UNIVERSITI PUTRA MALAYSIA

PAIN ASSESSMENT DURING ELECTROEJACULATION (EE) USING ELECTROENCEPHALOGRAPHY (EEG) IN BULLS

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FACULTY OF VETERINARY MEDICINE

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MUHAMMAD FARHAN BIN ZAINUDDIN

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.

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Pain Assessment During Electroejaculation (EE) Using Electroencephalography (EEG) in Bulls”, by Muhammad Farhan bin Zainuddin and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4901 - Project.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRAK

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

PENILAIAN KESAKITAN SEMASA PROSES ELEKTROEJAKULASI (EE) DALAM LEMBU MENGGUNAKAN ELEKTROENSEFALOGRAFI (EEG)

OLEH

Muhammad Farhan Bin Zainuddin

2023

Penyelia: DR. UBEDULLAH KAKA

Elektroejakulasi (EE) adalah salah satu kaedah yang digunakan untuk pengumpulan air mani lembu di ladang kerana kecekapan dan kebolehpercayaannya berbanding dengan kaedah lain seperti vagina tiruan. Kajian-kajian terdahulu telah menunjukkan ketidakupayaan untuk mengaitkan kesakitan secara konkrit dengan kaedah seperti ini. Sebaliknya, elektroensefalografi (EEG) digunakan sebagai kaedah objektif untuk mengukur aktiviti otak dan mengukur kesakitan secara objektif. Oleh itu, kajian ini direka untuk mengukur respons EEG terhadap peningkatan voltan semasa EE. Enam ekor lembu digunakan, dan mereka dipasang dengan dua elektrod perekat EEG pada lengkung zygomatic dan tulang temporal. Pembacaan EEG diambil selama 5 minit sebelum EE sebagai pengukuran asas dan pada peningkatan voltan seterusnya pada voltan 2 dan 4. Perubahan tingkah laku sepanjang proses juga direkodkan. Data kemudian dianalisis secara offline menggunakan Pembaca LabChart ADInstrument 8.1.25 dengan selang masa epoch 10 saat. Hasilnya menunjukkan peningkatan signifikan ($P \leq 0.05$) dalam frekuensi median (MF) dari 19.13+-10.03 pada asas, kepada 36.94+-21.88 untuk 2 volt dan 44.52+-14.87 untuk 4 volt. Demikian juga, kuasa total (Ptot) juga menunjukkan peningkatan yang signifikan dari asas 8.9+-3.25, kepada 10.13+-2.19 untuk 2 volt dan 11.29+-3.12 untuk 4 volt. Kebanyakan lembu menunjukkan lengkung punggung, penghasilan air liur, dan bergelut semasa prosedur EE. Walau bagaimanapun, tiada vokalisasi yang diperhatikan pada mana-mana lembu sepanjang prosedur. Secara keseluruhan, peningkatan respons MF dengan peningkatan voltan menunjukkan peningkatan kesakitan dengan voltan yang lebih tinggi. Oleh itu, dengan mengambil kira respons tingkah laku dan, disimpulkan bahawa EE mungkin merupakan kaedah yang menyakitkan untuk pengumpulan air mani pada lembu. Kajian akan datang perlu dilakukan untuk mengukuhkan penemuan kajian ini dengan menggunakan parameter lain seperti kortisol dan dengan julat voltan yang lebih tinggi.

Kata kunci: Elektroejakulasi, Elektroensefalografi , Kesakitan, Voltan

ABSTRACT

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

PAIN ASSESSMENT DURING ELECTROEJACULATION (EE) USING ELECTROENCEPHALOGRAPHY (EEG) IN BULLS

BY

Muhammad Farhan Bin Zainuddin

2023

Supervisor: DR. UBEDULLAH KAKA

Electroejaculation (EE) is one of the methods used for semen collection in the bull at farms for its efficiency and reliability compared to other methods like artificial vagina. Past studies have shown inability to concretely associate pain with such a method. On the other hand, electroencephalography (EEG) is used as an objective method to measure brain activity and to measure pain objectively. Therefore, this study was designed to measure the EEG response to increasing voltage during EE. Six bulls were used, and they were attached with two EEG adhesive electrodes on the zygomatic arch and the temporal bone. EEG reading was taken for 5 minutes before EE as the baseline measurement and at subsequent voltage increment at voltage 2 and 4. Behaviour changes throughout the process were also recorded. The data was then analysed offline using ADInstrument LabChart Reader 8.1.25 with 10 seconds epoch interval. The result showed significant ($P \leq 0.05$) increase in median frequency (MF) from 19.13+-10.03 at baseline, to 36.94+-21.88 for 2 volts and 44.52+-14.87 for 4 volts. Similarly, total power (Ptot) also showed significant increase from baseline 8.9+-3.25, to 10.13+-2.19 for 2 volts and 11.29+-3.12 for 4 volts. Most of the bulls showed arched back, salivation, and struggling during EE procedure. However, no vocalisation was observed in any of the bulls throughout the procedure. Overall, the increase in MF response with the increment voltage indicates increased pain with higher voltage. Thus, keeping in view, the behaviour response and, it is concluded that EE may be painful method for semen collection in bulls. Future studies needed to be done to consolidate the findings of this study with the use of other parameters like cortisol and with a higher range of voltage.

Keyword: Electroejaculation, Electroencephalography, Pain, Voltage

CHAPTER 1

INTRODUCTION

1.1 Background

Electroejaculation (EE) is a method used to collect semen samples from male animals, especially in bulls that are untrained to collect semen through an artificial vagina (Abril-Sánchez et al., 2019). The use of EE is always preferred in farms with a large population and are reliable without depending on seasonal reproductive pattern (Abril-Sánchez et al., 2019). However, there are some studies showing that bulls undergoing this procedure were in pain (Palmer, 2005) whereas some studies showing the vice versa (Whitlock et al., 2012). The first study acknowledges that the procedures are painful in male ruminants noted by changes in heart rate, respiratory rate, serum cortisol and haematological variables (Abril-Sánchez et al., 2019). Increases in cortisol after EE in bulls may indicate activation of the HPA axis as a result of stress brought on by nociception, or they may just be a natural component of the ejaculatory process and/or a reaction to physical exercise (Whitlock et al., 2012). Conversely, another study showed no difference in plasma concentrations of substance P which functions as mediators of noxious stimuli or stress indicating that the procedure is not painful (Whitlock et al., 2012).

To date, there is limited information and research available regarding brain activity in response to pain in EE procedures. The frequencies categorising the signals are designated as 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). The combination of delta and theta activity shows that in an unconscious state, there is active brain activity. Alpha and beta integration reveals active brain activity during a sensible state (Kumar et al., 2022). Studying these waves in animals can be a great tool to explore their response to stressors (Kumar et al., 2022).

During EE, a rectal probe is inserted after rectal assessment to assess seminal vesicle, prostate, pelvic urethra, inguinal ring and the ampullae. Once insertion of the rectal probe is successful, electrical simulation is controlled by the voltage input through the rectal probe. With each successful simulation indicated by penile protrusion, erection and ejaculation, the intensity of the voltage is increased (Ball & Furman, 1972). This study is intended to measure the EEG responses as gradual increase of voltage input through the rectal probe during EE procedure.

1.2 Hypothesis

The increase in median frequency (MF) and total power (Ptot) in EEG readings corresponding to higher voltage levels during electroejaculation (EE) suggests a potential relationship between voltage increments and heightened neural activity in bulls. This heightened neural response, accompanied by observed behavioral changes like arched back, salivation, and struggling, indicates a plausible association between increased voltage and discomfort during the semen collection process. Therefore, it is hypothesized that higher voltage settings during EE might induce elevated neural activity and subsequent discomfort in bulls.

1.3 Objectives

To evaluate and measure pain during electroejaculation (EE) using electroencephalography (EEG) in bulls.

CHAPTER 2

LITERATURE REVIEW

2.1 Bulls

Bos indicus (Linnaeus, 1758) is a subspecies derived from Auroch which originated from the indus valley. This species from the Bovidae family is known to be domesticated around 8000 years ago in south asia (Pérez-Pardal et al., 2018). One of the many breeds of Bos indicus is Malaysian Kedah Kelantan (KK) that accounts for more than 70% of the domestic beef production in Malaysia (Islam et al., 2022). Majority of the Malaysian KK cattle are characterised by brown coat colour with overall mean body length of 98.3±12.3 cm with significantly larger male body length compared to female (Islam et al., 2022). Malaysian KK cattle have higher reproductive performance based on calving interval, conception rate and semen quality albeit lower growth performance compared to other cattle breeds such as exotic breeds, synthetic breeds and crossbreeds (Islam et al., 2021). Thus, these characteristics paired with its high adaptability with the local environment and high tolerance against ticks and parasites (Jamaludin et al., 2014) are the majority breed used in the Malaysian beef industry.

2.2 Electroejaculation

Electroejaculation is a method used to collect semen by stimulating nerves associated with ejaculation response through electrical stimulation, especially the pudendal and hemorrhoidal nerves that stem from lumbo-sacral plexus and separate in the pelvic genital organ region (Ball & Furman, 1972). It was pioneered by Gunn in 1936 and has since been refined to bipolar electrodes implanted in rectal probes using material that covers opposite charge electrodes (Ball & Furman, 1972).

There are also other methods that can be used to collect sperm such as artificial vagina although with different effects to the sperm qualities. Previous studies have shown sperm collected in cattles using electroejaculation methods to have higher volume and pH albeit lower concentration and similar motility score compared with sperm collected through artificial vagina (León et al., 1991; Abril-Sánchez et al., 2019). This is further supported by another study done on Brahman bulls indicating greater semen volume and seminal plasma proteins for electroejaculation than for internal artificial vagina with no differences in sperm motility and percentage of normal sperm (Rego et al., 2015). However, in another study shown mithun (*Bos frontalis*) bulls have noted significant lower semen quality parameters, motility and velocity in both fresh and frozen thawed semen harvested through electroejaculation compared to artificial vagina with insignificant lower conception rate using electroejaculation sperm compared to artificial vagina (Nadaf et al., 2021). Nevertheless, electroejaculation is a preferred method used to collect sperm in animals and is very practical by eliminating the need to be trained to use an artificial vagina and can be done in large quantities (Abril-Sánchez et al., 2019).

2.3 Pain and Electroejaculation

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage". Acute pain is the initial tissue damage whereas chronic pain is associated with the healing process although less acute, can last several days before subsiding (Napolitano et al., 2020). Pain is caused by noxious stimuli which are carried out by nociception as defined by the International Association for the Study of Pain as "the neural process of encoding noxious stimuli”.

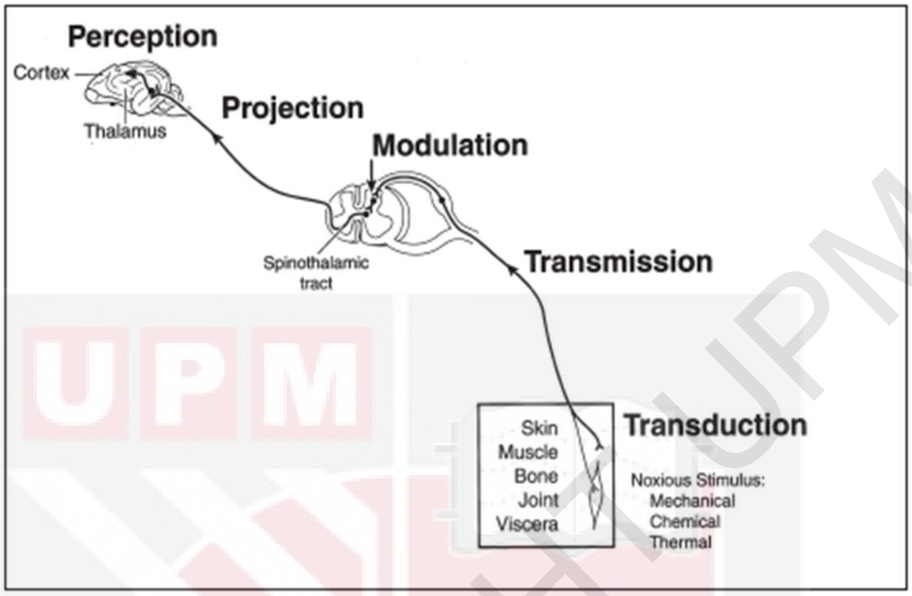

Figure 1 Schematic diagram of the pathways and physiologic processes involved in pain sensation.

(Muir III & Woolf, 2001)

However, whether EE causes pain or not is still debatable. On one hand electroejaculation is noted to be a painful procedure due to several reasons. Past studies have shown an increment of vocalisation along with mean plasma cortisol and progesterone associated with electroejaculation method in bulls (Whitlock et al., 2012). This is supported by other previous studies done on bulls during electroejaculation have shown an increment of vocalisation when high levels of electrical stimulation are used (Falk et al., 2001). Vocalization is agreed to be an easy observable behavioural and physiological response that can indicate distress and pain and can vary by both quantitatively and qualitatively (Watts & Stookey, 2000). In the case of mean plasma cortisol and progesterone however, previous study showed a similar hormone response (corticosteroid and progesterone) in bulls that undergo insertion of rectal electroejaculation probe with or without electrical stimulation (Welsh & Johnson, 1981; Palmer, 2005).

Conversely, previous studies have shown negligible differences in plasma concentration of substances P after ejaculation using electroejaculation in bulls indicating lack of pain associated with nociception (Whitlock et al., 2012). In this case, noxious stimuli are transferred by proteins in the nociceptors' membrane into a depolarizing electrical potential. From the central terminals of the nociceptive afferents, neurotransmitter which is substance P is released and activates dorsal horn neurons to relay the information to other parts of the brain for central processing (Tucker & Adcock, 2017). Further studies concluded that substance P concentration as a way to evaluate painful procedures and condition in cattles appears as an acceptable biomarker for nociception however the results are heterogeneous (Tschoner & Feist, 2022). The study also suggested conducting further research to assess factors other than nociception which may influence substance P concentrations and to establish a baseline reference range (Tschoner & Feist, 2022).

2.4 Electroencephalography

Electroencephalography (EEG) is the electrical activity and magnetic field produced by neurons and glial cells recorded from electrodes implanted at various points on the scalp (human) or head (other species) (Murrell & Johnson, 2006). During information processing, the cortical pyramidal neuron in the brain generates an electrical signal and these brain oscillations vary in frequency, amplitude, and timing, and they follow a certain spatiotemporal pattern (Babiloni et al., 2016).

Once EEG data is recorded, Fast Fourier transformation (FFT) which is a mathematical process is used to compute information and to change the EEG signal from time domain to frequency domain which in turn produces a power spectrum (Murrell & Johnson, 2006). The spectrum of the signal is classified based on its frequencies, which include delta (4.0 Hz), theta (4.1-8.0 Hz), alpha (8.1-12.0 Hz), and beta (12.1-30.0 Hz) (Raghazli et al., 2021). These waves may represent different states of brain activities. The combination of delta and theta activity shows that there is an active brain activity in an unconscious state. Meanwhile, alpha and beta wave integration reveals active brain activity during a sensible state (Kumar et al., 2022).

In humans, EEG has an advantage as a direct measurement and also high temporal precision of population-level neural activities (Cohen, 2017). The signals produced are detected through EEG and have been demonstrated for its ability to analyse multilevel mental stress in humans (Al-shargie et al., 2017). These are further supported by a previous study that shows the dependability of EEG results using only two frontal electrodes to predict stress and non-stress in humans (Attallah, 2020). Another research also inferred significant changes in some spectral EEG indices which are associated with psychosocial stress in humans (Vanhollebeke et al., 2022).

There are also other parameters that can be measured from EEG signals specifically from the power spectrum which are median frequency and total power (Murrell & Johnson, 2006). A rise in the EEG's spectrum's median frequency often implies stress or painful situations (Murrell & Johnson, 2006). This is also supported by another study showing how EEG is a reliable tool to assess pain and stress in a conscious state using MF (Harris et al., 2020). As a result, it is reasonable to believe that EEG has immense potential for studying an animal's brain response to diverse stressors (Freeman and Quiroga, 2012). Previous studies have shown electroencephalogram spectrum and EEG has been used to measure nociceptive response in dogs (Kaka et al., 2015, 2016, Kongara et al., 2010), horses (Murrell et al., 2005), ponies (Murrell et al., 2005), sheep (Otto & Gerich, 2001) and cattle (Zulkifli et al., 2014).

CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical Approval

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-U020/2023).

3.2 Animals

The experiment was conducted at Ladang 16 and a total of 6 bulls were used in this study. These bulls were fed with *Brachia Decumbens* grass and given ad-libitum drinking water source. The bulls are brought to a crusher for the EE procedure. Simple physical assessment is done prior to conducting EE procedure.

3.3 Electroencephalography

Each bull was prepared for the attachment of two conductive electrode patches which are located at the zygomatic process of the frontal bone and secondly at the mastoid area which is a method followed by Imlan et al. in 2021. Prior to the attachment, the fur areas was shaved and cleaned with 70% alcohol.

It is imperative to clean the area as any disturbance will cause improper electrode patch attachment which in turn may cause disturbance in the EEG signal. The electrode used was Kendall™ (Covidien 11c, 15 Hampshire Street, Mansfield 02048 USA) which uses adhesive hydrogel foam to form contact with the skin. The negative electrode was placed at the zygomatic process of the frontal bone while the positive electrode was placed at the cleaned mastoid process.

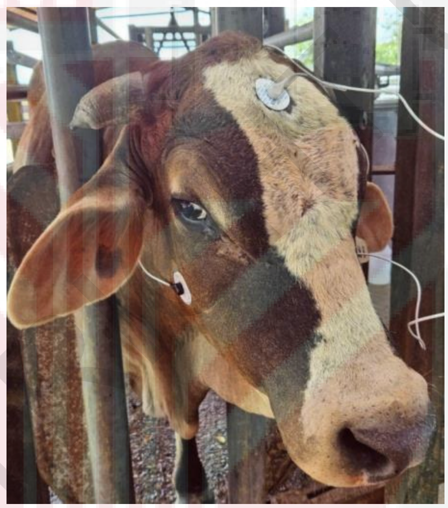

Figure 2 The position of electrodes on the zygomatic process of the frontal bone and mastoid process.

EEG signals were sampled at the rate of 1 kHz and individual power spectra of alpha (8.1 to 12 Hz), beta (12.1 to 20 Hz), delta (0.1 to 4 Hz) and theta (4.1 to 8 Hz) waves were calculated. Afterwards, the root mean square of each wave spectrum e.g., alpha, beta, delta and theta were calculated. Total power (Ptot) and median frequency (MF) was extracted from spectrum. The signals were sampled before EE procedure for 10 seconds interval as baseline reading, followed by 2 volts and 4 volts during EE procedure. Any interference which can be caused by muscle twitching at the head or rumination process was removed and excluded from measurement. The readings for 10 sec intervals along with readings from 6 bulls were then averaged and compared with base, 2 volts and 4 volts.

3.4 Electroejaculation

Firstly, the rectum was cleared from faecal material to prepare for electroejaculation probe insertion. The probe is measured at 51 mm in diameter and 330 mm in length with 3 longitudinal electrodes located at the ventral part of the probe. The electrical stimulation was supplied from the electroejaculator using Electrojac 6; Neogen® Corporation, Landing, IM48912; L24290812 and the voltage was increased according to the automatic setting. The voltage was increased systematically, and the cutoff point of this experiment was set to 10 volts although two of the bulls were found recumbent when the voltage reached at 6 volts.

3.5 Behavioural recording

Throughout the electroejaculation procedure, any changes in behaviour that is associated with pain were recorded. Videos and pictures were taken during the procedure and were used for evaluation later.

3.6 Statistical analysis

Data taken from EEG which were composed of alpha, beta, delta, theta, median frequency (MF) and total power (Ptot) was selected, and any artefact caused by muscle twitch was removed. The data was then analysed using SAS system, version 9.1 (SAS Inst. Inc., Cary., NC) by using analysis of variance running through a general linear model (GLM). Post Hoc test was done afterwards in order to measure significant difference between treatment groups which are baseline, 2 volts and 4 volts.

CHAPTER 4

RESULTS

Figure 3,4 and 5 shows the electroencephalogram during baseline, 2 volts and 4 volts, respectively. The highlighted grey area is 1 second epoch when median frequency (MF) and total power (Ptot) was extracted and analyzed in accordance with methods from previous studies (Kumar et al., 2023).

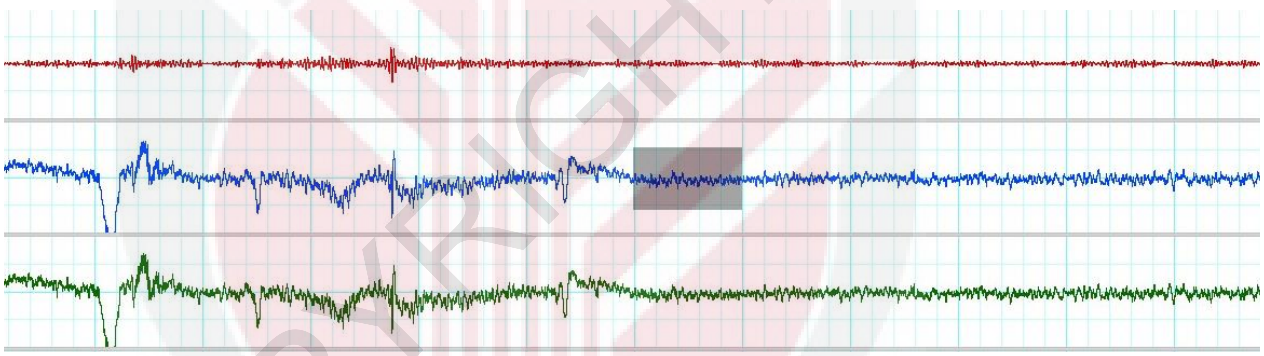

Figure 3 : Baseline chart view

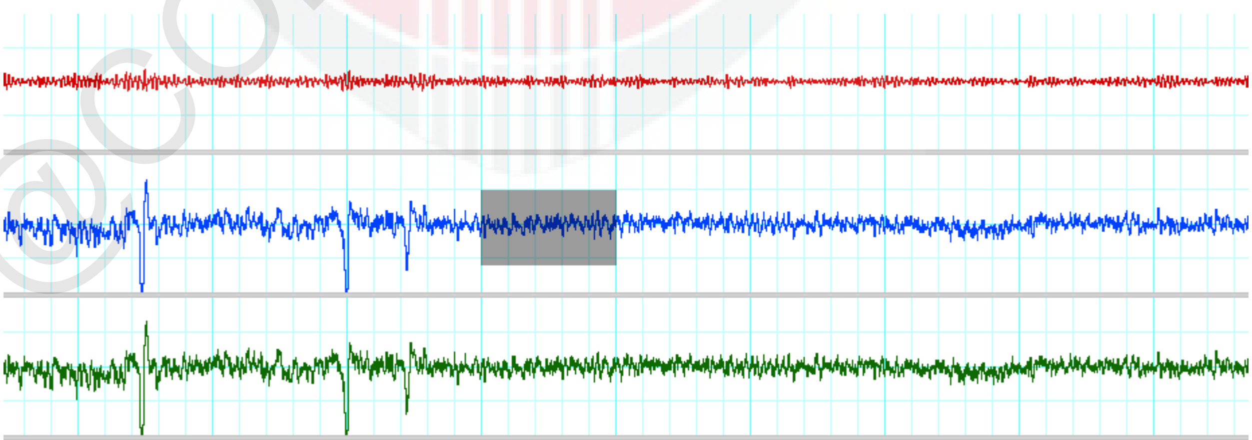

Figure 4 : 2 Volts chart view

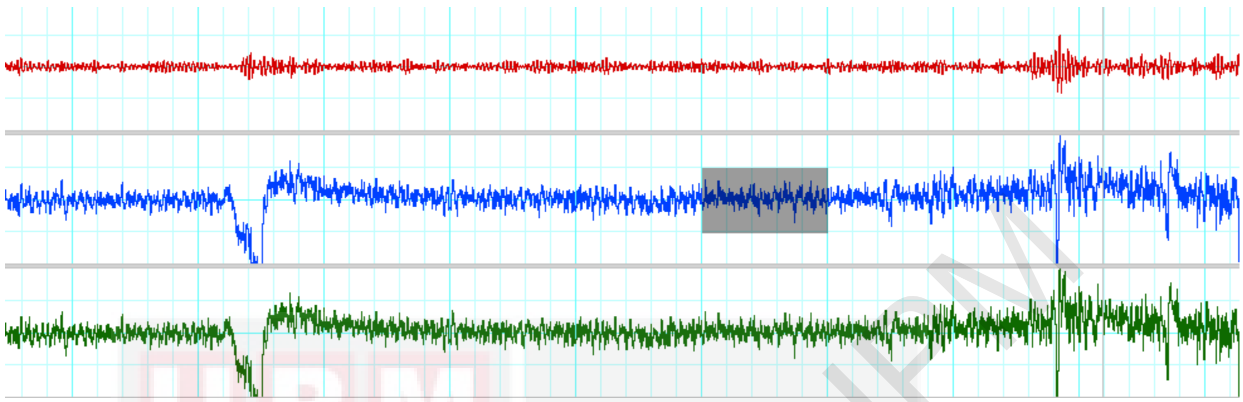

Figure 5 : 4 Volts chart view

Table 1 shows readings during baseline, 2 volts and 4 volts. while MF and Ptot are presented in Table 2.

Table 1 Mean ± SE (µV) of root mean square of alpha, beta, delta and theta waves for Baseline, 2 volts and 4 volts

Parameter	Variable			P value
	Baseline	2 Volts	4 Volts	
Alpha	1.066 ± 0.04	1.169 ± 0.06	1.245 ± 0.09	0.1497
Beta	2.097 ± 0.08^{b}	2.357± 0.11^{b}	2.96 ± 0.14^{a}	<0.0001
Delta	3.89 ± 0.35	3.69 ± 0.4	3.956 ± 0.34	0.8635
Theta	1.114 ± 0.04	1.173 ± 0.04	1.046 ± 0.04	0.0897

[a,b] Difference across the row with different superscript differed significantly at $p<0.05$

Beta frequency significantly increased ($p<0.05$) from baseline of 2.097 ± 0.08 to 2.96 ± 0.14µV at 4 volts. Alpha, Delta and theta frequencies did not show any changes.

Table 2 Electroencephalogram total power (Ptot)(µV) and median frequency (MF) (Hz) values (means ± SE) for Baseline, 2 volts and 4 volts

Parameter	Variable			P value
	Baseline	2 Volts	4 Volts	
Ptot	8.903 ± 0.42^{b}	10.127 ± 0.28^{ab}	11.294 ± 0.4^{a}	<0.0001
MF	19.137 ± 1.3^{a}	36.94 ± 2.83^{b}	44.52 ± 1.92^{c}	<0.0001

[a,b,c] Difference across the row with different superscript differed significantly at $p<0.05$

Ptot frequency significantly increase ($p<0.05$) from baseline of 8.903 ± 0.42 to 11.294 ± 0.4 at 4 volts. Meanwhile for MF there is significant increase ($p<0.05$) from baseline of 19.137 ± 1.3 to 36.94 ±2.83 at 2 volts to 44.52 ± 1.92 at 4 volts.

Table 3 Notable changes in behaviour (hypersalivation, arch back, struggling) throughout EE

Bull	Behaviour			
	Hypersalivation	Arch back	Struggling	Vocalisation
Bull 1	+ve	+ve	+ve	-ve
Bull 2	+ve	+ve	+ve	-ve
Bull 3	+ve	+ve	+ve	-ve
Bull 4	+ve	+ve	+ve	-ve
Bull 5	+ve	+ve	+ve	-ve
Bull 6	+ve	+e	+ve	-ve

All the bulls exhibited change in the behaviour with increase in voltage, such as, hypersalivation, arch back and struggling throughout the EE procedure (table 3).

CHAPTER 5

DISCUSSION

In humans, elevation of alpha waves where the frequency of the waves is in the width of 8-12 Hz have shown correlation with auditory and visual stimulations with memory-related activities (Başar et al., 2001). In animals it is suggested that alpha waves likely means that the animal are relaxed (Imlan et al., 2021). Therefore, this likely shows that the bulls are not in a calm state. Meanwhile for beta waves, there is significant increase ($p>0.05$) between 2 volts and 4 volts. Increased stimulation of the brain activity is indicated by the increase in beta waves in the EEG readings (Freeman & Quiroga, 2012). Reports have also shown that increase in beta waves are associated with increased brain activity during panic situations (Başar et al., 2001). In animal models specifically in goats, studies have shown elevation of beta brain activity during exposure to stress which are transportation to lairage and slaughter (Raghazli et al., 2021). Therefore, this likely shows that the bulls are undergoing stressful conditions.

The delta waves in this study have also not shown significant changes. A study done using rabbit models has shown delta waves in EEG reflect sleep propensity (Opp et al., 1997). A brain's default mode network is also related with delta waves (Meerwijk et al., 2015). In addition to that, theta wave shows insignificant changes when compared with baseline, 2 volts and 4 volts during electroejaculation. An increase in theta waves in humans would be related with high alertness and arousal (Başar et al., 2001). In animals, increased theta waves are apparent in goats' heightened emotional condition which can be because of exposure to the act of slaughter. (Kumar et al., 2023)

The total power of the EEG spectrum (Ptot) has shown significant increase when compared between baseline and 4 volts. This is alongside a significant increase in median frequency (MF) between baseline, 2 volts and also 4 volts. This is in line with previous studies that have shown correlation between Ptot and MF as the value increases in response to noxious stimuli or pain as recorded by Imlan et al. in 2020 using a cattle model. This is further backed up by the findings of a study stating notable significant increase in median frequency in cattle undergoing noxious stimuli due to slaughtering (Zulkifli et al., 2014). However, the significant changes of Ptot in association with MF as a response to noxious stimuli has not always been the case. A study has shown no changes in Ptot as a response to noxious stimuli in contrast with MF under anaesthesia using a dog model (Kaka et al., 2015). This is further supported by findings done by Karna et al. in 2020 where they found no significant changes in Ptot but significant increase in MF as a response to acute electrical stimulation in anaesthetized dogs. However, it must be noted that the present study design uses conscious bull. Therefore, this shows how Ptot are not directly related with MF although it might have connection with other elements of pain and stress (Kaka et al., 2015).

Another parameter that should be discussed would be changes in behaviour. In this study, we observed positive results for arched back, hypersalivation, struggling but no vocalisation as a behavioural parameter in response to EE. Animals that are subjected to unknown unfamiliar conditions or objects would be inclined to show signs of discomfort and stress (Baiee et al., 2018). This can be seen as recumbent, jumping, and kicking and arch back as reported by previous studies (Baiee et al., 2018). According to Baiee et al. (2018) it has been reported that 100% of bulls undergoing EE procedure have shown arched back similar to present study. Studies have also shown intense muscle contraction, vocalisation, struggling as an indication to pain during EE in bulls (Palmer, 2005). This is backed up by another research stating how arched back and vocalisation is a sign of pain in cattle (Gleerup et al., 2015). As a response to no vocalisation, Palmer (2005) noted that the absence of vocalisation in response to EEJ in bulls does not conclusively indicate the absence of pain, but it is more likely reflective of variations in pain perception among the animals and differences in the technique of the procedure itself.

CHAPTER 6

CONCLUSION

The findings from the present study have shown that EE is a painful procedure by using objective parameters based on EEG parameters which are median frequency (MF) and total power (Ptot). In depth, the increased use of voltage during EE causes increase in MF and Ptot which indicates that higher the voltage is used in EE, the more painful the bull is. This is backed up by using other subjective parameters which are changes in behaviour particularly seen as a response to pain which are arched back, hypersalivation and struggling.

CHAPTER 7

RECOMMENDATION

In order to create more accurate and reliable data, the study should include a larger sample size for further studies. This would reduce the margins of error, lower standards of deviation and enhance reliability of the data by minimising the impact of outliers or extreme values. Further study should also use a wider range of voltage during EE following the routine recommended method of EE. This would give more authentic data since it is similar to the procedure done in the industry. Lastly, this study model can be improved by increasing the parameters used to detect pain. For this, blood samples can be taken in order to measure stress hormones like adrenaline, noradrenaline and glucose.

CHAPTER 8

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