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Electroencephalographic changes during experimental pain induction in goats

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ABSTRACT

Electroencephalographic (EEG) studies were conducted during experimental pain induction in goats to find the association between routine managemental stress and EEG patterns. Duplicate EEG records from 4 adult (age 8 - 12 months, 10 ± 2 kg body mass) female goats (crossbred Jamunapari and local), using bipolar scalp electrodes were obtained. Pain was induced in a conscious and locally anaesthetised state using a rubber tourniquet applied at the base of the tail and fastened. Xylocaine 2% was used as an injection in the epidural space to achieve local anaesthesia. EEG recordings were taken at 0, 2 and 5 min. after pain application and at the same time intervals after removal of the tourniquet. The EEG frequencies upon pain induction in conscious and epidural anaesthetized states recorded were 38.50 ± 1.96, 33.88 ± 1.42, 31.25 ± 1.39 and 41.88 ± 0.91, 35.63 ± 0.94, 31.50 ± 0.82 Hz at 0, 2 and 5 min of application of pain stimulus, respectively. The frequencies ranged from 26 to 46 Hz in conscious and 27 to 45 Hz in epidural anaesthetized states, whereas amplitudes were higher after 2 min of pain application in both states. With tourniquet removal, the frequency increased significantly ($P<0.05$) after 2 min in a conscious state, whereas in an epidural anaesthetized state, it increased initially and then declined significantly ($P<0.05$). The amplitudes and frequencies during pain induction and removal were found to be significantly ($P<0.05$) different from each other and significantly ($P<0.05$) higher than in the control (27.75 ± 1.13 Hz and 5.80 ± 0.21μV). Prominent behavioural changes were observed with pain induction. Hence EEG changes can be used as a tool to understand and quantify painful distress in goat.

Key words: electroencephalography, pain induction, consciousness, anesthesia, goat

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Introduction

Electroencephalography, being part of electrophysiology, has been used to record the ‘brain waves’ influenced by different behavioural and physiological states

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(CUNNINGHAM, 1997). It is the graphical recording of electrical activity produced by firing neurons within the brain using a macro-electrode placed on the scalp (GUYTON and HALL, 2000**)**. It indicates the whole brain activity and the chemical actions of an entire array of neurons in the brain (NIEDERMEYER and LOPES DA SILVA, 1999). The normal electroencephalogram (EEG) gives a mental picture and serves as a frame of reference for various studies in different animals. Using EEG it enables us to know the different behavioural states and understand the feelings and thoughts of animals (BERGAMASCOA et al., 2005).

Veterinarians and researchers share equal responsibility to understand and minimize managemental stressors for better survival and increased production. Previously many attempts have been made to assess and recognize pain associated with routine animal husbandry procedures. The ability to quantify the degree of pain experienced by animals is important in order to assess and improve animal welfare. The tools conventionally used to assess the degree of distress caused by daily farm operations are behavioural symptoms (posture, vocalization and activity) and physiological changes (hormone levels, cardiac parameters, respiration rate, body temperature etc). These responses indirectly reflect stress response to pain in farm animals (MINTON, 1994). A number of studies in small animals have reported that EEG can be used as a research tool to study different behavioural states, normal and abnormal behaviours (ADDAE et al., 2000**),** neurological disorders (CROFT, 1962; KLEMM, 1989**)**, for distress evaluation or animal welfare (MINTON, 1994; COTTRELL and MOLONY, 1995**)** and can be utilized in combination with other behavioural and physiological measurements (BARNETT, 1997). Though animals cannot provide verbal reports, there are behavioural responses and EEG spectral changes with increased pain stimulation indicating distress in animals (BARNETT, 1997; GIBSON et al., 2009**).** EEG recording before, during and after castration, tail docking, mulesing, ear tagging, shearing and induced lameness in sheep have been found to have significant correlation to pain associated with an increase in EEG activity (MELLOR and MURRAY, 1989; MORRIS et al., 1997; JONGMAN et al., 2000). Therefore the present study was designed with the objective of utilizing a clinical model for evaluation of pain perception and concomitant EEG changes.

Materials and methods

The study was conducted on four apparently healthy crossbred (local X Jamunapari) female goats (10 ± 2 kg body mass). All the experiments were duly screened and approved by the Institutional Animal Ethics Committee, GBPUAT, Pantnagar. All the animals were habituated daily to the experimental conditions until consistent EEG recordings were obtained in conscious and epidural anaesthetized states. Fifteen minutes before the actual recordings, the animals were acclimatized to the recording conditions with the instrument placed in the ‘ON’ position and all leads attached to the animal.

A single channel student physiograph (Biodevice, Ambala Cantt, India) was used for EEG recording. Calibration of the physiograph was done by keeping amplifier sensitivity at 50 μV with calibration at 100 μV equal to 44 mm. Paper speed was adjusted to 50 mm/sec (1 mm equal to 0.02 s) and the time constant was 0.03 s.

Each animal was fitted with a set of bipolar (RO-LO i.e. right occipital, left occipital) lead systems in the occipital region, while the earth lead was applied at the tip of the nostril as reported previously (SUZUKI et al., 1990). The site for placement of electrodes was selected after examining the saggital sections of the skull obtained from a local slaughter house. The occipital region was just 1-2 cm caudomedial from the base of the horn. This site overlaid the occipital cortex consisting of most cortical neurons and had a minimum distance between nervous tissue and electrodes. The earth lead was secured on the dorsal aspect of the nostril, midway between the external nostrils and the eyes. The attached leads were secured in place by using a locally fabricated strap to suit the animal’s head. The hairs on the selected sites were shaved and freed from skin oils by using ethanol before application of the electrodes. Local anaesthesia (Xylocaine 2%) was injected subcutaneously into the underlying areas of skin immediately before lead placement, to minimize the artefacts arising from adjacent skeletal muscles **(**HOLLIDAY and WILLIAMS, 1999**)**. Leads (silver disc electrodes) were applied onto the scalp using EEG paste (bentonite paste).

Pain was induced in a conscious and locally anaesthetised state using a rubber tourniquet applied at the base of the tail and fastened tightly. Xylocaine 2% was injected into the epidural space to achieve local anaesthesia. During pain induction, the animals were manually restrained in right lateral recumbency. EEG recordings were made in both unrestrained and restrained positions until a steady trace was obtained. The behavioural changes were noted by visual observation.

After a stabilization period of 15 min, an EEG trace was recorded for 2 min. For pain induction, a rubber tourniquet was applied at the base of the tail and fastened tightly. EEG was recorded at 0, 2 and 5 min after application of the tourniquet. The rubber tourniquet was released and another set of EEG recordings were obtained at respective time intervals. After this, 0.5 to 1.0 mL of local anaesthesia (Xylocaine 2%) was injected in the epidural space between 1st and 2nd coccygeal vertebrae until complete tail flaccidity was achieved and pain was induced again as above. The EEG recording was obtained under local anaesthesia with a rubber tourniquet and after removal of the rubber tourniquet at the same time points. The same procedure for the EEG recording was followed in all four animals and at least two replicate recordings were taken for each event and animal.

EEG tracings recorded were visually analyzed for rhythm (frequency and amplitudes). The rhythm frequencies were calculated by counting all major and minor waves (MORRIS et al., 1997) and were expressed as the number of waves per second (Hz). The amplitudes were calculated as the height of the least and most waves in mm and then transformed to μV. The data from the experiment was statistically analyzed for analysis of variance (SNEDECOR and COCHRAN, 1994).

Results

The mean and SE of the data generated from eight records of four animals (two recordings from each animal) are presented in Table 1 and Fig.1.

Table 1. Electroencephalographic rhythm (mean ± SE) during pain induction (tourniquet application) in goats (n = 8)

	EEG Rhythm			
	Frequency	Amplitude	Frequency	Amplitude
Pre-Experimental condition (control)	27.75^{d} ± 1.13 (25 - 34)	5.80^{d} ± 0.21 (5.0 - 6.5)	--	--
Experimental condition / Time interval	Conscious state		Epidural anaesthesia	
Application of tourniquet				
0 min	38.50^{a} ± 1.96 (30 - 46)	7.64^{a} ± 0.61 (5.2-10.4)	41.88^{a} ± 0.91 (38 - 45)	9.81^{a} ± 0.79 (5.8 - 12.6)
2 min.	33.88^{b} ± 1.42 (28 - 37)	10.65^{b} ± 0.90 (6.0-14.4)	35.63^{b} ± 0.94 (31 - 39)	12.98^{b} ± 1.19 (6.5 -17.2)
5 min.	31.25^{c} ± 1.39 (26 - 38)	8.43^{a} ± 0.77 (5.2 - 10.4)	31.50^{c} ± 0.82 (27 - 34)	10.88^{a} ± 0.81 (5.8 -12.6)
Removal of tourniquet				
0 min	33.25^{a} ± 0.92 (30 - 37)	7.83^{a} ± 0.76 (5.0 -12.0)	37.75^{a} ± 2.04 (28 - 44)	8.54^{a} ± 0.69 (6.5-12.6)
2 min.	37.38^{b} ± 0.10 (32 - 40)	10.39^{c} ± 0.80 (7.3 -12.6)	34.38^{a} ± 1.93 (27 - 42)	11.61^{c} ± 0.59 (9.9-14.4)
5 min.	31.63^{a} ± 0.82 (28 - 35)	9.36^{b} ± 1.02 (6.4 - 14.4)	31.75^{b} ± 2.02 (23 - 38)	10.09^{b} ± 0.52 (9.2-13.00)

*Means with different superscript (a, b, c,…..) within a column differ significantly (P<0.05). *Figures within parenthesis indicate range.

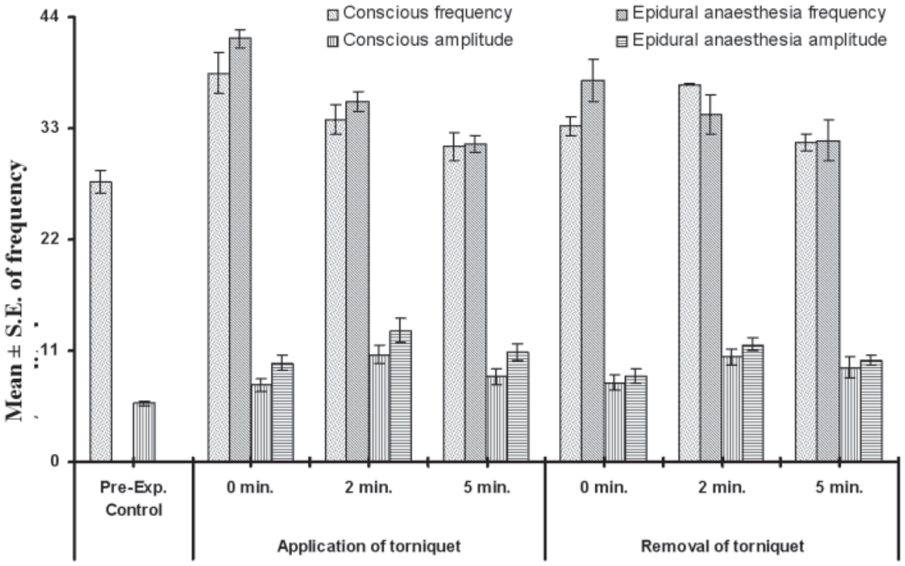

Fig. 1. Graphical representation of electroencephalographic rhythm during pain induction (tourniquet application) in goats.

The average frequencies (Hz) for conscious state with tourniquet application were recorded as 38.50 ± 1.96, 33.88 ± 1.42 and 31.25 ± 1.39 at 0, 2 and 5 min of application, respectively and with epidural anaesthesia the corresponding average frequencies were 41.88 ± 0.91, 35.63 ± 0.94 and 31.50 ± 0.82.

The average amplitudes (µV) recorded in a conscious state with tourniquet application were 7.64 ± 0.61, 10.65 ± 0.90 and 8.43 ± 0.77 at 0, 2 and 5 min intervals, respectively and with epidural anaesthesia the corresponding average amplitudes (µV) were 9.81 ± 0.79, 12.98 ± 1.19 and 10.88 ± 0.81 at respective time intervals. The average amplitude ranged between 5.2 to 14.4 µV during tourniquet application in a conscious state and 5.8 to 17.2 µV during epidural anaesthetized state.

The average frequencies recorded in a conscious state after tourniquet removal were 33.25 ± 0.92, 37.38 ± 0.10 and 31.63 ± 0.82 Hz at 0, 2 and 5 min, respectively and during the epidural anaesthetized state, after tourniquet removal, the corresponding average frequencies were 37.75 ± 2.04, 34.38 ± 1.93 and 31.75 ± 2.02 Hz for the respective periods. The average frequencies of tourniquet removal in a conscious state ranged from 28 to 40 Hz and in an epidural anaesthetized state ranged from 23 to 44 Hz.

The average amplitudes recorded in a conscious state with tourniquet removal were 7.83 ± 0.76, 10.39 ± 0.80 and 9.36 ± 1.02 μV at time intervals of 0, 2 and 5 min, respectively. The average amplitudes recorded in an epidural anaesthetized state with tourniquet removal were 8.54 ± 0.69, 11.61 ± 0.59 and 10.09 ± 0.52 μV at the respective time intervals. The average amplitudes on tourniquet removal in a conscious state ranged from 5.0 to 14.4 μV and in an epidural anaesthetized state they ranged from 6.5 to 14.4 μV.

The EEG pattern of pain induction (tourniquet application) in different states is depicted in Fig. 1. The EEG pattern initially showed low voltage fast activity (LVFA) with tourniquet application and as the pain continued it was transformed to high voltage slow activity (HVSA). The EEG pattern was similar in both states of tourniquet application, i.e. with and without anaesthesia. After the tourniquet was removed, the EEG patterns in conscious and epidural anaesthetized states showed low voltage fast activity (LVFA), but during the later stages of the epidural anaesthetized state there was an EEG pattern of high voltage and slow activity (HVSA) as in the case of the drowsy state that was described earlier (HUOZHA et al., 2009). The frequency during tourniquet application was high and categorized under the β frequency band.

The animals exhibited escape and avoidance behaviour during tourniquet application. This was characterized by frequent vocalization, restlessness with stretching of limbs, pupil dilatation, frequent turning of the head and neck towards the source of pain. Besides these behaviours, there was frequent defecation and urination. The behaviour continued as long as the tourniquet was applied in the conscious state and the signs disappeared with the removal of tourniquet. The animals showed that it was not permitted to touch the site of the applied tourniquet for a few seconds while the pain persisted. In the epidural anaesthetized state with the tourniquet application, the goats showed initial restlessness and discomfort, and as the pain continued the discomfort slowly disappeared.

The differences in frequency between the anaesthetized and conscious states were non-significant at various time intervals. Pain induction (tourniquet application) in the conscious and epidural (posterior epidural) anaesthetized states resulted in a significant ($P<0.05$) initial increase in frequency, which later decreased. The frequency of pain induction (tourniquet application) was high and was categorized under the β frequency band, which indicated that pain is associated with an increase in EEG activity. Similar observations were made by JONGMAN et al. (2000). The frequency recorded after tourniquet removal in each state revealed a significant ($P<0.05$) increase in amplitude after 2 min of removal in conscious state, whereas in the epidural anaesthetized state, an initial rise followed by a consistent decline of a significant ($P<0.05$) nature was observed. The frequencies with tourniquet application and removal in both the states were significantly ($P<0.05$) different and higher than the control frequency.

Comparison of EEG amplitudes (µV) during tourniquet application in both states indicated significantly ($P<0.05$) higher values after 2 min of application. Similarly, a significantly ($P<0.05$) greater amplitude after 2 min was observed after tourniquet removal in each state. The amplitudes during tourniquet application and removal were found to be significantly different and higher than the control amplitude.

Discussion

One of the characteristic observations of this experiment found that application of a tourniquet (both during consciousness and epidural anaesthesia) resulted in a significant ($P<0.05$) increase in EEG frequencies and amplitudes with respect to the control. This reflected that the distress caused by tourniquet application was adequately represented in EEG records and suggested the involvement of superficial cortical neurons. Similar reports have been provided on distress related EEG changes in sheep (ONG et al., 1997) and poultry (GENTLE and HUNTER, 1990).

The results of pain induction (tourniquet application) in different states (Fig 1) indicated that even under local anaesthesia, pain persisted since the amplitudes and frequencies were of a similar order. This might be due to the fact that the epidural blockade may only restrict the sensory pathway to the brain while the area responsible for processing sensory information is situated deep in the cerebral cortex. The scalp electrodes applied in this study were only able to pick up potentials from superficial neurons of the occipital cortex. Pain appeared to be caused by a stimulus, which is actually or potentially noxious to tissues. If the stimulus evokes a painful sensation, which was noxious or potentially noxious to tissue, it provokes an escape and emotional response of an avoidance nature (KITCHELL, 1987; GIBSON et al., 2009). It is possible that the somatosensory cortex may be more involved in changes in pain perception. Other areas of the brain, the thalamus in particular, may be a more suitable site to attempt to measure acute or chronic pain. The duration of pain varies with the type of pain inducer applied. The pain measurement using EEG may not be sensitive enough to discriminate between degrees of mild pain, but it has been found to be a good measure of chronic and severe pain (ONG et al., 1997).

Behavioural changes depicting the states of consciousness without and with pain induction were noticed and found to vary from each other. The persistence of pain stimulus even after removal of the pain source might have occurred due to the sensory adaptation of the nerves supplying the area of pain application. Goats in pain exhibited these painful behaviours, correlating with perceived pain intensity in a conscious state, indicating pain**.** This reflects a reflex or secondary response to pain and has been a useful indicator of pain in animals (KITCHELL, 1987).

EEG, while having methodological limitations, also has interpretative difficulties due to errors caused by bodily movements and environment artefacts. In the case of pain stress studies, there is difficulty in locating pain centres in the brain and separating pain response from motivational status (fear or anxiety). Notwithstanding these problems, changes in the EEG frequency spectrum appear to be useful indicators of management stress and could even be utilized in combination with other behavioural and physiological measurements (KITCHELL, 1987; BARNETT, 1997; JOHNSON, 2007). A multipolar electrode system, covering a wider area of the cerebral cortex, might reveal better EEG changes associated with pain distress.

It could be concluded that the present study provided an indication for potential use of EEG in animal welfare, stress evaluation and distress management in goat. Both EEG and behavioural changes are useful to minimise painful experiences, since it is in the farmer's best interest to ensure his animals' wellbeing for economic reasons as well. However, using spectral analysis in multipolar electrode system with wireless electrodes could provide a better insight for further studies in animal welfare.

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SAŽETAK

Provedena su elektroencefalografska (EEG) istraživanja kod pokusno izazvane boli u koza sa svrhom da se ustanovi utjecaj stresa uzrokovanoga proizvodnim procesom na elektroencefalogram. Analizirani su rezultati nakon dvokratnoga postupka EEG-a primjenom bipolarnih elektroda na kožu lubanje u četiri odrasle koze (križane Jamunapari i lokalne) u dobi od 8 do 12 mjeseci, tjelesne mase 10 ± 2 kg. Bol je bila izazvana čvrstim stezanjem gumenoga poveza na bazi repa u koza bez anestezije i u koza s epiduralnom anestezijom. Za regionalnu je anesteziju bio upotrijebljen 2 %-tni ksilokain ubrizgan u epiduralni prostor. EEG je bila provedena 0., 2. i 5. minute nakon izazivanja boli te 0., 2. i 5. minute po prestanku bolnoga podražaja. Frekvencija EEG nakon bolnog podražaja u koza bez anestezije iznosila je 38,50 ± 1,96 Hz 0. minute, 33,88 ± 1,42 Hz 2. minute i 31,25 ± 1,39 Hz 5. minute, a u koza s lokalnom anestezijom 41,88 ± 0,91 Hz, 35,63 ± 0,94 Hz i 31,50 ± 0,82 Hz. Frekvencije su se kretale od 26 do 46 Hz u koza bez anestezije, a 27 do 45 Hz u koza s primijenjenom epiduralnom anestezijom, dok su amplitude bile veće nakon 2 minute bolnog podražaja u obje skupine. Frekvencije su se značajno povećale ($P<0,05$) dvije minute nakon prestanka bolnog podražaja u koza bez lokalne enestezije, dok su se u onih s epiduralnom anestezijom početno značajno povećale, a zatim značajno smanjile. Amplitude i frekvencije za vrijeme i nakon bolnog podražaja bile su međusobno značajno različite, a također i značajno veće nego u kontrolnih koza (27,75 ± 1,13 Hz and 5,80 ± 0,21 µV). Značajne promjene u ponašanju životinja bile su uočene nakon bolnog podražaja. Promjene u elektrokardiogramu mogu se rabiti za proučavanje boli i određivanje jačine boli u koza.

Ključne riječi: elektroencefalografija, indukcija boli, anestezija, koze