UNIVERSITI PUTRA MALAYSIA

ELECTROENCEPHALOGRAPHIC CHANGES IN RESPONSE TO ELECTRICAL STIMULATION AND ACEPROMAZINE IN MINIMALLY ANAESTHETISED DOGS

YAP COCO

lp
FPV 2023 30

ELECTROENCEPHALOGRAPHIC CHANGES IN RESPONSE TO ELECTRICAL STIMULATION AND ACEPROMAZINE IN MINIMALLY ANAESTHETISED DOGS

YAP COCO

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan.

2023/2024

@COPYRIGHT UPM

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Electroencephalographic Changes in Response to Electrical Stimulation and Acepromazine in Minimally Anaesthetised Dogs", by Yap Coco and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirements for the course VPD 4999 – Final Year Project.

DR. UBEDULLAH KAKA
DVM (SAU, Pakistan), MSc (SAU, Pakistan), PhD (UPM)
Department of Companion Animal Medicine and Surgery,
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Supervisor)

PROF. DR. GOH YONG MENG
DVM (UPM), PhD (UPM)
Department of Veterinary Preclinical Sciences/
Research Management Centre,
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Co-Supervisor)

@COPYRIGHT UPM

ASSOC. PROF. DR. CHEN HUI CHENG

DVM (UPM), MVM (UPM), DVSc (Guelph)

Department of Companion Animal Medicine and Surgery,

Faculty of Veterinary Medicine,

Universiti Putra Malaysia

(Co-Supervisor)

@COPYRIGHT UPM

ACKNOWLEDGEMENT

Completing my Final Year Project (FYP) has been a journey marked by guidance, support, and collaboration, for which I owe immense gratitude to a group of remarkable individuals.

First and foremost, I want to extend my heartfelt gratitude to my supervisor, Dr. Ubedullah Kaka. His guidance, understanding, patience, and encouragement have been invaluable throughout this journey. In times when things seemed chaotic, Dr. Kaka's support and reassurance made a significant difference. Thank you for making sure that things not only got done but also for reminding me that challenges are not as overwhelming as they may seem.

I would also like to extend my gratitude to my co-supervisors, Prof. Dr. Goh Yong Meng and Assoc. Prof. Dr. Chen Hui Cheng. Their scholarly advice and valuable insights are important in shaping this project. Their expertise has not only provided clarity and direction but has also significantly contributed to the success of my work.

I want to express special acknowledgement to Dr. Azalea Hani Othman and Zul for in completing the EEG data extraction and analysis. Their contributions have truly enhanced the overall quality of the project. The completion of experiments would not have been possible without the support of the UVH staff. Their collaborative efforts and assistance were integral to the execution of my research objectives, and I extend my sincere thanks for their contributions.

To my friends and family, I owe a debt of gratitude for their unwavering support and encouragement. Their belief in my abilities and the understanding during the challenging phases of the project provided the motivation necessary to persevere.

In a heartfelt tribute to the silent companions in my life, the doggies, I extend my deepest thanks. May they always be remembered with love and gratitude for their contribution

©COPYRIGHT UPM

to the project. In acknowledging their involvement, I also express my sincere hope that they find eternal peace, free from suffering and pain.

@COPYRIGHT UPM

TABLE OF CONTENTS

© COPYRIGHT UPM

LIST OF FIGURES

© COPYRIGHT UPM

LIST OF TABLES

@COPYRIGHT UPM

LIST OF ABBREVATIONS

ACE	Acepromazine
ANOVA	Analysis of Variance
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CNS	Central Nervous System
ECG	Electrocardiogram
EEG	Electroencephalography
EPSP	Excitatory Postsynaptic Potential
EtCO2	End-tidal Carbon Dioxide
EtHal	End-tidal Halothane
FFT	Fast-Fourier Transformation
GABA	Gamma-aminobutyric acid
GLM	Generalised Linear Model
HSD	Tukey's Studentized Range
IPSP	Inhibitory Postsynaptic Potential
MF	Median Frequency
MPSJ	Majlis Perbandaraan Subang Jaya
NMDA	N-methyl-D-aspartate
Ptot	Total Power
RAS	Reticular Activating System
RMS	Root Mean Square
SpO_2	Oxygen saturation in blood
TET	Tetanus Pulse Train

@COPYRIGHT UPM

ABSTRAK

Abstrak daripada kertas projek yang dikemukan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD – 4999 (Projek Ilmiah Tahun Akhir)

PERUBAHAN ELEKTROENSEFALOGRAFI AKIBAT RANGSANGAN ELEKTRIK DAN ACEPROMAZINE PADA ANJING YANG DIBIUS SECARA MINIMA

Oleh

Yap Coco

2023

Penyelia: Dr. Ubedullah Kaka

Elektroensefalografi (EEG) telah digunakan untuk menilai keberkesanan analgesik ubat-ubatan yang bertindak pada sistem saraf pusat (CNS) pada haiwan-haiwan yang telah dibius. Peningkatan frekuensi median (MF) dengan pengurangan jumlah kuasa (Ptot) telah dilaporkan apabila haiwan mengalami kesakitan (nociception). Kebanyakan penyelidikan mendapati analgesik sedatif atau ubat yang bertindak pada CNS menghalang peningkatan MF apabila dirangsang oleh rangsangan elektrik. Walaubagaimanapun, persoalan telah dikemukakan bahawa perubahan MF ini adalah akibat sedasi yang menekan pada CNS. Acepromazine (ACE) ialah fenotiazin yang bersifat sedatif tanpa kesan analgesik. Oleh itu, kajian ini bertujuan untuk menyiasat kesan sedatif ACE ke atas EEG apabila dirangsang elektrik. Enam anjing diberikan ACE secara intravena (IV), bermula dari 0.05 mg/kg, diikuti dengan 0.1 mg/kg dan 0.2 mg/kg dengan waktu selang 20 minit. Anjing dibius dengan propofol pada 5 mg/kg IV dan dikekalkan dengan halotana pada kepekatan antara 0.85 hingga 0.95% (Model Anestesia Minima). Rangsangan elektrik dihasilkan dengan menggunakan perangsang saraf periferi N272 pada 40 mA dan 50 Hz selama 5 saat. Setiap haiwan berfungsi sebagai kawalan untuk sendiri. Garis dasar EEG direkodkan sebelum sebarang ACE atau rangsangan elektrik. Seterusnya, EEG direkodkan sebelum ACE dan selepas ACE semasa rangsangan elektrik. Keputusan ini

@COPYRIGHT UPM

menunjukkan bahawa MF pasca rangsangan meningkat ($p < 0.05$) dalam ketiga-tiga rawatan ACE. Sebaliknya, Ptot pasca rangsangan tidak berubah dalam semua kumpulan rawatan ($p > 0.05$). Oleh itu, ACE tidak menghalang peningkatan MF dan tiada kesan ke atas Ptot selepas dirangsang elektrik. Kesimpulannya, kesan analgesik agen bertindak berpusat yang dinyatakan oleh MF EEG mungkin bukan disebabkan oleh sedasi.

Kata kunci: acepromazine, elektroensefalografi, rangsangan elektrik, kesakitan (nociception), sedasi, model anestesia minima

@COPYRIGHT UPM

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD4999 - Final Year Project.

ELECTROENCEPHALOGRAPHIC CHANGES IN RESPONSE TO ELECTRICAL STIMULATION AND ACEPROMAZINE IN MINIMALLY ANAESTHETISED DOGS

By

Yap Coco

2023

Supervisor: Dr. Ubedullah Kaka

Electroencephalography (EEG) has been reported as a tool to evaluate analgesic effect of centrally acting drugs in minimally anaesthetised animals. Notable increase in median frequency (MF) coupled with total power (Ptot) reduction were reported when animals experienced nociception. Most research found sedative analgesics and other centrally acting drugs prevent increase or even suppress MF in response to noxious stimulation. However, a question raised as effect MF associated with analgesia could be due to sedation caused by CNS depression. Acepromazine (ACE) is a phenothiazine having sedative but no analgesic effect. Therefore, this study aims to investigate the sedative effect of ACE on EEG in response to noxious electrical stimulus. Six dogs were subjected to incremental doses of ACE intravenously (IV), starting from 0.05 mg/kg, followed by 0.1 mg/kg and 0.2 mg/kg with interval of 20 minutes. Anaesthesia was induced with propofol at 5 mg/kg IV and maintained with halothane at stable concentration between 0.85 to 0.95% (Minimal Anaesthesia Model). Noxious electrical stimulus was delivered with a peripheral nerve stimulator N272 at 40 mA and 50 Hz for 5 seconds. Each animal served as its own control. Baseline EEG was recorded before any ACE or noxious electrical stimulation. EEG were recorded prior to ACE and

©COPYRIGHT UPM

following ACE during noxious electrical stimulation. Result showed that post-stimulation MF increased significantly ($p < 0.05$) in all treatment groups. In contrast, there was no significant ($p > 0.05$) difference in post-stimulation Ptot in all treatment groups. Thus, ACE neither suppressed nor prevented increase in MF and has no effect on Ptot in response to noxious electric stimulus. MF is associated with nociception, whereas Ptot does not. It is concluded that analgesic effect of the centrally acting agents expressed by MF of EEG may not be due to sedation.

Keywords: acepromazine, electroencephalography, electrical stimulation, nociception, sedation, minimal anaesthesia model

@COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

Electroencephalography (EEG) is a neurophysiological technique that records the electrical activity of the cerebral cortex by using electrodes placed on the skull or head (Murrell & Johnson, 2006). It has been reported as an instrument to measure the antinociceptive effect of centrally acting drugs in minimally anaesthetised animals and it remains as an ongoing interest among the researchers (Kaka *et al.*, 2015; 2016; Kongara *et al.*, 2010; 2012). When animals in light plane anaesthesia experienced nociception, it has been reported that EEG spectrum shifted towards higher frequency and lower amplitude, coupled with increased median frequency (MF) in response to noxious stimulation (Murrell *et al.*, 2003). However, total power (Ptot) has been reported to be not directly related to MF, changes in Ptot may represent a different component of nociception than MF (Kaka *et al.*, 2015; 2016; Karna *et al*., 2020).

Researchers found that sedative analgesics and other centrally acting drugs such as ketamine, lidocaine, morphine, and various others prevent increase or even suppress MF in response to noxious stimulation (Haga & Ranheim, 2005; Johnson *et al.*, 2005a; Kaka *et al*., 2015; Karna et al., 2020; Kongara *et al.*, 2010, 2012). However, meloxicam, an analgesic without central mode of action, has reported to have no effect on MF suppression (Kaka *et al.*, 2015). This raises a question that the analgesic effect of the centrally acting agents expressed by MF of EEG could also be due to depression of central nervous system (CNS) caused by sedation as most centrally acting drugs mentioned have both sedative and analgesics effect. To date, the question remains unanswered.

To address the research question, it is necessary to use sedative drugs without any analgesic effect to investigate EEG changes in response to noxious stimulation. Acepromazine (ACE) is a phenothiazine agent with sedation but no analgesic effect (Plumb, 2011). This study aimed to investigate the sedative effect of ACE on EEG changes in response to electrical noxious

©COPYRIGHT UPM

stimulation in minimally anaesthetised dogs. It was hypothesized that ACE would not have any effect on EEG nociceptive indices. The information from this study will further provide insight on association between MF and nociception.

@COPYRIGHT UPM

CHAPTER 2

LITERATURE REVIEW

2.1 Neurophysiological basis of EEG

Electroencephalography (EEG) is a neurophysiological technique that records the electrical activity originating from the cerebral cortex through the electrodes that placed on the skull or head of the animals (Murrell & Johnson, 2006). The product of the EEG is known as electroencephalogram, a graphical plot of the voltage changes over the function of time, which indicative of the animals' brain activity. These voltage changes recorded in EEG do not arise from action potentials; rather, they result from the summation of the excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs). This is due to the longer lasting duration of postsynaptic potential, which can reach up to 10 ms, enable these potential changes to be summed up and detected extracellularly by the electrodes on the scalp, in contrast to the action potentials that only last about 1 ms (Kirschstein & Köhling, 2009).

The generation of postsynaptic potential in EEG is attributed to the cortical pyramidal neurons, characterized with a large somatic body and long apical dendrites oriented perpendicular to the cortical surface (Greene, 2002; Murrell & Johnson, 2006; Müller-Putz *et al.*, 2015). The long apical dendrites of pyramidal cells projects upward to the cortical surface while basal dendrites project horizontally from the cell body to form the synapses with other neurons via corticocortical and thalamocortical nerve fibres (Klein, 2020). The corticocortical afferents are evenly distributed and form synapses with pyramidal cells in all cortical layers. Pyramidal cells in superficial layers synapses with the non-specific thalamocortical afferents, whereas pyramidal cells in deeper cortical layers synapses with specific thalamocortical afferents. Due to the proximity to the scalp, it has been suggested that most postsynaptic potential recorded in EEG has been proposed to originate from non-specific thalamocortical afferents as it located more superficially in the cortical layers (Kirschstein & Köhling, 2009).

©COPYRIGHT UPM

The synapses can be classified as excitatory or inhibitory based on the type of neurotransmitter and receptor. In inhibitory synapses, the presynaptic calcium influx triggers the release of gamma-aminobutyric acid (GABA) from presynaptic vesicle that diffuse across the synaptic cleft and subsequently binds to the postsynaptic GABA receptors. This leads to the influx of chloride ions and efflux of potassium ions resulting in generation of IPSPs. In contrast to GABAergic synapse, excitatory synapses release glutamate as the neurotransmitter. Upon binding to postsynaptic AMPA and NMDA receptors, glutamate induces influx of sodium and calcium ions resulting in generation of EPSPs (Kirschstein & Köhling, 2009; Purves *et al*., 2001).

Generation of EPSPs and IPSPs in the postsynaptic neurons results in the formation of the current sinks and sources in the extracellular fluid (Murrell & Johnson, 2006). The location and nature of these postsynaptic change will determine the polarity of voltage changes recorded at the scalp. In EEG, a downward deflection indicates a positive voltage change induced by input from deep cortical EPSPs and superficial IPSPs. Conversely, an upward deflection indicates a negative voltage change attributed to the inputs from superficial EPSPs and deep cortical IPSPs (Kirschstein & Köhling, 2009; Klein, 2020). However, the magnitude of changes caused by a single EPSPs and IPSPs are too attenuated to be recorded as EEG waves. Hence, a substantial number of neurons need to synchronize simultaneously, producing the postsynaptic potentials in same direction to allow the summation of EEG waves that are measurable at the scalp (Kirschstein & Köhling, 2009).

©COPYRIGHT UPM

2.2 Analysis of EEG data

To allow interpretation of the raw EEG signals, Fast Fourier Transform (FFT) technique is commonly used to generate spectrum of signals by transforming the raw EEG signals from time domain into frequency domain (Murrell & Johnson, 2006).The EEG signals can be further classified into four common brain waves based on the frequency bands, denoted by delta (0 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 12 Hz) and beta (> 12 Hz). These brain waves have a different functionality according to the frequency band as reported in humans as tabulated in Table 2.1 (Ibrahim & Shamsuddin, 2023).

Table 2.1: Functionality of the human brain waves according to the frequencies.

Brain wave	Frequency (Hz)	Functionality
Delta	0 – 4	Unconscious, deep sleep or under deep anaesthesia
Theta	4 – 8	Early phase of sleep or dreaming. Associated with working memory
Alpha	8 – 12	Rest but awake. Greater alpha activity associated with loss in attentiveness and attention
Beta	> 12	Alert and involvement of information processing and integration. Greater beta activity associated with activation of neurological system and increased body's energy level

[Adapted from Ibrahim & Shamsuddin. (2023)]

Analysis of different EEG brain waves has been adopted in animal EEG (Pellegrino & Álvarez, 2023; Sabow *et al.*, 2017; 2019; Zulkifli *et al.*, 2014). In EEG of normal adult dogs, the brain waves typically fall between the range of theta and alpha activity (6 – 12 Hz), with alpha rhythm being the predominant brain wave (Pellegrino & Álvarez, 2023). In livestock animals, increase in beta wave has been reported when they subjected to conscious pain (Zulkifli *et al*., 2014; Sabow *et al*., 2017; 2019). However, the division and functionality of brain waves is not well established yet in animals. Careful interpretation must be made when inferred these functionalities across different animal species. A review by Murell and Johnson (2006) also

©COPYRIGHT UPM

suggested the interpretation based on arbitrary EEG frequency spectrum similarly across different species may provide wrong assumption.

@COPYRIGHT UPM

2.3 Nociception indices of EEG

It has been reported that EEG can be utilized as a tool to objectively measure the nociception in animals under minimal anaesthesia model, including horses (Murrell *et al.*, 2003), lambs (Gibson *et al.*, 2007; Johnson *et al.*, 2005a), deer (Johnson *et al.*, 2005b), dogs (Kaka *et al.*, 2016) and pigs (Kells *et al.*, 2017; Reiser *et al.*, 2022). Two common nociception indices derived from the EEG power spectrum are MF and Ptot. The MF is defined as frequency below which 50 % of the total power of EEG is located whereas Ptot is the total area under the curve (Murrell and Johnson, 2006). Murrell *et al.* (2003) reported increased MF and reduced Ptot when the minimally anaesthetised horses subjected to noxious stimuli during castration. However, this is not a common finding as there are few studies unable to identify the inversely proportional relationship between MF and Ptot (Kaka *et al.*, 2015; Karna *et al.*, 2020; Kongara *et al.*, 2010). Nevertheless, MF still recognised as a reliable EEG-derived indicator for nociception as reported in many studies (Gibson *et al*., 2007; Johnson *et al.*, 2005; Murrell *et al.*, 2003; Kongara *et al.*, 2014; Kaka *et al.*, 2015; Karna *et al.*, 2020).

These EEG-derived nociception indices, together with the use of minimal anaesthesia model, allowing the application of EEG in comparing the efficacy of the analgesics effect of centrally acting drugs or sedative analgesics. It has been reported that these centrally acting drugs able to prevent the increase or even suppress the MF in response to noxious stimuli (Haga & Ranheim, 2005; Johnson *et al.*, 2005a; Kongara *et al.*, 2010, 2012; Kaka *et al.*, 2015; Kongara *et al.*, 2020). However, the effect of sedation caused by these drugs on MF is still not know yet.

©COPYRIGHT UPM

CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical Approval and Consent

The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM) with reference number of UPM/IACUC/AUP-U029/2023 (Appendix I).

3.2 Animals

Six dogs (n = 6) with body weight of 10.5 ± 2.26 kg, comprising of 4 females and 2 males with healthy appearance were selected in this study. All dogs were obtained from Pusat Kurungan Haiwan Majlis Perbandaraan Subang Jaya (MPSJ) in Taman Perindustrian Puchong Utama, Selangor. The animals were then transferred and kept in individual dog kennel in at the Faculty of Veterinary Medicine, UPM, one day before the start of experiment. Animals were provided with feed twice per day and free access to water. The dog kennel was cleaned once per day.

3.3 Experimental design

Each animal served as its own control for the baseline EEG. Data were collected before and after the treatment with ACE. All dog received treatment of incremental dose of ACE intravenously, starting from 0.05 mg/kg to 0.1 mg/kg and 0.2 mg/kg with the interval of 20 minutes.

3.4 Anaesthesia protocol

The dogs were fasted 12 hours prior to the anaesthesia. Intravenous catheterisation of right cephalic vein was performed using 20-gauge intravenous indwelling catheter. Anaesthesia was induced with propofol at 5 mg/kg intravenously. Following induction, animals were intubated using endotracheal tube and connected to anaesthetic machine (SurgiVet). The dogs

©COPYRIGHT UPM

were maintained at end-tidal halothane concentration (EtHal) between 0.85% and 0.95% using minimal anaesthesia model (Murrell *et al.*, 2003; Johnson *et al.*, 2005a; Murrell & Johnson, 2006). All dogs breathed spontaneously and were positioned on the right lateral recumbency. Intravenous fluid was administered at 10 ml/kg/hour using Hartmann's solution. Warm air blanket was used to maintain the body temperature of animals. Pulse oximeter was placed on the dogs' tongue to monitor the oxygen saturation in the blood (SpO_2). Blood pressure cuff was placed on the left antebrachium of the dogs to monitor the blood pressure. Heart rate, respiratory rate, EtHal and end-tidal carbon dioxide (EtCO2) were monitored using a multi-parameter monitor (GE Healthcare B40 Patient Monitor V2 PTO Model).

3.5 Noxious electrical stimulation

The noxious electrical stimulation was produced by a peripheral nerve stimulator NS272 (Fisher & Paykel Healthcare). Two 23-gauge, 1-inch disposable needles were placed subcutaneously at the lateral aspect of distal metatarsus distance 2 cm apart to serve as electrodes which connected to the device with terminal alligator clips (Figure 3.1). External mode and tetanus pulse train (TET) function (Figure 3.2) were selected to deliver the transcutaneous noxious electrical stimulus with current of 40 mA and tetanus frequency of 50 Hz for 5 seconds at left hindlimb using the device as described by Kaka *et al.* (2016). Noxious electrical stimulus was applied 2 minutes after the start of EEG recording for pre-treatment group. For the treatment group, the noxious electrical stimulus was given 5 minutes following the administration of the ACE.

©COPYRIGHT UPM

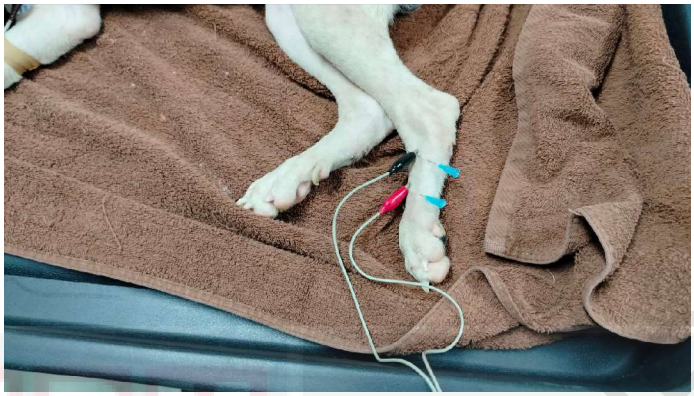

Figure 3.1: Placement of electrodes for transcutaneous noxious electrical stimulus.

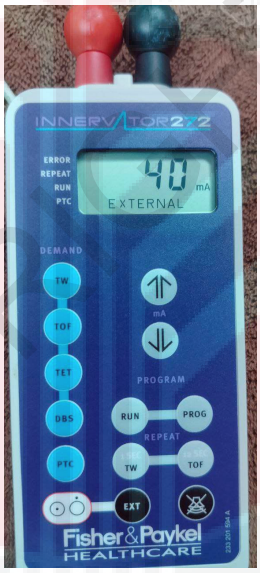

Figure 3.2: External mode and tetanus pulse train (TET) function of peripheral nerve stimulator NS272 to deliver transcutaneous noxious electrical stimulus at current of 40 mA and tetanus frequency of 50 Hz for 5 seconds.

©COPYRIGHT UPM

3.6 EEG recording

Shaving of the fur with a minimum diameter of 5 cm on the zygomatic process of the left frontal bone, left mastoid process and area caudal to occipital process by clipper and shaver was performed to allow the placement of electrodes. The shaved areas were cleaned with a gauze soaked with 70 % isopropyl alcohol to remove tiny furs and dirt debris that adhered to the skin. Three sterile disposable adhesive white foam electrode patches with solid hydrogel (Ceracarta Top Trace) were placed at zygomatic process of left frontal bone, left mastoid process and caudal to occipital process respectively to serve as inverting, non-inverting, and ground electrodes for recording of EEG (Kaka *et al.*, 2015) as shown in Figure 3.3. Electrocardiogram (ECG) was recorded in standard lead II configuration by placing the negative electrode on the right forelimb below the elbow point while positive electrode was placed on the loose skin at medial aspect of left hindlimb around the stifle joint.

Figure 3.3: Placement of inverting (negative), non-inverting (positive) and ground electrodes on the head to record EEG.

The EEG was sampled at the rate of 1 kHz by connecting a personal laptop installed with Chart 5.0 data acquisition software (ADInstruments Pty Ltd) to a Powerlab 26T data acquisition system hardware (ADInstruments Pty Ltd) as shown in Figure 3.4. The raw EEG signals were analysed and subjected to Fast Fourier Transformation (FFT) using Chart 5.0

@COPYRIGHT UPM

data acquisition software (ADInstruments Pty Ltd) which then further filtered into resampled EEG using a low-pass digital filter with a cut-off frequency of 200 Hz. The raw EEG was then filtered into delta (0.1 to 4 Hz), theta (4.1 to 8 Hz), alpha (8.1 to 12 Hz) and beta waves (12.1 to 20 Hz) by the band-pass digital filter based on their specific bandwidth frequency. Once the stabilisation of the anaesthesia achieved by maintaining the EtHal between 0.85 to 0.95 %, Baseline EEG was recorded before any intervention including administration of ACE and noxious electrical stimulation. EEG signals (Figure 3.5) were recorded prior to ACE and following ACE administration during noxious electrical stimulation.

The EEG signals were analysed offline using the Chart 5.0 data acquisition software (ADInstruments Pty Ltd). The EEG data were processed and extracted in the 10-seconds block from consecutive non-overlapping 1-second epoch. The wave signals 10 seconds before and 2 seconds after noxious electrical stimulation were excluded due to mechanical and electrical interference. The 10-seconds block before and after the noxious electrical stimulation, excluding the wave signals under mechanical and electrical interference were taken for data analysis. The root mean square (RMS) of Ptot and each wave form were calculated for consecutive non-overlapping 1-second epoch. The MF was derived automatically from the spectrum at upper frequency of 200 Hz using LabChart 8.1.27 Reader (ADInstruments Pty Ltd).

@COPYRIGHT UPM

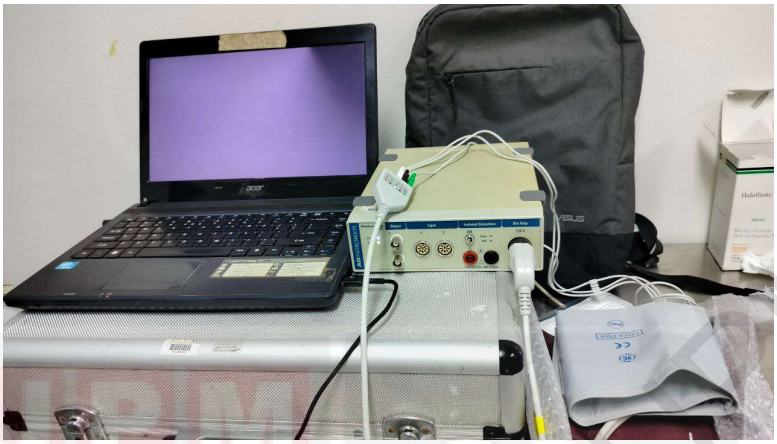

Figure 3.4: Recording of EEG Using Powerlab 26T Data Acquisition System Hardware (ADInstruments Pty Ltd).

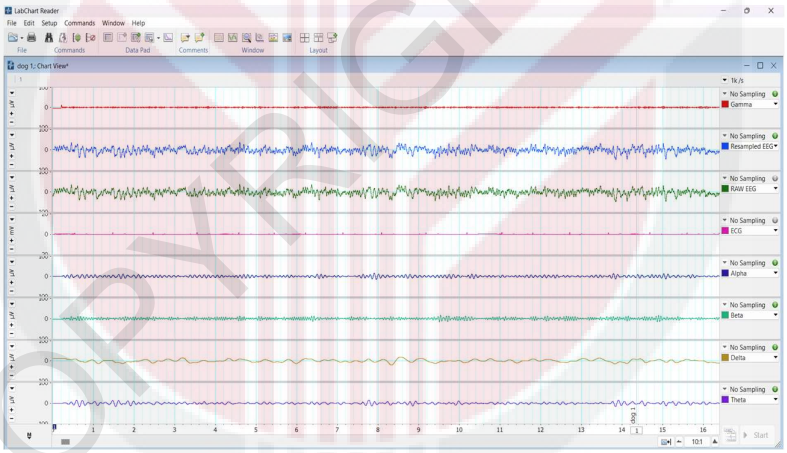

Figure 3.5: The Chart View of the Chart 5.0 Data Acquisition Software (ADInstruments Pty Ltd) which displayed EEG signals recorded in the eight channels.

@COPYRIGHT UPM

3.7 Statistical analysis

The data are presented in the mean ± standard deviation and was analysed using SAS software version 9.1 (SAS Inst. Inc.) with generalised linear model (GLM) procedure. Comparison within and between treatment groups was performed, and significant differences among means were determined using Tukey's Studentized Range (HSD) post-hoc test. All data analysis were conducted at a 95% confidence level and only considered statistically significant if $p < 0.05$.

@COPYRIGHT UPM

CHAPTER 4

RESULTS

4.1 The EEG

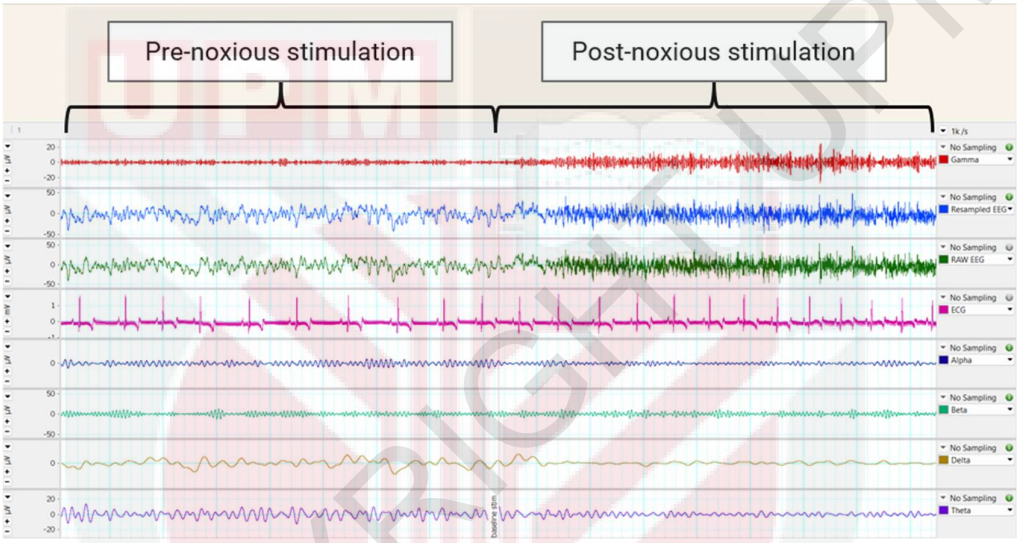

Figure 4.1: Comparison between EEG signals before and after noxious electrical stimulation in Chart View of the Chart 5.0 Data Acquisition Software (ADInstruments Pty Ltd).

The Figure 4.1 illustrates a comparison between EEG signals before and after the noxious electrical stimulation in the Chart View. A noticeable shift can be appreciated in EEG signals, transitioning from high-amplitude and low-frequency to low-amplitude and high-frequency pattern in response to the noxious electrical stimulation.

©COPYRIGHT UPM

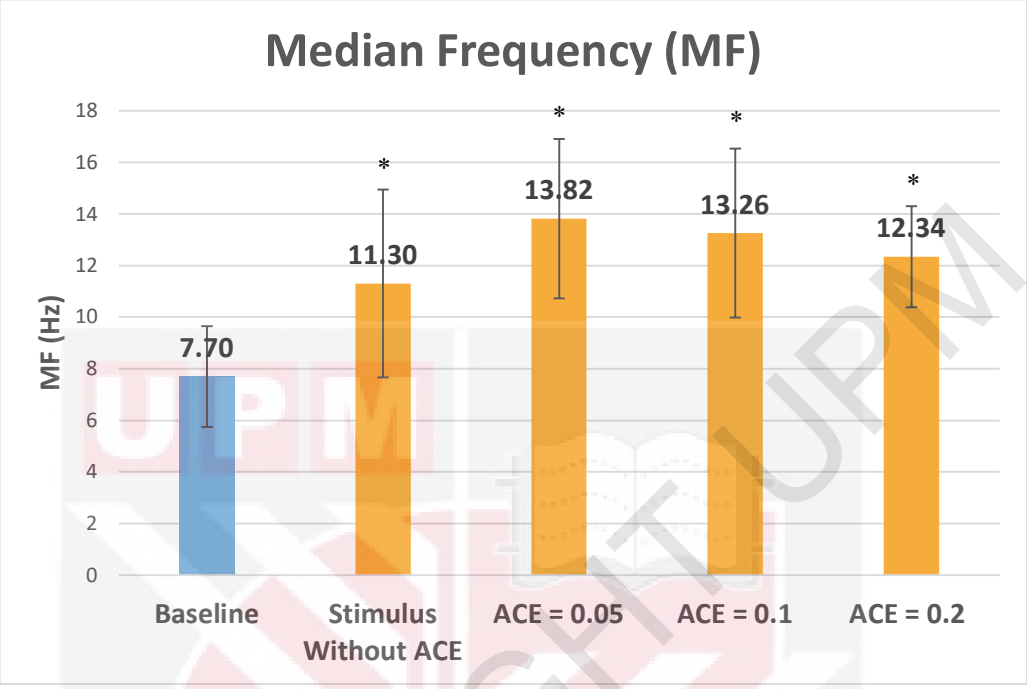

Figure 4.2: Comparison between baseline and post-stimulation MF without ACE and with ACE = 0.05 mg/kg, ACE = 0.1mg/kg, ACE = 0.2 mg/kg. The asterisk (*) denotes a significant difference in the mean value of post-stimulation MF compared to baseline MF at $p < 0.05$.

The Figure 4.2 represents the changes in post-stimulation MF in pre-treatment and post-treatment groups in comparison to baseline MF. Generally, it depicts an upward trend in changes in MF across all treatment groups in response to the noxious electrical stimulation. The post-stimulation MF increased significantly ($p < 0.05$) at all treatment groups compared to baseline MF.

@COPYRIGHT UPM

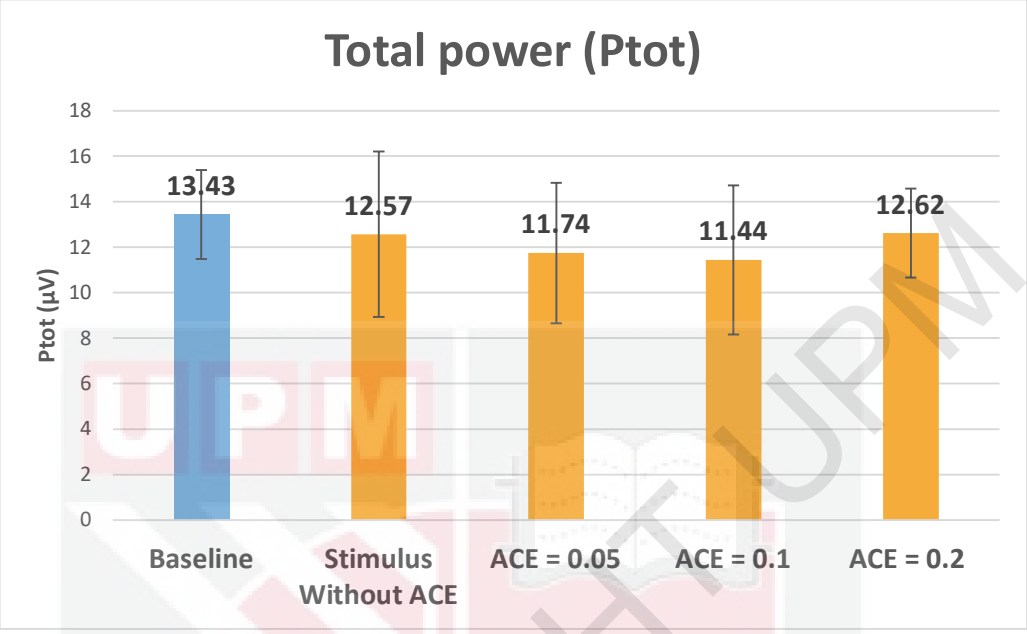

Figure 4.3: Comparison between baseline and post-stimulation Ptot without ACE and with ACE = 0.05 mg/kg, ACE = 0.1mg/kg, ACE = 0.2 mg/kg. The asterisk (*) denotes a significant difference in the mean value of post-stimulation Ptot compared to baseline Ptot at $p < 0.05$.

The Figure 4.3 depicts the changes in post-stimulation Ptot in all treatment groups in comparison to the baseline Ptot. In contrast to MF, there is a downward pattern between baseline and post-stimulation Ptot across all treatment groups. However, the changes between the baseline and post-stimulation Ptot are not statistically significant ($p > 0.05$) for all treatment groups. This shows there is no statistically differences between baseline and post-stimulation Ptot for all treatment groups.

@COPYRIGHT UPM

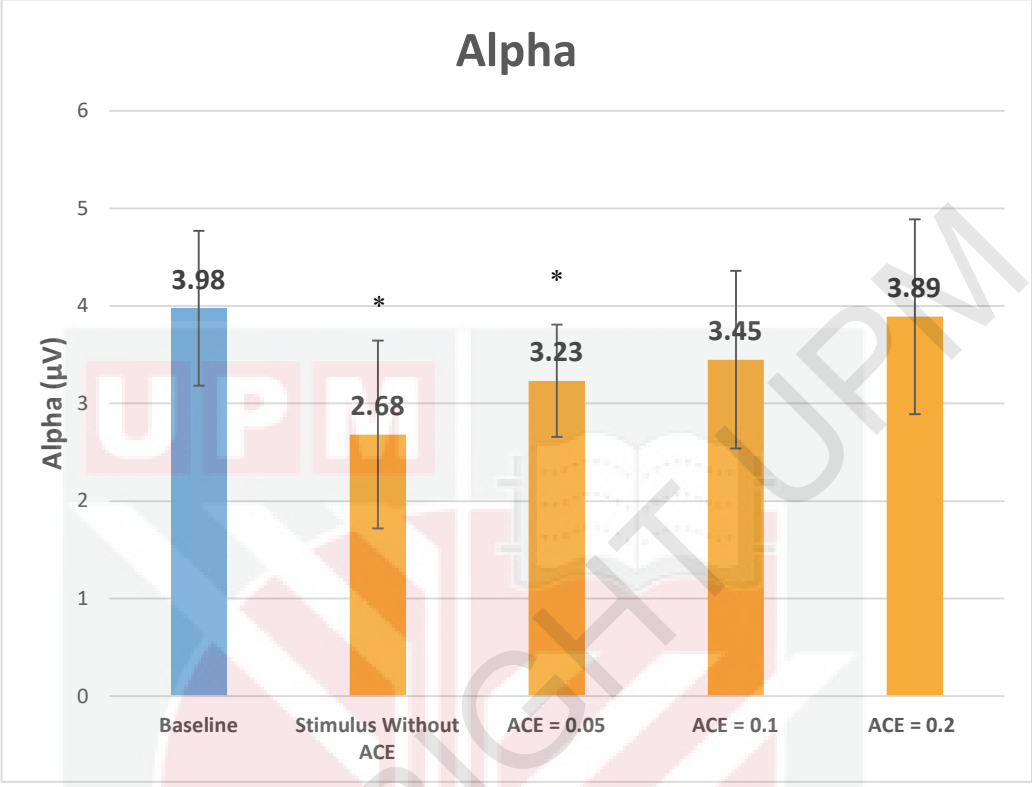

Figure 4.4: Comparison between baseline and post-stimulation RMS of alpha wave in dogs without ACE and with ACE = 0.05 mg/kg, ACE = 0.1mg/kg, ACE = 0.2 mg/kg. The asterisk (*) denotes a significant difference in the mean value of post-stimulation alpha wave compared to baseline alpha wave at $p < 0.05$.

The Figure 4.4 shows the changes in post-stimulation RMS of alpha wave in all treatment groups in comparison to the baseline RMS of alpha wave. The post-stimulation alpha wave decreases in all treatment groups in comparison to the baseline alpha wave but only significant without ACE and with ACE = 0.05 ($p < 0.05$).

©COPYRIGHT UPM

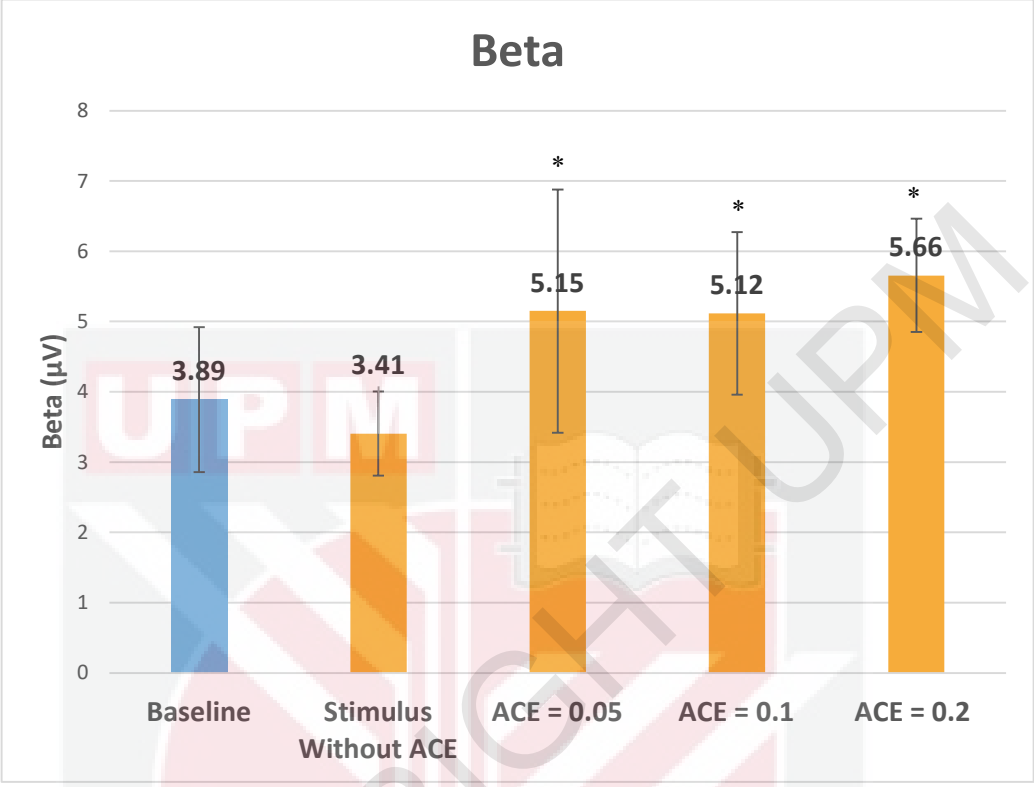

Figure 4.5: Comparison between baseline and post-stimulation RMS of beta wave in dogs without ACE and with ACE = 0.05 mg/kg, ACE = 0.1mg/kg, ACE = 0.2 mg/kg. The asterisk (*) denotes a significant difference in the mean value of post-stimulation beta wave compared to baseline beta wave at $p < 0.05$.

The Figure 4.5 illustrates the changes in post-stimulation RMS of beta wave in all treatment groups in comparison to the baseline RMS of beta wave. Notably, there is a significant increase in post-stimulation beta wave in all ACE treated groups compared to baseline beta wave ($p < 0.05$).

@COPYRIGHT UPM

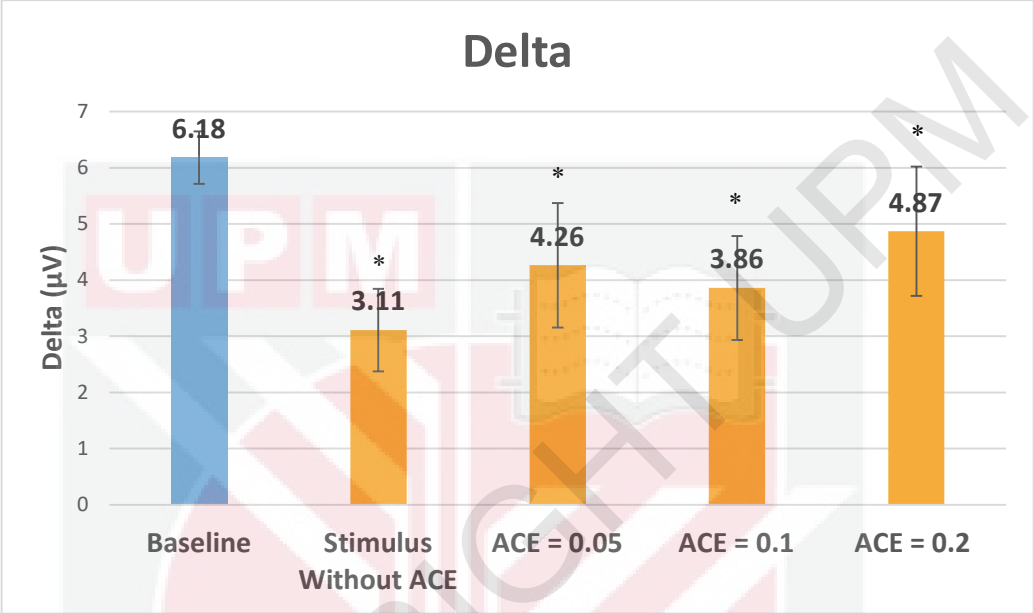

Figure 4.6: Comparison between baseline and post-stimulation RMS of delta wave in dogs without ACE and with ACE = 0.05 mg/kg, ACE = 0.1mg/kg, ACE = 0.2 mg/kg. The asterisk (*) denotes a significant difference in the mean value of post-stimulation delta wave compared to baseline delta wave at $p < 0.05$.

The Figure 4.6 represents the changes in post-stimulation RMS of delta wave in all treatment groups in comparison to the baseline RMS of delta wave. In contrast to beta wave, the post-stimulation delta wave decreased significantly in all treatment groups when compared to baseline delta wave ($p < 0.05$).

©COPYRIGHT UPM

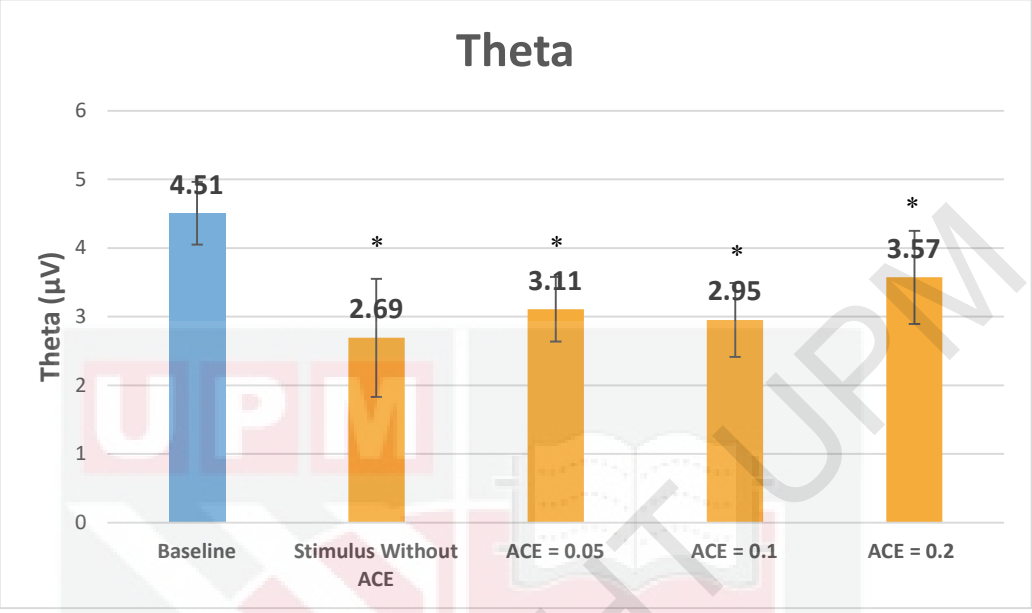

Figure 4.7: Comparison between baseline and post-stimulation RMS of theta wave in dogs without ACE and with ACE = 0.05 mg/kg, ACE = 0.1mg/kg, ACE = 0.2 mg/kg. The asterisk (*) denotes a significant difference in the mean value of post-stimulation theta compared to baseline theta at $p < 0.05$.

The Figure 4.7 reveals the changes in post-stimulation RMS of theta wave in all treatment groups in comparison to the baseline RMS of theta wave. Generally, there is a significant drop in the RMS of theta wave after noxious electrical stimulation in all treatment groups when compared to the baseline ($p < 0.05$).

@COPYRIGHT UPM

4.2 Physiological parameters

Table 4.1: Comparison between pre-stimulation and post-stimulation heart rate and respiratory rate in dogs before and after treatment with ACE = 0.05 mg/kg, ACE = 0.1mg/kg, ACE = 0.2 mg/kg.

Stimulus	Pre-treatment	ACE = 0.05	ACE = 0.1	ACE = 0.2
		Heart Rate		
Before	102.00 ± 15.406	117.00 ± 22.554	113.25 ± 17.056	111.75 ±17.933
After	128.25* ± 17.821	125.75* ± 21.030	124.25* ± 19.500	124.50* ± 27.135
		Respiratory Rate		
Before	15.00 ± 6.633	13.50 ± 6.658	13.25 ± 5.560	13.25 ± 5.560
After	23.25 ± 13.022	14.50 ± 6.807	14.50 ± 4.509	15.00 ± 3.266

The asterisk (*) denotes a significant difference in the mean value of post-stimulation compared to pre-stimulation mean value at $p < 0.05$.

Table 4.2 reveals the mean of pre-stimulation and post-stimulation heart rate differed statistically significant across all treatment group ($F(1,3) = 20.979$, $p = 0.001$) based on repeated measure ANOVA. Post-hoc analysis with Bonferroni adjustment revealed the heart rate increased significantly after noxious electrical stimulation ($p < 0.05$). In contrast, the mean of the respiratory rate did not statistically differ before and after noxious electrical stimulation ($p > 0.05$).

@COPYRIGHT UPM

CHAPTER 5
DISCUSSION

In this study, desynchronization or arousal has been observed in all dogs when subjected to electrical noxious stimulation, showing shifting of EEG waves from a high-amplitude and low-frequency pattern to a low-amplitude and high-frequency pattern. This EEG change is a typical EEG response to various noxious stimulations, as reported in various studies, observed in dogs (Kaka *et al.*, 2015; 2016; Kongara *et al.*, 2010), as well as other animals, such as red deer (Johson *et al.*, 2005b), horses (Murrell *et al.*, 2003); rats (Peng *et al.*, 2010) and calves (Gibson *et al.*, 2007) and cattle (Zulkifli *et al.*, 2014). The arousal response has been suggested to be associated with cerebral processing of noxious stimuli or experience of pain in conscious individuals (Murrell *et al.*, 2007). Peng *et al.* (2010) suggested activation of cerebral cortex may be attributed to direct activation of ascending arousal system by the noxious stimuli. The cerebral cortex would not be activated if only involving the spinothalamic tract (Peng *et al.*, 2010), as somatic sensory information can be projected to reticular formation of brain stem via different circuits including spinothalamic, spinoreticular, spinomesencephalic and postsynaptic dorsal column tracts (Muir III & Woolf, 2001). Additionally, a study by Murrell *et al.* (2007), concluded electrical stimuli are the most reliable and repeatable way to produce noxious stimulation; conversely, the mechanical and thermal stimulation produce variable EEG responses, despite ability of most nociceptors to detect multiple types of noxious stimuli by most nociceptors.

Previous studies reported that antinociceptive effect of most centrally acting agents or sedative analgesics prevent the increase in MF in response to noxious stimulation (Haga & Ranheim, 2005; Johnson *et al.*, 2005a; Kaka *et al.*, 2015; Karna *et al.*, 2020; Kongara *et al.*, 2010; 2012), however, the effect of sedation on these MF changes is not known yet. In current result, post-stimulation MF increased significantly in all treatment groups ($p < 0.05$). The significant increase in MF after noxious electrical stimulation suggests that the dogs experienced

@COPYRIGHT UPM

nociception. As a reliable indicator for nociception, increase in MF has been reported as a common EEG response to nociception among various animal species (Gibson *et al.*, 2007; Johnson *et al.*, 2005; Murrell *et al.*, 2003; Kongara *et al.*, 2014; Kaka *et al.*, 2015; Karna *et al.*, 2020). Additionally, the physiologically parameters, specifically the heart rate also raised significantly after noxious electrical stimulation. Similar findings have been reported in sheep and goats showing a rise in heart rate and blood pressure in response to noxious stimulation (Haga *et al.* 2001; Otto & Gerich, 2001).

Unlike MF, there is no statistically significant changes in Ptot in response to noxious electrical stimulation across all treatment groups when comparing to baseline Ptot in present study ($p < 0.05$). Previous study by Murrell *et al.* (2003), found an increase in MF is accompanied with decrease in Ptot following noxious stimulation in castrated horses. Conversely, many studies have found that the changes in Ptot is not inversely proportional to MF when response to noxious electrical stimulation (Kaka *et al.*, 2015; Karna *et al.*, 2020; Kongara *et al.*, 2010). It has been suggested the reduction in Ptot is possibly related to inadequacy of anaesthesia and depletion in EtHal due to noxious stimulation (Murrell *et al.*, 2003). Karna *et al.* (2020) also proposed that the changes in Ptot may represent a different component of nociception when compared to MF. Further research is required to investigate the role of Ptot in EEG response.

The post-stimulation beta wave increased significantly in ACE-treated groups when compared to baseline beta wave. It is suggested that increased beta wave activity in response to electrical stimulus is due to nociception. Similar increase in beta activity has reported in livestock animals during conscious pain (Zulkifli *et al.*, 2014; Sabow *et al.*, 2017; 2019). The post-stimulation alpha wave decreases in all treatment groups in comparison to the baseline alpha wave but only significant without ACE and with ACE = 0.05 ($p < 0.05$). In this study, there is a significant drop in RMS of both delta and theta waves after noxious electrical stimulation in all treatment groups when compared to baseline data ($p < 0.05$). These findings are consistent

©COPYRIGHT UPM

with study by Haga and Ranheim (2005), in which there is a decrease in delta, theta and alpha bands following castration in three-weeks-old piglets under halothane anaesthesia without any analgesia. Hence, this study proposed there will be increase in beta activity and reduce in alpha, delta and theta activity in EEG when animals subjected to noxious stimulation.

@COPYRIGHT UPM

CHAPTER 6
CONCLUSION

The findings from present study shows ACE does not prevent or suppress the MF as other centrally acting drugs or sedative analgesics when animals subjected to noxious stimuli. Additionally, the current result shows ACE has no effect on Ptot regardless of dosage during the noxious electrical stimulation. Increase in beta activity and reduce in alpha, delta and theta activity has been shown in this study as response to noxious stimulation. In conclusion, ACE neither suppressed nor prevented increase in MF and has no effect on Ptot in response to noxious electric stimulus. MF is associated with nociception, whereas Ptot does not. It is concluded that analgesic effect of the centrally acting agents expressed by MF of EEG may not be due to sedation

@COPYRIGHT UPM

CHAPTER 7
RECOMMENDATIONS

It is recommended that future study would implement the same study design incorporates different group of sedative drugs to evaluate the effect of sedation on EEG. This includes benzodiazepine drugs such as diazepam, midazolam or zolazepam or alpha 2 adrenoceptor agonist drugs such as xylazine and detomidine. It is also recommended to have a washout period between the administration of different dosage of ACE to avoid potential accumulative effects of the AC

@COPYRIGHT UPM

REFERENCES

Gibson, T. J., Johnson, C. B., Stafford, K. J., Mitchinson, S. L., & Mellor, D. J. (2007). Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal*, *55*(4), 152–157. https://doi.org/10.1080/00480169.2007.36760

Greene, S. A., 2002. Monitoring the Electroencephalogram. In: Veterinary Anesthesia and Pain Management Secrets. Hanley & Belfus, Inc., Philadelphia, pp. 131-134.

Haga, H. A., Tevik, A., & Moerch, H. (2001). Electroencephalographic and cardiovascular indicators of nociception during isoflurane anaesthesia in pigs. Veterinary Anaesthesia and Analgesia, 28(3), 126–131. doi:10.1046/j.1467-2987.2001.00051.x

Ibrahim, M. S., Kamat, S. R., & Shamsuddin, S. (2023). The Role of Brain Wave Activity by Electroencephalogram (EEG) in Assessing Cognitive Skills as an Indicator for Driving Fatigue: A Review. Malaysian Journal on Composites Science and Manufacturing, 11(1), 19-23. https://doi.org/10.37934/mjcsm.11.1.1931

Johnson, C. B., Wilson, P. R., Woodbury, M. R., & Caulkett, N. A. (2005). Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (Cervus elaphus): electroencephalographic responses. *Veterinary Anaesthesia and Analgesia*, *32*(2), 61–71. https://doi.org/10.1111/j.1467-2995.2005.00228.x

Johnson, C., Stafford, K., Sylvester, S., Ward, R., Mitchinson, S., & Mellor, D. (2005). Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal*, *53*(6), 433–437. https://doi.org/10.1080/00480169.2005.36589

Kaka, U., Goh, Y. M., Chean, L. W., & Chen, H. C. (2016). Electroencephalographic changes associated with non-invasive nociceptive stimulus in minimally anaesthetised dogs. *Polish Journal of Veterinary Sciences*, *19*(4), 675–683. https://doi.org/10.1515/pjvs-2016-0086

Kaka, U., Hui Cheng, C., Meng, G. Y., Fakurazi, S., Kaka, A., Behan, A. A., & Ebrahimi, M. (2015). Electroencephalographic Changes Associated with Antinociceptive Actions of Lidocaine, Ketamine, Meloxicam, and Morphine Administration in Minimally Anaesthetized Dogs. *BioMed Research International*, *2015*, 1–10. https://doi.org/10.1155/2015/305367

Karna, S. R., Chambers, P., Johnson, C. B., Singh, P., Stewart, L. A., Lopez-Villalobos, N., & Kongara, K. (2020). Effect of combinations of morphine, dexmedetomidine and maropitant on the electroencephalogram in response to acute electrical stimulation in anaesthetized dogs. *Journal of Veterinary Pharmacology and Therapeutics*, *43*(6), 538–546. https://doi.org/10.1111/JVP.12889

Kells, N. J., Beausoleil, N. J., Sutherland, M. A., Morrison, R. M., & Johnson, C. B. (2017). Electroencephalographic assessment of oral meloxicam, topical anaesthetic cream and cautery iron for mitigating acute pain in pigs (Sus scrofa) undergoing tail docking.

@COPYRIGHT UPM

Veterinary Anaesthesia and Analgesia, *44*(5), 1166–1174. https://doi.org/10.1016/J.VAA.2017.02.004

Kirschstein, T., & Köhling, R. (2009). What is the Source of the EEG? *Clinical EEG and Neuroscience*, *40*(3), 146–149. https://doi.org/10.1177/155005940904000305

Kongara, K., Chambers, J., & Johnson, C. (2012). Effects of tramadol, morphine or their combination in dogs undergoing ovariohysterectomy on peri-operative electroencephalographic responses and post-operative pain. *New Zealand Veterinary Journal*, *60*(2), 129–135. https://doi.org/10.1080/00480169.2011.641156

Kongara, K., Chambers, J. P., & Johnson, C. B. (2010). Electroencephalographic responses of tramadol, parecoxib and morphine to acute noxious electrical stimulation in anaesthetised dogs. *Research in Veterinary Science*, *88*(1), 127–133. https://doi.org/10.1016/J.RVSC.2009.05.012

Kongara, K., McIlhone, A., Kells, N., & Johnson, C. (2014). Electroencephalographic evaluation of decapitation of the anaesthetized rat. *Laboratory Animals*, *48*(1), 15–19. https://doi.org/10.1177/0023677213502016

Klein, B.G. and Rossmeisl, J.H. (2020) 'The Electroencephalogram and Sensory-Evoked Potentials', in Cunningham's Textbook of Veterinary Physiology. Sixth. St. Louis, MO: Elsevier, pp. 158–165.

Murrell, J. C., & Johnson, C. B. (2006). Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics*, *29*(5), 325–335. https://doi.org/10.1111/J.1365-2885.2006.00758.X

Murrell, J. C., Johnson, C. B., White, K. L., Taylor, P. M., Haberham, Z. L., & Waterman-Pearson, A. E. (2003). Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia*, *30*(3), 138–146. https://doi.org/10.1046/J.1467-2995.2003.00138.X

Murrell, J. C., Mitchinson, S. L., Waters, D., & Johnson, C. B. (2007). Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia*, *98*(3), 366–371. https://doi.org/10.1093/bja/ael377

Müller-Putz, G. R., Riedl, R., & C., S. (2015). Electroencephalography (EEG) as a Research Tool in the Information Systems Discipline: Foundations, Measurement, and Applications. Communications of the Association for Information Systems, 37, pp-pp. https://doi.org/10.17705/1CAIS.03746

Muir III, W. W., & Woolf, C. J. (2001). Mechanisms of pain and their therapeutic implications. Journal of the American Veterinary Medical Association, 219(10), 1346-1356. https://doi.org/10.2460/javma.2001.219.1346

Otto, K. A., & Gerich, T. (2001). Comparison of simultaneous changes in electroencephalographic and haemodynamic variables in sheep anaesthetised with halothane. *Veterinary Record*, *149*(3), 80–84. https://doi.org/10.1136/vr.149.3.80

Pellegrino, F., & Gómez Álvarez, C. M. (2023). Electroencephalographic features of the developing brain in 72 dogs under xylazine sedation: a visual and statistical analysis. *Frontiers in Veterinary Science*, *10*. https://doi.org/10.3389/fvets.2023.1150617

@COPYRIGHT UPM

Peng, Y.-Z., Li, X.-X., & Wang, Y.-W. (2010). Effects of Parecoxib and Fentanyl on nociception-induced cortical activity. *Molecular Pain*, *6*, 3. https://doi.org/10.1186/1744-8069-6-3

Plumb, D.C. (2011) Plumb's veterinary drug handbook. 7th edn. Stockholm, Wisconsin: PharmaVet.

Purves D, Augustine GJ, Fitzpatrick D, et al., editors. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Excitatory and Inhibitory Postsynaptic Potentials. Available from: https://www.ncbi.nlm.nih.gov/books/NBK11117/

Reiser, J., Kreuzer, M., Werner, J., Saller, A. M., Fischer, J., Senf, S., Deffner, P., Abendschön, N., Groll, T., Grott, A., Miller, R., Bergmann, S., Erhard, M. H., Ritzmann, M., Zöls, S., Schneider, G., Steiger, K., & Baumgartner, C. (2022). Nociception-Induced Changes in Electroencephalographic Activity and FOS Protein Expression in Piglets Undergoing Castration under Isoflurane Anaesthesia. *Animals*, *12*(18). https://doi.org/10.3390/ani12182309

Sabow, A. B., Goh, Y. M., Zulkifli, I., Ab Kadir, M. Z., Kaka, U., Adeyemi, K. D., Abubakar, A. A., Imlan, J. C., Ebrahimi, M., & Sazili, A. Q. (2019). Electroencephalographic and blood parameters changes in anaesthetised goats subjected to slaughter without stunning and slaughter following different electrical stunning methods. *Animal Production Science*, *59*(5), 849. https://doi.org/10.1071/AN17486

Sabow, A. B., Nakyinsige, K., Adeyemi, K. D., Sazili, A. Q., Johnson, C. B., Webster, J., & Farouk, M. M. (2017). High frequency pre-slaughter electrical stunning in ruminants and poultry for halal meat production: A review. *Livestock Science*, *202*, 124–134. https://doi.org/10.1016/j.livsci.2017.05.021

Zulkifli, I., Goh, Y. M., Norbaiyah, B., Sazili, A. Q., Lotfi, M., Soleimani, A. F., & Small, A. H. (2014). Changes in blood parameters and electroencephalogram of cattle as affected by different stunning and slaughter methods in cattle. *Animal Production Science*, *54*(2), 187. https://doi.org/10.1071/AN12128

@COPYRIGHT UPM

APPENDICES

Appendix I: IACUC Approval Letter

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date:	01st August 2023
AUP No.:	UPM/IACUC/AUP-U029/2023
Project Title:	Electroencephalogram Changes Associated with Sedative Effect of Acepromazine in Anesthetized Dogs in Response to Electrical Stimulation.
Principal Investigator:	Dr. Ubedullah Kaka
Members:	Yap Coco, Adam Naim Bin Aimi Azwani
Attending Veterinarian:	Dr. Ubedullah Kaka
Committee Decision:	The committee has reviewed and approved the proposed animal utilisation protocol, subject to relevant permit and/ or owner's consent.
Project Classification:	Acute
Category of Invasiveness:	B
Source of Animals:	**DBKL Pound,** Chan Sow Lin, 55200 Kuala Lumpur,
Number of Animals Approved:	6 Dog
Housing:	Small Animal Student Surgery Operating Theater, University Veterinary Hospital, 43400 UPM Serdang, Selangor.
Duration	01st August 2023 – 30th December 2023

Ethical approval is required in the case of amendments to the approved animal utilisation protocol (AUP). Please apply using Form 105. Kindly submit a final/annual report (Form 106) upon study completion, or before expiry of approval.

PROF. DATO' DR. MOHD AZMI MOHD LILA
Chairman
Institutional Animal Care and Use Committee
Universiti Putra Malaysia

Pejabat Timbalan Naib Canselor (Penyelidikan dan Inovasi), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
Pejabat Timbalan Naib Canselor (P&I) 603-9769 1002, Pejabat Pentadbiran TNCPI 603-9769 1608, Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) 603-9769 1610, Pejabat Pengarah, Putra Science Park (PSP) 603-9769 1291
http://www.tncpi.upm.edu.my

@COPYRIGHT UPM