Alteration of Electroencephalographic Responses to Castration in Cats by Administration of Opioids

Kavitha Kongara, Lorna Johnson, Nikki J Kells, Craig B Johnson, Venkata SR Dukkipati & Sheryl L Mitchinson

***Abstract*—The aim of this study was to investigate the effect of opioids on electroencephalogram (EEG) indices of nociception in cats undergoing castration. Cats were randomly assigned to receive one of the four treatments (n=8); 0.2 mg/kg morphine, 0.005 mg/ kg fentanyl, 0.01 mg/kg buprenorphine or 0.2 mg/kg butorphanol, administered subcutaneously (SC) at the time of pre-anesthetic medication. Anesthesia was induced with intravenous propofol and maintained with halothane in oxygen. EEG was recorded continuously in a three electrode montage. Median frequency (F_{50}), total power (P_{TOT}) and 95% spectral edge frequency (F_{95}) derived from the EEG power spectra recorded prior to skin incision (baseline) were compared with those recorded during the ligation of the spermatic cords of both testicles. During the ligation of testicle 1, the mean F_{50} of cats that received buprenorphine and butorphanol was significantly ($p<0.05$) higher, compared with baseline values. During the ligation of testicle 2, the cats in the butorphanol and fentanyl groups showed significantly ($p<0.05$) higher F_{50} values, compared with that of the morphine group as well as with their respective values during the ligation of testicle 1. P_{tot} values decreased significantly ($p<0.05$) in all the treatment groups (excluding morphine after the removal of testicle 2), compared with baseline values. Morphine treated cats had significantly ($p<0.05$) higher P_{tot} values than cats in the buprenorphine and fentanyl groups during the removal of both testicles. The F_{95} of the EEG did not differ between the two groups during the ligation of either testicle ($p>0.05$). These results indicate that opioid analgesics, acting at different opioid receptors with variable affinity, produce changes in the EEG responses that reflect their anti-nociceptive efficacy. This study demonstrates the usefulness of the EEG as a valid tool for evaluating analgesic efficacy in cats, as shown in other species of animals in previous studies.**

Index Terms – Electroencephalogram, castration, analgesia, cat, opiods, morphine, fentanyl, butorphanol, buprenorphine

I. Introduction

PROVISION of adequate analgesia following surgical procedures or trauma is a vital element of animal welfare. Currently, very limited approved medication is available for the treatment of cat pain compared with those approved in other species, especially dogs. This is likely, in large part, due to the lack of validated techniques to reliably identify and assess cat pain [1]. Owing to their unique temperament and minimal expression of pain behaviors, assessing and treating pain based on behavioral observation is less effective [1]. This often leads to underestimation of the level of pain that a cat experiences.

Development of objective and repeatable methods for assessment and management of pain in cats is essential [2]. Electroencephalography (EEG) has been used to objectively assess pain and analgesia in other animals [3]. Changes in EEG activity correlate with verbal reports of pain experience in people, and with behavioral responses to nociceptive stimuli in conscious sheep [4, 5]. The commonly used quantitative variables of the EEG power spectrum in response to nociception are median frequency (F_{50}), 95% spectral edge frequency (SEF or F_{95}) and total EEG power (P_{tot}) [6, 7]. The F_{50} and F_{95} are the frequencies below which 50% and 95%, respectively, of the total power of the EEG is located, and the P_{TOT} is the total area under the EEG power spectrum curve. For further details of these variables see the review by Murrell and Johnson [3].

Opioids are effective in controlling perioperative pain in veterinary patients. Quantitative analysis of EEG power spectra has been used to evaluate the efficacy of opioids and α2- agonist analgesics in anaesthetized dogs, and to correlate EEG changes with the pharmacokinetic properties of these agents [8-10]. However, no studies have been undertaken on the use of EEG in measuring the analgesic efficacy of opioids (or any other analgesics) in response to noxious stimulation in the cat.

Ovariohysterectomy and castration are the most common surgical procedures in small animal practice. These can cause significant postoperative pain [11]. Since these elective surgeries are performed routinely on healthy and pain-free animals, the efficacy of analgesic drugs or assessment techniques can be reliably evaluated, assuming that the resulting pain is solely due to surgery [12].

Manuscript received January 9, 2014.

K. Kongara (phone: +64-6-3569099; fax: +64-6-3505714; e-mail: k.kongara@ massey.ac.nz) is with the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.

L. Johnson is with the Universal College of Learning, Palmerston North, New Zealand.

N.J. Kells, C.B. Johnson, V.S.R. Dukkipati & S.L. Mitchinson are with the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.

DOI: 10.5176/0000-0003_1.1.5

The aim of the present study was to investigate the effects of four opioids on EEG indices of nociception in cats undergoing castration surgery. Also, the validity of the EEG to evaluate the anti-nociceptive efficacy of opioids in cats was tested. The hypothesis tested was that opioids administered prior to castration would change the EEG indices of nociception, and that EEG can be used as a reliable method to assess analgesia in cats.

II. Materials and Methods

A. Experimental Design

The study was approved by the Massey University Animal Ethics Committee. Thirty two mixed breed cats admitted for castration were recruited for the trial. The mean body weight of the cats was 3.5 kg (range, 1.6 to 4.2 kg). The age of the cats ranged from 5 to 12 months. Only clinically normal cats, as assessed by routine physical examination and clinical biochemistry and hematology, were included in the study. Cats were randomly assigned to receive one of the four treatments (n=8 per treatment group): 0.2 mg/kg morphine (Morphine Sulfate Injection, Mayne Pharma Pty Ltd, Mulgrave, Victoria, Australia), 0.005 mg/kg fentanyl (Fentanyl citrate injection, Abbott laboratories, Chicago, IL, USA), 0.01 mg/kg buprenorphine (Temgesic® injection, Reckitt Benckiser pharmaceutical Inc., West Ryde, NSW, Australia) or 0.2 mg/kg butorphanol (Torbugesic® Butorphanol Tartrate, Fort dodge Australia Pty Limited, NSW, Australia). These drugs were administered subcutaneously (SC), at the same time as pre-anesthetic medication.

B. Anesthesia

All animals received 0.05 mg/kg acepromazine SC (Acezine 2, Delta Veterinary Laboratories, NewSouth Wales, Australia) as pre-anesthetic medication. Respective test drugs were also administered at this time. Forty five minutes after pre-anesthetic administration, anesthesia was induced with intravenous (IV) propofol (Propofol Injection, Mayne Pharma Pty Ltd, Mulgrave, Victoria, Australia) to effect and maintained with halothane in oxygen. The concentration of halothane was adjusted to keep the cat at a suitable plane of surgical anesthesia, as judged by the anesthetist. As soon as the cat was anaesthetized and its airway, breathing and circulation had been checked, a pulse oximeter (Pulse Ox, Fisher & Paykel Healthcare Ltd, Auckland, NZ) and Doppler transducer (Doppler flow detector, Parks medical electronics Inc, Oregon, USA) with an appropriate cuff for each cat, were attached to monitor arterial hemoglobin oxygen saturation and blood pressure non-invasively. Airway gases were sampled continuously from the end of endotracheal tube connected to the T-piece, using an anesthetic gas analyzer (Hewlet Packard M1025B, Hewlet Packard, Hamburg, Germany). All animals breathed spontaneously throughout anesthesia and end-tidal CO_2 tension was maintained between 4.60 and 5.92 kPa (35 and 45 mmHg), using intermittent positive pressure ventilation. Body temperature was monitored in all animals using an esophageal thermistor probe and was maintained between 37 and 38°C with a circulating warm water blanket heating device. Respiratory rate, heart rate, end-tidal halothane tension (ET_{Hal}) and end-tidal CO_2 tension (ET_{CO2}) of each cat were also monitored using an anesthetic agent monitor (Hewlett Packard M1025B, Hewlett Packard, Hamburg, Germany).

C. EEG and Electrocardiogram (ECG) Recording

EEG was recorded using an Apple Macintosh personal computer installed with Chart 5.2.2 recording software and connected to Powerlab 4/20 data recording system (Powerlab™ data acquisition system, AD Instruments Ltd, Sydney, Australia). Three 27 SWG stainless steel needle electrodes (Medelec, Radiometer, Auckland, New Zealand) were placed subcutaneously, with the inverting electrode over the zygomatic process of the left frontal bone, the non-inverting electrode over the left mastoid process and the ground electrode caudal to the occipital process [3]. The EEG recording was started as soon as the cat was stabilized under anesthesia, with a sample rate of 1 kHz and a pass band of 0.5–400 Hz using an amplifier and analogue to digital converter (Alert System, Medlec, Surrey, UK). All animals were castrated using a routine scrotal approach. EEG data from 180-second blocks immediately preceding the skin incision were sampled as the baseline time period (at least 40 min after induction of anesthesia). Data from 180-second blocks following clamping of the spermatic cord of each testicle were measured as testicle 1 (T_1) and testicle 2 (T_2). Data were averaged over the 180-second blocks at each data point for statistical comparisons.

EEG epochs contaminated by movement artifacts were manually rejected from analysis of raw EEG data. Data were multiplied using a Welch window and fast Fourier transformation applied to each epoch, generating sequential power spectra with 1 Hz frequency bins. F_{50}, F_{95} and P_{tot} of the EEG power spectra were calculated using a Spectral Analyser (CB Johnson, Massey University, Palmerston North, NZ, 2002) and used for statistical comparisons.

D. Statistical Analyses

EEG variables (F_{50}, F_{95} and P_{TOT}), recorded during a three minute baseline period (before surgery began) and three minutes during the removal of each testicle (T_1 and T_2), were compared between treatment groups as well as between the two surgical periods within a group, using generalized linear mixed model analysis [13] in SAS® 9.3 (SAS Institute Inc. Cary, NC, USA). The linear mixed model included the fixed effects of treatments, time and their interaction, and random effects of animals. Baseline values were treated as a covariate. The covariance error structure for repeated measures over the two surgical periods (T_1 and T_2), within animals within group was determined using Akaike's information criterion. A first-order autoregressive model was found to be the most appropriate error structure. EEG variables were tested for normality using Shapiro-Wilk, Kolmogorov-Smirnov, Anderson-Darling, and Cramér-von Mises tests in SAS® 9.3. Since the residuals of data

were not normally distributed, log-transformed variables were used in the analyses and the resulting least square means in logarithmic scale were used for graphical presentations. Heart rate, respiratory rate, ET_{Hal} and ET_{CO2} of the cats recorded at 5-minute intervals during surgery were also analyzed as repeated measures employing linear mixed model analysis in SAS® 9.3.

III. Results

During the removal of T_1, the mean F_{50} of cats in the buprenorphine and butorphanol groups increased significantly (p<0.05), compared with their respective baseline values (Fig. 1). Although the F_{50} of cats in the morphine and fentanyl groups had slightly elevated during the removal of T_1, their mean values were not significantly (p>0.05) different to their respective baseline values. During the removal of T_2, a further increase in F_{50} was evident, with the mean values in all treatment groups being significantly (p<0.01) higher than their respective baseline values. Additionally, the mean F_{50} at T_2 in the butorphanol and fentanyl groups was significantly (p<0.05) higher than that at T_1 and that of the morphine group at T_2.

Mean P_{tot} during the removal of T_1 as well as T_2 in all the four groups (excluding morphine after the removal of T_2) was significantly (p<0.05) lower, compared with respective baseline values (Fig. 2). Also, cats in the buprenorphine and fentanyl groups had significantly (p<0.05) lower P_{tot} values during the removal of both testicles, compared to those in the morphine treated cats. However, there were no significant (p>0.05) differences in the P_{tot} means between the two surgical time-points in all the four groups.

There were no significant differences (p>0.05) in mean F_{95} either between treatment groups or between time-points within groups (Fig. 3).

No significant (p>0.05) differences between the treatment groups were observed with respect to the overall mean

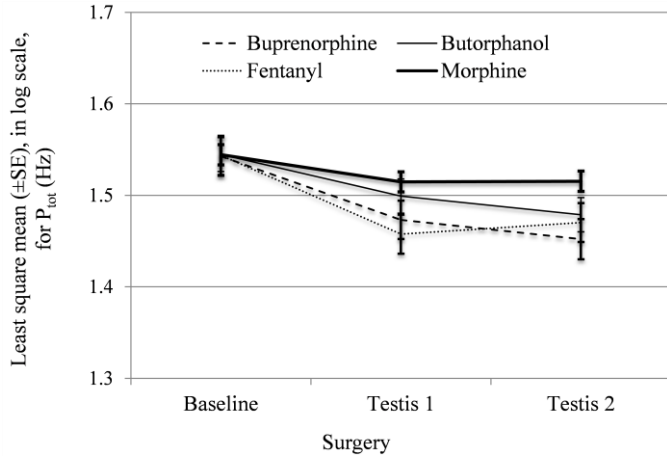

Figure 2. Least square mean (± standard error), in logarithmic scale, for P_{tot} EEG variable before and during removal of testicles 1 and 2 in cats administered four opioids.

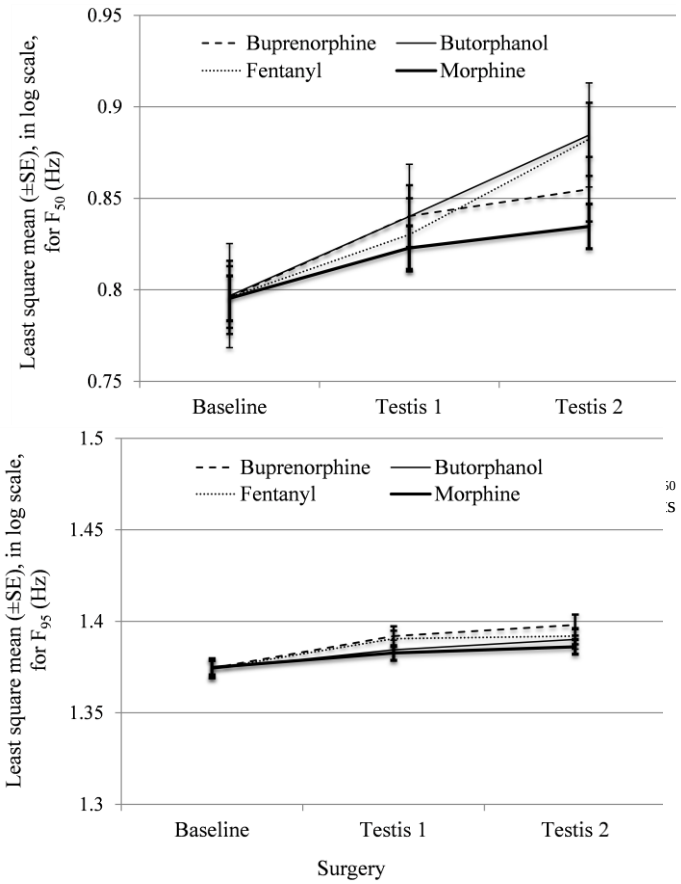

Figure 3. Least square mean (± standard error), in logarithmic scale, for F_{95} EEG variable before and during removal of testicles 1 and 2 in cats administered four opioids.

respiratory rate, heart rate, blood pressure, ET_{CO2} and or ET_{Hal} (data not shown).

IV. Discussion

The aim of this study was to investigate the effects of opioids on EEG indices of nociception in cats undergoing castration. The study further validates the use of EEG to assess the anti-nociceptive efficacy of opioids in cats. Significant increases in F50, with concomitant decreases in P_{tot} of the EEG, were evident during the removal of testicles 1 and 2. During the removal of T1, cats treated with buprenorphine and butorphanol had a significantly higher F50, compared with that of the baseline period. A significant increase in F_{50} has previously been demonstrated to be associated with nociception in castrated dogs and ponies [7, 14]. During castration, tension on the spermatic cord and cremaster muscle might cause visceral nociception [11]. Buprenorphine is a partial agonist at μ-opioid receptors and considered to have a slow onset of action and a lesser degree of anti-nociception when administered subcutaneously [15, 16]. Buprenorphine administered by IV and intramuscular (IM) routes produced better and faster postoperative analgesia than by SC and oral trans-mucosal routes in cats undergoing ovariohysterectomy [17]. Pharmacokinetic-pharmacodynamic (PK-PD) studies in

conscious cats have demonstrated that SC buprenorphine results in an erratic absorption and disposition whereas its IV and IM administration follows a standard disposition, absorption and elimination pattern [18]. To date, there are no PK-PD data available for SC buprenorphine in cats under anesthesia. From the available studies it appears that the route of administration is important for buprenorphine to produce analgesia. In the current study, subcutaneously administered buprenorphine (0.01 mg/kg) would not have reached a plasma concentration (85-100 minutes after administration) high enough to prevent afferent noxious transmission during the removal of the testicles, as reflected by the observed increase in F_{50} of the EEG. The SC route is easier to administer drugs than IV injection and considered to be less painful than IM injection [19].

Butorphanol has been considered as a partial antagonist at μ-opioid receptors, and produces analgesia through its k-agonist activity [20]. It has been demonstrated to produce better analgesia against acute visceral pain (e.g. interstitial cystitis) than somatic pain [21, 22], and is considered to be a poor analgesic for surgical pain management [23]. In the present study, cats administered 0.2 mg/kg of butorphanol SC showed no reduction in afferent transmission of noxious input during the removal of both testicles. Pure μ-opioid agonist agents can effectively reduce or prevent afferent noxious transmission when administered pre-emptively [24]. Because of its antagonistic action at μ-receptors, butorphanol was not expected to reduce the raise in F_{50} during castration, especially compared with morphine (which is a full agonist at μ-receptors).

Fentanyl is a short- acting μ-opioid agonist with a greater potency than morphine. It is commonly administered as a continuous rate infusion, transdermal patch or IV bolus in small animals. In the present study, mean F50 of the cats in the fentanyl group increased significantly ($p<0.05$) during removal of T2 compared to that of T1 and baseline time-periods. Following an IV administration (10 μg/kg), fentanyl was shown to reach a maximum plasma concentration by 2 minutes and the concentrations were undetectable after 95 min in conscious cats [15]. No reports are available for PK-PD data for SC fentanyl in cats. A study in greyhound dogs found that SC fentanyl was rapidly absorbed, reaching a peak plasma concentration in 0.24 h (14.4 min), with a mean terminal elimination half-life of 2.97 hours [25]. Plasma concentrations greater than 1 ng/mL were found to be effective in cats [15]. In the present study, fentanyl (5 μg/kg) administered SC 85-100 min prior to the incision reduced afferent noxious transmission during the removal of T1 but not during T2 removal (seen as an increase in F50). This could likely be due to the result of rapidly declining levels of fentanyl, leading to ineffective reduction in the afferent transmission of the noxious stimuli, following the removal of T_2, through the already sensitized neurons (due impulses from the removal of T_1).

Morphine is a full agonist at μ-opioid receptors and has been used extensively in cats at doses of 0.1 to 0.2 mg/kg that provided effective analgesia [26]. In the present study, there were no significant changes in F_{50} in morphine treated cats during the ligation of T_1. This is similar to the findings in dogs that demonstrated blunting of changes in F_{50} with SC morphine, following castration and electrical stimulation [14, 27]. During ligation of T_2, a slight but significant increase in the mean F_{50} (p=0.056) was found in morphine treated cats in this study. Limited metabolism and increased sensitivity of neurons following T_1 removal could have caused a marginal increase in afferent transmission during removal of T_2 in the morphine treated cats.

The magnitude of increase in mean F_{50} during the removal of T_1 and T_2 in morphine treated cats was significantly lower than that of other treatment groups. Onset of analgesia occurs about 45–60 minutes after its SC administration [26]. In the present study, morphine administered 85–100 minutes prior to the incision would have reached sufficient levels in the plasma to reduce the transmission of noxious input more effectively than other opioids. Total EEG power (P_{tot}) of cats (Fig. 2) treated with buprenorphine, butorphanol and fentanyl decreased significantly from baseline following the removal of both testicles. Also, and the mean P_{tot} in the morphine group was significantly higher than that of the buprenorphine and fentanyl groups. This finding is consistent with that of a study [14] in dogs administered morphine pre-operatively. A decrease in P_{TOT} has been linked to nociception [7] and related to a decrease in depth of anesthesia during noxious stimulation. It is likely that P_{tot} represents a different aspect of nociception than F_{50} [7].

There were no significant differences in the mean F_{95} responses (Fig. 3) either between the treatment groups or between time-points, within groups. Changes in F_{95} might be more representative of the level of general central nervous system depression rather than nociception [27]. This finding is consistent with that of the findings in dogs administered analgesics preemptively [14, 27]. In the present study, dose rates of the opioids were chosen based on their experimental or clinical use in cats [26].

Significant increase in F_{50} and a concomitant decrease in P_{tot} were evident in cats administered buprenorphine, butorphanol and fentanyl, following noxious stimulation. These EEG changes, which are indicators of nociception across different species of animals [7, 14, 28-32] were significantly less in cats administered morphine. These results indicate that opioid analgesics, acting at different opioid receptors with variable affinity, produce changes in the EEG that reflect their analgesic efficacy. In addition, this study demonstrates the usefulness of the EEG as a valid tool for evaluating analgesic efficacy in cats, as shown in other species of animals in previous studies.

ACKNOWLEDGEMENT

Financial assistance for the study, in the form of Lewis Fitch research funds, provided by the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, New Zealand is gratefully acknowledged.

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Dr. Kavitha Kongara
BVSc(Cert. ECFVG-AVMA), MVSc, PhD
Lecturer in Veterinary Anatomy
Institute of Veterinary, Animal and Biomedical Sciences
Private Bag 11 222, Palmerston North 4442, New Zealand
E-mail: K.Kongara@massey.ac.nz