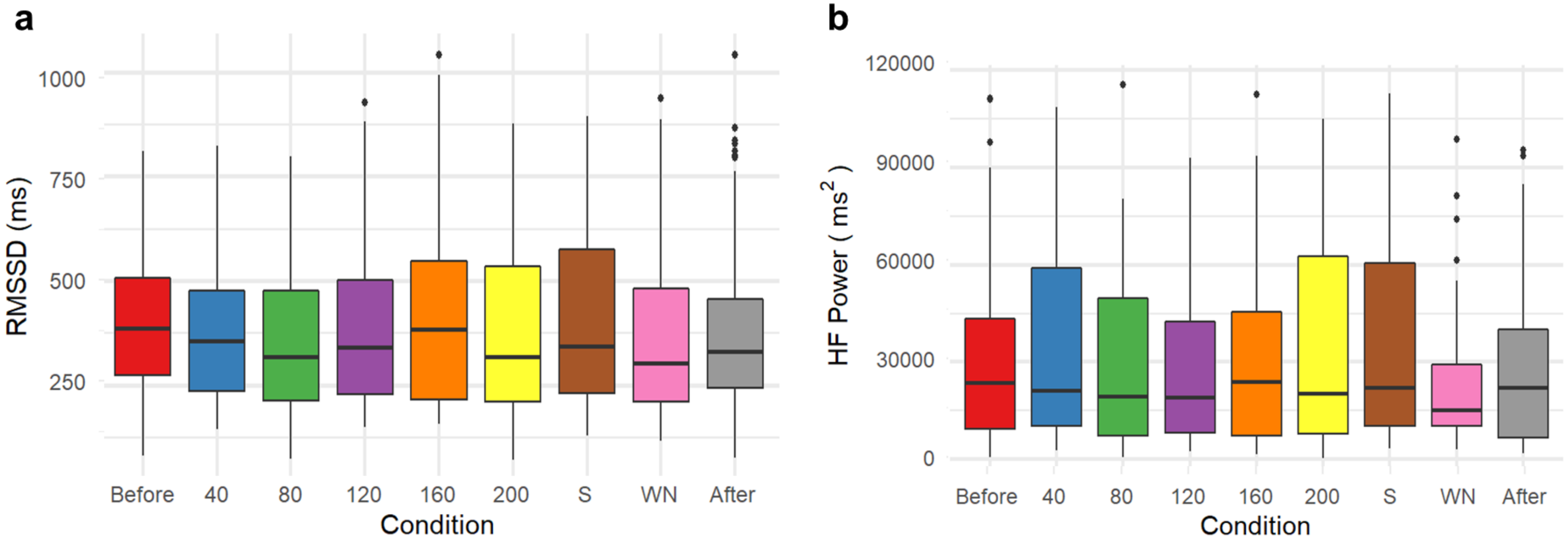


Figure 3-3 Boxplots (dog metronome) showing that RMSSD (a) and HF Power (b) did not differ when dogs were exposed to each of the randomly ordered enrichment conditions (silence (S), white noise (WN) and metronome beats 5 different tempos 40-200 bpm). RMSSD (Before (401±158 ms) and After (375±191 ms) (mean ± SD)) and HF power (Before (41,672± 43,139 ms) and After (35,172± 38,429 ms) (mean ± SD)) were not significantly (p=0.06) different

Investigation of the effects of metronome tempo (dogs) There was no significant difference in RMSSD ($F_{6,334} = 2$, p=0.06) or HF Power ($F_{6,334} = 0.4$, p=0.85) across the range of metronome tempos. RMSSD and HF power were not significantly different (p=0.06) between the two periods of no enrichment (Figure 3-3).

Investigation of the effects of pitch (dogs) There was a significant (p<0.001) increase in mean RMSSD and HF Power between the periods of no enrichment before and after the auditory enrichment (Figure 3-4). There were no significant differences in RMSSD ($F_{6,400} = 1.6$, p=0.15) or HF Power ($F_{6,400} = 0.9$, p=0.51) across the 5 different keys of K448, white noise or silence.

Investigation of the effects of tempo (horses) Overall, auditory enrichment (music/silence/white noise) with changed tempos did not significantly affect RMSSD ($F_{1,832} = 0.6$, p=0.46) or HF Power ($F_{1,832} = 0.1$, p=0.74) in horses compared to the control condition of no enrichment recorded the previous day (Figure 3-5). There was no significant difference between the 7 experimental conditions for RMSSD ($F_{6,232} = 1.2$, p=0.34) or HF Power ($F_{6,232} = 1.5$, p=0.17) RMSSD was not significantly different between 'Before' and 'After' (Figure 3-6 a), but HF power was significantly lower 'After' (8,985 ± 15,580 ms^2) when compared to 'Before' (13,509 ± 24,448 ms^2) (mean ± SD), p=0.04 (Figure 3-6 b). Location made no significant difference RMSSD ($F_{1, 5} = 2.0$, p=0.22) or HF Power ($F_{1, 5} = 1.6$, p=0.26)

Investigation of the effects of pitch (horses) Overall, auditory enrichment (music/silence/white noise) with changed pitches did not significant affect RMSSD ($F_{6, 232} = 1.2$, p=0.33) or HF Power ($F_{6, 232} = 1.5$, p=0.17) (Figure 3-7). There was no significant difference between the 7 enrichment conditions for RMSSD ($F_{6, 232} = 1.0$, p=0.44) or HF Power ($F_{6, 232} = 0.5$, p=0.79). RMSSD and HF power were not significantly different between 'Before' and 'After' (Figure 3-8 a and b). Location made no significant difference RMSSD ($F_{1, 5} = 3.5$, p=0.12) or HF Power ($F_{1, 5} = 2.5$, p=0.17).

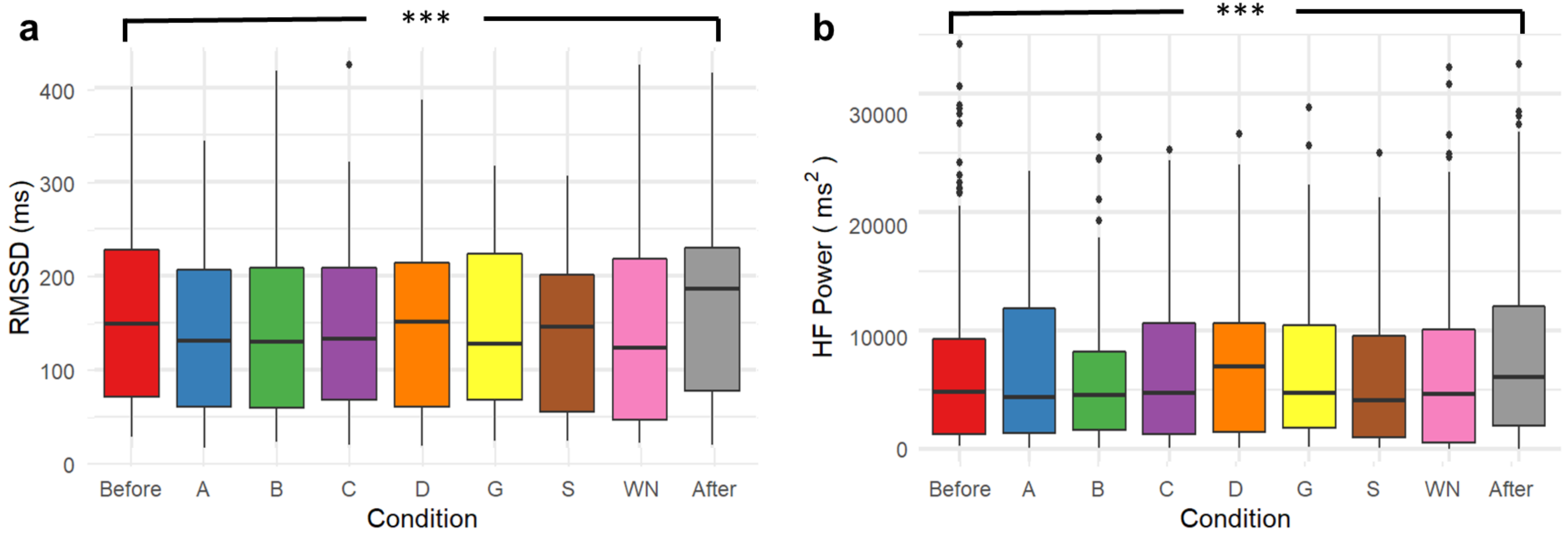


Figure 3-4 Boxplots (dog pitch) showing that RMSSD (a) and HF Power (b) did not differ when dogs were exposed to each of the randomly ordered enrichment conditions (silence (S), white noise (WN) and 5 different K448 Keys A,B,C,D and G). RMSSD (Before 153±95 ms, After 211±163 ms (mean ± SD)) and HF Power (Before 7,512± 8,315 ms^2, After 16,038± 26,242 ms^2 (mean ± SD)) were significantly ($p<0.001$) higher 'After' when compared to 'Before'

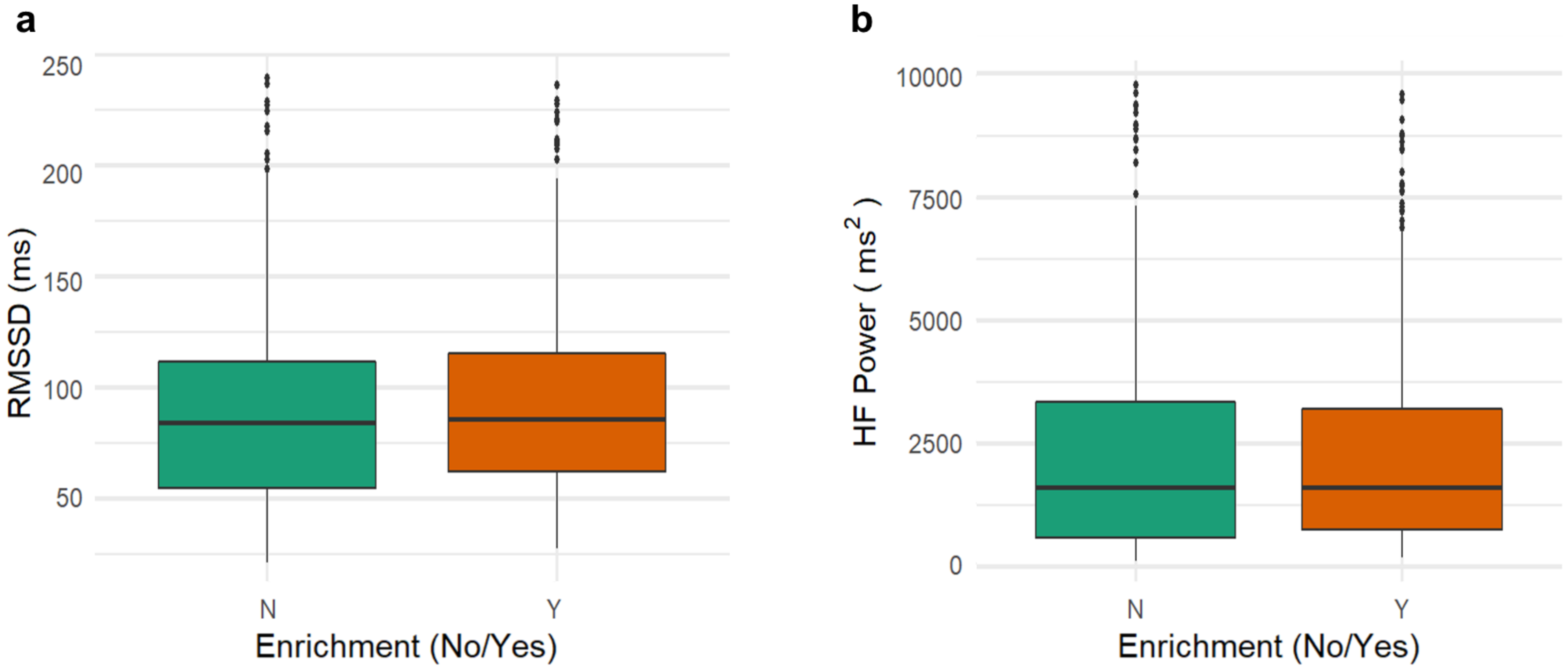


Figure 3-5 Boxplots (horse tempo) showing no significant difference between the effect of no auditory enrichment (N) compared to auditory enrichment (Y) on RMSSD (a) and HF Power (b)

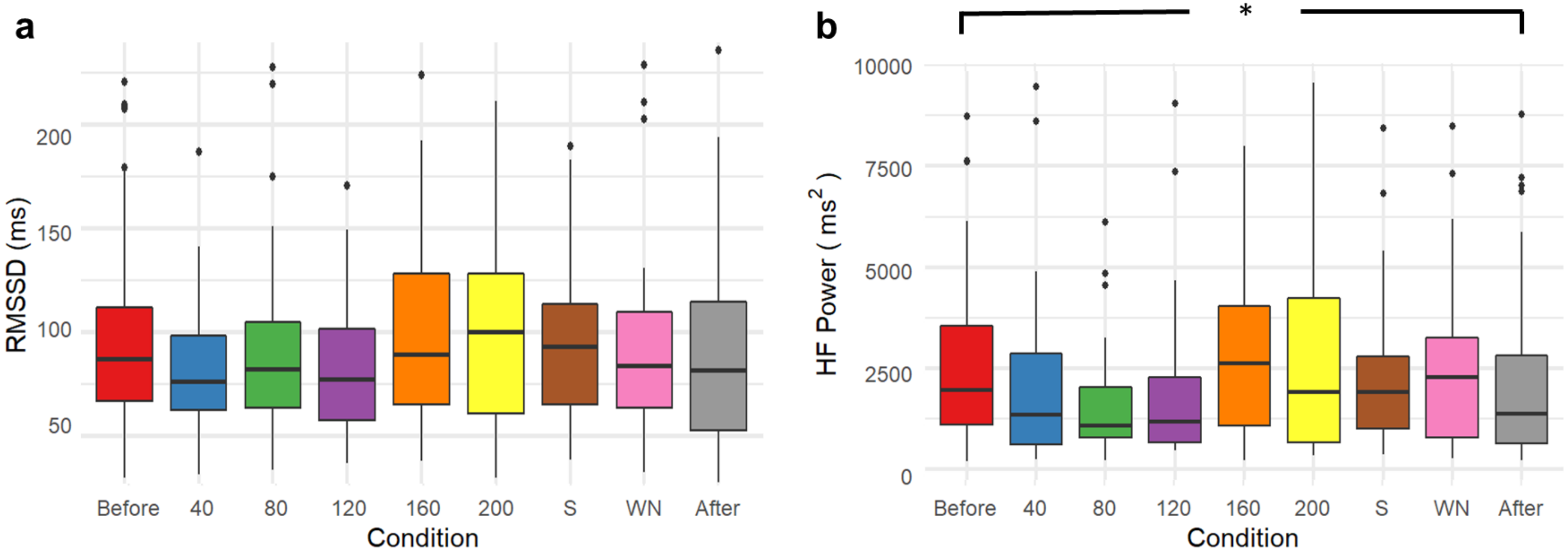


Figure 3-6 Boxplots (horse tempo) showing that RMSSD (a) and HF Power (b) did not differ when horses were exposed to each of the randomly ordered enrichment conditions (silence (S), white noise (WN) and Mozart played at 5 different tempos 40-200 bpm). HF power was significantly ($p=0.04$) lower 'After' when compared to 'Before'

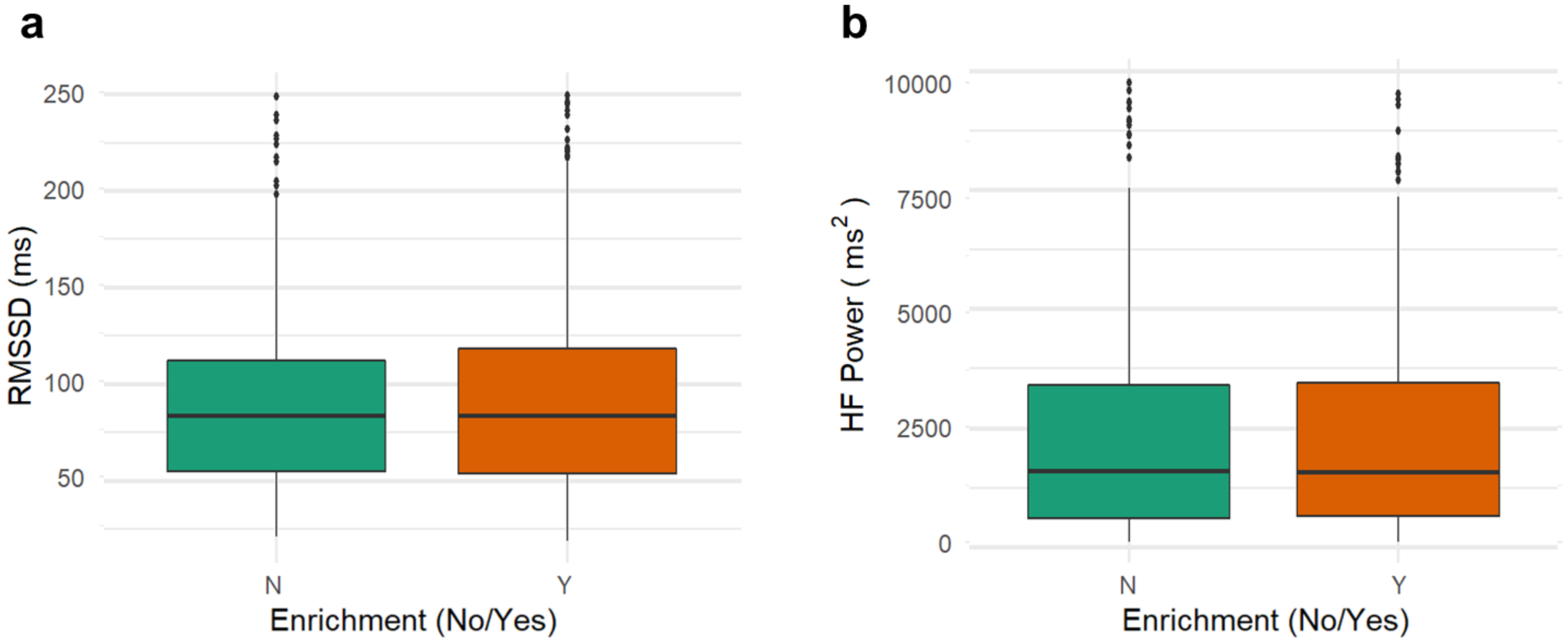


Figure 3-7 Boxplots (horse pitch) showing no significant difference between the effect of no auditory enrichment (N) compared to auditory enrichment (Y) on RMSSD (a) and HF Power (b)

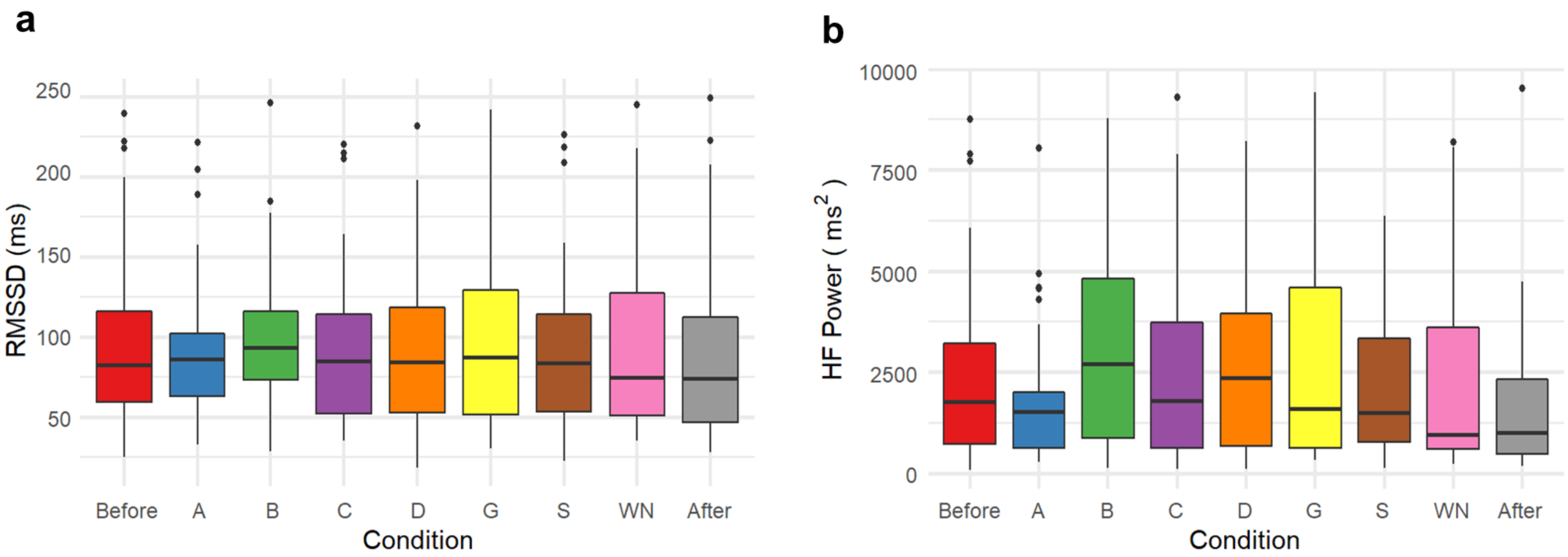


Figure 3-8 **Boxplots (horse pitch) showing that RMSSD (a) and HF Power (b) did not differ when horses were exposed to each of the randomly ordered enrichment conditions (silence (S), white noise (WN) and 5 different K448 Keys A,B,C,D and G)**

3.4 Discussion

This study set out to explore the impact of pitch and tempo on the vagal tone of two non-vocal learning species, horses and domestic dogs. Changing the pitch or tempo of the music provided as auditory enrichment made no significant difference to arousal levels of either dogs or horses, as quantified by two measures of HRV (RMSSD and HF Power). HRV was also not significantly different when dogs were exposed to various tempos of a metronome beat.

Auditory enrichment resulted in an overall arousal-reducing effect for dogs in a kennels environment when compared to the control condition when no enrichment was provided. Due to the impact of COVID-19 and the subsequent loss of access to any of the Scottish SPCA ARRCs, the remaining studies (horse pitch and tempo; dog pitch and metronome tempo) were conducted in alternative environments including the University of Glasgow's Animal Behaviour Centre and a private stable block. In contrast to the noisy, working kennel environments of the SBOHVM and Scottish SPCA AARCs, these were typically quieter environments with minimal background noise or disruption. In these environments, auditory enrichment made no significant difference to HRV of dogs or horses when compared to the control conditions of no enrichment. This incidental finding is suggestive that acoustic masking is at least part of the mechanism by which auditory enrichment is having an effect.

3.4.1 Tempo (Dogs)

The finding of significant reduction in arousal seen in dogs in a noisy (kennels) environment, when auditory enrichment of different tempos was provided, compared to the control condition of no enrichment, is in agreement with the previous work with dogs in rescue centres that has shown arousal-reducing benefits of a range of auditory enrichments (Amaya et al., 2020; Bowman et al., 2017; Brayley & Montrose, 2016; Wells et al., 2002). There was, however, no significant difference between any of the auditory conditions, suggesting three key findings. Firstly, there was no significant difference in arousal between any of the five tempos of Mozart K448 (40-200 bpm), suggesting lack of any effect of tempo which is supported by the findings of Amaya *et al.* (2020) who found no effect of changing the tempo of music on the arousal behaviour of dogs. Secondly, there was no significant difference in arousal between white noise and any of the five tempos of Mozart K448. This suggests that auditory enrichment in general rather than music *per se* is having an arousal reducing effect. Thirdly, and most unexpectedly, there was no significant difference in arousal between the 1 min periods of silence and any of the other auditory conditions, but they were different from the control condition of silence the previous day. HRV parameters change within a few seconds of a change in stimuli, so, although 1 min is a relatively short epoch for quantifying HRV, there should not be a carry-over effect from previous auditory stimuli, so it may be that the silence is having the effect. A similar result was seen in humans by Bernardi et al. (2006) who saw a reduction in arousal below baseline during 2 min periods of silence randomly placed during music presentation. They suggested that a pause may be a crucial aspect of the arousal-reducing effects of music but did not hypothesise a mechanism.

The effect of tempo on behavioural arousal of dogs has been investigated in a shelter environment (Amaya et al., 2020) and an attempt has been made to entrain dog's HR to a musical tempo (King et al., 2022). Neither of these studies were able to demonstrate an effect of tempo, but both studies used music rather than a simple beat. To remove the element of musical complexity, a similar experimental protocol was run, but this time using metronome beats (40-200 bpm) instead of various tempos of Mozart K448. This was conducted in the quiet environment of the University of Glasgow's Animal Behaviour Centre. For the collection of these data, a baseline recording was not taken the previous day, as the primary aim was to compare the auditory conditions with each other rather than with a baseline, as the general principle of arousal reduction had already been demonstrated. A 15 min period of no-enrichment was included at the start of the data collection and 10 min of no-enrichment was included at the end so the time series of HRV across 60 min could be plotted in 1 min increments. As with the tempo study conducted using Mozart K448, there was no significant difference in arousal between the five tempos, and there was no significant difference between the tempos, white noise or periods of silence. This is suggestive of a lack of tempo effect, even when musical complexity is removed.

One of the constraints of conducting data collection in any ARRC is the number of variables that cannot be controlled. To take account of circadian and ultradian rhythms of HRV (Stein et al., 2006) the control condition for the data collected in a kennel environment was an un-enriched 35 min with data collected at the same time of day that data for the experimental condition was going to be collected. Although this accounted for the circadian and ultradian rhythms of HRV, because data collection was conducted on two different days, it did introduce other variables. The dog's routine should have been the same, but with different staff on duty on different days and the potential variance in background noise each day, conditions were unlikely to be identical. Even something as simple as different weather on the two days could have impacted on arousal, particularly when HRV is so sensitive to small changes. It is therefore possible that a baseline control condition recorded the previous day is not a reliable control in this environment, particularly when effect sizes are small. When looking at the model selection and performance, it is of note that both location and individual ID had a larger impact on the outcome measure (HRV) than the musical conditions under test. This is to be expected as the different environments were likely to provide different baseline HRV values as demonstrated in Chapter 2, and large between subject HRV variation is also to be expected. However, changing the musical condition had no significant effect on individuals, regardless of their location or their resting HRV. Another key difference between the two studies is that one used Mozart and the other used metronome beats. It could therefore be suggested that musical complexity is required to have an enriching effect, however, both studies also used the same non-music control (white noise), and this was seen to have an effect compared to baseline in the K448 study but not in the metronome study. If musical complexity was the key, it would be expected that there would be a difference in arousal between white noise and the metronome beat (i.e., only a reduction in arousal during white noise as seen in the K448 study) but this difference was not seen. What is certain is that no cardiac entrainment was evident in either environment.

3.4.2 Pitch (Dogs)

Domestic dogs, like humans, show abstract pitch-size association, meaning dogs can tell the size of an object from the pitch of the sound it emits (Korzeniowska et al., 2022). This phenomenon, referred to as the pitch-size crossmodal correspondence, is an adaptive trait conserved across many species, as it gives an indication as to the size of another animal purely from the pitch of their vocalisations (Pisanski et al., 2017). Dogs therefore have some perception of pitch, and this was confirmed by Amaya et al. (2020) who investigated the effects of pitch on behaviour of shelter dogs. They found that low pitch music increased ‘alertness’ behaviour when compared to high pitch music, mirroring the results from human studies, which at the very least demonstrates that dogs perceived the different pitches of music differently. So, the question is, why is there no evidence of an effect of different pitches in the current study? The Amaya et al. (2020) study differs from the current study in the length of exposure to music. They used 10 days of exposure for 2 h 40 min each day, in two 5 day blocks, with a 2 day break in between. Each individual track was played on loop for 10 min. It is possible that the five repetitions of 1 min loops repeated for just 40 min used in the current study was not long enough to see an effect. This is an unlikely explanation, as ANS responses to changing stimuli are rapid, and Trochidis and Bigand (2013) saw a change in effect using auditory stimuli of 36 s in duration. The other difference is that Amaya et al. (2020) used a behavioural ethogram rather than physiological measures. Of the 20 behaviours recorded, only the four tail position behaviours were significantly different. With no significant differences seen in any other body posture, behaviour or vocalisations measured, and with no physiological measurements taken, arguing that the dogs were more alert during low pitched music might demonstrate that dogs are sensitive to pitch, but only express this through very specific behavioural responses rather than a generalised change in arousal.

The ability of dogs to show abstract pitch-size association (Korzeniowska et al., 2022) but for the differences in pitch of music to have little or no effect points to dogs having an absolute pitch perception, but poor relative pitch perception (Hoeschele, 2017). Absolute pitch perception is the ability to categorise a sound as high pitch or low pitch, and this is the requirement for abstract pitch-size association (Hoeschele, 2017). Relative pitch perception requires perception of the relationship between pitches. For example, humans can recognise a tune when the key has been changed – the pitch of the entire tune is different, but the relative difference in pitch between the notes is still the same (Hoeschele, 2017). Songbirds have this ability (Hulse et al., 1990; MacDougall-Shackleton & Hulse, 1996), but only with conspecific songs and not human music (Bregman, Patel, et al., 2012). Having been investigated in a range of species including monkeys and rats (D'Amato, 1988), the only non-human mammals where relative pitch perception has been demonstrated are dolphins (Ralston & Herman, 1995) and ferrets (Yin et al., 2010), but only after extensive training. It appears that most non-human animals rely more heavily on the absolute pitch of sound elements and are less responsive to relative pitch changes (Yin et al., 2010), and this may explain why the ability of dogs to show abstract pitch-size associations does not correlate to a perception of pitch within music.

3.4.3 Horses

The results of both the tempo and pitch studies mirrored those of the dog studies conducted in a quiet environment. There was no significant difference in arousal between any of the experimental conditions (tempo or pitch) nor was there a significant difference between the experimental conditions and the control condition of no enrichment taken the day before. The studies using horses were only conducted in quiet, relaxed, familiar environments and therefore, there could be no comparison between the effects of auditory enrichment in quiet versus noisy environments.

The hearing of horses is similar to humans, with a comparable frequency / intensity profile, with horses having better high-frequency hearing, while humans have better low and mid frequency hearing (Heffner & Heffner, 1983). The auditory enrichment used in these studies, played at 65 dB, could therefore be heard equally well by horses, dogs and humans. Horses are visual communicators, relying far more on body language than vocalisation for conspecific communication (Ladewig, 2019) and are not as dependent on human vocalisations as dogs, as human / horse interaction is mainly through physical contact and body language (Proops & McComb, 2010). However, it is likely that horses, like most animals, have an abstract sense of pitch, and their ability to differentiate positively and negatively valanced human vocalisations (Smith et al., 2018) is possibly evidence of this, as the horses offered the correctly valenced response to low pitched growls and high-pitched laughter. As a non-vocal learning species with a comparable auditory acuity to dogs, the similarity in results is unsurprising although direct comparison between the horses and dogs' studies is difficult due to the different environments that the studies took place in.

3.4.4 Noisy versus Quiet Environments

An incidental finding of this series of studies is that in a noisy environment, there was the expected arousal-reducing effect of auditory enrichment when compared to the un-enriched control condition, but in quiet environments, this effect was not seen in either dogs or horses. As arousal-reduction was being measured, it could be argued that these animals were as relaxed as possible in the quiet environment and therefore could not get any more relaxed. This could hold true for the horses, as they were all recorded in their own stables with familiar horses in adjoining stables. However, the dogs in the Animal Behaviour Centre were isolated in a novel environment, so despite not showing any overt separation anxiety, were unlikely to be completely relaxed. On average, the HRV of the dogs in the Animal Behaviour Centre was higher than the dogs in the ARRC suggesting less arousal, but there was considerable individual variance with one of the lowest mean HRVs coming from a dog in the Animal Behaviour Centre. The ARRC may be considered to be a stressful environment as it is unpredictably noisy, but it was familiar to the dogs at the time of test (all had been there at least 3 weeks), whereas the Animal Behaviour Centre was a novel environment for every dog tested. It is therefore unlikely that any of the dogs involved in the study in any of the locations were so relaxed they could not be relaxed further. This incidental finding is in line with previous studies on dogs in relatively quiet environments such as in a veterinary hospital (McDonald & Zaki, 2020) and a study of separation anxiety (Kinnaird & Wells, 2022) found only weak evidence demonstrating that exposure to music reduced arousal, in this instance using behaviour as an outcome measure. An alternative hypothesis might therefore be that auditory enrichment in a noisy environment is acting as an acoustic mask, as proposed by Kriengwatana et al. (2022) - a principle that is routinely used to increase focus and productivity in open-plan offices (Renz *et al.*, 2018). However, as the music in this study was played at 65 dB, far below the peak values of 90 dB sometimes seen in an ARRC, acoustic masking may not be as simple as “covering up” aversive noise.

3.4.5 Limitations

The first and most major limitation of this study is that it was carried out on two different species, conducted at different times, using different study populations at different locations over a three-year period. Although the incidental finding of an effect of novel auditory enrichment in a noisy environment but not in a quiet one is interesting, it was not a hypothesis that was being specifically tested, so there are more variables than just the level of background noise. It is therefore impossible to draw a specific conclusion about the influence of background noise on the effectiveness of auditory enrichment.

The order of presentation of each stimulus was initially randomised, but each animal received the stimuli in the same (randomised) order. This ensured that each experiment condition was identical but does mean there could have been an order effect that was not accounted for. Randomising for each animal would have ensured that any differences in effect were due to the auditory condition. For the horse study, all horses went through the study in the same order: Day 1 - control, Day 2 – Tempo Test, Day 3 - Pitch Test. This leads to the possibility of not accounting for any carry-over effect, and as the horses were tested in two groups, swapping the Temp and Pitch days for one of the groups may have been worthwhile.

3.5 Conclusion

Changing the pitch and tempo of music provided as auditory enrichment made no significant difference to the HRV of either horses or dogs, and this contrasts with the human literature on the effect of musical pitch and tempo. When musical complexity was removed, metronome beats at the same tempos as used in the music tempo study also made no significant difference to the HRV of dogs, but this was not tested in horses. These results are suggestive of more limited auditory perceptive abilities in horses and dogs than in humans, and this calls into question how they may be perceiving music. The incidental finding of a lack of arousal-reducing effect in quieter environments indicates that, in the possible absence of musical perception or appreciation, at least part of the mechanism by which auditory enrichment is having an effect may be acoustic masking, and this warrants further investigation.

Chapter 4 Enhancing auditory enrichment for domestic dogs using the owner’s voice

4.1 Introduction

Companion dogs require efficient processing of speech content and the ability to recognize specific humans, as highlighted by Miklósi (2014). Dogs have been selected for their preference towards humans, thus tend to form close social bonds with their owners (Hart, 1995; Miklósi, 2014), and their exposure to a human social environment has made them highly receptive to speech. Dogs can comprehend both non-linguistic cues such as emotional tone and linguistic cues such as lexicality in speech (Andics et al., 2016; Andics et al., 2014; Gábor et al., 2020; Kaminski et al., 2004). Studies have also found that dogs are capable of distinguishing between male and female voices (Ratcliffe & Reby, 2014) and can pair their owner's voice and face (Adachi et al., 2007). Recent research has also suggested that dogs can differentiate between unfamiliar speakers, both in their behaviour and neural activity (Boros et al., 2020; Root-Gutteridge et al., 2019) and can differentiate their owner’s voice from that of strangers (Adachi et al., 2007; Gábor et al., 2019). It is therefore plausible that for most dogs, a positively valenced association has been made with their owner’s voice. This commercially contracted study tested the hypothesis that music based on the tone and inflection of the owner’s voice, with/without clips of the owner’s voice, would have a greater effect on arousal compared to Mozart K448 (previously demonstrated in Chapter 3 to reduce arousal in a noisy environment) and a non-music control (white noise).

4.2 Materials and Methods

4.2.1 Subjects

The study was conducted using 11 dogs of mixed breeds (5 male, 6 female, 7.6 ± 4.2 years) owned by staff and students of the University of Glasgow, and took place at the University of Glasgow’s Animal Behaviour Centre.

This study was approved by the University of Glasgow School of Veterinary Medicine Ethics and Welfare Committee and was conducted in line with the International Society for Applied Ethology’s ethical guidelines (Sherwin et al., 2017). Informed consent was obtained from all owners.

4.2.2 Data Collection

Owners provided voice clips of common phases such as “good boy” and “it’s OK” via WhatsApp voice notes. The voice clips were then passed to the commercial company that had commissioned the study, who generated the auditory enrichment to be tested. At the Animal behaviour centre at the University of Glasgow, dogs were exposed to a) commercially produced bespoke electronic music based on the owner’s voice, b) the bespoke music used in a, interspersed with clips of the owner’s voice, c) Mozart (K448) and d) white noise. The four auditory conditions were played for 5 min in a pseudo-randomised order for a total of 40 min, each auditory condition played twice. Effect was assessed as changes in heart rate variability (HRV) (RMSSD and HF Power) recorded using the Actiheart 5, as described in Chapter 2. Activity levels were recorded using the Actiheart 5’s inbuilt tri-axial accelerometer, and reported as an arbitrary measure of activity, with larger numbers equating to increasing activity. Each dog’s behaviour was also monitored and recorded using CCTV cameras, as described in Chapter 5.

4.2.3 Statistics

All data were analysed relative to the control condition of Mozart K448. Null Hypothesis Significance Testing was conducted using a mixed effects linear model in lme4 (Bates et al., 2015) using R 4.1.2 with dog ID fitted as the random effect. Equivalence testing was conducted in Jamovi 2.2.5 using TOST paired samples t-tests, d=0.8 (The Jamovi Project, 2022). Data were processed in 5 min epochs except for the visualisation of the time series, for which the data were processed in 1 min epochs. Between dog differences were modelled using the ANOVA function of a linear model.

4.3 Results

All three forms of auditory enrichment were significantly equivalent in effect to the control condition of Mozart K448 in relation to RMSSD, HF Power and Activity (Table 4-1). Activity levels recorded by the Actiheart 5 were very low throughout the study (Figure 4-1c), with video analysis showing dogs mainly lying on the dog bed in the centre of the room or lying by the door.

Table 4-1 Equivalence table showing no significant group effect (t-test p>0.05) and a significant equivalence within the two one sided test (TOST) upper and lower bounds (p<0.05) when comparing the three auditory conditions with the control condition of K448

Condition	Test	RMSSD (p)	HF Power (p)	Activity (p)
Track	t-test	0.112	0.326	0.362
	TOST Upper	0.024	0.006	<0.001
	TOST Lower	<0.001	<0.001	0.005
White Noise	t-test	0.234	0.432	0.998
	TOST Upper	<0.001	0.004	<0.001
	TOST Lower	0.010	<0.001	<0.001
Voice Clips	t-test	0.458	0.801	0.227
	TOST Upper	0.003	<0.001	<0.001
	TOST Lower	<0.001	0.001	0.010

The results showed that there was no significant effect of the changing auditory conditions with regard to RMSSD ($F_{1,3} = 2.7$, p=0.054), HF Power ($F_{1,3} = 0.6$, p=0.616) or Activity ($F_{1,3} = 1.1$, p=0.379) (Figure 4-1a-c). Between dogs, there was significant difference in RMSSD ($F_{10,77} = 89.7$, p<0.001) and HF Power ($F_{10,77} = 90.3$, p<0.001), RMSSD data are shown in Figure 4-2. There was no significant difference in Activity between dogs ($F_{10,77} = 2.8$, p=0.005).

The time series analysis shows a significant variance in RMSSD ($F_{1,39} = 1.8$, p=0.004), independent of the auditory condition (Figure 4-3), but no significant variance in HF Power ($F_{1,39} = 0.9$, p=0.566) or Activity ($F_{1,39} = 1.1$, p=0.287), nor a change in arousal over the course of the 40 min recording.

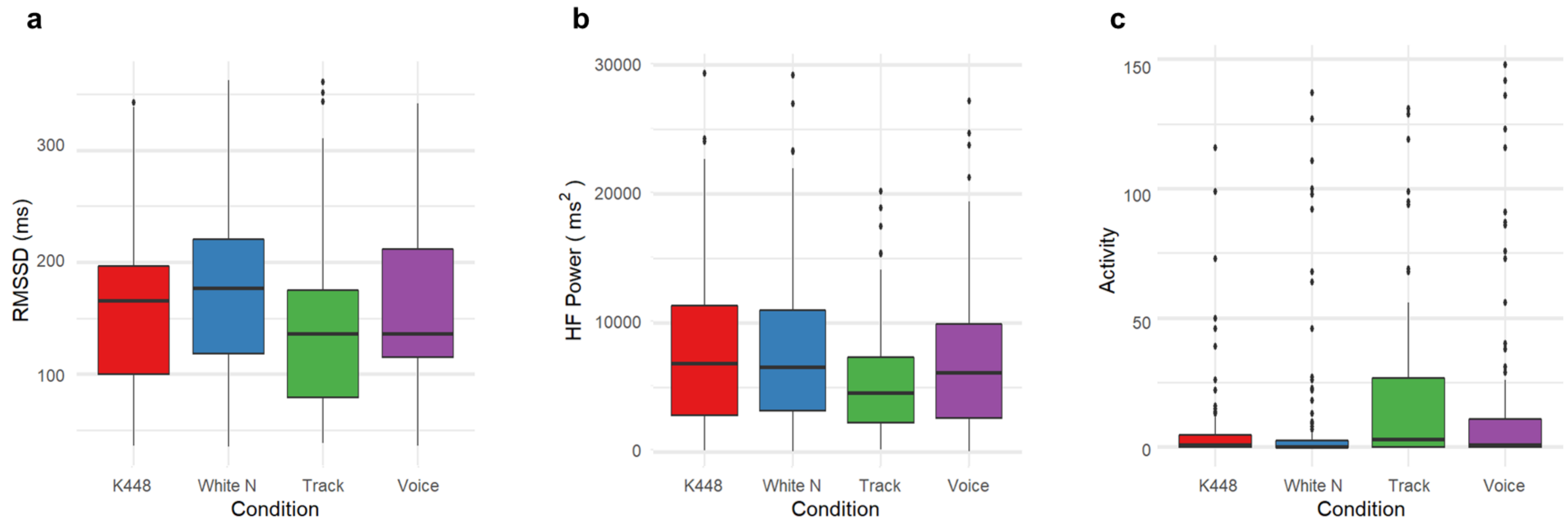


Figure 4-1 Boxplots showing that RMSSD (a) ($F_{1,3}$ = 2.7, p=0.054), HF Power (b) ($F_{1,3}$ = 0.6, p=0.616) and Activity (c) ($F_{1,3}$ = 1.1, p=0.379) did not significantly differ when dogs were exposed to each of the randomly ordered enrichment conditions (Mozart (K448), white noise (White N), the bespoke track (Track) and the bespoke track with clips of the owner’s voice (Voice))

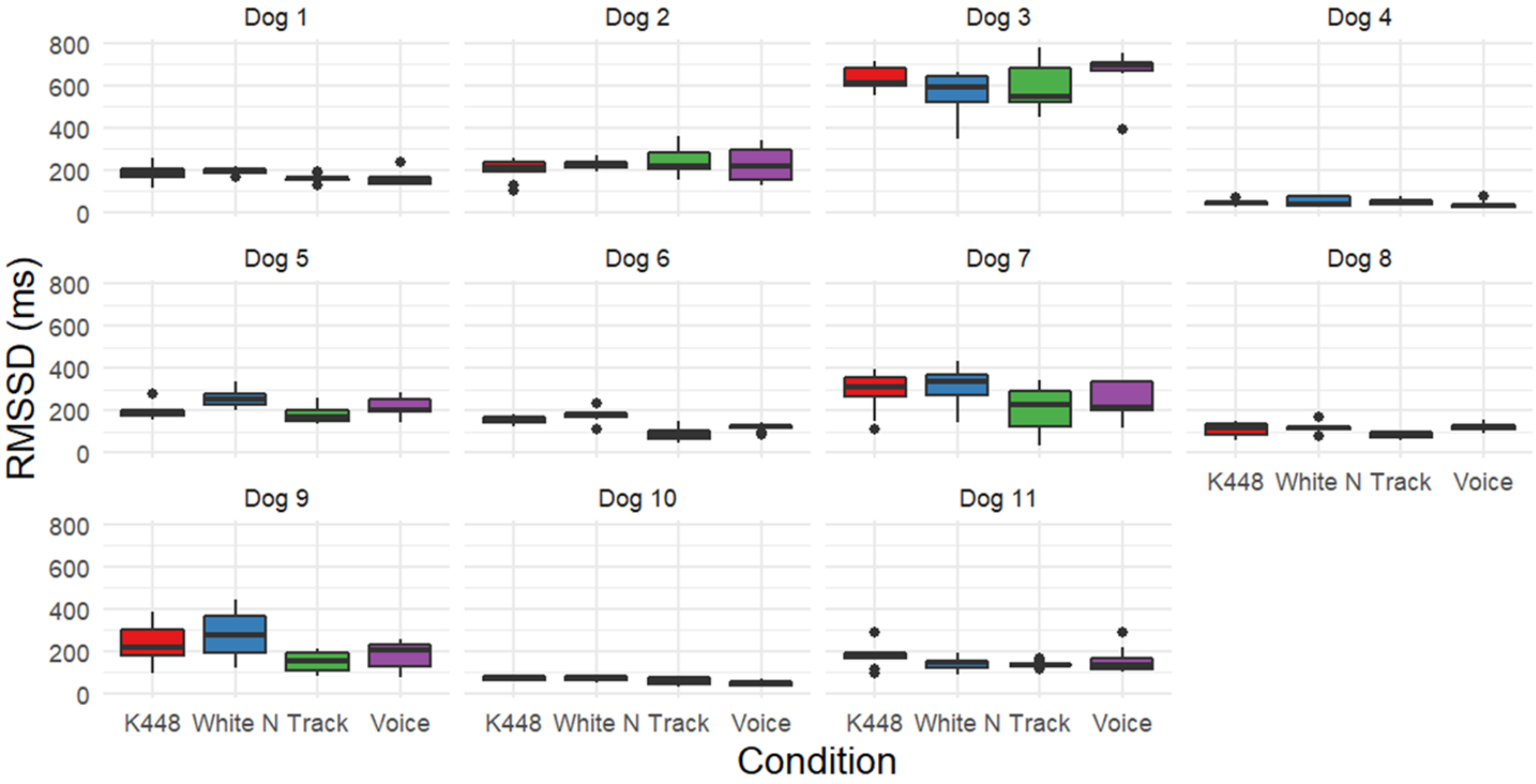


Figure 4-2 **Boxplots showing that RMSSD varied significantly between dogs ($F_{10,77} = 89.7$, $p<0.001$)**

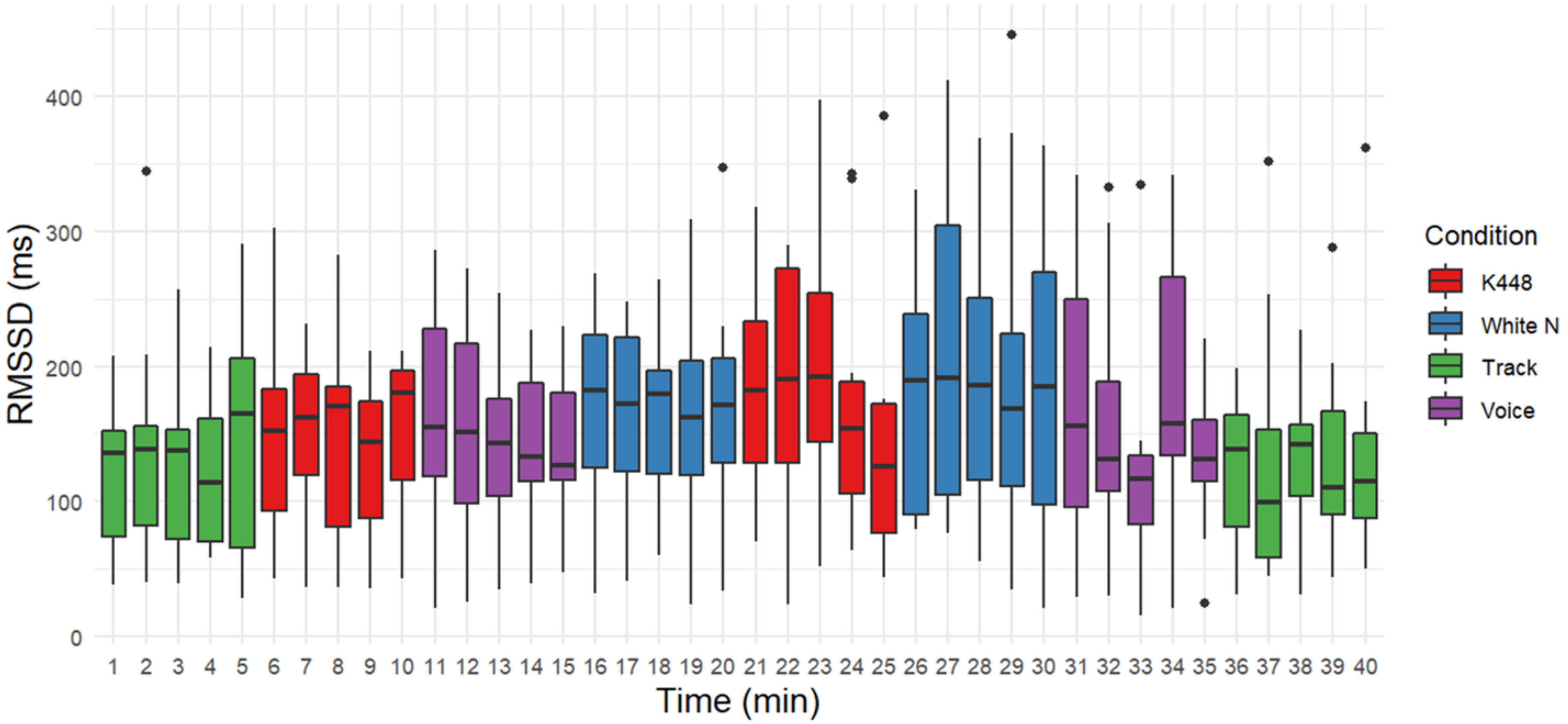


Figure 4-3 Boxplot showing that RMSSD varied significantly ($F_{1,39}$ = 1.8, p=0.004) over time, independent of the Condition

4.4 Discussion

This study sought to examine the effect of using the characteristics of the owner's voice to make bespoke music for individual dogs and examined the effect of adding clips of the owner's voice into that bespoke music. Dogs can recognise their owner's voice (Adachi et al., 2007) and it is plausible that for most dogs, a positively valenced association has been made with that voice. The hypothesis for this study was 2-tailed, as it was feasible that exposure to elements of the owner's voice could either increase or decrease arousal, as the association made with the owner's voice could either be one of calming reassurance or one of excitement. However, all three auditory conditions were significantly equivalent. It was possible that exposure to the owner's voice could elicit the negative emotion of frustration (McPeake et al., 2019) due to being able to hear the owner but not be able to see them. It was likely that different effects would be seen in individual dogs, with some becoming calmer, some more excited and some frustrated. However, this is not supported by the data in Figure 4-2 and the only observable behavioural changes throughout the study were a look towards the speaker on hearing a clip of the owner's voice, but only for 3 of the 11 dogs, and only on the first presentation of the owner's voice clip. This was unexpected, as habituation / dishabituation paradigms such as those used to test the differentiation of human voices by dogs (Root-Gutteridge et al., 2019) rely on animals performing a behavioural response to novel stimuli, and such responses are routinely reported in other studies. Figure 4-2 also demonstrates very clearly the between-subject variance in resting HRV. As discussed in Chapter 2, this is to be expected and shows the difficultly of relying on a single measure of HRV rather than the change in HRV to draw any conclusions about arousal state. To check for any order effect a time series analysis was conducted (Figure 4-3), and this suggests that the dogs where not becoming either more or less aroused due to the experimental set-up i.e., habituating to the experimental environment, nor was there any order effect.

The main limitation of this study is that, as a commercially contracted study, the methods used to produce the bespoke music are not clear. If an increased effect had been seen of the bespoke music, it would be hard to determine which specific aspect of the music was generating that effect. It is also notable that this study was conducted in a quiet environment with already calm dogs. There is a degree of selection bias in that the dogs that were volunteered for this study would cope with being left alone in an unfamiliar environment and would not suffer from separation anxiety. Running this study in an aversively noisy environment or with dogs prone to separation anxiety may have demonstrated a differential effect between the bespoke music and the control conditions.

4.5 Conclusion

Whilst dogs are conditioned to their owner’s voice, the results of this study do not support either the owner’s tone of voice, or tone combined with specific phrases, providing a greater effect on arousal compared to Mozart K488. These results could support the hypothesis that auditory enrichment operates through a very simple mechanism.

Chapter 5 Beat perception in a non-vocal learner: Can dogs discriminate between isochronous beats of different tempos?

5.1 Introduction

When considering how and why musical auditory enrichment affects arousal in dogs, it is important to consider not only what they are physically capable of hearing, but also what they can perceive. Perception is important because understanding species-specific capabilities informs which specific aspects of the auditory environment are useful to manipulate and could lead to more effective targeting of species-specific auditory enrichment. Humans can perceive a variety of aspects within a musical composition including tempo and pitch, but this might not be the case for other species, particularly non-vocal learners. Following the early work that demonstrated an arousal-reducing effect of playing music to dogs in a rescue centre (Wells et al., 2002), some of the subsequent work has looked at the question of whether musical genre can affect the ability of music to influence arousal (Bowman et al., 2017; Brayley & Montrose, 2016; Kogan et al., 2012). This anthropocentric view assumes that dogs tell different genres apart, and that all music of a particular genre is similar enough to have the same effect, and this has been specifically contested with regard to 'classical' music (see review by Lindig et al. (2020)). Although musical discrimination, which may be required to differentiate between genres, has been widely studied in non-human species, including fish (Chase, 2001), rats (Celma-Miralles & Toro, 2020), non-human primates (McDermott & Hauser, 2007) and birds (Dooling et al., 2002; Porter & Neuringer, 1984), there is no such work specifically related to dogs. While much of the work in non-human species has focused on elucidation of the origins of musicallity (Honing et al., 2015), a characteristic defined as "*the capacity that underlies the human ability to perceive, appreciate, and produce music*" (Hoeschele et al., 2015), many of the abilities that generate a perception of music are not uniquely human (Hoeschele et al., 2015). As an example, carp (*Cyprinus carpio)* are able to distinguish between classical and blues music (Chase, 2001) and different classical composers (Shinozuka et al., 2013). There is evidence, however, that musicality, at least in non-vocal learners, may, if present at all, be restricted to the most basic element; the discrimination of rhythmic regularity (Celma-Miralles & Toro, 2020).

Before considering if a dog perceives different auditory categories, it is important to first consider what a dog can hear. Brainstem-evoked response audiometry (BERA), an objective neurophysiological method for the evaluation of the hearing threshold, gives a clear indication of the limits of canine hearing in terms of volume and frequency (Ter Haar et al., 2002; Ter Haar et al., 2010). As detailed in Chapter 1, the work of Heffner (1983) demonstrated that the maximum frequency that dogs can hear is higher than that of humans (approximately 64 kHz versus 20 kHz), although frequencies of 64 kHz need to be at around 80 dB or above to be heard by dogs. When comparing the frequency / intensity curves for humans (Herman, 2016) and dogs (Heffner, 1983) the area occupied by speech and music overlaps with what dogs can hear at 30 dB and above, so it can be concluded that the full range of human speech and music is audible to dogs, even when it is very quiet (see Chapter 1 for a full assessment of the comparative hearing of humans and dogs). However, it is less clear what dogs perceive, particularly their ability to discriminate different sounds, as this is dependent on species-specific neural processing. Categorical perception is the ability to identify the boundaries of categories (e.g., fast tempo versus slow tempo; high pitch versus low pitch), and focuses on where inclusion in a particular category begins and ends (Damper & Harnad, 2000). It was demonstrated in Chapter 3 that the arousal state of dogs (in an ARRC) and horses (home stables) was not influenced by the tempo at which a piece of complex classical music was played. This differs from humans, where cardiac entrainment (with HR increasing and decreasing to match a changing musical tempo) routinely occurs (Karageorghis & Priest, 2008; Van Dyck et al., 2017). The lack of effect in dogs could indicate that, unlike humans, they cannot differentiate between different tempos (i.e., their categorical perception of tempo is poor).

The abilities to perceive music and language may be correlated, as the same neural networks may be used for both (ten Cate & Honing, 2022). It has been demonstrated that dogs can discriminate between different speakers and vowel sounds (Root-Gutteridge et al., 2019), and between English and Spanish (Mallikarjun et al., 2022). Dogs have also been shown to be able to discriminate between the distress vocalisations of human infants and puppies (Root-Gutteridge et al., 2021). While these studies indicate that dogs clearly have some auditory discriminatory abilities, with regard to languages, it is unclear what specific cues are being used by dogs to tell them apart (Mallikarjun et al., 2022) and, as discussed in Chapter 3, it has not been determined whether dogs express aspects of musical discrimination including perception of tempo and pitch, which may underpin any language perception they have. When training dogs, fast short auditory cues such as whistle blasts innately produce physical activity whereas slow long auditory cues reduce physical activity (McConnell, 1991) which indicates at least some temporal perception (length and/or timing of cues), although, as with language, it is impossible to identify what specific aspect of the cue the dogs are attending to. The evidence for language discrimination in dogs is therefore not evidence of categorical perception of either pitch or tempo.

Limited evidence of musical discrimination is provided by the observation that the response of dogs can be influenced by certain aspects of the music to which they are exposed. For example, it has been reported in dogs that some forms of music (specifically heavy metal) can result in increased expression of anxiety related behaviours (Kogan et al., 2012). Given that beat perception is a fundamental element of musicality (Wittmann & Pöppel, 1999), determination of whether dogs have this basic ability may allow greater understanding of what is perceived by dogs and maximise the effectiveness of auditory enrichment paradigms through selection of appropriate stimuli.

A difficulty with studies of musical discrimination/perception in non-verbal subjects is the inability to self-report what has been perceived. A technological solution is the use of fMRI, and this has been used been used extensively for discrimination studies in dogs (Chiu, 2019; Prichard et al., 2018; Szabó et al., 2020) but there is a considerable training burden associated with getting dogs to remain still in the scanner whilst awake and unrestrained (Berns & Cook, 2016). A more accessible approach is to assess behavioural markers such as entrainment (movement in time with a beat). Entrainment is readily seen in humans and can be used to assess if an animal can perceive beats. There is evidence for entrainment in parrots, sea lions and elephants (Hoeschele et al., 2015) but it has only ever been seen in vocal mimicking animals (i.e., animals that can use an auditory experience to modify their vocalisations (Janik & Slater, 1997)) (Schachner, 2010). Vocal mimicry does not necessarily imply the replication of human language in the way that is seen in parrots, as elephants have also been reported to imitate the noise of a passing truck (Poole et al., 2005). The drawback of using entrainment as a measure of beat perception, however, is that the inability to show entrainment does not necessarily mean the absence of beat perception, as entrainment also relies on connections between the auditory and motor cortices (Patel & Iversen, 2014), rather than just being a factor of the auditory cortex itself. Therefore, an inability to entrain could be seen in animals that have a conscious perception of the beats but lack the neural connections to coordinate movement with the auditory input. An alternate method to assess beat perception is provided by the assignment of different meanings to auditory stimuli of differing tempos and then observation of the animals' behaviour when presented with these stimuli. Such approaches are the go/no-go paradigms (Alegre et al., 2004) and cognitive bias testing (Harding et al., 2004), where an animal learns to perform an action when presented with one stimulus but inhibit that action (behavioural inhibition) when presented with a different stimulus. The no-go arm of a go/no-go paradigm can be punished or non-punished. A non-punished paradigm raises fewer ethical concerns; however, commission errors (responding to the no-go stimulus) may reflect generalisation where there is no punishment (Yechiam et al., 2006) rather than a lack of ability to discriminate. A less cognitively demanding approach which does not rely on behavioural inhibition and is unaffected by generalisation is a rewarded go/go paradigm, where two different stimuli require the subject to enact two different actions (Eagle et al., 2008).

The use of go/no go and go/go testing paradigms is possible with dogs, as the nature of the use of dogs as companion animals relies on the fact that they are capable of learning instrumental actions (i.e. touching a target to get a reward) (Yin et al., 2008). This ability should then be able to be harnessed to test whether dogs can discriminate between two beats by making that instrumental action, the conditioned response to a specific stimulus, through a process of Pavlovian instrumental transfer (PIT) (Corbit & Balleine, 2015). PIT arises from the combination of two learning processes, Pavlovian and instrumental conditioning, and specifically refers to the ability of a Pavlovian stimulus that predicts a reward to instigate or magnify an instrumental response for the same reward (Holmes et al., 2010). The use of a behavioural output should ensure that test subjects are required to make a clear and deliberate choice, when presented with a beat that is associated with a reward and should remove ambiguity from the results. PIT is cognitively demanding (Corbit & Balleine, 2015), and although it has been demonstrated in rats, (Corbit et al., 2007), to the author's knowledge, it has never been reported in dogs.

This study aimed to test the hypothesis that dogs can discriminate between metronome tempos of 50 bpm and 200 bpm. The objectives where:

1. To establish if dogs have the capacity to perform PIT, using a training phase of instrumental and classical conditioning.

2. To test, using a rewarded go/go 2-choice selection test, whether dogs can discriminate between metronome tempos of 50 bpm and 200 bpm.

5.2 Materials and Methods

5.2.1 Subjects

The study was conducted using 14 dogs of mixed breeding (4 male, 10 female, 4 ± 2.1 years, 19 ± 7.7 kg (mean ± SD)), owned by staff and students at the University of Glasgow recruited following an open call. Dogs were excluded from participation in the study if they had any history of hearing impairment or known neurological deficit. The study was approved by the University of Glasgow School of Veterinary Medicine Ethics and Welfare Committee, conducted in line with the International Society for Applied Ethology's ethical guidelines (Sherwin et al., 2017), and informed consent was obtained from each owner. Each dog was expected to go through stages of training and would be tested, individually, over the course of a single day. There was attrition at every stage of the study, with dogs failing to meet the criteria required for progression to the next step of the study and testing (detailed at each stage). Only one dog (male, 2 years old, 14 kg, Springer Spaniel) progressed to the final stage of perception testing.

5.2.2 Data Collection

Location The study was conducted at the University of Glasgow's Animal Behaviour Centre, in a 5.5 m x 3.5 m indoor testing arena where dogs could receive a remotely administered food reward from either side of the arena (Figure 5-1a). A Bluetooth speaker system (JBL Flip 3) was suspended above the centre of the arena (Figure 5-1b) at a height of 2.2 m. Metronome beats were played through this at a sound pressure level of 65 dB when measured 1 metre above floor height, i.e., at approximately the height of the ears of a large dog. A microphone was mounted on the wall to capture any vocalisation behaviour (Figure 5-1c). The animal's behaviour during the study was monitored remotely using 2 CCTV cameras (Digital Direct Security, Huntingdon, UK) with overlapping arcs to cover the entire test arena (Figure 5-2).

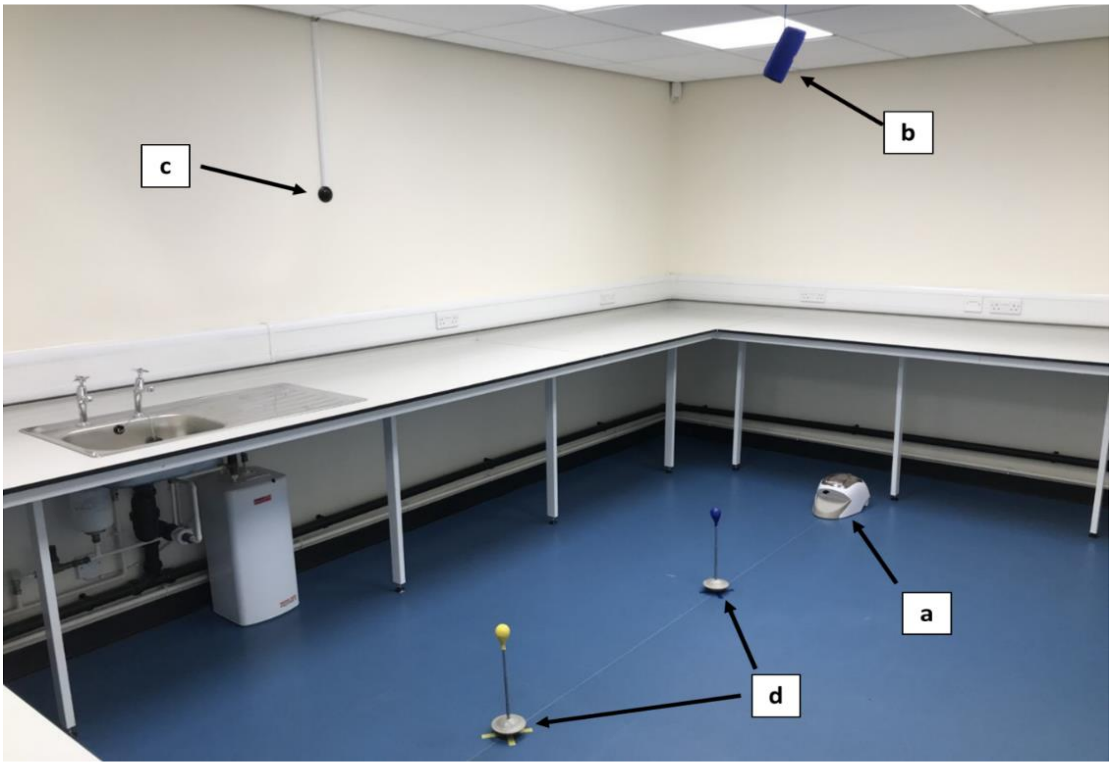


Figure 5-1 The layout of the test arena of the University of Glasgow's Animal Behaviour Centre showing the location of one of the two food dispensers (a), the Bluetooth speaker (b), the microphone (c), and the two training targets 1.3 m apart (d)

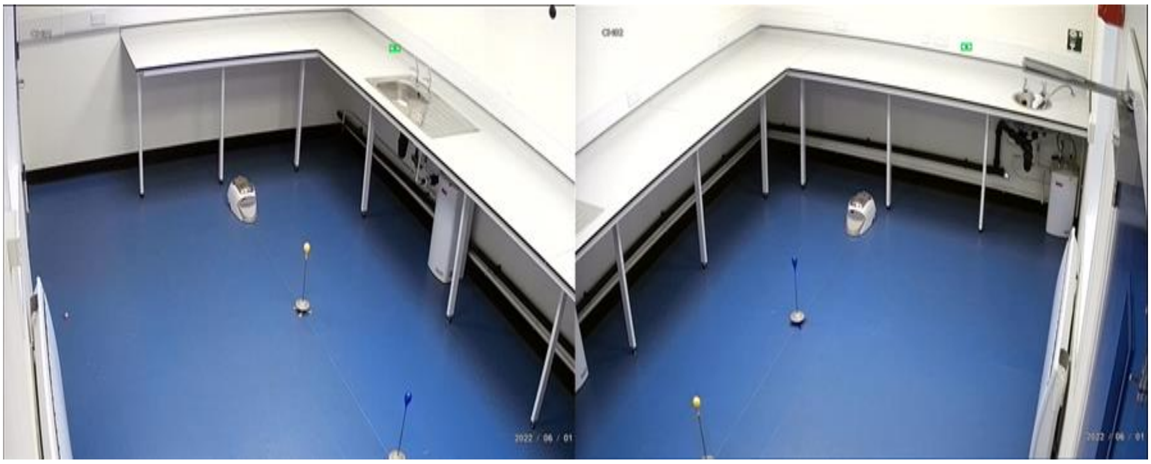


Figure 5-2 Screen shot taken from the CCTV system showing the full coverage of the test arena. Note both targets are visible on both cameras, so they can always be seen, regardless of the approach angle of the dog

Equipment The positive reinforcement throughout was a food reward that could be delivered via a pair of Treat & Train remote food dispensers (PetSafe, Knoxville, USA) (Figure 5-3). The food reward used was part of the dog's normal dietary intake and was supplied for each dog by their owner. For morning sessions, the dog had half its normal morning feed before arrival, and the food reward used was the second half of the morning feed. For afternoon sessions, the food reward was either the dog's normal mid-day feed or half of its evening feed, depending on its normal routine. Therefore, the dog's dietary intake of food, in both type and quantity, remained unchanged over a 24 hour period. The Treat & Train target (a foam ball on an extendable rod, herein known as 'the target') is shown at Figure 5-3. The standard colour of the foam target is red, but for the purpose of this study they were coloured yellow and blue, using electrical tape to match dogs' visual acuity (Byosiere et al., 2018). Two colours were used so that for the test phase, the targets were visually different, as well as being in different locations within the test arena.

Figure 5-3 The two PetSafe Treat & Train food dispensers with the yellow and blue training targets and the paired hand-held dispenser remote controls

Pavlovian Instrumental Transfer The PIT paradigm required two periods of conditioning and a test, but first required familiarisation of the test subjects with the test arena and the Treat and Train food dispenser. Familiarisation was achieved whilst the experimenter was in the test arena with the dog, and food was dispensed at random intervals from the Treat and Train dispenser. Within 10 min from the start of the familiarisation, most dogs associated the noise of the dispenser operation with food in the Treat and Train dispenser tray. Two dogs would not approach the dispenser after 30 min of familiarisation and did not progress to the next stage of the study. Immediately after the familiarisation, training commenced. PIT training took place in two phases followed by a test phase, and followed the hierarchical theory of transfer, where only in the presence of the stimulus (S) would the food-seeking response (R) give an effective outcome (O) (S+R=O) (Hogarth et al., 2014). To demonstrate PIT, dogs were trained with a single target (yellow) and single tempo (50 bpm).

> 1 **Instrumental conditioning** Each dog was introduced to the target and whilst the base end of the target was held by the experimenter, the dog was encouraged to touch the target. The instant the target was touched by the dog’s nose, a food reward was dispensed from the Treat and Train (O) using a remote control operated by the experimenter. This series of events was repeated until the dog actively sought the target when it was held in different positions within the test arena, at which point the target was fitted to the heavy base and the experimenter stepped away. Once the dog has independently touched the target (instrumental response (R)) and then approached the food dispenser 5 times in succession in the presence of the experimenter, the experimenter left the room and, whilst monitoring the dog via CCTV, operated the dispenser remotely for a further 5 consecutive target presses (R) / rewards (O). A variable reinforcement schedule was then introduced whereby up to 4 target presses (R) remained unrewarded, before the next target press (R) did result in a reward (O), thus requiring the dogs to demonstrate persistence in the face of violated expectations. Training was conducted for 2 hours in eight 10 min blocks, with a 5 min break between each block. One dog failed to successfully complete the instrumental conditioning and therefore did not progress to the rest of the study.

2 **Pavlovian conditioning** During this phase of training the target was removed from the test arena and the dog was trained to associate a 50 bpm metronome (S) with a food reward appearing from the dispenser (O). This phase lasted approximately 30 min during which time the dog was free to explore the test arena. During the Pavlovian conditioning, 2 min of 50 bpm were played 3 times, with 2 min of silence at the start, between each presentation of 50 bpm and at the end. During each 2 min of 50 bpm, a food reward was dispensed every 5-10 sec, regardless of the location or actions of the dog. After a 10 min break, the 14 min protocol was repeated, and this was done a total of 5 times (in total 2 hours). Four dogs failed to engage with the food dispensers during the Pavlovian conditioning phase of training and so did not progress within the study.

3 **Pavlovian to Instrumental Transfer (PIT) Test** The remaining 7 dogs entered the PIT test phase. As with the Pavlovian conditioning phase, 2 min of silence and 2 min of 50 bpm were played, each repeated twice (total 8 min). For the test phase, the target was reintroduced. The paradigm was now that touching the target during both silence and when the 50 bpm were played was unrewarded, but the number of touches under each condition were counted and compared (Corbit & Balleine, 2015). Success for each dog was defined as numerically more target touches during the 50 bpm condition than during silence.

Perception Testing Only one dog was able to demonstrate PIT and progressed to perception testing. Perception testing was composed of a period of consolidation training followed by the test itself. The consolidation training started with a brief revision of the instrumental conditioning (this time using the blue target and a Treat and Train dispenser at the other end of the room), both targets and dispensers were left in the room. Without the experimenter present, the dog's instrumental actions were able to be rewarded (left target, left dispenser; right target, right dispenser). Again, a variable reinforcement schedule was used, with the dog progressing to the next stage once it had switched targets after showing brief perseverance following an unrewarded action, to demonstrate an understanding that both targets were equally likely to be rewarding. The next stage of the consolidation training was a repeat of Pavlovian conditioning with the addition of the second dispenser and use of a 200 bpm metronome auditory stimulus. The order of auditory stimulation was silence - 50 bpm - silence - 200 bpm (2 min of each), each tempo was played twice, and the test finished with 2 min of silence (total test time,18 min). The relevant dispenser to the tempo being played dispensed a food reward every 5-10 sec, regardless of the location or actions of the dog. After a 12 min break, the sequence was repeated for 5 repetitions (2.5 hours).

After completion of the consolidation training, the dog entered the test phase, which was a modification of the training phase, with a single dispenser placed along the side wall at a point equidistant between the two targets. This was to prevent the dog remaining at the end of the room that had just been rewarded. The targets were moved further towards either end of the room and positioned where the dispensers had been during the previous phase (Figure 5-4).

The two auditory stimuli were played in a pseudo-randomised order (each stimuli repeated no more than 4 times). Each test ended when the dog made an initial selection (left or right target) or after 30 sec if no selection was made. A correct selection resulted in a food reward from the dispenser. An incorrect response or no response was not rewarded, but was also not punished. A total of 125 presentations were made in 5 sessions of 25 (over approximately 10 min), with a brief break between each session. The total test took approximately 60 min. In a modification of the go/no-go protocol used by Meule (2017), results were recorded as reaction time (latency to make a response), omission errors (failure to respond) and commission errors (incorrect selection).

The design that had been planned for perception testing was that dogs would have been pseudo-randomised into two cohorts to give an even distribution of age, sex and breed; half the sample having 50 bpm indicate left and 200 bpm indicate right, with this reversed for the other half, but this was not required as only one dog progressed to this stage of the study.

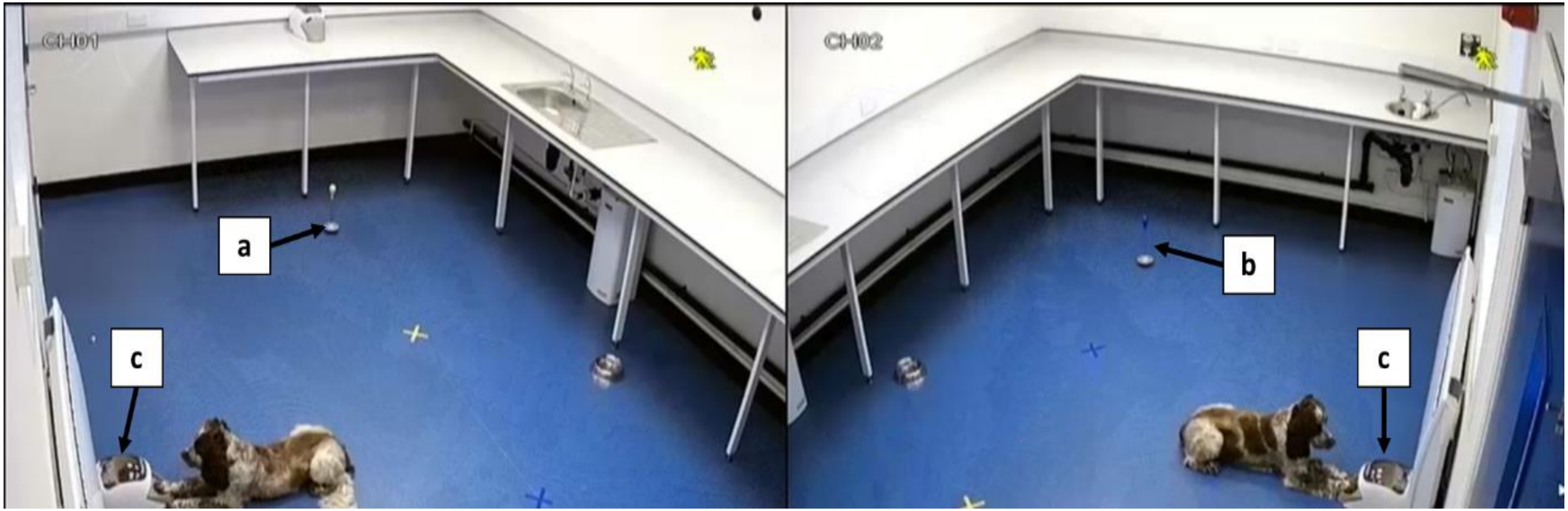


Figure 5-4 The modified room layout for the perception testing showing the position of the yellow target (a), the blue target (b) and the single food dispenser (c) where the dog waited between each test

5.2.3 Statistics

A Shapiro-wilk test found that the latency data did not conform to normality. A generalized linear model (GLM) in nlme (Pinheiro & Bates, 2023) fitted with a gamma distribution was used to examine the variance between reaction time across the 5 sessions of the perception test. Plotting the residuals confirmed that the model assumptions were met. The number of correct responses were calculated as a percentage of correct responses per trial, with success being considered as giving a correct response better than by change (>50%). All modeling and descriptive statistics were conducted using R 4.0.2 (Core Team, 2021). As n=1 for the perception test, a mixed effects model was not required.

5.3 Results

Pavlovian to instrumental transfer Table 5-1 shows the stages of conditioning and testing successfully completed by each dog. The notes give an indication as to why each dog did not progress and was removed from the study.

Table 5-1 Successful completion of each stage of conditioning and testing by individual dog. A ✓ indicates that a stage was successfully completed, a ✗ indicates that a stage was attempted but not completed (dog withdrawn), and n/a indicates a stage that was not attempted

Dog	Familiarisation	Instrumental Conditioning	Pavlovian Conditioning	PIT Testing	Perception Testing	Notes
Jura	✗	n/a	n/a	n/a	n/a	Scared of dispenser
Sally	✗	n/a	n/a	n/a	n/a	Scared of dispenser
Oscar	✓	✗	n/a	n/a	n/a	Used target as a toy
Ari	✓	✓	✗	n/a	n/a	Ignored food
Kiwi	✓	✓	✗	n/a	n/a	Ignored food
Mavis	✓	✓	✗	n/a	n/a	Ignored food
River	✓	✓	✗	n/a	n/a	Ignored food
Arwen	✓	✓	✓	✓	✗	Failed PIT Test
Boo	✓	✓	✓	✓	✗	Failed PIT Test
Brodie	✓	✓	✓	✓	✗	Failed PIT Test
Leyla	✓	✓	✓	✓	✗	Failed PIT Test
Pippa	✓	✓	✓	✓	✗	Failed PIT Test
Rainbow	✓	✓	✓	✓	✗	Failed PIT Test
Iroh	✓	✓	✓	✓	✓	

Table 5-2 shows the performance of the 7 dogs that undertook the PIT test. Only one dog, Iroh, passed the PIT test.

Table 5-2 Individual dog performance on the PIT test, showing total number of touches under each condition and a summary Pass/Fail

Dog	Target touches		Pass / Fail
	Silence	50 bpm	
Iroh	7	15	Pass
Arwen	7	1	Fail
Boo	2	0	Fail
Brodie	3	0	Fail
Leyla	3	0	Fail
Pippa	2	1	Fail
Rainbow	4	0	Fail

Perception Testing Table 5-3 summarises the correctly selected target and average reaction time for each session of perception testing, for the one dog that completed this phase. There were no omission errors on any of the 5 sessions. The overall correct selection rate was 47%, with a mean reaction time of 2.4 sec.

Table 5-3 Percentage of correct responses, mean reaction time (s) and SD of reaction time (s) for each of 5 sessions, n=1

Session	1	2	3	4	5
Correct (%)	68	52	40	36	40
Reaction (s)	2.6	2.3	2.3	2.6	2.1
Reaction (SD)	0.7	0.5	0.5	1.4	0.4

The percentage of correct responses was less than chance (>50%) in sessions 3,4 and 5 and significantly lower in session 4 (p= 0.0026) when compared to session 1. Reaction time from the presentation of the stimuli to the dog selecting a target was not significantly different across all 5 sessions ($F_{4,120}$ =1.68, p=0.16).

5.4 Discussion

This study set out to test the categorical perception of tempo in domestic dogs by means of a rewarded 2-choice go/go paradigm. The test paradigm was dependent on the dogs being able to demonstrate PIT, something that had not previously been reported in dogs. Of the 14 dogs engaged in the study, under the conditions of this experiment, only seven dogs met the criteria to undergo PIT testing, and of those, only one dog passed that stage and progressed to the beat perception testing. This dog was only able to discriminate between metronome beats at 50 and 200 bpm with an average 47% success, but only limited inference can be drawn from these findings.

Unfortunately, only one of the dogs recruited to this study was able to progress through the conditioning and PIT testing to the perception test. This may indicate that the test paradigm chosen was too cognitively demanding for dogs, within the timescale of this study. Previous auditory discrimination studies in non-humans have used less cognitively demanding habituation-dishabituation paradigms. In these simpler studies, a novel stimulus which elicits a behavioural response (head turn, ear flick etc.) is applied until the animal habituates to that sound and stops responding, at which point a different auditory stimulus is introduced with the assumption that if the animal now offers a behavioural response, it must be able to discriminate between the two sounds. Such an experimental paradigm has been used with cats to demonstrate their ability to discriminate human vocalisations (nonsense words and their own name) (Saito et al., 2019). Root-Gutteridge et al. (2019) also used a similar approach to demonstrate the ability of dogs to discriminate human vowel sounds. However, it was not a suitable approach for the current study as the habituation-dishabituation paradigms approach is dependent on the animal offering a behavioural response to a novel auditory stimulus as opposed to differentiation between two auditory stimuli. Also, in both the studies conducted by Saito et al. (2019) and Root-Gutteridge et al. (2019) they relied on novel human vocalisations (nonsense words) attracting the attention of the animal. The success of animals to differentiate between sounds in these studies may be a manifestation of the fact that for domesticated animals, all human vocalisations will potentially have acquired some implicit meaning. This means that even if the words are novel, the animals may react to the tone of voice used, but in our study where the only change was in the number of beats provided, dogs may be unable to differentiate between the two metronome tempos. This possibility gains a little support from the results of the study which investigated auditory enrichment including music based on the frequency profile of their owners' voices and clips of the owners' voices (Chapter 4). In that study, a behavioural change was observed whereby the dog looked at the speaker on hearing a clip of the owner's voice, suggesting that, as seen by Saito et al. (2019) and Root-Gutteridge et al. (2019), dogs were able to differentiate human vocalisations, however, this response was only seen for 3 of the 11 dogs tested, and the response only occurred on the first presentation of the owner's voice clip. Having not seen any evidence of habituation-dishabituation using music or voice clips during the studies in the previous chapters and Appendix B, rather than rely on a behavioural response to stimuli that has no meaning or benefit for the animal, a more complex rewarded go/go two-choice paradigm was used in which the subject had to select one of two actions (touching the left or right foam target) depending on the auditory stimulus presented.

In this study only 7 dogs successfully completed the two PIT conditioning phases and made it through to the PIT test and of those, only 1 successfully completed it. This lack of success in the dogs of the study population is surprising as PIT in rats using a similar training and assessment regime to that of the current study is well established (Cartoni et al., 2016; Laurent & Balleine, 2015). PIT is a cognitively intensive concept (Corbit & Balleine, 2015). The success of this one dog may indicate that it is a population outlier or a 'gifted dog' with regard to its cognitive ability, a phenomenon that has previously been reported (Fugazza et al., 2021). In that study Fugazza et al. (2021) noted that while some dogs were capable of correct identification of up to 59 named toys with a success rate of up to 91.5%, only 1 out of 36 naïve dogs were able to learn the names of 2 new toys after 3 months of intensive training.

PIT requires that the test subject shows a high degree of perseverance, as it needs to keep pressing the target when it is no longer rewarded i.e., an extinction paradigm. High levels of perseverance when presented with an extinction paradigm has been reported in horses that display stereotypical behaviour (Hemmings et al., 2007), a condition which is in part mediated by basal ganglia dysregulation (McBride & Parker, 2015). Hemmings et al. (2007) suggested that both the stereotypical behaviour and the increased perseverance were due to a failure to monitor the striatal action-outcome loop as described by Thorn et al. (2010). It is therefore possible that for the dogs in the current study that disengaged with the target quickly after that action became unrewarded, this is a neurotypical response. That said, the owner of the one dog that passed the PIT test, and thus demonstrated increased perseverance, did not report any stereotypical behaviours, so their PIT success may be independent of any basal ganglia dysregulation. Perseverance in domesticated dogs presented with an unsolvable task has been reported to be poor compared to wolves (Rao et al., 2018), possibly because they have become very dependent on their human caregivers. Udell (2015) also showed domestic dogs had poor perseverance on a solvable task, having only 5% success compared to 80% on the same task for wolves. The same study showed that the domestic dogs spent a significantly longer amount of time looking at the nearest human, interpreted as seeking help. This apparent conditioned inhibition of independent problem-solving behaviour may explain why, in the current study, once the instrumental action was unrewarded and no human was present, 6 of the 7 dogs gave up on the task, leading to more target presses during the initial ‘silent’ condition than in the subsequent ‘tempo’ condition, as the silent condition was always presented first.

An alternate explanation for the lack of success in performing PIT in the current study is the need for more/longer training. In this study the training all took place over a single day. While this training regime was similar in duration to the 30 min instrumental / 75 min Pavlovian paradigm used successfully by Corbit et al. (2007) to teach PIT to rats, it was not successful in the dogs in the current study. This is also in contrast with most domestic dog training programmes wherein it is unusual to attempt to teach dogs something once. Indeed, it has been reported that learning acquisition is greater in dogs following a single training session 1 or 2 times per week compared to dogs trained daily or several times per day (Demant et al., 2011). Furthermore, even in rats, complex maze training often uses more protracted training regimes, for example, Brandner et al. (2000) used 36 trials over 5 days. Therefore, while PIT may be learnt by rats in one day (Corbit et al., 2007) an extended training schedule might be more successful for both rats and dogs and should be considered if PIT is to be further investigated in dogs.

Relative to the poor success rate of the training and testing in the current study, it is worth considering why only 7 of the original 14 dogs made it as far as PIT testing. There are a variety of possible factors that may have played a role, including past experience, fear and motivation. Two dogs (both young female Collies) would not approach the food dispenser, one of which would not even enter the room it was in, having heard it dispense food once. One of these dogs lived with a cat, and according to the owner, the cat was fed from a similar device as the one being used in the test, and the dog had learnt from bitter experience not to go near it – a variable not accounted for in the exclusion criteria. The third dog to be excluded (male, 1.5 years, Springer Spaniel) failed the instrumental conditioning phase because he was more motivated by the target than the food and would pull the target from its base and run around the room with it. No amount of food reward could distract this dog from his new toy, and this aspect of motivation was not considered in the initial inclusion criteria. Four dogs (female, mixed breeds and under 5 years) did not progress within the study at the Pavlovian conditioning stage as they ignored the food coming from the dispenser. This was unexpected, as all 4 dogs were sufficiently food motivated to succeed on the instrumental training. A possible explanation for this is contra-freeloading, the phenomenon that animals will work for a food reward in preference to freely available food (Inglis et al., 1997), that has most recently been demonstrated in goats (Zobel & Nawroth, 2020). The initial recruitment for the current study asked for “intelligent, trainable dogs” and it is a plausible hypothesis that contra-freeloading is evident in dogs, particularly those considered to be of above average intelligence by their owners, as were the ones in this study. Contra-freeloading has never been tested for in domestic dogs, but it has been reported in wolves (da Silva Vasconcellos et al., 2012) and thus may be an inherent aspect of canine behaviour. Contra-freeloading is worth considering for future experimental designs, and recruitment of average-intellect, food-motivated dogs for this study may have delivered very different results.

A further factor that may have influenced the success of PIT training in the current study is intra-species variability in dogs' cognitive ability (Fugazza et al., 2021). Intra-species cognitive variability has been reviewed in farm animal cognitive research, with the use of multilevel models recommended to account for individual variation (Bushby et al., 2018). Selection bias of subjects (i.e., the specific recruitment of intelligent, trainable animals) is therefore very important if results are to be generalised to the population. This is a perpetual issue using domestic dogs as a study species with the large variation in both background and character traits that are seen to a lesser extent in laboratory models where experimental groups are far more homogeneous, but it is something rarely controlled for in study design.

The perception test was repeated 5 times with a short break between each, and as correct responses were rewarded, each test also constituted continued training. It was therefore expected that performance may improve over the course of repeated testing. This was not the case for the one subject tested, and in contrast, there was a reduction in performance over the course of the test. Latency to select did not change over the course of the 5 sessions, which would suggest that the dog was not making quicker decisions, or that he was getting tired or bored which would have resulted in longer latencies. The relative consistency in latency times and overall performance suggests that success in the first session was purely by chance. It did appear that the strategy used by this dog changed in the final 3 sessions, with long periods of alternating from left to right targets, regardless of the stimulus and regardless of whether the previous response was correct. It is hard to explain this change in strategy, particularly as it was far less rewarding, but it possibly does indicate a strong choice history bias in dogs (Fründ et al., 2014; Urai et al., 2019) whereby a current selection is heavily biased by the previous choices made.

The key limitation of this study is the n=1, meaning that only very limited inference can be drawn from the results. As discussed above, the experimental paradigm was either too cognitively demanding or was not trained for long enough. Domestic pet dogs may also not be the best subjects for complex training and testing tasks, as there is considerable variance in background and experience which is hard to control for.

5.5 Conclusion

As a non-vocal learning species, it could be hypothesised that dogs lack any concept of complex musicality and would have limited, if any, beat perception. While this hypothesis is supported by the findings in Chapters 3 and 4, unfortunately only limited inference can be drawn from the results of the study presented in the current chapter which aimed to test categorical perception in relation to beat/tempo, as the training may have been cognitively too demanding and/or not long enough. This resulted in only one dog undertaking the beat perception test. If dogs do lack the ability to perceive rhythmic regularity within auditory stimuli, it does not detract from the previously demonstrated ability of music to affect physiology and behaviour, but rather indicates that effects are mediated through an alternate mechanism, explored in Chapter 6.

Chapter 6 Conditioning to strengthen the effects of auditory enrichment for dogs in rescue centres

6.1 Introduction

Passive listening to music and other auditory enrichments can reduce anxiety in humans (Graff et al., 2019) as well as behaviours and other markers of stress and anxiety in a range of non-human species (Barcellos et al., 2018; Bowman et al., 2015; Crouch et al., 2019; Hampton et al., 2019; Kühlmann et al., 2018; Williams et al., 2017; Zapata Cardona et al., 2022). Whilst the benefits to welfare of auditory enrichment with music have been reported in a variety of ways including anxiety reduction, increased relaxation and reduced stress, many of these are hard to accurately define and quantify. Furthermore, the effect sizes (Cohen's d) with regard to reductions in stress/anxiety in many animal studies are small (d=0.28, Zapata Cardona et al. (2022); d=0.3, Hartman and Greening (2019); d=0.5, Bowman et al. (2015)). One of the challenges in improving these effect sizes is that the mechanisms by which auditory enrichments have positive effects (primarily reducing physiological arousal) are still unclear.

In human studies, the anxiolytic effects of passively listening to music are larger when participants select their own music (Binns-Turner et al., 2008; Gillen et al., 2008; Krout, 2007; Wulff et al., 2017), with effect sizes of 0.77 (medium) where participants choose their own music (Binns-Turner et al., 2008), but only 0.21 (small) when the music was chosen by the researcher (Graff et al., 2019). It has been proposed that such an increase in effect size is related to an expectation of benefit (Benedetti et al., 2005), but it may also be related to positive association, possibly made through Pavlovian conditioning (Gorn, 1982).

The possibility that the effects of auditory enrichment in dogs may also be strengthened by conditioning is supported by the observation that dogs diagnosed with separation anxiety show a greater reduction in anxiety related behaviours (barking and door scratching) when played music that they associate with their owners' presence, compared with novel classical music (Bernardini and Niccolini (2015), although this has not been reported in other species.

This study tested the hypothesis that conditioning dogs to a specific piece of music, during a time of day when their physiological arousal was already reduced, would increase the ability of that music to reduce physiological arousal during a more stressful time of day. The first objective was to test this hypothesis quantitatively in a controlled environment to determine if conditioning could be used as a means to reduce arousal at measured by HRV within a rescue centre environment. The second objective was to qualitatively test whether a conditioned response to a specific piece of music, developed in Scottish SPCA Animal Rescue and Rehoming Centres (ARRC), could be used to reduce unwanted behaviours of dogs during their first 14 days following rehoming.

6.2 Materials and Methods

6.2.1 Subjects

Experimental Study. The conditioning study was conducted at three Scottish SPCA Animal Rescue and Rehoming Centres (ARRC), using a total of 19 dogs of mixed breeding. All Scottish SPCA accommodation comprises an indoor kennel and outdoor run, and the dogs are provided with a plastic bed, blanket, water bowls (inside and outside) and a variety of toys including rope chews, Kongs® and balls. There are 20 kennels in each block, and there is no visual contact with other dogs. The only selection criterion was no history of aggression. The average age of the dogs was 7.4 ± SD 3.5 years (range 2-14 years) and the mean weight was 21.3 ± SD 9.6 kg (range 6-35 kg). There were 11 male dogs of which eight were entire and three were neutered and eight female dogs of which four were entire and four were neutered. The average length of stay in the kennels at time of testing was 97 ± SD 81 days (range 17 - 213 days). A 20th dog was recruited for the study but was excluded for repeatedly removing the HRV equipment.

Qualitative Validation. To assess the utility of the conditioned response to help dogs settle after placement (rehoming or fostering), new dog owners were asked to fill in a questionnaire. The survey was open to new owners and fosterers of dogs that left the care of the Scottish SPCA between 1 April and 6 November 2022, a total of approximately 100 dogs of mixed breeds and sex. The survey asked owners to reflect on the first 14 days following rehoming.

6.2.2 Data Collection

Experimental Study. A nine-day protocol was run, involving a baseline measurement, a first test, a conditioning phase and a second test as detailed below and in Figure 6-1. The protocol was run for 5 dogs at a time.

Day 0 (Monday) – Baseline. IBI data were recorded (12:00-12:30), with no music being played.

Day 1 (Tuesday) – First test. IBI data were recorded (12:00-12:30) whilst dogs were exposed to a novel piece of music (Mozart K448) played on a 30 min loop at 65 dB.

Days 1-7 (Tuesday – Monday) - Conditioning Phase. Mozart K448 was played on loop for 30 min (16:30 – 17:00), coinciding with the quietest time of day when the dogs were normally relaxed. No IBI data were collected during the conditioning phase.

Day 8 (Tuesday) – Second test. IBI data were again collected (12:00-12:30), whilst the Mozart K448 was played in the kennels.

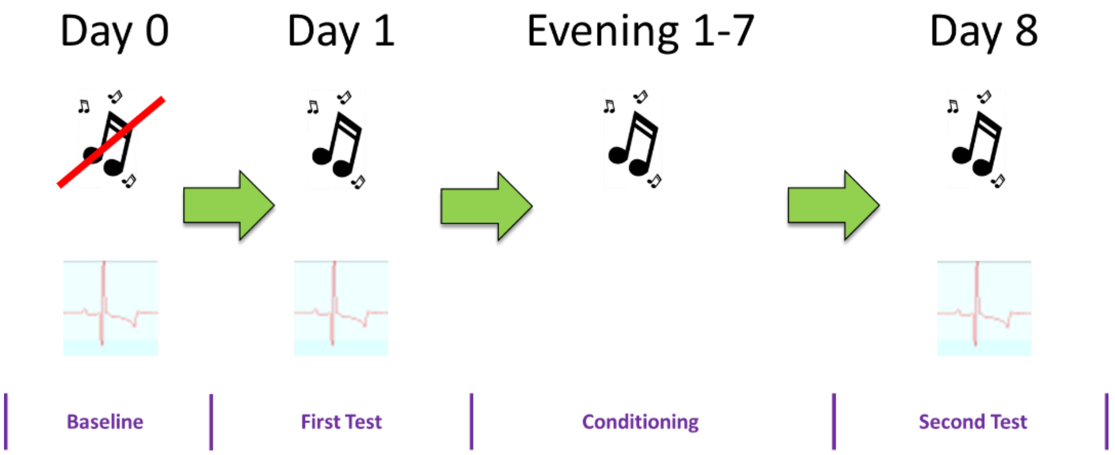


Figure 6-1 Infographic of 9-day protocol showing the baseline HRV recording on Day 0, the first test on Day 1, the conditioning phase of Evenings 1-7 and the second test on Day 8.

IBI data were collected using Actiheart 5 ECGs, using the methods described in Chapter 2. To fit the devices, each dog was removed in turn from its kennel and brought to a side room by a member of staff, where the device was fitted by the experimenter, a procedure that took approximately 10 minutes. The last dog was returned to their kennel at least 30 minutes before the start of recording. All five devices were set to record at the same time. After each data recording session, the devices were removed from the dogs in their kennels. Only one of the 20 dogs recruited managed to remove the device themselves and was therefore excluded from the study. Beat correction was conducted manually on the data from each dog in the Actiheart 5 software, and the corrected IBI data exported to a .csv file for processing in Kubios (Standard) 3.5.0 software. No automated artefact correction was applied. Each 30 min recording was analysed in 1 min epochs, giving a time series of 30 readings per dog per recording. The results were exported as a .csv file for statistical analysis in R Studio (running R version 4.1.2).

Qualitative Validation. The nine Scottish SPCA centres were divided into an experimental group (Aberdeen, Dumbarton, Dundee, Glasgow and Lanarkshire) and a control group (Ayrshire, Caithness, Edinburgh and Inverness). The allocation was not randomised to ensure an even mix of small and large centres in each group. Centres in the experimental group were asked to play "Tomorrow starts today" by Dave Thomas Junior (track 10 on the Scottish SPCA's "Paws. Play. Relax." album) in the kennel block on 30 min loop from 16:30 to 17:00 every day. This album was chosen as all the centres had access to it and it was already being recommended to new owners. The final track on the album was selected as firstly, the album was specifically structured for the final track to be the most relaxing, and secondly, it was easier to remember which track was to be played. A key advantage of using this track as opposed to Mozart K448 used in previous studies was that there is only one version of it available. Owners searching online for K448 could find various versions, all subtly different in tempo and timbre. Centre staff in the experimental group were asked to brief new owners that they should play "Tomorrow starts today" at any time their dog appeared to be distressed or was struggling to settle into their new home. All new owners in both the experimental and control groups were given a short information leaflet about the study as part of the rehoming pack, as well as a business card with QR codes linking to a 2 min explanatory video to enhance engagement, and an on-line survey. All new owners were reminded about the survey during the standard 14 day post rehoming follow-up by Scottish SPCA centre staff. The on-line survey was made up of 39 questions based on a modified C-BARQ (Hsu & Serpell, 2003) and included general questions about the use of music within the home. This was hosted on the University of Glasgow's on-line survey platform (Jisc) and the full question set is in Appendix B. In order to limit selection bias to those with internet access (Bowling, 2005), paper copies of the survey were also available at each Scottish SPCA ARRC.

6.2.3 Statistics

Experimental study. Data were analysed using a GLMM (lme4 - (Bates et al., 2015)) and the effsize package (Torchiano, 2020) in R 4.1.2 to determine the size and statistical significance of any difference in effect resulting from the conditioning between Days 0 and 1, Days 1 and 8, and Days 0 and 8. Dog ID was modelled as the random effect. To determine if age, sex, weight, and gonadal status influenced the effectiveness of the music to reduce physiological arousal, these variables were included in the model as fixed effects.

Qualitative Validation. Insufficient questionnaires were completed to perform any statistical analysis.

6.3 Results

Experimental Study. There were no significant effects of age, sex, weight or gonadal status on the effectiveness of auditory enrichment on Day 1 compared to Day 0 or on the effects of conditioning (Day 8 versus Day 1).

Novel music led to significantly higher RMSSD compared to when no music was played (Day 1: 95 ± 12.8 ms versus Day 0: 75 ± 9.3 ms, t=2.63, p=0.0196) (Figure 6-2). Conditioning led to significantly higher RMSSD on Day 8 compared to day 1 (Day 8:118 ± 14.5 ms versus Day 1: 95 ± 12.8 ms, t=2.45, p=0.0272) (Figure 6-2a) and HF Power (Day 8: 5919 ± 1478 ms^2 versus Day 1: 4813 ± 1242 ms^2, t=2.96, p=0.0097) (Figure 6-2b). The effect size (Cohen's d) of initial presentation of the music was 0.5 (medium) for both RMSSD and HF Power. However, on Day 8, the effect size increased to 'large' for both RMSSD (1.1) and HF Power (0.8) (Figure 6-3).

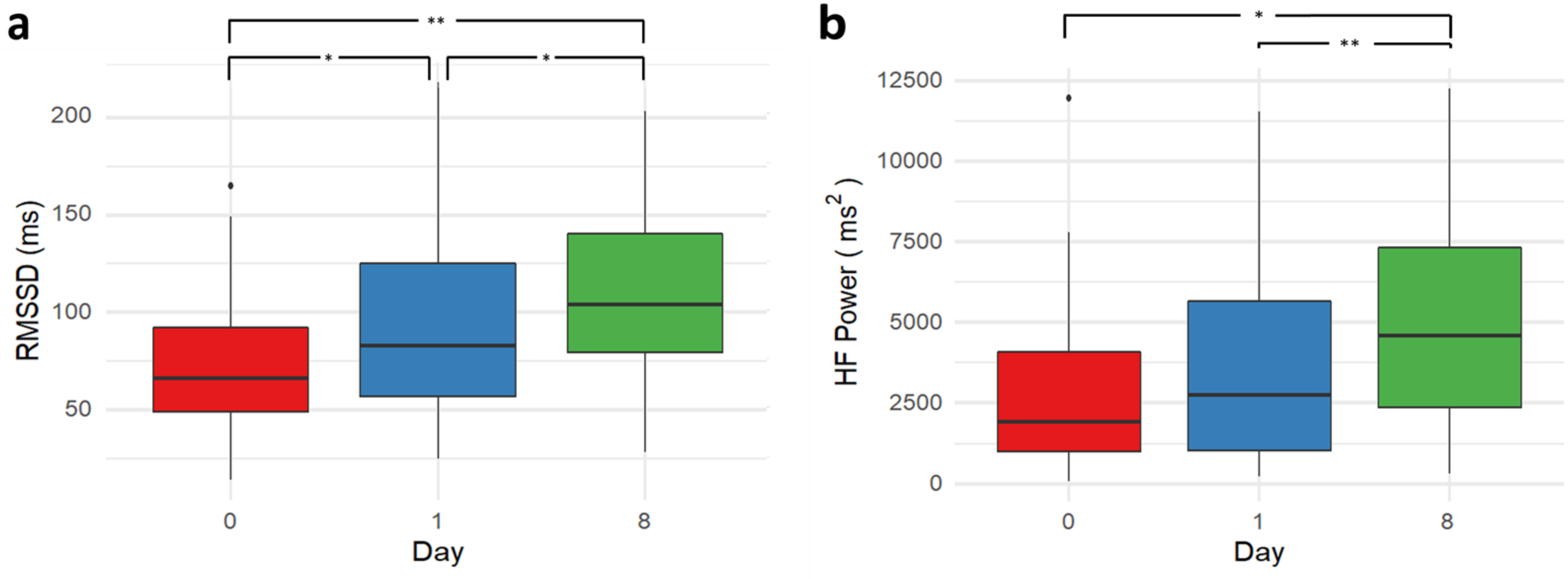


Figure 6-2 Results for RMSSD (a) and HF Power (b) comparing Day 0 (no music), Day 1 (first play of novel music) and Day 8 (second play of same music following conditioning) (P<0.05 (*), P<0.01 (**))

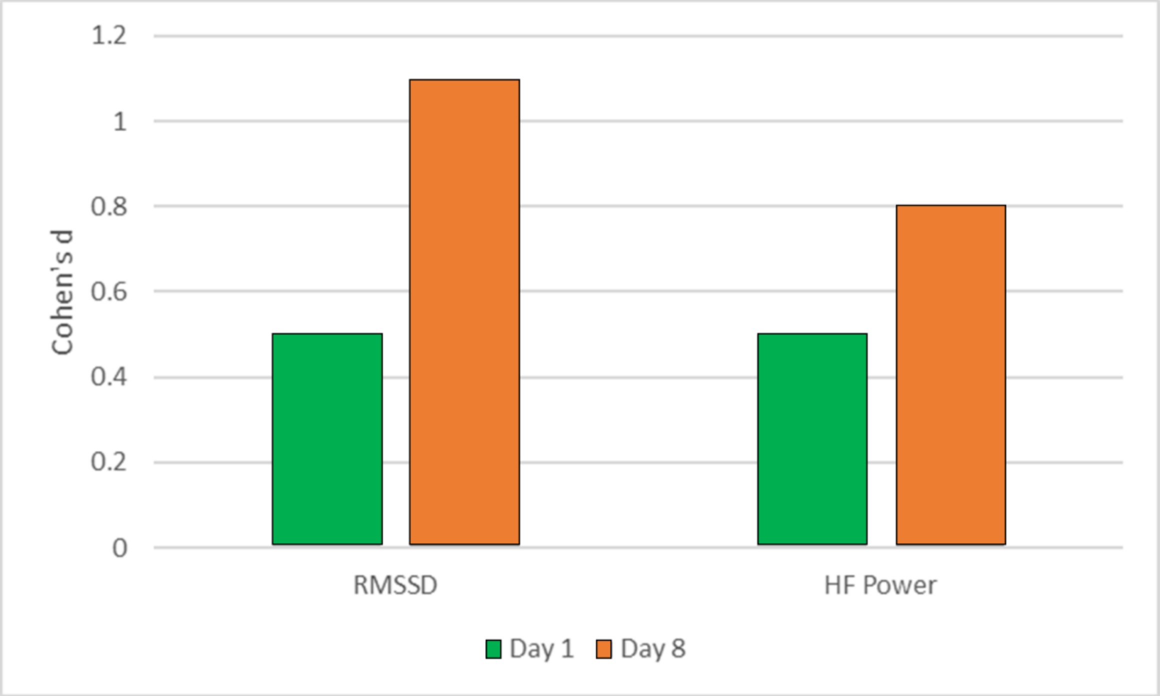


Figure 6-3 Effect size (Cohen's d) of music for two measures of HRV (RMSSD and HF power) when novel music is first played (Day 1) and after 7 days of conditioning (Day 8).

Qualitative Validation. Only one survey was returned, and this was for a dog that was rehomed before the start of the study.

6.4 Discussion

This study has demonstrated that dogs can form an association between a specific piece of music and a time when their arousal is already reduced, and in doing so, this increases the effectiveness of that music to reduce arousal at a different time of day. These findings suggest a key mechanism by which music is having an arousal reducing effect in dogs.

The positive benefits from passively listening to music are widely accepted in both the human and animal literature. This intrinsically calming effect of musical auditory enrichment was also demonstrated in the current study by the increase in both RMSSD and HF Power, indicative of an increase in parasympathetic activity, with the first play of music on Day 1 when compared to the unenriched baseline measures. It should be noted, however, that calming music is not effective at reducing arousal in all circumstances, for example, Kinnaird and Wells (2022) found that classical music had only a moderately calming effect on dogs when they were separated from their owners. The conclusion reached by the authors was that auditory enrichment may have a limited ability to reduce measures of short-term acute stress. In reviewing the evidence of music improving emotional wellbeing in animals, Snowdon (2021) commented on the inconsistency of results, even within the same species. When considering these inconsistencies, it is worth considering the triggers to the stress related behaviours. In cases of separation anxiety, the stress related behaviours are triggered by the absence of the owner whereas in ARRCs the environment is noisy (we have recorded sound pressure levels peaking at over 90 dB), but this noise is nether constant nor predictable, and is often due to the vocalisations of other residents who are unknown to the subjects.

The effect of musical auditory enrichment in noisy environments, discussed in Chapter 3, is relatively small, and here we have shown a Cohen's d of 0.5 (medium effect) for the initial play of novel music, and this aligns with the effect sizes seen in previous studies such as Bowman et al. (2015). By conditioning the dogs to associate that same piece of music with a time of day when they were already calm and relaxed, the effect size on HRV when the same music was played at a time when arousal levels were generally high was increased to 'large' for both RMSSD (1.1) and HF Power (0.8). Previous work has shown a reduction in the relaxing effects of music after successive exposure to the same music, potentially due to habituation (Bowman et al., 2015). This was not seen in the current study despite repeated exposure to the same piece of music for 8 days. In the current study, the dogs were repeatedly exposed to a relatively short period of music (30 min) when they were already at their most relaxed, thereby conditioning the dogs to associate this music with a relaxed internal state. In the study by Bowman et al. (2015), the dogs were exposed to the same 6.5 hour playlist throughout seven consecutive days, the music being played when dogs were both resting and active, so there was no opportunity to form an association between a defined internal state and a specific piece of music.

The results of this study indicate that conditioning can be used to increase the effects of music to decrease arousal in a noisy environment. The conditioned increase in the effects of musical auditory enrichment may add to any masking or smoothing effects of music when used in a noisy environment. Association of music which lowers arousal, however, could also be effective in situations such as separation anxiety, where music has previously been found to be of limited effect (Kinnaird & Wells, 2022). Furthermore, it could be effective in additional situations where the trigger is not aversive sounds that require masking including, but not limited to, visits to the vet; car journeys and moving to a new home.

For the Scottish SPCA, one of the concerns is dogs that are returned to them shortly after being rehomed because they struggle to settle into their new environment. For the human caregivers, rehoming a dog from a rescue centre is regarded as an entirely positive experience, but the dogs may not view it in the same way. All they know is that everything in their life has just changed again, and if the dog has previously come from a sub-optimal environment, they may be far more pessimistic about changes and novel experiences (for a review see Lagisz et al. (2020)). From the results of the current study, it is a logical hypothesis that once dogs are conditioned to associate a specific piece of music with being calm and relaxed, that music could go with them to their new homes and could be used by their new owners to help ease the transition to an environment where everything else has changed.

Having experimentally demonstrated a proof of concept, the second aim of this study was to validate those findings in a real-world setting by conducting an extended period of conditioning in the ARRCs and then testing the effectiveness of that specific track to reduce arousal of dogs during their first few days in a new home. In the five ARRCs that were conducting the conditioning, it was reported (personal communication with manager of the Aberdeen ARRC) that a number of the dogs had learnt that the conditioning music meant bedtime, so when it started to play at 16:30, they would bring themselves in from their runs and get into their beds. While this was a benefit for staff, others reported fatigue of listening to the same track on a 30 min loop. As the responses of the dogs reported at the Aberdeen ARRC were triggered when the music started playing, it is feasible that after an initial conditioning phase, only a single play of the track (rather than the full 30 min) would be required to achieve the same response. To further mitigate staff fatigue, rather than conditioning to a single track on a 30 min loop, a 30 min play-list could be used, providing that each night it was the same playlist in the same order, starting in the same place, with the first track in the play-list being the ‘go-to’ track recommended for new dog owners for stress mitigation in the home environment. As it is still unclear exactly what aspect of the music that dogs are attending to, it is not possible to predict if conditioning would take longer with a play-list as opposed to a single track on loop, but it would appear to be a good compromise between speed of conditioning and staff well-being.

It is very disappointing that the uptake of the survey was so poor, despite the question-set being kept deliberately short and simple (Liu & Wronski, 2018). Completion rates for surveys are improved by regular reminders (Callegaro et al., 2009), but due to GDPR considerations, the researcher had no contact with those rehoming the dogs. Therefore, all briefings, documentation and reminders were conducted by the Scottish SPCA staff. The ‘personal touch’ is also important for survey engagement (Nulty, 2008), and in an attempt to replicate this, the lead researcher made a 2 min video explaining both the purpose of the research and how participation in the study would help to improve animal welfare. A QR code linking to this video was added to the study business cards, and a link was also included in the owner leaflet, however, by the close of the survey, this video had only been viewed seven times. The Scottish SPCA now has an established fostering scheme, and for future studies fosterers may be more likely to engage with efforts to improve animal welfare, although the barrier between the researcher and the target audience imposed by GDPR will need to be addressed if a meaningful response to a survey is to be generated.

This study has demonstrated the potential of increasing the calming effect of music using positive associations, but there are still a lot of unknowns about the most effective use of associations. Here, the conditioning was conducted over seven days, but a longer period of conditioning may produce a stronger effect, although Demant et al. (2011) showed that training dogs every day led to lower learning acquisition than training conducted once a week. Due to the potential for habituation (Bowman et al., 2015), it is also possible that a longer period of conditioning would lead to a progressively reduced effect, particularly if conditioning was conducted every day, so once conditioned, a variable reinforcement schedule may be needed to reduce the risk of habituation as proposed by Bowman et al. (2017). For this study, the effect of conditioning was tested immediately after the conditioning phase. We therefore do not know anything about time to extinction i.e., how many times a conditioned piece of music can be used in an aversive environment before it becomes ineffective, with the only comparable work being that of Bowman et al. (2017), who showed an habituation effect after seven days. In the current study, the dogs were repeatedly exposed to a relatively short period of music (30 min) when they were already at their most relaxed, thereby conditioning the dogs to associate this music with a relaxed internal state. Whereas in the study by Bowman *et al.* (2017), the dogs were exposed to the same 6.5 hour playlist throughout 7 consecutive days, the music being played when dogs were both resting and active, so there was no opportunity to form an association between a defined internal state and a specific piece of music. This leads onto the potential problem of reversal learning. If the conditioned piece of music is used in an aversive environment, the dog may learn to associate that music with the aversive situation rather than being calm and relaxed, a phenomenon similar to reversal learning paradigms used to test cognitive flexibility (Izquierdo et al., 2017). As a single exposure to an aversive event can bring about long-term behavioural changes (Belda et al., 2004), it is plausible that the reversal learning of a negative association like this may take place quicker than the original formation of the positive association, as is seen in other learning paradigms (Baumeister et al., 2001).

The main limitation of this study is that, due to resource constraints, the experimental design compared a baseline HRV measure with an initial play of novel music, and then compared the initial play of novel music with the effect of that music following conditioning. This effectively means that there was no control condition and therefore the change in effect size cannot be definitively attributed to the conditioning rather than, for example, habituation to the experimental set-up. Ideally, a second group of dogs would have been run through the same paradigm, without undergoing conditioning i.e., they would only hear the music on Day 1 and Day 8. However, due to the design of the kennels, it would not have been possible to expose only some dogs to the conditioning and not others.

6.5 Conclusion

Auditory enrichment has an inherent ability to reduce arousal in dogs in noisy environments. Conditioning dogs for seven days to associate a specific auditory enrichment with a quiet time of day increased the effectiveness of that enrichment to reduce arousal when used during a more stressful time of day. Longer periods of conditioning and different reinforcement schedules may further increase this arousal-reducing effect. Forming a positive association with a specific auditory enrichment offers the potential for using auditory enrichment to reduce arousal in environments where it is currently ineffective, improving the quality of life for dogs in rescue centres and beyond.

Chapter 7 General Discussion

7.1 Introduction

The research presented in this thesis addresses the broad question of how to strengthen the previously demonstrated arousal reducing effects of auditory enrichment, specifically for dogs in an Animal Rescue and Rehoming Centre (ARRC) context. Due to the restrictions imposed by the COVID-19 pandemic and the resultant loss of access to the ARRCs, this study was also extended to include horses as another non-vocal learning species in which an arousal reducing effect of auditory enrichment has also previously been shown. Three aspects of how to strengthen the arousal reducing effects of auditory enrichment were examined, with the aims to: understand what specific aspects of auditory enrichment had the greatest influence on arousal in dogs and horses; determine if dogs have the auditory perceptive abilities that justify any assumptions of musical appreciation, and; establish if a positive association with specific music can influence how that music can manipulate arousal in dogs.

7.1.1 What specific aspects of auditory enrichment has the greatest influence on arousal in dogs and horses?

The objectives underpinning this first aim were to: 1) compare the effect on arousal of different tempos of the same music; 2) compare the effect on arousal of different pitches of the same music; and 3) investigate the effect of play-back of the owner's voice within music on the arousal of dogs. Chapter 3 explored the relationship between arousal, as measured by HRV, and the changing of pitch and tempo. Having found no influence of either tempo or pitch in either dogs or horses, the use of the owner's voice to create bespoke music for dogs was investigated in Chapter 4. This determined that bespoke music, bespoke music with clips of the owner's voice, Mozart K448 and white noise all had an equivalent effect on arousal as measured by HRV. It has therefore not been possible to determine a specific aspect of auditory enrichment to which dogs or horses are attending, or indeed, whether they are attending to any specific aspects. Intuitively, this is an unexpected finding, as both pitch and tempo profoundly affects arousal in humans (Collier & Hubbard, 1998; Hevner, 1937; Jaquet et al., 2014; Trochidis & Bigand, 2013) and therefore leads into the second aim of this thesis.

7.1.2 Do dogs have the auditory perceptive abilities that justify any assumptions of musical appreciation?

Chapter 5 sought to address the second aim of this thesis. The first objective was to develop a two-choice go/go selection paradigm to test dogs' ability to discriminate between two different auditory conditions. A relatively complex two-choice go/go selection paradigm was used for the reasons discussed in Chapter 5, and this required the dogs to demonstrate Pavlovian to instrumental transfer. Only one of the fourteen dogs recruited for the study successfully completed the pre-training and went on to complete the test phase. This may have been either because the test paradigm selected was too complex for dogs to master or because the length of pre-training was insufficient. With only one dog being able to take the test, the objective of developing a test of discrimination has, at best, only been partially achieved.

The next objective under this aim was to determine the extent of dogs' categorical perception of tempo, examined with metronome beats, and then extending the same testing to complex music of different tempos. For the single dog tested, the go/go selection test worked well and gave unambiguous results when using the metronome beat, and showed that, for that dog, there was an inability to distinguish between 50 and 200 bpm. However, with such limited inference being drawn from the results of a single dog, the study was not continued to test complect music of different tempos.

The final objective under this aim was to determine the extent of dogs' categorical perception of pitch, examined with pure tones and complex music. The intension was to use the same two-choice go/go selection paradigm used to test tempo discrimination, but as the development of this test paradigm was only partially successful, tests of pitch discrimination were not attempted.

7.1.3 Can a positive association with specific music influence how that music can manipulate arousal in dogs?

Chapter 6 sought to address the final aim of this thesis. The first objective was to quantitatively test if the arousal-reducing effect of auditory enrichment for dogs could be enhanced by the formation of a positive association with it in an ARRC setting. Conditioning dogs for seven days to associate a specific auditory enrichment with a quiet time of day increased the effectiveness of that enrichment to reduce arousal when used during a more stressful time of day. However, as there was not the ability to concurrently run a control condition, only limited inference can be drawn from these results. To further validate these initial findings, the second objective sought to test the ability of previously conditioned auditory enrichment to aid the transition to a new environment for dogs on being rehomed. All nine Scottish SPCA ARRCs were involved in the study, with questionaries being given out to every owner of a newly rehomed dog over a period of six months (approximately 100 dogs). Unfortunately, there was extremely poor engagement with the survey, and no usable data were generated.

7.2 Heartrate Variability as a Measure of Arousal

The primary metric for quantifying effect throughout this thesis has been HRV, specifically RMSSD and HF Power. Chapter 2 validated the two HRMs used for data collection (Polar V800 and Actiheart 5) in both horses and dogs against a clinical 5-lead ECG. This validation confirmed that, not only was the beat detection of these two devices accurate in the target species, but the attachment method for each device was also reliable. Using liberal amounts of ECG gel, the Polar chest straps made a good electrical contact without the need to clip the fur or conduct any other skin preparation. As dogs (particularly in the ARRCs) where more active, the security of the Polar chest strap was improved with the application of veterinary cohesive bandage over the top of the Polar strap. The work presented in Chapter 2 justifies high confidence in the HRV data reported in this thesis. The HRV data are accurate, but an important question to address is the interpretation of those data, and what they tell us about physiological arousal and affective state. HRV analysis can also be time consuming, so it is also important to assess what advantage, if any, HRV gives over simply reporting the HR. The mechanisms underpinning HRV are outlined in Chapter 1 and shows that

HRV is an accurate reflection of the functioning of the PNS (rest and digest) side of the ANS, whereas HR is determined by the ANS as a whole. As HR increases, HRV will always decrease, as a faster HR by necessity has shorter IBIs, with less scope for variance between them. For gauging short-term changes in arousal, HR and HRV provide the same information (increase or decrease in physiological arousal), however, as HRV only reflects the activity of the PNS, the results from HRV can be reported as an increase or decrease in vagal tone. In the study of human physiology and athletic performance, HRV is measured at a resting HR and set breathing rates (Shaffer & Ginsberg, 2017), (i.e., the participant is asked to breath at a specific rate), and this allows direct comparison of changes in HRV over and above the influence of RSA and HR. This is unfeasible for animal studies, but a solution may be to plot HRV against HR and then investigate the change in intercept between conditions, there-by removing the influence of HR. This novel approach may be worth considering for future studies using HRV.

As discussed in Chapter 1, HRV is a physiological measure, and as demonstrated in Chapter 2, in insolation it can tell us very little about the animal’s affective state. This is a key limitation of HRV but is also true of all physiological measures. This does not diminish the utility of HRV as a measure of effect, but care must be taken as to how to report the findings and to not make assumptions about an animal’s affective state that are not justifiable. As outlined in Chapter 1, ‘success’ throughout this thesis was defined as ‘reduced physiological arousal’, and to quantify this, HRV is an ideal metric as it can provide non-invasive continuous recording showing minute-by-minute changes in PNS activity.

7.3 Music for Non-Vocal Learners

The lack of effect of changing tempo and pitch on dogs and horses reported in Chapter 3 is unexpected, given the fact that it is well documented in human subjects (Collier & Hubbard, 1998; Hevner, 1937; Jaquet et al., 2014; Trochidis & Bigand, 2013). This could suggest that the mechanism of action through which music has an anxiolytic effect is not as complex in dogs or horses as it is in humans. The origins of musicality have been studied in a wide range of species, mainly as a vehicle to investigate the evolution of language (ten Cate & Honing, 2022). As discussed in Chapter 3, the neural processing required for a vocal language (to be a vocal learner / vocal mimic) is intrinsically linked to the processing required to perceive complex musical precepts such as beat and pitch. Dogs and horses are not vocal learners, nor do they have any ability for rhythmic entrainment, and this calls into question whether they have any musicality or musical appreciation. This lack of musicality is supported by the lack of change in effect by the changing of pitch and tempo in Chapter 3 and the, the lack of effect from different types of music in Chapter 4 and the, albeit limited, testing of beat perception in Chapter 5. If non-vocal learners do lack any musicality, it may indicate that the genre of music selected may be less important than previously suggested.

7.4 The Power of Association

The complex response of humans to music may be due in part to an expectation of benefit, as described by Hall et al. (2015) in other therapies. In humans, if there is a belief that slower music is more relaxing, that belief may play a large part in the physiological response. No human test subject is going to be completely naïve to music, so when presented with slow music, it is probably impossible to tell how much of the effect is placebo. The fact that human studies have found a more powerful relaxing effect from patients using their own selection of relaxing music rather than the selection of the clinician (Binns-Turner et al., 2008; Gillen et al., 2008; Krout, 2007), further implicates at least an element of expectation of benefit, which may manifest as a conditioned response. It is very hard to justify a placebo effect or an expectation of benefit in non-humans, as this is dependent on the ability for a complex narrative construction which, although present in some higher primates (Cheney & Seyfarth, 2008), the only evidence for it in dogs is willingness to trust people who have proved to be trustworthy in the past (Pelgrim et al., 2021). However, a similar, although all together simpler mechanism was demonstrated in Chapter 6 where an association with calmness was made with a specific piece of music through a process of classical conditioning. Without any requirement for narrative construction, a reduction in arousal was achieved as a conditioned response. Within the ARRCs, this was demonstrated by an increase in the effect size of the music on HRV parameters, but there was also anecdotal evidence from the ARRC staff of behavioural responses e.g., dogs coming in from their runs and getting into bed when the music started playing. The intention was that having formed a positive association within the ARRCs, this specific music would go with the dogs upon rehoming with the hope that it would help ease the transition to a new environment, and the lack of engagement with the study by new owners was very disappointing.

7.5 Acoustic Masking

A large part of the functioning of all brains is prediction (Clark, 2013) and the response to an aversive stimulus is heightened when those predictions are violated (Shankman *et al.*, 2011). Unfortunately, the acoustic environment within an ARRC is loud and unpredictable, with periods of silence randomly interspersed with conspecific barking and anthropogenic noise. The consequences of this acoustic unpredictability, with regard to anxiety and stress, may be heightened in ARRCs, as their design often means that dogs have a restricted view of the environment beyond their own kennel and run. As such, dogs may rely, to a greater extent, on auditory cues to gather information and make predictions about their environment. Furthermore, in an ARRC environment, olfactory cues may be blunted by strong extraneous odours such as bleach (Waggoner *et al.*, 1998). Therefore, the auditory enrichment used in this environment may act to smooth the auditory landscape in the noisy environments, making it less unpredictable as opposed to "covering up" auditory stimuli that trigger aversive responses. Interestingly, if this was to be the explanation for the observed effect then it could be argued that the type of auditory enrichment used in such situations is not as important as its role to reduce the variation between silence, and low and high amplitude noise, and this is supported by the finding in the current studies, and the study in Chapter 4, that there is no difference in effect between white noise and the music samples tested. Previous studies on dogs in relatively quiet environments such as in a veterinary hospital (McDonald & Zaki, 2020) and a study of separation anxiety (Kinnaird & Wells, 2022) found only weak evidence demonstrating that exposure to music reduced arousal, and it may be because in those environments, there was nothing aversive to mask.

7.6 Future Work

This thesis has generated several unanswered questions that are worthy of investigation. The role of masking and / or smoothing has not been directly tested, with this hypothesis generated from the apparent lack of effect in a quiet environment. As it is unlikely that the mechanism involved is simply the covering-up of aversive sounds (based on that music played in the ARRCs was never as loud as the 80-85 dB barking), the role of acoustic smoothing i.e., making the acoustic environment more predictable, warrants investigation. The first stage of this needs to be testing of a noisy, predictable environment and assess if it is less arousing than a noisy, unpredictable one.

The power of forming a positive association with a specific piece of music has been demonstrated in a noisy environment, and the next logical step is to test if such associations can increase the effectiveness of music to have an arousal-reducing effect in quieter environments where aversive sounds may not be the trigger to a stress response. This may prove to be particularly helpful in situations such as separation anxiety, where playing music has been shown to have limited intrinsic effect (Kinnaird & Wells, 2022).

The understanding of species-specific perception is also very important, and Snowdon (2021) emphasised the importance of any auditory enrichment matching an animal's perceptive abilities, yet this is something that is rarely considered. Chapter 5 sought to establish to what extent dogs have any beat perception, but with only a single dog progressing as far at testing, this work needs pursuing in a way that achieves a larger sample size, potentially using habituation / dishabituation paradigms. The logical continuation of this is to test further discrimination protocols to establish to what aspects of music dogs are attending. The two different tempos investigated in Chapter 5 were both regular, unlike the work in rats by Celma-Miralles and Toro (2020) who investigated the discrimination of temporal regularities in sequences of sounds and suggested that this trait may have ancient evolutionary roots. Temporal regularities may therefore be simpler to discriminate than regular beats of differing tempos, so this may be worthy of future investigation in dogs.

It is notable that in this thesis, together with all the published auditory enrichment literature, (except examples pertaining to rodents e.g., Castelhano-Carlos and Baumans (2009)), the focus had been on the auditory spectrum of humans (up to approximately 20 kHz (Herman, 2016)). This is in part because all but the most highly specialised audio equipment operates in this range. Dogs can hear at much higher frequencies than humans (up to approximately 64 kHz (Heffner, 1983)), and where previous studies have ignored the 20 – 64 kHz range, opportunities for enrichment / mitigation remain to be exploited.

7.7 Conclusion

The over-arching aim of this thesis was to strengthen the previously demonstrated arousal-reducing effects of auditory enrichment for dogs and horses. As non-vocal learning species, it is likely that both dogs and horses have limited, if any, musicality, so the effects reported are unlikely to derive from any musical appreciation. This is supported by the results of Chapters 3, 4 and 5, where the influence of specific musical attributes could not be demonstrated in either species. The work presented in Chapter 6 indicates that forming a positive association with music may be a useful tool in strengthening the effect, and its use in stressful environments where aversive noise is not the trigger is worthy of further investigation. The incidental finding of a lack of effect seen in quiet environments in contrast to the demonstrated effect in noisy environments is suggestive that masking or smoothing of the acoustic environment may be important to the way auditory enrichment is having an arousal-reducing effect, and this is a principle that has been previously demonstrated in human subjects.

The key findings from the literature and the experimental studies throughout this thesis, summarised in Figure 7-1, are that, although auditory enrichment has an arousal-reducing effect in noisy environments for both humans and non-humans, and in quiet environments for humans, for non-humans there is limited effect in quiet environments. Forming a positive association with a specific piece of music may increase the effect for non-humans in a noisy environment and has the potential to make auditory enrichment more effective for non-humans in a quiet environment.

Auditory enrichment in the form of anthropogenic music is a potentially powerful tool to reduce arousal for a range of domestic animal species, and it is both inexpensive and easy to implement. Understanding the mechanisms through which music is having an arousal-reducing effect will inform better tailoring of auditory enrichment for specific circumstances.

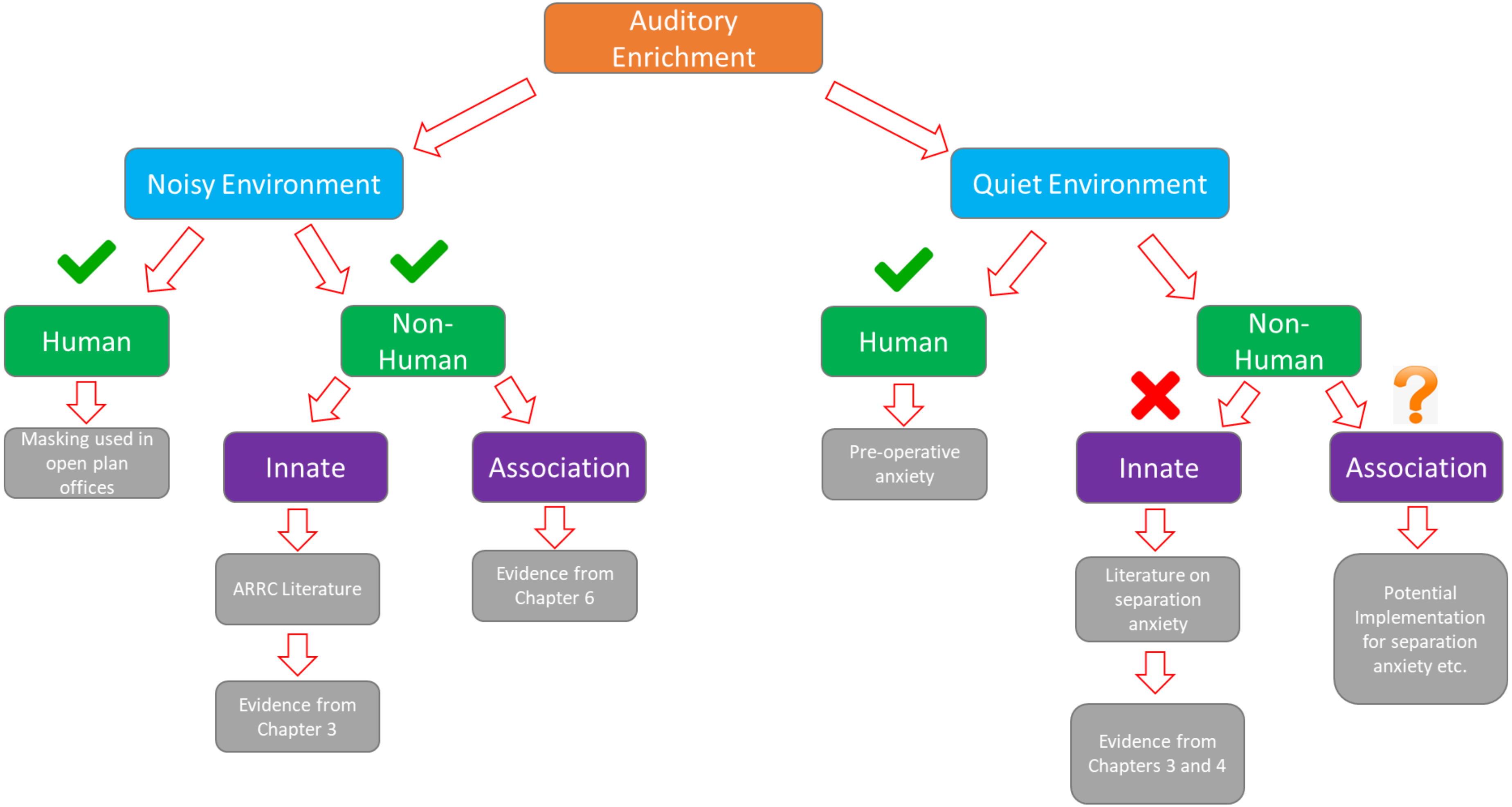


Figure 7-1 **Infographic summarising the key finding of this thesis, both from the literature and from the experimental studies. Music has an innate arousal reducing effect in humans and non-humans in noisy environments, and in humans in quiet environments. Associations may strengthen the effect for non-humans in noisy environments and could prove to be an effective solution for non-humans in quiet environments.**

Appendix A Bland-Altman test of agreement against Televet 100 with 0.9 sec correction applied (Equine Data, Group S, n=13)

Table A1 – Bland-Altman test of agreement for RMSSD data

Device/Correction	Bias (ms) (95% CI)	Lower LOA (95% CI)	Upper LOA (95% CI)
Polar.0	-30.50 (-46.91 to -14.09)	-353.58 (-381.66 to -325.52)	292.58 (264.51 to 320.65)
Polar.0.9	-10.99 (-15.23 to -6.75)	-94.49 (-101.74 to -87.23)	72.51 (65.25 to 79.76)
Polar.0.6	-4.09 (-6.90 to -1.27)	-59.53 (-64.35 to -54.72)	51.36 (46.54 to 56.17)
Polar 0.45	-1.48 (-4.17 to 1.21)	-54.47 (-59.07 to -49.86)	51.51 (46.91 to 56.11)
Polar 0.4	0.48 (-2.09 to 3.06)	-50.14 (-54.54 to -45.75)	51.11 (46.71 to 55.51)
Polar.0.3	5.40 (2.49 to 8.30)	-51.81 (-56.78 to -46.84)	62.61 (57.64 to 67.58)
AH 0	-3.82 (-7.39 to -0.24)	-74.19 (-80.29901 to -68.07)	66.55 (60.43 to 72.66)
AH 0.9	-0.26 (-1.56 to 1.03)	-25.79 (-28.01 to -23.57)	25.27 (23.05 to 27.48)
AH 0.6	0.88 (-0.92 to 2.69)	-34.60 (-37.68 to -31.52)	36.36 (33.28 to 39.45)
AH 0.3	5.86 (3.16 to 8.56)	-47.34 (-51.96 to -42.72)	59.06 (54.44 to 63.68)

Table A2 – Bland-Altman test of agreement for HF Power data

Device/Correction	Bias (ms) (95% CI)	Lower LOA (95% CI)	Upper LOA (95% CI)
Polar.0	-11096.4 (-20151.3 to -2041.5)	-189363.3 (-204851.3 to -173875.2)	167170.4 (151682.4 to 182658.5)
Polar.0.9	-1681.42 (-2642.02 to -720.84)	-20592.87 (-22235.91 to -18949.82)	17230.01 (15586.96 to 18873.06)
Polar.0.6	-525.20 (-1137.30 to 86.90)	-12575.80 (-13622.77 to -11528.83)	11525.41 (10478.44 to 12572.38)
Polar 0.45	-371.36 (-855.09 to 112.37)	-9894.74 (-10722.15 to -9067.34)	9152.02 (8324.62 to 9979.42)
Polar 0.4	-89.87 (-504.89 to 325.15)	-8260.48 (-8970.36 to -7550.61)	8080.74 (7370.87 to 8790.61)
Polar.0.3	471.15 (-32.35 to 974.65)	-9441.45 (-10302.67 to -8580.23)	10383.75 (9522.54 to 11244.97)
AH 0	-619.42 (-1349.36 to 110.52)	-14989.92 (-16238.44 to -13741.39)	13751.08 (12502.55 to 14999.60)
AH 0.9	44.95 (-50.91 to 140.81)	-1842.32 (-2006.29 to -1678.35)	1932.22 (1768.25 to 2096.18)
AH 0.6	209.32 (-123.14 to 541.77)	-6335.90 (-6904.56 to -5767.25)	6754.54 (6185.88 to 7323.19)
AH 0.3	682.63 (232.47 to 1132.79)	-8179.76 (-8949.73 to -7409.78)	9545.01 (8775.04 to 10314.99)

Table A3 – Bland-Altman test of agreement for HR data

Device/Correction	Bias (ms) (95% CI)	Lower LOA (95% CI)	Upper LOA (95% CI)
Polar.0	-0.16 (-0.27 to -0.06)	-2.28 (-2.46 to -2.09)	1.95 (1.77 to 2.14)
Polar.0.9	-0.19 (-0.27 to -0.11)	-1.78 (-1.92 to -1.64)	1.40 (1.26 to 1.54)
Polar.0.6	-0.13 (-0.20 to -0.05)	-1.56 (-1.68 to -1.44)	1.31 (1.18 to 1.43)
Polar 0.45	-0.11 (-0.18 to -0.04)	-1.50 (-1.62 to -1.38)	1.28 (1.15 to 1.40)
Polar 0.4	-0.09 (-0.14 to -0.04)	-1.05 (-1.14 to -0.97)	0.87 (0.79 to 0.95)
Polar.0.3	0.87 (0.79 to 0.95)	-1.11 (-1.20 to -1.02)	0.93 (0.84 to 1.02)
AH 0	-0.01 (-0.03 to 0.01)	-0.40 (-0.43 to -0.36)	0.38 (0.34 to 0.41)
AH 0.9	-0.02 (-0.04 to -0.01)	-0.32 (-0.35 to -0.30)	0.28 (0.25 to 0.30)
AH 0.6	-0.03 (-0.04 to -0.01)	-0.35 (-0.38 to -0.32)	0.30 (0.27 to 0.32)
AH 0.3	-0.04 (-0.06 to -0.02)	-0.43 (-0.47 to -0.40)	0.36 (0.32 to 0.39)

Appendix B Post Rehoming Behaviour Questionnaire

SECTION 1: Music and Ownership

1. Which Scottish SPCA centre did you rehome your dog from?

Aberdeenshire	
Angus, Fife & Tayside	
Ayrshire and South West Scotland	
Caithness and Sutherland	
Dunbartonshire & West Scotland	
Edinburgh & Lothians	
Glasgow	
Highlands & Islands	
Lanarkshire	

2. How long have you had this dog for?

Less than 2 weeks	
2 – 4 weeks	
1 – 3 months	
3 – 6 months	
More than 6 months	

3. Were you advised of a specific track that you dog would find relaxing?

Yes	
No (go to Question 9)	

4. Have you used this specific track?

Never	
Less than once a week	
Several times a week	
Every day	
More than once a day	

5. If you never or rarely used the specific track, is it because: (tick all that apply)

The dog hasn’t needed it	
I don’t have a device to play the track	
I don’t like that specific track	
I never play music in the house	
This dog doesn’t like it	
Other animals in the house don’t like it	
Any other reason – please give details	

6. What behaviour or event prompts you to play this specific track?

Hyperactivity	
Constant barking	
Showing fearful behaviour	
At bedtime	
Leaving the dog on their own	
Other – please give details	

7. Do you think playing this track has resulted in less problems or better behaviour? By circling a number on the following 5-point scales (0=No Change, 4=Complete Change), please indicate your own dog's recent change in behaviour in relation to this track being played **(please circle only one number)**:

No Change:	**Mild—Moderate change**	**Complete Change:**
little or no change in behaviour	0..............1..............2..............3..............4	Problem behaviours completely removed

8. How long does this change in behaviour last for after the music stops?

Only while the track is playing	
Less than 30 min	
30 min – 60 min	
1 – 3 hours	
More than 3 hours	
N/A	

9. What type of music do you generally play in the home? (tick all that apply)

None		Music Radio		Talk Radio	
Blues		Funk		Punk	
Classical		Grunge		Reggae	
Country		Heavy Metal		Rock	
Dance		Hip Hop		R & B	
Disco		House		Ska	
Drum and Bass		Jazz		Soul	
Dubstep		Opera		Theatrical	
Emo		Pop		Trance	
Folk		Prog Rock		Other	

10. What is your preferred way to access music?

CD	
Download to phone/tablet	
Download to another device	
Direct from a streaming site	

11. How many dogs have you previously owned dogs?

0	
1 - 3	
4 - 6	
More than 6	

12. How many other dogs do you currently own?

0	
1 - 3	
4 - 6	
More than 6	

13. How many other dogs have you previously rehomed from a shelter?

0	
1 - 3	
4 - 6	
More than 6	

14. Is there anything else you do to relax your dogs?

SECTION 2: Excitability

INSTRUCTIONS: Some dogs show little reaction to exciting events, while others become highly excited at the slightest novelty. By circling a number on the following 5-point scales (0=Calm, 4=Extremely excitable), please indicate, **in the first 14 days following re-homing**, your own dog's tendency to become excitable in the following circumstances **(please select only one number)**:

15. Just before being taken for a walk.

Calm:	**Mild—Moderate excitability**	**Extremely excitable:**
little or no special reaction	0..............1..............2..............3..............4	over-reacts, hard to calm down.

16. Just before being taken on a car trip.

Calm:	**Mild—Moderate excitability**	**Extremely excitable:**
little or no special reaction	0..............1..............2..............3..............4	over-reacts, hard to calm down.

SECTION 3: Fear and Anxiety

INSTRUCTIONS: Dogs often show signs of anxiety or fear when exposed to particular sounds, objects, persons or situations e.g., crouching or cringing with tail tucked between the legs; whimpering or whining, freezing, trembling, or attempting to escape or hide. Using the following 5-point scales (0=No fear, 4=Extreme fear), please indicate, **in the first 14 days following re-homing**, your own dog's tendency to display fearful behaviour in the following circumstances **(please select only one number)**:

17. When approached directly by an unfamiliar person while away from your home.

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

18. In response to sudden or loud noises (e.g., thunder, vacuum cleaner, car backfire, road drills, objects being dropped, etc.).

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

19. When an unfamiliar person tries to touch or pet the dog.

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

20. In response to strange or unfamiliar objects on or near the pavement (e.g., plastic rubbish bags, leaves, litter, flags flapping, etc.)

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

21. When approached directly by an unfamiliar dog.

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

22. When first exposed to unfamiliar situations (e.g., first car trip, first time in elevator, first visit to the vet, etc.).

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

23. When barked, growled, or lunged at by an unfamiliar dog.

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

24. When having nails clipped by a household member.

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

25. When groomed or bathed by a household member.

No fear/anxiety: No visible signs of fear	**Mild—Moderate fear/anxiety** 0..............1..............2..............3..............4	**Extreme fear:** cowers; retreats or hides, etc.

SECTION 4: Separation-related behaviour.

INSTRUCTIONS: Some dogs show signs of anxiety when left alone, even for short periods of time.

In the first 14 days following re-homing, how often has your dog shown each of the following signs of anxiety when left, or about to be left, on its own **(please select only one box per question)**:

		Never	Seldom	Sometimes	Usually	Always
26	Restlessness / agitation / pacing					
27	Barking or whining					
28	Chewing / scratching at doors, floor, windows, curtains, etc.					

SECTION 5: Attachment and Attention-seeking.

INSTRUCTIONS: Most dogs are strongly attached to their people, and some demand a great deal of attention and affection from them. In the first 14 days following re-homing, how often has your dog shown each of the following signs of attachment or attention-seeking **(please check only one box per question)**:

		Never	Seldom	Sometimes	Usually	Always
29	Tends to follow you (or other members of the household) about the house, from room to room.					
30	Tends to sit close to, or in contact with, you (or others) when you are sitting down					

SECTION 6: Miscellaneous problems

INSTRUCTIONS: Dogs display a wide range of miscellaneous behaviour problems in addition to those already covered by this questionnaire. In the first 14 days following re-homing, please indicate how often your dog has shown any of the following behaviours **(please select only one box per question)**:

		Never	Seldom	Sometimes	Usually	Always
31	Chews inappropriate objects.					
32	Urinates against objects/furnishings in your home.					
33	Urinates when left alone at night, or during the daytime.					
34	Defecates when left alone at night, or during the daytime.					
35	Hyperactive, restless, has trouble settling down.					
36	Playful, puppyish, boisterous.					
37	Active, energetic, always on the go.					
38	Chases own tail/hind end.					
39	Barks persistently when alarmed or excited.					

SECTION 7: Additional Information

Please include any additional information regarding your re-homed dog that you think may be relevant and that has not already been covered on the questionnaire:

Thank you for providing this helpful information!

Thank you so much for taking part in this survey, the results of which will hopefully help to improve the welfare of dogs both in the rescue centres and once they have been rehomed.
If you would like to be sent a copy of any final published report, or if you have any further questions about this study, please email Richard.Mott@glasgow.ac.uk within 6 months of completing the questionnaire. As the questionnaires are completed anonymously, unfortunately we cannot give feed-back on individual dogs.
https://glasgow-research.onlinesurveys.ac.uk/scottish-spca-rehoming-survey

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