UNIVERSITI PUTRA MALAYSIA

ELECTROENCEPHALOGRAPHIC CHANGES ASSOCIATED WITH CLASSICAL MUSIC THERAPY ON PREOPERATIVE STRESS IN CANINE UNDERGOING ELECTIVE SURGERY

MURNI BALQIS BINTI MOHD PAKRI

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MURNI BALQIS BINTI MOHD PAKRI

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It is hereby certified that we have read this project paper entitled "Electroencephalographic Changes Associated with Classical Music Therapy on Preoperative Stress in Canine Undergoing Elective Surgery," by Murni Balqis binti Mohd Pakri and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 - Project.

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DEDICATIONS

“It is not just humans who are musical. Animals too. Not only they make music, they hear it in unique fashion.”

-Mitch Albom, The Magic Strings of Frankie Presto

I dedicate this thesis to the music of my life: My mom, my late father, my family, my friends and all the humans and animals who join the band. Thank you for lifting the curtain on my passions!

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And to all the dogs involved in this project, thank you for listening to the music I chose specially for all of you. Thank you for the experience.

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LIST OF ABBREVIATIONS

CDSS	Clinic Dog Stress Scale
EEG	Electroencephalogram
HPA	Hypothalamic-pituitary-axis
SAM	Sympathetic-adrenal-medullary
ACTH	Adrenocorticotropic hormone
CRH	Corticotropin-releasing hormone
C-BARQ	Canine Behavioural Assessment and Research Questionnaire
OSI	Oxidative Stress Index
FFT	Fast Fourier Transformation
BPM	Beats per minute
MF/ F50	Median frequency
Ptot	Total power
MABP	Mean Arterial Blood Pressure

ABSTRAK

Abstrak daripada kertas projek yang dikemukan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

PERUBAHAN ELEKTROENSEFALOGRAFI YANG BERHUBUNG DENGAN TERAPI MUZIK KLASIK TERHADAP TEKANAN PRABEDAH KE ATAS KANIN YANG MENJALANI PEMBEDAHAN ELEKTIF

Oleh

Murni Balqis binti Mohd Pakri

2022

Penyelia: Dr. Ubedullah Kaka

Persekitaran baharu seperti hospital veterinar, persekitaran praoperasi dan penjaga yang tidak dikenali dianggap sebagai tekanan tehadap anjing. Tindak balas tekanan boleh mempunyai implikasi yang memudaratkan pada fisiologi mereka dan mungkin mengelirukan parameter klinikal. Selain itu, anjing yang tertekan boleh menjadi cabaran ketika pengendalian dan menimbulkan risiko pekerjaan yang serius kepada kakitangan veterinar. Oleh itu, adalah penting untuk menguruskan tekanan dengan menggunakan kaedah yang praktikal dan cekap. Dalam eksperimen semasa, terapi muzik klasik digunakan sebagai rangsangan pendengaran untuk mengurangkan tekanan praoperasi pada anjing. Enam anjing telah didaftarkan dalam kajian ini. Kadar denyutan jantung, tekanan darah, penilaian tingkah laku dan bacaan electroencephalogram (EEG) direkodkan serta-merta apabila anjing itu tiba sebagai pra-muzik (T0). Kemudian, muzik klasik pilihan, berjulat antara 70-130 denyutan seminit (BPM) dimainkan selama lebih 20 minit. Selepas itu, parameter diulang dan direkodkan sebagai muzik pasca (Tm). Penilaian tekanan adalah berdasarkan kadar denyutan jantung kuantitatif, tekanan darah, EEG dan Skala Tekanan Anjing Klinik (CDSS) yang disahkan. Keputusan menunjukkan bahawa muzik mengurangkan kadar denyutan jantung daripada 126±8.29 kepada 90±11.79. Tekanan darah juga menurun dengan sistolik, diastolik dan min masing-masing 119.8±16.15, 65.4±19.90, 82.8±18.35 sebelum muzik kepada 114.6±11.48, 58.6±8.44 dan 77.2±9.68 selepas muzik. Kekerapan Median (MF) untuk EEG menurun daripada 35.37±18.37 pra muzik kepada 13.15±12.15 selepas muzik. Jumlah kuasa EEG (Ptot) berkurangan daripada 31.36±16.43 kepada 21.83±1.51. Penilaian tingkah laku menggunakan pemarkahan CDSS menunjukkan penurunan skor untuk postur badan (1.33±0.516 hingga 0.50±0.548), postur telinga (1.67±1.506 hingga 0.33±0.516), pandangan (2.50±0.837 hingga 0.83±0.408), pernafasan (2.00±0.894 hingga 0.50±0.837) dan bibir (1.67±0.516 hingga 0.17±0.408). Walaupun nilai min menurun, tiada perbezaan yang signifikan secara statistik diperhatikan disebabkan saiz sampel yang kecil. Oleh itu, keputusan keseluruhan kajian ini mencadangkan bahawa terapi muzik klasik berkesan dalam mengurangkan tekanan praoperasi pada anjing yang menjalani pembedahan elektif. Hasil kajian ini menjamin kajian masa depan dengan saiz sampel yang lebih besar.

Kata kunci: elektroensefalografi; tekanan; terapi muzik klasik; anjing; kebajikan

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Project.

ELECTROENCEPHALOGRAPHIC CHANGES ASSOCIATED WITH CLASSICAL MUSIC THERAPY ON PREOPERATIVE STRESS IN CANINE UNDERGOING ELECTIVE SURGERY

By

Murni Balqis binti Mohd Pakri

2022

Supervisor: Dr. Ubedullah Kaka

New surroundings such as veterinary hospitals, preoperative settings and unfamiliar caretakers are perceived as stressful for dogs. The stress responses can have detrimental implications on their physiology and may confounds clinical parameters. Additionally, stressful dogs can be challenging to handle and pose serious occupational risks to veterinary personnel. Therefore, it is crucial to manage the stress by using practical and efficient methods. In current experiment, classical music therapy was used as an auditory stimulus to alleviate the preoperative stress in dogs.

Six dogs were enrolled in this study. The heart rate, blood pressure, behaviour assessment and electroencephalogram (EEG) readings were recorded immediately as the dogs arrived as pre-music (T0). Then, classical music of chosen, ranged from 70-130 beats per minute (BPM) was played for over 20 minutes. Afterwards, the parameters were repeated and recorded as post-music (Tm). Stress evaluation was based on the quantitative heart rate, blood pressure, EEG and a verified Clinic Dog Stress Scale (CDSS). Results showed that music reduced the heart rate from 126±8.29 to 90±11.79. Blood pressure also decreased with systolic, diastolic and mean of 119.8±16.15, 65.4±19.90, 82.8±18.35 pre-music to 114.6±11.48, 58.6±8.44 and 77.2±9.68 post-music respectively. The Median Frequency (MF) for EEG decreased from 35.37±18.37 pre-music to 13.15±12.15 post-music. The EEG total power (Ptot) reduced from 31.36±16.43 to 21.83±1.51. The assessment of behaviour using CDSS scoring showed decrease of score for body posture (1.33±0.516 to 0.50±0.548), ear posture (1.67±1.506 to 0.33±0.516), gaze (2.50±0.837 to 0.83±0.408), respirations (2.00±0.894 to 0.50±0.837) and lips (1.67±0.516 to 0.17±0.408). Although the mean values decreased, no statistically significant differences were observed due to small sample size. Thus, the overall results of this study suggested that classical music therapy is effective in reducing the preoperative stress in canine undergoing elective surgery. The result of this study warrants future studies with larger sample size.

Keywords: electroencephalography; stress; classical music therapy; canine; welfare

CHAPTER 1

INTRODUCTION

New surroundings such as veterinary hospitals and unfamiliar caretakers are perceived to be stressful for dogs and these adverse encounters can have a lasting impact on the animals (Spitznagel *et al*., 2017; Lloyd., 2017). Despite the stress response being an adaptive process that enable animals to respond quickly to chronic or extreme stress or is frequently referred to as distress, it can be harmful to the animals and thus, may lead to poor welfare (Wielebnowski., 2003).

Psychogenic stress is natural in animals (Juodžentė., 2018) and preoperative events could be one of the situations causing the stress (Pittman *et al*., 2011). These stressful events can impair an animal's welfare and have an impact on a variety of physiological functions, including the activation of the hypothalamic-pituitary-adrenal axis (HPA). In addition, cellular responses to stressful events might result in biochemical and molecular alterations. The body also releases the chemicals cortisol and adrenaline while under stress. The immune system is suppressed by cortisol while the heart and respiratory rates are increased due to the release of adrenalin (Beerda *et al*., 1997; Casey., 2002; Csoltova *et al*., 2017; Juodžentė., 2018).

Extensive data retrieved from experimental, clinical and epidemiological research shows that acute and chronic psychogenic stress can have detrimental implications in both human and non-human animals, contributing to increased morbidity and death in patients (Hekman *et al*., 2014; *Glaser et al*., 2005).

Therefore, managing preoperative stress is important in reducing the influence towards postoperative pain intensity, decreasing the requirement for anaesthetic and analgesic (Hole *et al*., 2015) and giving better welfare to the animals.

Music therapy by means of classical pieces has been proven to give positive outcomes towards the health of humans, including for preoperative stress (Pittman *et al*., 2011). Entrainment, a method of playing music at a specific pace to synchronise physiological reactions, has been performed successfully in humans (Bradt, 2010). Music, especially classicals, are therapeutic. They are also cost effective and are easy to be employed. Kogan *et al.* (2012) shows that classical music affects the behavioural changes on kennelled dogs in which those exposed to the music spent more time sleeping and reduced vocalisation compared to those without exposure to music. In the same study, heavy metal music appeared to increase the dogs' body shaking which is a suggestive behaviour of nervousness.

King *et al*. (2022) measured the stress parameters in dogs such as salivary cortisol and IgA, temperature, pulse, respiration and the dogs' behaviour during veterinary visit in which each dog would experience two mock veterinary visits, one with music and one without music. According to the findings of the study, the customised music therapy may be effective in modifying these physiological parameters during veterinarian consultations, which may lead to dogs feeling less anxious. In addition, lesser stress will also enable the surgeon or the assistant in handling the animals prior to sedation with ease without higher risk of bite or scratch injury.

In Malaysia, the research or application of classical music therapy on animals, especially canine is still inadequate. It was hypothesized that music would have significant impact on the physiological parameters of dogs. Moreover, EEG has been reported to be an important research tool to objectively measure pain and stress, however, studies on EEG and stress are very limited in small animals. Therefore, the purpose of this study is to demonstrate the positive impact of music therapy on the dogs using EEG, during the preoperative period of the elective surgery. Due to its therapeutic effect and non-invasive approach, the response of music therapy on the pre-operative stress in canine was assessed in this study in terms of the brain activity by using electroencephalogram (EEG), heart rate, blood pressure and behaviour.

CHAPTER 2

LITERATURE REVIEW

2.1 Stress

Stress, also known as a condition of impaired homeostasis, can result from stressors, which include physiological or psychological influences. In these circumstances, the animal responds by activating mechanisms that sets off physiologic, immunological and behavioural actions that aid its adaptations to the new environment (Berteselli, 2005). In variety of animals including dogs, acute stress is associated by increased activity of the hypothalamic-pituitary-adrenal (HPA) axis (Dess *et al*., 1983; Gue *et al*., 1988) and the sympathetic-adrenal-medullary (SAM) system (Anderson and Brady, 1972; Engeland *et al*., 1990; Gaebelein *et al*., 1977).

As in acute stress circumstances, chronic stress may result in greater central activation of the HPA axis, which may or may not result in increased cortisol (the major glucocorticoid in dogs) release. Excessive levels of glucocorticoids will suppress the HPA axis's central (Beerda *et al*., 1998; Feldman and Weidenfeld, 1995) and pituitary levels (Beerda *et al*., 1998; Keller-Wood and Dallman *et al*., 1984) and the pituitary may develop greater resistant to the adrenocorticotropic hormone (ACTH) to which releasing vasopressin (Beerda *et al*., 1998; Koch and Lutz-Bucher *et al*., 1985) and corticotropin-releasing hormone (CRH) (Beerda *et al*., 1998; Tizabi and Aguilera *et al*., 1992). Figure 2.1 below summarises the regulation of the hypothalamus-pituitary-adrenal axis.

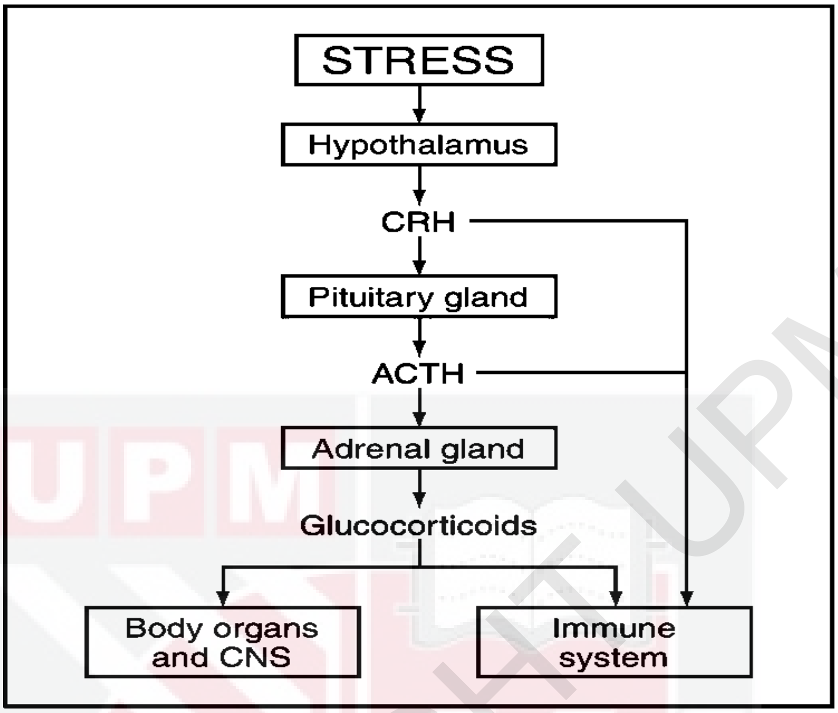


Figure 2.1: The regulation of the hypothalamus-pituitary-adrenal axis

There have been a lot of studies involving animals such as dogs in relation to stress. Kartashova *et al*. (2021) analysed more than 100 papers to highlight the techniques for stress evaluation in various clinical and experimental settings, as well as techniques for reducing stress. Based on the review, although it appears that stress in dogs has been extensively researched, there are currently no widely acknowledged guidelines for the prevention, control and treatment of stress in animals, nor have there been any discovery to the standard quantitative and qualitative signs of stress in this animal. In addition, the possibility of misinterpreting data when measuring stress responses in terms of wellbeing exists. Real stress responses may be biased and misinterpreted as a result of anticipated reactions and responses to the collection of samples that are not particular to stress (Beerda *et al*., 1996; Lynch and McCarthy, 1969). Therefore, the use of the non-invasive sampling techniques may cause the subject of the study only a small amount of disruption, aiding to partially minimise anticipatory or stress-related nonspecific reactions (Beerda *et al*., 1996).

When dealing with stressed animals, it's important to consider more than just physiological parameters in order to identify stress more accurately. There have been several theories put out as to why dogs become fearful and aggressive after receiving veterinarian care. Several of the factors include the dog's age, any underlying illnesses or treatments, previous visits to the vet, the owner's disposition as well as the amount of the dogs' physical activity (Kartashova *et al*., 2021; Lansberg *et al*., 2011; Packer *et al*., 2019; Sundman *et al*., 2019). A recent retrospective analysis using the data from the Canine Behavioural Assessment and Research Questionnaire (C-BARQ) discovered that breed group, prior involvement in roles or activities (such as breeding, showing, and hunting), source, weight, the presence of other dogs in the home and whether the owners were first-time dog owners were all associated with owner-reported fear during a veterinary examination (Stellato *et al*., 2021; Edwards *et al*., 2019).

Previously, Juodžentė *et al*. (2018) evaluated the long-term isolation of dogs from its owner and the unfamiliar surroundings towards their susceptibility to psychogenic and oxidative stress. From this study, longer stays in the unfamiliar environment without their owners resulted in substantial increases in OSI (Oxidative Stress Index) and cortisol levels, which might cause systemic disorders to occur and hinder the healing of wounds. According to Stellato *et al*. (2021), the response to separation from owners (Riemer *et al*., 2016), the dog's prior history of painful or stressful experiences (Doring *et al*., 2009) and the dog's prior history of exposure to novel stimuli, such as new people (Vas *et al*., 2005), new animals, environments and handling (Garnier *et al*., 1990), are additional factors that merit further investigation and are hypothesised to affect dogs.

2.2 Stress stimuli

2.2.1 Physiological stressors

According to an article by Notari (2005), physiological stressors put the body's integrity and physiological balance in jeopardy. This balance must be kept within a relatively small range of parameters, including those of temperature, extracellular sodium concentration or blood sugar level. Any attempt to upset this equilibrium is handled by the homeostatic system. Stressors that directly challenge physiological balance produce responses in order to meet set points. These stressors could be considered physiological stressors because they directly affect physiological functions and their effects are controlled by autonomic body receptor systems: viscerosensory pathways that activate subcortical autonomic circuits and directly affect stress-related motor neurons (Kovács *et al*., 2005), frequently without a lot of cortical input unless these systems are unable to handle the stress.

Hunger, thirst or discomfort brought on by cold or hot temperatures are examples of physiological stressors and they can have an impact on a variety of physiological indicators such as blood sugar levels, body temperature, and blood pressure. These difficulties cause the paraventricular nucleus' CRH-secreting neurons to become active, which starts a cascade of stress hormones. In order to trigger the adaptive responses (cardiovascular, respiratory, and thermoregulatory) and those of the sympatheticoadrenal system, both the neuroendocrine response and an autonomic efferent projection are triggered (Notari, 2005).

2.2.2 Psychological stressors

Higher-order brain circuits process psychological stressors, also known as emotional stressors, which involve learning, emotional, and cognitive processes. In the paraventricular nucleus (PVN), parvocellular neurosecretory motor neurons are activated in a manner like how physiological stresses are. These stimuli include social conflicts, incorrect handling, frightening stimuli, personal perceptions of being unable to cope, among many more. Visceral reactions to psychological stressors also involve modifications in the heart rate, breathing, digestion, and body temperature.

Psychological stressor such as sheltering from a predator call for various bodily and behavioural coping mechanisms. The first problem calls for a method that fosters social environment adaptation and coping while maintaining stability during change. The latter may call for an active coping strategy (escape) or passive coping approach (immobility) (Keay and Bandler, 2001). The centrally mediated, coordinated responses are triggered by the subjective experience of physical suffering or disability (including HPA-axis and autonomic activations). Although it may appear that psychological and physical stressors activate different pathways at a functional neuroanatomical level, stressors are frequently compound, both psychological and physical, therefore it is difficult to draw a clear distinction between them (Kovács *et al*., 2005).

2.3 Electroencephalography

Electroencephalography (EEG) is a real-time graphic representation of the small (of the microvolt range) spontaneous electrical currents of neurons that emerge from the cerebral cortex through electrodes positioned in various locations on the scalp in humans or head in many other species (Murrell and Johnson, 2006; Kaka *et al*., 2015). This approach can be used to test nociception because it is non-invasive, stress-free and painless (Sabow *et al.*, 2016). Animals' nociception and pain responses have been studied using EEG over the years (Grint *et al*., 2015; Kaka *et al*., 2015; Sabow *et al*., 2016). Previously, nociceptive response in horses (Murrell *et al*., 2003), dogs (Kongara *et al*., 2010; Kongara *et al*., 2013; Kaka *et al*., 2015), sheep (Otto and Gerich., 2001), pigs (Haga and Ranheim., 2005) and cattle (Zulkifli *et al*., 2014; Gibson *et al*., 2007) have been successfully measured using changes in the electroencephalogram spectrums and EEG has also been demonstrated to produce fast, sensitive and accurate results (Bo *et al*., 2003; Coetzee, 2013).

Fast Fourier Transform (FFT) techniques are used to analyse the frequency from the EEG recording and to determine the spectrum of the signals (Ang *et al*., 2017; Singh and Kanda, 2017). The frequencies of the signals are divided into four categories: delta frequency (4.0 Hz), theta frequency (4.1–8.0 Hz), alpha frequency (8.1–12.0 Hz), and beta frequency (12.1–30.0 Hz) (Raghazli *et al*., 2021). The slow-wave delta and theta frequency shows the sleepy state. A rise in high-frequency beta oscillation signals an animal's increased brain activity (Freeman and Quiroga, 2013), whereas alpha waves appear during mild and moderate stress (Jena, 2015). The combination of delta and theta activity shows that the unconscious state is accompanied with active brain activity. According to Seo and Lee (2010), the combination of alpha and beta activity shows active brain activity while in a conscious state.

The cortical pyramidal neuron in the brain generates an electrical signal during the processing of information (Babiloni *et al*., 2015; Bergamasco *et al*., 2006). The frequency, amplitude and timing of these brain oscillations are all variable, and they follow a particular spatiotemporal pattern. The animal's emotion may be reflected in the pattern (Cohen, 2017). These neural oscillations were measured using the EEG during the stressful period (Cohen, 2017; Sabow *et al*., 2018). In human, numerous investigations have demonstrated that EEG could deliver information on the amount of personal stress. EEG activity and human psychological stress levels were significantly correlated (Alshargie and Tang, 2017; Hou *et al*., 2015). Therefore, it is reasonable to assume that EEG has enormous potential to study an animal's brain reaction to various stressors (Freeman and Quiroga, 2013). Furthermore, EEG is a practical and non-invasive technique that can provide information on the physiological status of the animal (Jun and Smitha, 2016; Sabow *et al*., 2018) and for animal welfare management measures to be effective, more knowledge on animals' stress responses is required.

2.4 The canine auditory system

An animal's ability to hear is crucial to its survival. Hearing helps one to identify sounds and pinpoint their source. The ability of the animal to determine the source of the sound is ideal and crucial (Carlson, 2004). Dogs' ears play a significant role in beginning of the auditory process. The pinnae assist in directing sounds into the ear canal, where the organ of Corti's cell receptors transmit auditory information to the brain. When a sound is heard, neuronal impulses carry it to the sensory thalamus, where it is processed in parallel by the auditory cortex and amygdala (Levine, 2005).

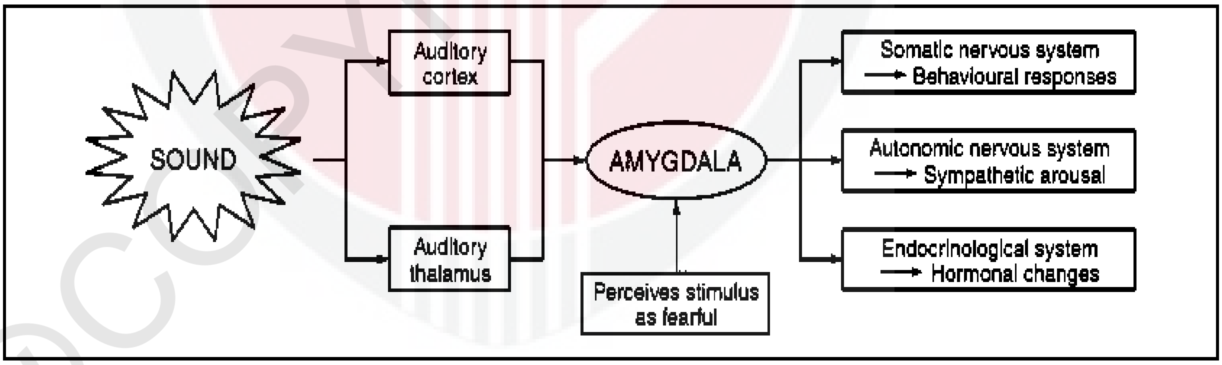


Figure 2.2: The mechanism of sound processing through the central nervous system which subsequently leading to an animal's behavioural reaction.

In addition to being activated by the auditory pathway as shown in the Figure 2.2 above, the amygdala is also activated when an animal experiences fear, stress, or anxiety. Animals typically exhibit an orienting response in response to sound, and many start exhibiting this reaction as immediately as they hear a sound. In this response, the brain becomes more awake while the body gets quieter. The heart rate may slow down for the brief period that an orienting reaction lasts (Leeds and Wagner, 2008). This is also consistent with a study by Fukuzawa and Kajino (2018) on the canine auditory stimulation of dogs receiving auditory stimulation for the first time, which discovered that the maximum heart rate was significantly higher during exposure to heartbeat sound than it was afterwards and that both heartbeat sound and classical sound tended to decrease average heart rate.

2.5 Music therapy in canine species

Music therapy uses special musical aspects such as the tone, rhythm, melody, harmony, dynamic and tempo in order to promote or assist movement, constructive relationships and/or enhanced emotional or cognitive states (Haase, 2012). Humans have been demonstrated to benefit greatly from music therapy, which includes alleviation from pain, lowered heart rate, blood pressure and anxiety levels, among other benefits (Witte *et al*., 2020; Umbrello *et al*., 2019). In veterinary medicine, there is considerable interest in implementing music therapy approaches from the human domain to enhance the health and welfare of companion, performance, and production animals (Lindig *et al*., 2020) due to the affordability and simplicity of administering music to domestic animals (Alworth and Buerkle, 2013).

Although the precise mechanics of these treatments are not fully known, one hypothesis is that music serves as a distractor stimulus, diverting the patient's attention from unpleasant or painful sensations to something good and pleasurable (King *et al*., 2022). In another study, (Nilsson, 2008) proposed that the widely accepted hypothesis explaining how music can relieve pain, anxiety and stress is that it serves as a distraction, drawing the patient's attention away from unpleasant stimuli and toward something uplifting. The patient might enter his or her "own world" when listening to music since it gives their mind something comforting and familiar to think about. With music, the dog's anxious-inducing noises can be obscured through the covering up of stressful sounds, such as the beeping of the monitor, vets and surrounding people's movements. This capability of linking music to feelings of peace and emotional well-being can be a useful tool for changing the dog's behaviour and preventing its surrender (Bernardini and Niccolini, 2015).

Based on the analysis by McDonald and Zaki (2020), it has been demonstrated that classical music has the power to drastically affect behaviours and physiological factors linked to the canine stress response, including heart rate variability, volume of vocalisation and amount of rest time. Findings from Fukuzawa and Kajino (2018) showed that although it was not statistically significant, the average heart rate drops throughout the heartbeat and classical music periods. Following exposure to the heartbeat therapy, maximum heart rate was noticeably lower. In another study involving two dog populations from a boarding facility and a shelter, when exposed to classical music, both populations spent substantially more time resting and less time vocalising (Kogan *et al*., 2012). These demonstrations of positive stimulation offer compelling evidence that musical exposure affects dogs' behavioural characteristics, with classical music commonly being reported to have a calming effect in potentially stressful settings including boarding kennels, rescue shelters and veterinarian offices (Lindig *et al*., 2020

CHAPTER 3
MATERIAL AND METHODS

3.1 Ethical statement

The research protocol in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Universiti Putra Malaysia (Approval no: UPM/IACUC/AUP-U028/2022).

3.2 Experimental design

The situation was set to mimic the dogs coming for elective surgery such as orchiectomy or ovariohysterectomy. Six volunteer dogs, aged between 1 year old to 7-year-olds were brought individually into the Student Surgery room, University Veterinary Hospital, Universiti Putra Malaysia. Heart rate, blood pressure, behaviour assessment and electroencephalogram were taken immediately as the dog arrived as the baseline (pre-music). Then, classical music was played on speaker for over 20 minutes. The beats per minutes (BPM) of the chosen classical music ranged from 70 to 130 BPM to match the tempo of the estimated resting heartbeat of the dogs as adapted from a study by King *et al*. (2022). After 20 minutes, the EEG measurements, heart rate, blood pressure and behaviour assessment were repeated and recorded as post-music. Throughout the duration of the study, all dogs were handled with minimal restraint according to the canine restraint SOP developed by the Office of the University Veterinarian and reviewed by Virginia Tech IACUC.

3.3 Electroencephalogram analysis

Electroencephalogram (EEG) is a non-invasive procedure in which tiny metal discs (electrodes) are connected to the scalp to assess the electrical activity in the brain (Murrel and Johnson, 2006). Fur areas of 7 cm in diameter between the medial canthi of the eyesand on the mastoid process were shaved and cleaned with 70% alcohol before placing the hydrogel conductive adhesive sterile disposable electrodes (Covidien llc, Mansfield, MA, USA) (Kaka *et al.*, 2016). An inverting (negative) electrode was placed on zygomatic process of the frontal bone, while a non-inverting (positive) electrode was placed on the mastoid process (Murrell & Johnson, 2006; Othman *et al.*, 2020). The electroencephalogram was recorded at a sampling rate of 1 kHz and raw EEG was resampled with low pass filter of 200 Hz into delta frequency (0.1 to 4 Hz), theta frequency (4.1 to 8 Hz), alpha frequency (8.1 to 12 Hz), and beta frequency (12.1 to 20 Hz) (Raghazli *et al.*, 2021). Care was taken in ensuring that the total impedance of the circuitry was less than 5 kOhms (Kaka *et al.*, 2016).

The signals were analysed offline using Chart 5.0 software (ADInstruments, Bella Vista, NSW, Australia) and were subjected to fast Fourier transformation (FFT). FFT is commonly used to quantify information within the raw EEG signal, and it is a mathematical process that changes the raw EEG signal from the time domain to the frequency domain, generating a power spectrum as shown in Figure 3.1. Simple descriptors including total power (Ptot, the total area under the curve) and median frequency (F50, the frequency below which 50% of Ptot lies) can be derived from the power spectrum (Murrell & Johnson, 2006).

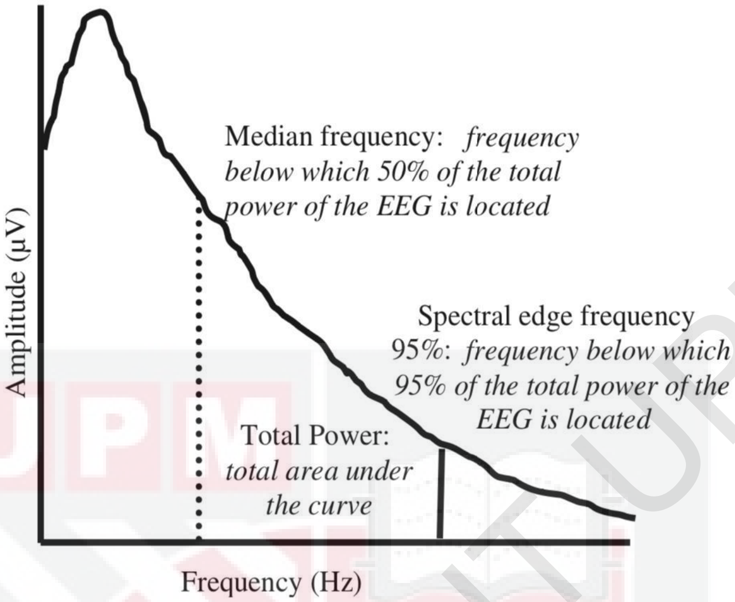


Figure 3.1: The EEG power spectrum including the total power and the median frequency

One-minute block EEG data were collected at baseline (pre-music), immediately after arrival and after 20 minutes into the music therapy (post-music). Each block was calculated for consecutive non-overlapping 1-s epochs (Figure 3.2). The root mean square of Ptot was calculated and the F50 was derived (Murrell & Johnson, 2006).

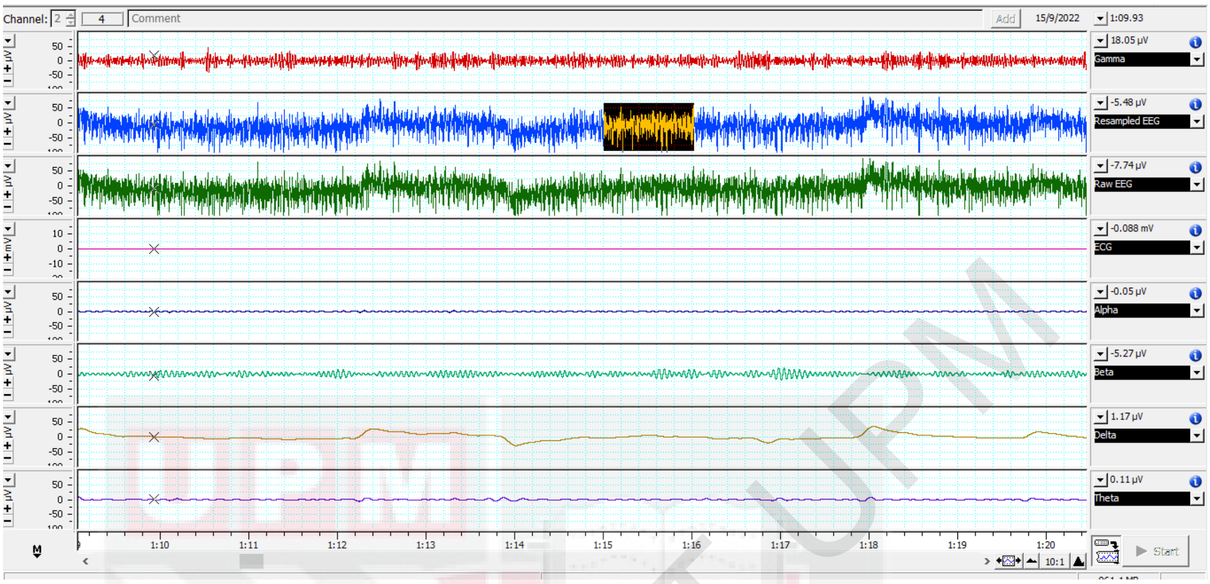


Figure 3.2: The “chart view” sample of Chart 5.0 software (ADInstruments) representing electroencephalography (EEG) activity of dog immediately after arrival at the Student Surgery room, UVH. The highlighted area (black background) is an example of a 1-s epoch (0:00:01:15 to 0:00:01:16) of EEG signal that was subjected to fast Fourier transformation (Murrell & Johnson, 2006).

3.4 Heart rate and blood pressure analysis

Heart rate was taken immediately as the dog arrived by using a 3M Littmann Classic III stethoscope. Non-invasive systolic, diastolic and mean blood pressure were measured using a multiparametric monitor (GE Healthcare, Helsinki, Finland). Blood pressure cuff of 40% to 60% circumference of the dog’s antebrachium was used to measure blood pressure (Kaka *et al*., 2016).

3.5 Clinic Dog Stress Scale (CDSS) analysis

The measuring of pain or stress is challenging since animals cannot verbally express their emotions to human caretakers (Lush and Ijichi, 2018; Reid *et al*., 2013). Therefore, some set of scale is needed in order to aid in the evaluation of their stress. In this study, Clinic Dog Stress Scale (CDSS) was used in which it evaluates the body regions that are involved in the stress response such as body posture, ear posture gaze, respirations, lips, activity and vocalization (Overall, 2013).

3.6 Statistical analysis

The data was tested for normal distribution using a Shapiro-Wilk test using SPSS Statistics Version 25 software (IBM Corporation, New York, USA). T test was used to determine the differences of values between the pre-music and post-music (n=6). The F50 and Ptot was analysed by using The SAS System. Tukey’s Studentized Range (HSD) Test was done to test the differences among sample means for significance.

CHAPTER 4

RESULTS

4.1 Electroencephalogram

Figure 4.1 shows the “chart view” sample of Chart 5.0 software (ADInstruments) representing electroencephalography (EEG) activity of dog for pre-music while Figure 4.2 represents the EEG activity of dog for post-music. The highlighted area (black background) is a 1-s epoch of EEG signal that was subjected to fast Fourier transformation (Murrell & Johnson, 2006). From these chart view, Ptot and the median frequency, F50 were calculated (Murrell & Johnson, 2006).

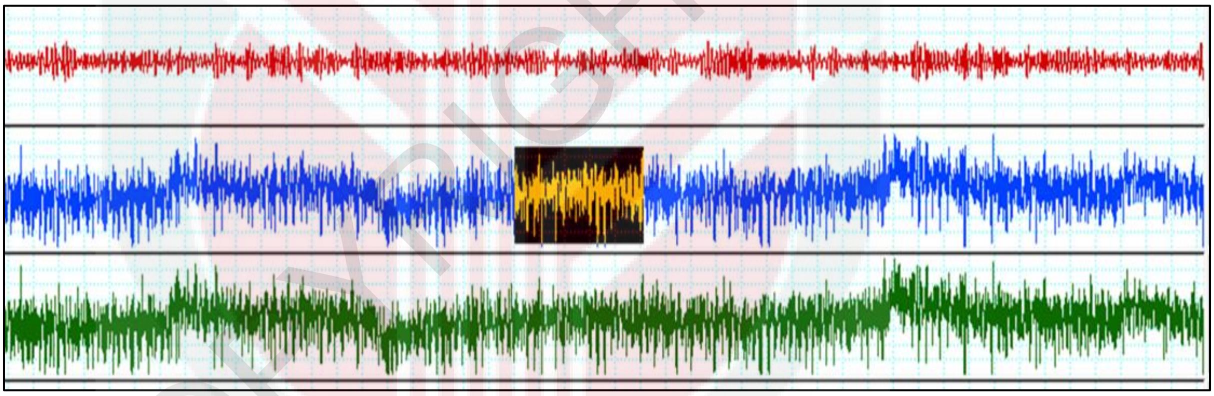


Figure 4.1: Pre-music chart view

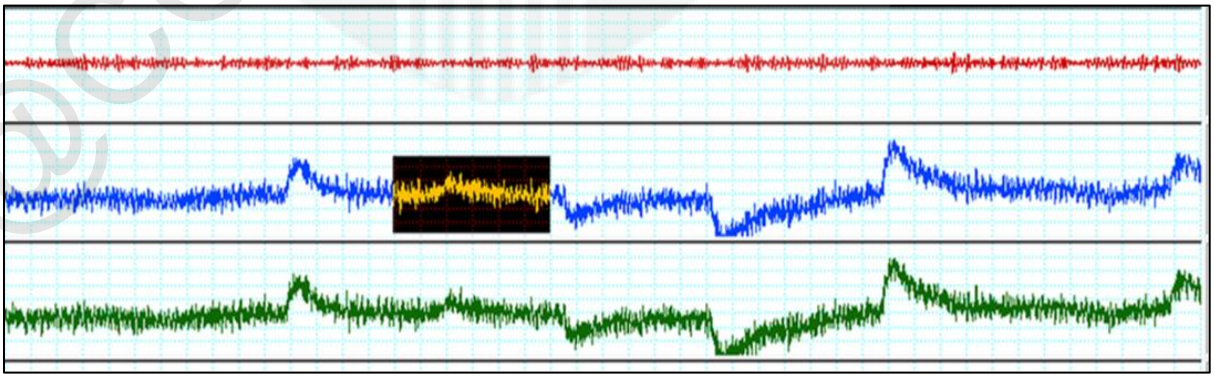


Figure 4.2: Post-music chart view

Figure 4.3 shows the total power (Ptot) or the total area under the power spectrum curve for pre-music and post-music. In this study, the Ptot of the dogs was higher (35.37 ± 2.36) immediately after arrival which significantly decreased (p = 0.0167) after the treatment with music (21.75 ± 0.86).

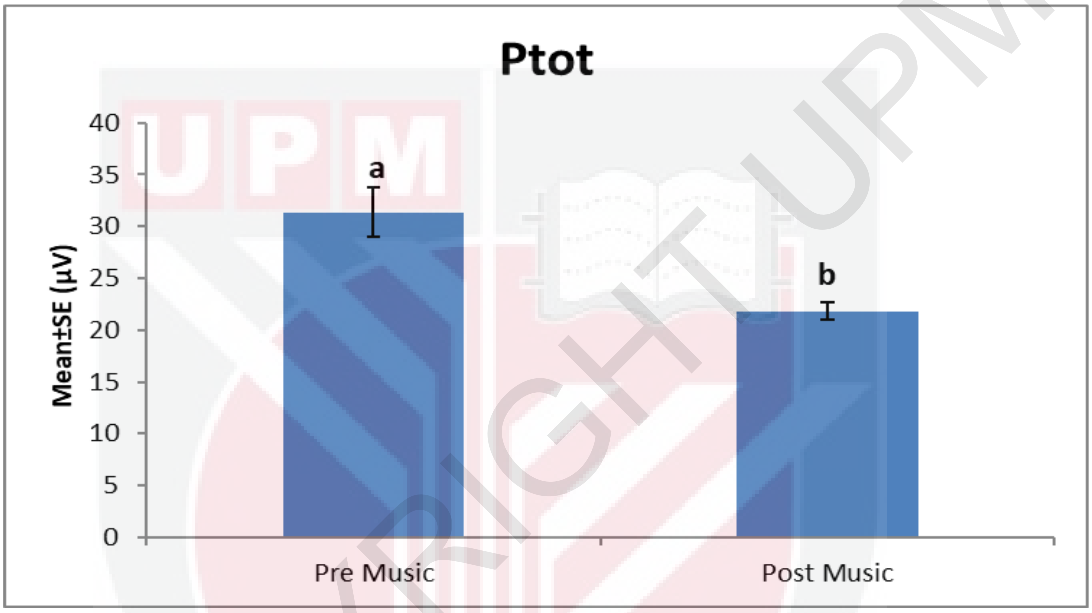


Figure 4.3: Total power (Ptot)

Figure 4.4 shows the median frequency (MF) or the frequency below which 50% of Ptot lies under the power spectrum curve for pre-music and post-music. In this study, the MF of the dogs was higher (35.37 ± 3.10) immediately after arrival which significantly decreased ($p < 0.0001$) after the treatment with music (13.28 ± 2.20).

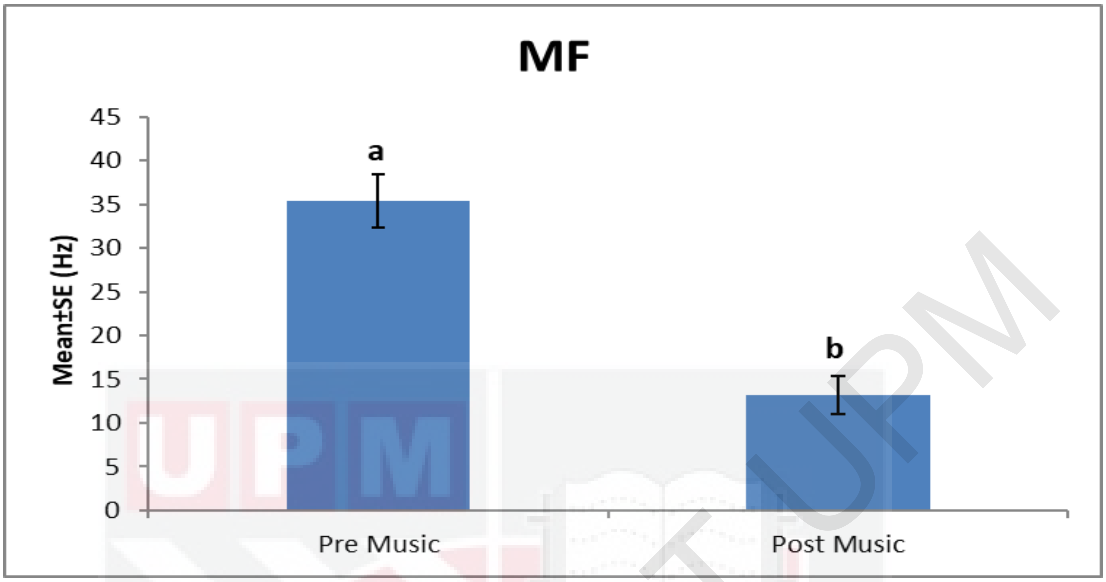
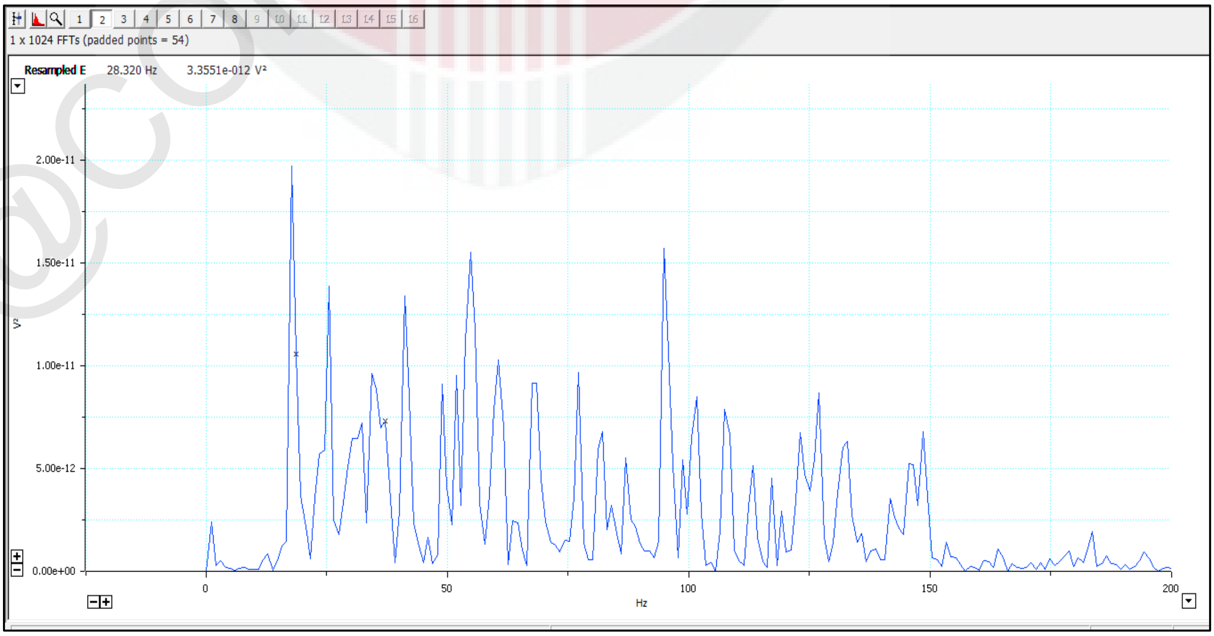


Figure 4.4: Median frequency

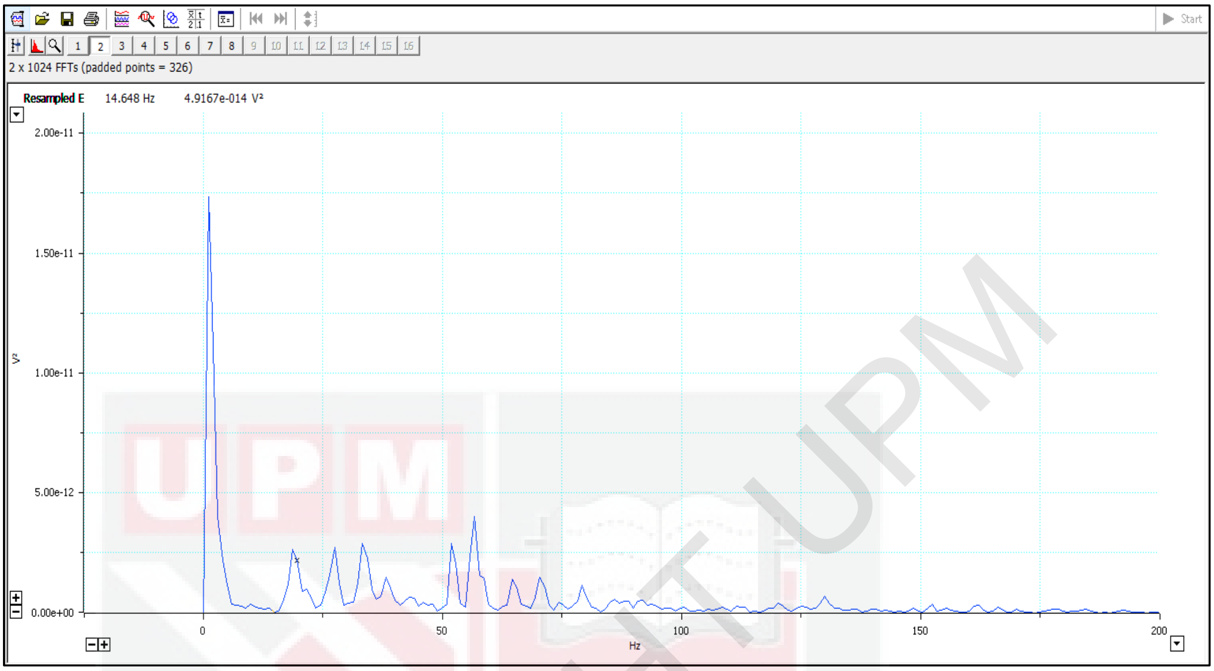
Figure 4.5 and Figure 4.6 show the chart in which median frequency under the power spectrum was derived from for pre-music and post-music, respectively. From these two figures, it was observed that the amplitude for pre-music is higher than post-music.



1 x 1024 FFTs (padded points = 54)

Resampled E 28.320 Hz 3.3551e-012 V²

Figure 4.5: Pre music median frequency



2 x 1024 FFTs (padded points = 326)

Resampled E 14.648 Hz 4.9167e-014 V²

Figure 4.6: Post music median frequency

4.2 Heart rate

Figure 4.7 shows the heart rate differences for each of the dog during the baseline (pre-music) compared to the post-music. It was observed that the heart rate during pre-music is higher than the post-music for all the dogs.

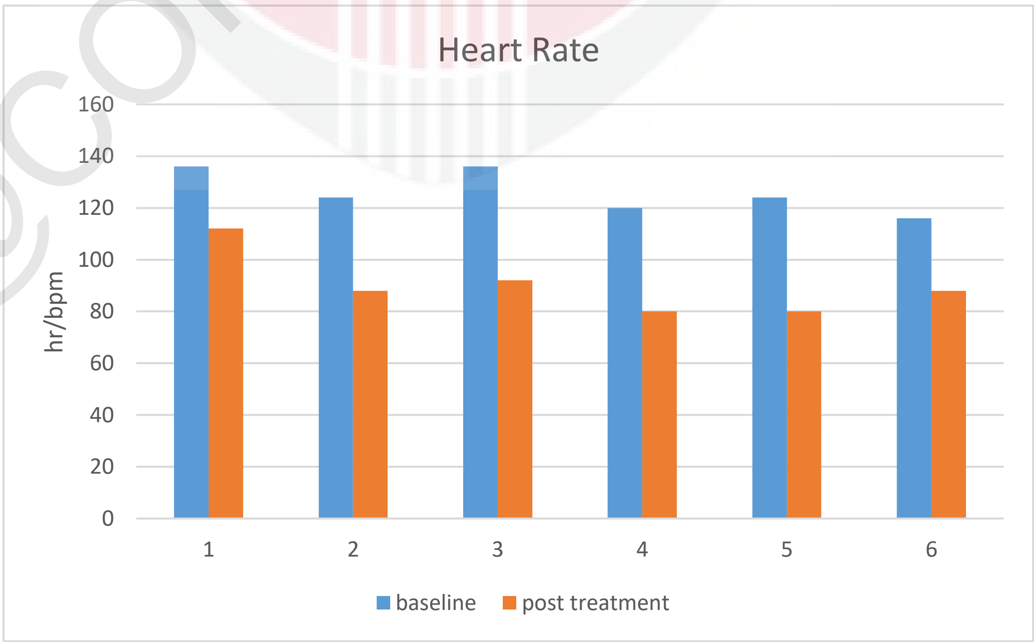


Figure 4.7: The heart rate differences between each dog

Table 4.1 shows the mean heart rate score at pre-music versus post-music. In this study, the mean heart rate of the dogs was higher (126.00 ± 8.29) immediately after arrival which significantly decreased (*p* = 0.0001) after the treatment with music (90.00 ± 11.79).

Table 4.1: Paired Samples t-test of the heart rate (n=6)

	Mean	Std. deviation	Sig. Value (*p*)
Pre-music	126.00	8.29	0.0001
Post-music	90.00	11.79	

4.3 Blood pressure

Table 4.2 shows the mean blood pressure during pre-music and post-music while Table 4.3 shows the average blood pressure for each of the dog during pre-music and post-music. It can be observed that the mean values of the mean arterial blood pressure (MABP) for pre-music (82.8 ± 18.35) and post-music (77.2 ± 9.68) do not show significant differences (*p* >0.05).

Table 4.2: Mean and standard deviation (SD) of systolic, diastolic and MABP of blood pressure

	Pre-music			Post-music		
	Systolic	Diastolic	MABP	Systolic	Diastolic	MABP
Mean	119.8	65.4	82.8	114.6	58.6	77.2
SD	16.15	19.91	18.35	11.48	8.44	9.68

Table 4.3: Average blood pressure

	Pre-music			Post-music		
	Systolic	Diastolic	Mean	Systolic	Diastolic	Mean
Dog 1	119	78	94	130	71	94
Dog 2	104	43	62	114	53	72
Dog 3	133	55	79	113	50	72
Dog 4	69	41	48	119	67	86
Dog 5	139	93	108	118	63	77
Dog 6	104	58	71	98	56	71

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4.4 Clinic Dog Stress Scale (CDSS)

Table 4.4 shows the paired samples t-test of the Clinic Dog Stress Scale (CDSS). From the test, the parameters that are statistically significant (*p* <0.05) includes the body posture, ear posture, gaze and lips while respiration, activity and vocalization showed non-significant result of (*p* >0.05).

Table 4.4: Paired Samples t-test of the CDSS (n=6)

Pair	Parameter	Pre-music and Post-music	Mean	t-value	Sig. Value (p)
1	Body posture	Pre-music	1.33	2.712	.042
		Post-music	.50		
2	Ear posture	Pre-music	1.67	3.162	.025
		Post-music	.33		
3	Gaze	Pre-music	2.50	5.000	.004
		Post-music	.83		
4	Respiration	Pre-music	2.00	3.503	.017
		Post-music	.50		
5	Lips	Pre-music	1.67	6.708	.001
		Post-music	.17		
6	Activity	Pre-music	.83	1.581	.175
		Post-music	.17		
7	Vocalization	Pre-music	.50	1.581	.175
		Post-music	.17		

CHAPTER 5
DISCUSSION

The positive and calming effect of music on canine species has been proven in numerous studies (Kogan *et al*., 2012; Fukuzawa and Kajino, 2018; King *et al*., 2022). In the present study, the EEG was used as one of the tools to identify stress in dogs in relation to classical music therapy and preoperative stress. From the experiment, the classical music therapy showed a significant result (p <0.05) between the pre-music and post-music. For EEG, the total power (Ptot) and the median frequency were used as the variables in describing the stress in the dogs. Interestingly, it was observed that both variables showed lower values during post-music compared to pre-music as shown in the results. In addition, by comparing the EEG spectrum in chart view as shown in Figure 4.1 and Figure 4.2, the difference of the amplitude in these two figures may suggest the changes from higher stress level to a calmer state. From these results, it can be derived that the dogs may have higher stress during the pre-music and become calmer after 20 minutes of music therapy. To the authors' knowledge, there is no previous studies using EEG as a stress indicator with music therapy reported to compare the results.

In this experiment, a significant difference in the mean heart rate was observed after the classical music therapy. Entrainment, a method of playing music at a specific pace to synchronise physiological reactions (Bradt, 2010) was used in this study to synchronise the beats per minute (BPM) of the music to the BPM of the dogs' heart rate. From the results, it could be possible that the heart rate was successfully entrained with the data showing a statistically significant results (p <0.05) between the pre-music and post-music. In a study by Fukuzawa and Kajino (2018), although the result was not statistically significant as achieved in current study, the average heart rate decreased throughout the classical music periods.

In this study, blood pressure did not differ significantly between pre-music and post-music. One factor that could affect the non-significance in this matter is that the mean blood pressure estimates were selected by combining the blood pressure from all the dogs in the study, not the specific individual dog who participated. During the experiment, there were two dogs that did not cooperate while taking the pre-music blood pressure, probably due to unfamiliar caretakers and novel environments. Their movements could have affected the accuracy of blood pressure measurements, resulting in the lowest blood pressure values in the ranges of hypotension. Beerda *et al*., (1997, 2000) states that the alteration in blood pressure is a neurophysiological sign of stress and it is a matter of fact, normal physiological reactions. This change in blood pressure were observed in a study by Bragg *et al*., (2015) which showed significant increment in the mean blood pressure when taken in hospital environment compared to the mean blood pressure taken at home.

In the current study, Clinic Dog Stress Scale (CDSS) was used as a scale to quantify the level of stress experienced by the dogs (King *et al*., 2022). The behaviours that showed statistically significant ($p < 0.05$) were the body posture, ear posture, gaze and lips while the respiration, activity and vocalization did not show significance. During the experiment, it can also be observed that the dogs progressively showed a calmer state and more relaxed towards the end of the music therapy in compared to the beginning of the therapy. Previous study by King *et al*., (2022) showed some slight differences in body posture and gaze, in which dogs with music treatment had lower CDSS score compared to those without music treatment in the control group. These scores suggest music may have had a positive impact. One difference between the current study and the one by King *et al*., (2022) is that, in their study, the evaluation of behaviours were done from video recordings while live scoring was done in this study. This could have an impact towards the results as some of the behaviours might be obscured due to the limitation of the video angle and the quality. Therefore, higher agreements could have been achieved by this study in relation to the real time live scoring.

CHAPTER 6
CONCLUSION

The findings from present study suggest that new surroundings such as veterinary hospitals and new caretakers are stressful for dogs, and therefore, classical music therapy can be used to reduce the stress. At the same time, the results of this study could also be valuable in enhancing the welfare of the dogs as stated in the American Veterinary Medical Association Animal Welfare Principles, “Procedures related to animal housing, management, care and use should be continuously evaluated, and when indicated, refined or replaced” (AVMA, 2006). With the fact that classical music is therapeutic, cost effective and are easy to be employed, it offers the opportunity to create a less stressful environments for the dogs at hospitals and clinics.

As mentioned in the literature review, the precise mechanism of music therapy is not fully known, but from the observation in this study, classical music managed to serve as a distractor stimulus and drew the dogs’ attention away from surrounding stressors. In addition, the music really showed calming effect in general over the course of the treatment and this is important for the dogs’ physical and mental well beings. In conclusion, the overall results of this study suggested that classical music therapy is effective in reducing the preoperative stress in canine undergoing elective surgery.

CHAPTER 7
RECOMMENDATIONS

Based on the results of this study, to provide a more accurate and reliable data with higher agreements, larger sample size should be opted in the further studies. In addition, this study was limited to only five weeks in which the recruitment for the subjects were also constrained. Therefore, more time allocations should be provided in future study for a better outcome. Also, further research can address the use of different species such as feline in a same study design.

This study was conducted to mimic the situations of the dogs coming in for elective surgery such as ovariohysterectomy or orchiectomy. In order to view the impact of classical music therapy in real case perspective, it is recommended that the recruitments are to be made from the dogs scheduled for the surgery. The study design should also be suited to the new location in which the parameters are to be taken.

Finally, it is also recommended that future study would implement the same study design with the addition of new treatments and parameters such as different genre of music, different exposure time to music and stress hormones (adrenaline and noradrenaline) as new stress parameter.

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APPENDICES

APPENDIX 1

Tukey's Studentized Range (HSD) Test for Alpha, Beta, Delta, Theta, Ptot and MF

Treatment	Variable	Mean	Standard Error
Pre-music	Alpha	2.29	0.28
	Beta	5.47	0.44
	Delta	17.7	1.54
	Theta	3.71	0.42
	Ptot	31.36	2.36
	MF	35.37	3.10
Post-music	Alpha	1.53	0.11
	Beta	2.75	0.12
	Delta	16.48	0.91
	Theta	2.69	0.22
	Ptot	21.75	0.86
	MF	13.28	2.20

APPENDIX 2

The Heart Rate for Each Dog

Dog 1:

	Baseline			Post Treatment		
	Systolic	Diastolic	MABP	Systolic	Diastolic	MABP
	116	80	95	88	66	73
	119	88	101	146	72	103
	118	94	102	140	82	106
	118	63	84	141	76	100
	126	66	90	139	61	88
Average	119	78	94	130	71	94

Dog 2:

	Baseline			Post Treatment		
	Systolic	Diastolic	MABP	Systolic	Diastolic	MABP
	112	44	63	98	58	74
	105	45	65	111	69	85
	105	44	63	124	44	64
	97	42	60	120	49	71
	103	39	57	116	47	68
Average	104	43	62	114	53	72

Dog 3:

	Baseline			Post Treatment		
	Systolic	Diastolic	MABP	Systolic	Diastolic	MABP
	125	49	71	128	48	69
	128	51	74	101	50	69
	144	63	91	111	49	71
	132	57	82	113	52	75
	138	55	79	113	53	75
Average	133	55	79	113	50	72

Dog 4:

	Baseline			Post Treatment		
	Systolic	Diastolic	MABP	Systolic	Diastolic	MABP
	71	53	58	132	72	93
	74	39	44	118	52	75
	62	30	42	108	62	80
	-	-	-	118	81	95
Average	69	41	48	119	67	86

Dog 5:

	Baseline			Post Treatment		
	Systolic	Diastolic	MABP	Systolic	Diastolic	MABP
	125	89	102	137	77	87
	130	83	99	93	55	68
	169	102	123	136	75	91
	135	84	105	109	46	62
	136	106	115	-	-	-
Average	139	93	108	118	63	77

Dog 6:

	Baseline			Post Treatment		
	Systolic	Diastolic	MABP	Systolic	Diastolic	MABP
	77	44	55	99	64	77
	116	43	62	114	50	72
	119	87	97	80	55	62
Average	104	58	71	98	56	71

APPENDIX 3

Paired Samples Statistics

Paired Samples t-test of The Heart Rate

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Baseline	126.00	6	8.295	3.386
	Post_Treatment	90.00	6	11.798	4.817

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Baseline - Post_Treatment	36.000	8.390	3.425	27.195	44.805	10.510	5	.000

APPENDIX 4

Paired Samples t-test of The Blood Pressure

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Baseline_systolic	111.33	6	25.272	10.317
	Post_tx_systolic	115.33	6	10.424	4.256

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Baseline_systolic - Post_tx_systolic	-4.000	26.465	10.804	-31.773	23.773	-.370	5	.726

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Baseline_diastolic	61.33	6	20.403	8.329
	Post_tx_diastolic	60.00	6	8.295	3.386

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Baseline_diastolic - Post_tx_diastolic	1.333	18.673	7.623	-18.262	20.929	.175	5	.868

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Baseline_mean	77.00	6	21.707	8.862
	Post_tx_mean	78.67	6	9.374	3.827

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Baseline_mean - Post_tx_mean	-1.667	22.527	9.197	-25.307	21.974	-.181	5	.863

APPENDIX 5

Paired Samples t-test of The Clinic Dog Stress Scale (CDSS)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	B4M_Body_posture	1.33	6	.516	.211
	PM_Body_posture	.50	6	.548	.224
Pair 2	B4M_Ear_posture	1.67	6	1.506	.615
	PM_Ear_posture	.33	6	.516	.211
Pair 3	B4M_Gaze	2.50	6	.837	.342
	PM_Gaze	.83	6	.408	.167
Pair 4	B4M_Respiration	2.00	6	.894	.365
	PM_Respiration	.50	6	.837	.342
Pair 5	B4M_Lips	1.67	6	.516	.211
	PM_Lips	.17	6	.408	.167
Pair 6	B4M_Activity	.83	6	1.329	.543
	PM_Activity	.17	6	.408	.167
Pair 7	B4M_Vocalisation	.50	6	.548	.224
	PM_Vocalisation	.17	6	.408	.167

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	B4M_Body_posture - PM_Body_posture	.833	.753	.307	.043	1.623	2.712	5	.042
Pair 2	B4M_Ear_posture - PM_Ear_posture	1.333	1.033	.422	.249	2.417	3.162	5	.025
Pair 3	B4M_Gaze - PM_Gaze	1.667	.816	.333	.810	2.524	5.000	5	.004
Pair 4	B4M_Respiration - PM_Respiration	1.500	1.049	.428	.399	2.601	3.503	5	.017
Pair 5	B4M_Lips - PM_Lips	1.500	.548	.224	.925	2.075	6.708	5	.001
Pair 6	B4M_Activity - PM_Activity	.667	1.033	.422	-.417	1.751	1.581	5	.175
Pair 7	B4M_Vocalisation - PM_Vocalisation	.333	.516	.211	-.209	.875	1.581	5	.175