Research Article

Electroencephalographic electrode montage in goats: topographical, radiological, and physiological assessment

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Abstract: The paucity of information on assortment of EEG leads in goats has hampered analysis of brain regional activity during different physiological, pathological, and ethological conditions in this species. Hence, the present study was designed to develop a montage with suitable combinations of EEG electrode arrays in goats. Ten specimens of freshly slaughtered goat heads (crossbreed of Jamunapari) were procured and preserved at –20 °C. Frozen samples were sliced sagittally and transversely to corroborate the link between intended topographic landmarks for EEG leads over the scalp and underlying provinces of the brain. Based on these observations, nine sites for EEG electrode placement were finalized so as to include the entire cerebral cortex. These electrode placement sites were further checked through another set of five goat heads. The electrode sites were then extrapolated to live animals for radiological (orthogonally) and physiological validation. The montage derived on basis on these electrode placement sites provided maximum brain coverage and they were found suitable for EEG in goats. Parietal and vertex areas of the brain were found to be most active during the resting phase with the right hemisphere exhibiting more activity than the left. The results indicated right hemisphere conservatism and an attentional process during the resting phase in goats.

Key words: Electroencephalography, montage, goat, skull topography, skull radiology

1. Introduction

Several neurophysiological techniques are commonly employed in animals and humans to assess brain functioning. Mental states are generated by neural processes that also produce an associated electroencephalogram (EEG). EEG topography is used to triangulate the source of electrical activity. Major problems associated with the efficacy of EEG in domestic animals are variability of skull shape and size, and presence or absence of cornua and quantum of paranasal and frontal sinuses, thereby reducing topography for placement of electrodes. The majority of work has been reported on bipolar lead systems in rats and mice (1,2) or sometimes four epidural stainless steel screw electrodes implanted bilaterally over the frontal and parietal cortices with a reference electrode over the occipital cortex in rats (3). Based upon combinations of fourteen electrodes with eight channels, reference and bipolar montages have been utilized in clinical cases in dogs (4). For evaluation of halothane

anesthesia in horses and ponies during castration, the active electrode was placed on the zygomatic process, the reference electrode over the parietal suture rostral to divergence of temporal muscles from the midline, and the ground electrode caudal to the poll (5). Electroencephalographic and behavioral alterations were studied in kids subjected to stress of physical separation from their biological dams utilizing a five lead-based montage (6).

Electroencephalographic studies using a bipolar electrode system have also been utilized in goats (11–13,16). Information on standardization of lead placement and montage development for analysis of brain regional activity in goats under different physiological states is lacking in the literature. Hence, the present study was planned with the objective to standardize a suitable montage of EEG lead combinations, anatomically and radiologically, under a multiple electrode system in goats and validate it physiologically.

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2. Materials and methods

2.1. Anatomical considerations

Fifteen heads of local × Jamunapari goats of about 1–1½ years of age, procured from a local slaughterhouse, were immediately washed under running tap water to remove excess blood. About 20 mL of 10% formalin-phenol [formalin (40%): 9 mL, phenol: 1 mL, distilled water: to make 100 mL] solution was injected through carotid arteries within 10 min of slaughtering. Another 5 mL of solution was injected very slowly with the help of a blunt long needle through the foramen magnum into the fourth ventricle. Thereafter, the heads were dipped in 10% formalin–phenol solution for 3 days, washed under running tap water, and preserved in a deep freeze at –20 °C for 3 days. Ten of the fifteen heads were taken out and the following measurements (in centimeters) were obtained with the help of vernier calipers:

1. The distance between inion and midpoint between two medial canthi.

2. The distance between two medial canthi of eye.

3. The distance between two lateral canthi of eye.

4. The distance between middle base of two external auricular orifices.

The heads (n = 10) were sliced at two planes, i.e. one saggital and four transverse planes with the help of an electric saw. The first transverse slash was made at level of the line joining the median canthi of the eye and the second at the line joining the lateral canthi. The third slash was made just posterior to the horn and the fourth slash at a line joining the middle of the two auricular orifices. The maximum length and depth (cm) of the brain, cerebrum, and cerebellum were recorded in the sagittal section (14). The maximum depth and width (cm) of the brain in various transverse slices were also measured. From recordings of the sagittal plane, the ratio between the length of the cerebrum as well as the length of the cerebellum to the total length of the brain was deduced. The ratio between the maximum width of the brain under the lateral canthi of the eye with the distance between the two lateral canthi and the maximum width of the brain in the middle of the two external auricular orifices with the distance between the bases of the two ears was also deduced. The above measurements were used to draw an arithmetic relationship between the underlying brain structures and putative surface points on the skull to standardize the following electrode placement sites (Figure 1):

F3: Left frontal electrode: Over left frontal cortex of brain.

F4: Right frontal electrode: Over right frontal cortex of brain.

Cz: Vertex electrode: Over vertex at midpoint between two hemispheres.

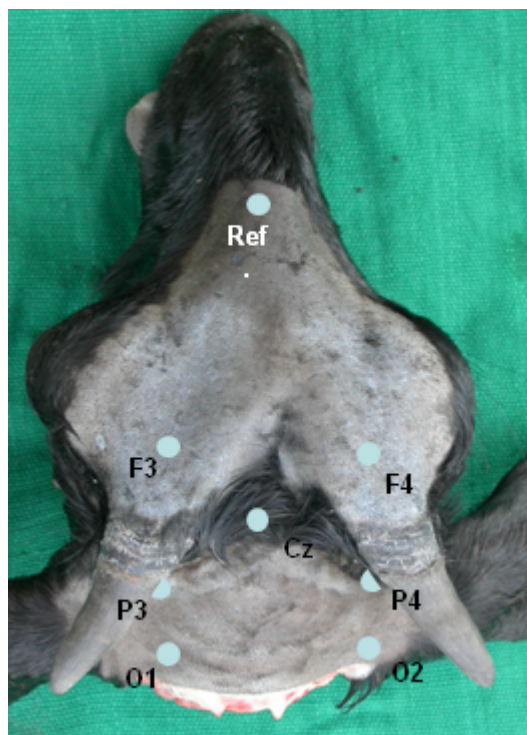P3: Left parietal electrode: Over left parietal cortex of brain.

Figure 1. Proposed sites of electrode placement (the head is slightly tilted towards the left).

P4: Right parietal electrode: Over right parietal cortex of brain.

O1: Left occipital electrode: Over left occipital cortex of brain.

O2: Right occipital electrode: Over right occipital cortex of brain.

G: Ground Electrode: Caudal to the external occipital protuberance.

R: Reference Electrode: On the bridge of the nose.

In the rest of the heads (n = 5), the data generated from the above study were compared and collated and, in addition to sectioning at the above-discussed planes, two para-sagittal sections, one at the line joining F3 to O1 and another at the line joining F4 to O2 were also obtained to confirm the positions (Figure 2). One of the heads was sectioned in oblique horizontal plane on a line from the bridge of the nose to the proposed location of occipital electrodes to extract out the brain. Measurements and repeated replacements of various slices were also performed on this head.

2.2. Radiological considerations

The putative electrode placement sites derived from the arithmetic relationship were validated by radiographic screening of live goat heads. Seven apparently healthy (local × Jamunapari) female goats of about 1–1½ years of age weighing between 19 and 26 kg (mean weight 22.14
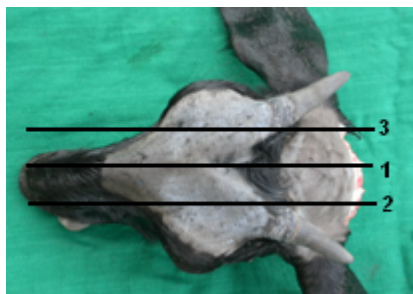
Figure 2. Alignment of median (1) and parasagittal (2 and 3) sections of the head.

kg) were selected. Dorso-ventral and lateral radiographs of the heads were taken in a 60 mA X-ray machine (Fixed Anode, M/s Allengers Medical Systems, Chandigarh, India). The exposure factors used for the dorsoventral radiograph were 60 kVp, 9.6 mAs and those for the lateral view were 55 kVp, 9.6 mAs and FFD remained constant at 90 cm during all exposures. The putative sites of electrode placement as above were marked with lead markers (A to I) after positioning the head of the animals on the X-ray table as described earlier (15).

2.3. Animals and experimental setup
The physiological experiments were conducted on seven apparently healthy female goats exhibiting the diestrous phase of the estrous cycle. During the entire experimental period the goats were allowed to graze from 1000 to 1300 and 1400 to 1700 and were supplemented with concentrate diet @ 0.25 kg/goat per day with surplus tree leaves and free access to drinking water. Routine deworming and a vaccination protocol were followed. Prior to the start of the experiment, all the goats were acclimatized to the environment of the EEG recording room and handling personnel as described (16). They were trained for other routine EEG recording practices such as posture adaptation on unrestrained sternal recumbency, placement of recording electrodes, and recording period and time daily from 0700 to 1000 for at least 15 days. All recordings were done in same the EEG recording room, which was well lit, sound attenuated, glass paneled, and electrically and wind shielded. The air temperature was maintained at 22.00 ± 2.00 °C and relative humidity was 60.00 ± 2.00%. All experiments performed were video as well as manually recorded. The sites for placement of electrodes, as deduced through anatomical and radiological studies, were cleaned and shaved every alternate day to minimize interference from hairs and maintain impedance at a low level (<10 kΩ).

2.4. Electroencephalographic recordings
A seven-channel monopolar montage was used: F3-Ref., F4-Ref., Cz-Ref., P3-Ref., P4-Ref., O1-Ref., and O2-

Ref. (even number: right, odd number: left hemisphere; F: Frontal; Cz: Vertex; P: Parietal; O: Occipital; Ref.: Reference electrode) to record brain bioelectric activity. Sternal recumbency was followed to facilitate eructation of gas, since lateral recumbency has been reported to obstruct cardia and produce acute gastric tympany. Leads were connected to the head box of the instrument RMS *Brain View Plus* (Recorder & Medicare Systems, Ambala Cantt., India). Sensitivity of the instrument was maintained at 7.5 µV/mm; sweep: 30 mm/s; time constant 0.02 s; Hf = 70 Hz; Lf = 1 Hz, notch filter inserted. Video settings were as follows: Video Card: Bt878TV card; Compression: MPEG-4 Video Codec V2 with a frame rate of 30/s. A standardized silver disc electrode (1 cm diameter) filled with bentonite paste (EEG paste) for maintaining conductivity was used. Five minutes after the electrodes were placed the instrument was switched 'ON' to recording mode with automatic simultaneous 'switching on' of video recording to time lock events. The data generated were stored in an acquisition station (RMS SSEEG 2.1 acquisition, RMS *Brain View* Plus). During the EEG recording, behavioral activities were recorded manually also. Recorded EEG tracings and data free from artifacts were selected and stored for further analysis. All experiments were duly screened and approved by the Institutional Animal Ethics Committee, GBPUAT, Pantnagar.

Special emphasis was given to eliminate possible errors due to ocular movements, muscular activity like ear or eyelid movement, and inadvertent movement of the handler and animals. EEG traces during eyes open condition were selected by corroborating visuals of video records and manual records. Three records of acceptable qualitative EEG (qEEG) of 3 s were utilized for spectral analysis.

2.5. Spectral analysis
Spectral analysis of each record was done with the help of a server (RMS *Super Spec*) utilizing integrated software (fast Fourier transformation). Fast Fourier transformation was performed for the complete frequency spectrum (0.1 to 30 Hz) to generate total power (P_{tot}, µV²) while relative power (RP, %) was generated over different frequency bands including delta (0.1 to 4.0 Hz), theta (4.1 to 8 Hz), alpha (8.1 to 12.0 Hz), and beta (12.1 to 30 Hz) to know the relative strength of each bandwidth within the spectrum as in humans, though applicable in animals as well (17). These parameters of bioelectric activity were generated for each electrode and each condition. Data from each electrode were analyzed separately. The mean of three records for each experiment was calculated to generate a single value for a particular animal from a particular electrode. The Kolmogorov–Smirnov test was employed for analysis of data for its normal distribution. Thereafter, one-way ANOVA was performed with the help of STPR

3 (Standard Program 3) developed in-house to generate statistically significant differences between electrodes within a condition. The data are presented as mean ± standard error and statistical significance was fixed at P < 0.05.

3. Results

3.1. Anatomical considerations
The mean distances between the various points on the head as well as the width and depth of the brain in various sections are presented in Table 1.

The mean length of the brain was found to be 8.40 ± 0.118 cm, while the length of the cerebrum was 6.45 ± 0.093 cm and the length of the cerebellum was 3.18 ± 0.030 cm. No brain portion could be visualized in the transverse section obtained at the level of the median canthi of the eye. The width of the brain at the level of lateral canthi of eyes was 3.54 ± 0.109 cm, while the width of the brain at the line joining the middle of the external auricular orifices was 4.97 ± 0.084 cm.

The ratios between various points on the head and underlying brain dimensions are presented in Table 2. The representative slices in transverse sections of goat heads (n = 10) are presented in Figure 3 to 5.

The ratio of width of the brain at the level of the line joining the middle of the two external auricular orifices to the distance between the bases of the two ears was 0.5378, thereby suggesting that 53.78% of distance was occupied by the brain between these two points.

3.2. Radiological considerations
Representative dorsoventral and lateral radiographs are presented in Figures 6 and 7, respectively. The various bones and features of the cranial cavity were identified as per the description offered (18). In both views, the cranial vault was discernible but superimposition produced by the rami of the mandible in the dorsoventral view restricts visualization of the wings of the cranial vault; however, this view well delineates the portion of the cranium where the cerebrum, pons, and medulla oblongata were situated. This view delineates the occipital region with a prominent

Table 1. Dimensions of brain and head at various locations on the surface (mean ± SE of ten heads).

Attribute	Distance (cm)
Distance between two lateral canthi of eye	11.26 ± 0.079
Width of brain at lateral canthi of eye	3.54 ± 0.109
Distance between two base of ears	9.24 ± 0.168
Width of brain at line joining middle of external auricular orifices	4.97 ± 0.084
Distance between center point of medial canthi of eye and inion	12.42 ± 0.196
Total length of brain	8.40 ± 0.118
Length of cerebrum	6.45 ± 0.093
Length of cerebellum	3.18 ± 0.030

Table 2. Ratio of different attributes of the brain and head dimensions (mean ± SE, n = 10).

Attribute	Ratio
Width of brain at lateral canthi of eye to Distance between two lateral canthi of eye	0.3143
Width of brain at line joining middle of external auricular orifices to Distance between two bases of ears	0.5378
Total length of brain to Distance between center point of medial canthi of eye and inion	0.6763
Length of cerebrum to Distance between center point of medial canthi of eye and inion	0.5193
Length of cerebellum to Distance between center point of medial canthi of eye and inion	0.2777

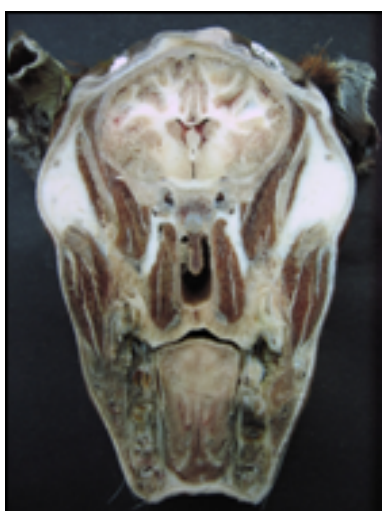**Figure 3.** Transverse sections of the head at the level of the lateral canthi of the eye (nasion side).

Figure 4. Transverse section of the head at the level posterior to the horn (nasion side).

Figure 5. Transverse section of the head at the level of the middle of the ear (inion side).

Figure 6. Dorsoventral radiograph of the goat skull showing the positions of the different EEG electrodes.

Figure 7. Lateral radiograph of the goat skull showing the positions of the different EEG electrodes.

foramen magnum. The lateral wall on each side was formed by temporal and parietal bones.

In lateral view, the cranium vault (*cavum crani*) of the goat was delineated. The boundaries of the cranial cavity were formed by the roof, base, caudal wall, rostral wall, and lateral walls. The roof of the skull, the calvarium, was formed by frontal and parietal bones and caudally interparietal bones. The interparietal bones were located between the squamous part of occipital bone and the parietal bone. The convexity of the frontal bone was oriented longitudinally. The rostral two-thirds of the base of the cranium was formed by the sphenoid bone and the caudal third by the basioccipital. The caudal wall was formed by the occipital bone. All paranasal sinuses were prominently visible in lateral view with prominent cornual processes.
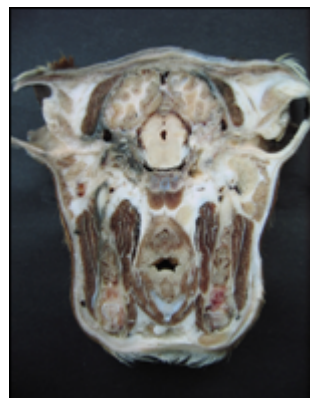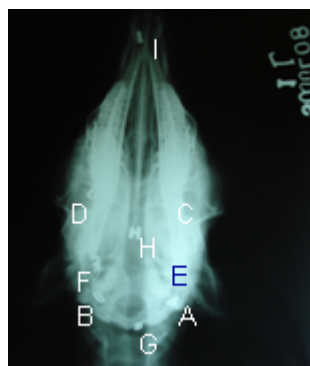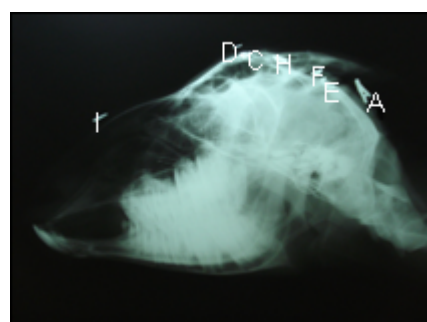
3.3. Physiological considerations

As depicted in Table 3, total power (P_{tot}, μV^2) in the vertex electrode was significantly ($P < 0.05$) higher as compared to the occipital and frontal electrodes and nonsignificantly higher as compared to the parietal electrodes. P_{tot} depicted in the P3 electrode did not differ significantly from the O1, though P_{tot} in the P4 electrode was significantly ($P <$

Table 3. Electroencephalographic attributes during somatic stimuli in goats (mean ± SE, n = 7).

Electrodes		F4	F3	Cz	P4	P3	O2	O1	CD (P < 0.05)
Total power (µV²)		791.7[ab] ± 31.2	744.0[a] ± 45.9	1708.3[c] ± 134.6	1624.0[c] ± 280.6	1511.2[cd] ± 131.3	1103.2[ae] ± 38.5	1158.5[bde] ± 48.0	405.22
Relative power (%)	Delta	67.16 ± 1.75	65.08 ± 1.76	60.58 ± 1.52	64.50 ± 2.37	63.36 ± 0.66	61.78 ± 2.66	61.58 ± 2.10	NS
	Theta	16.50[ad] ± 1.28	19.36[bcde] ± 1.58	21.75[b] ± 1.36	16.44[ac] ± 1.27	17.17[ae] ± 1.36	17.25[a] ± 3.49	15.10[a] ± 1.22	3.85
	Alpha	6.98 ± 0.73	6.84 ± 0.61	8.77 ± 0.86	7.94 ± 1.22	8.53 ± 0.84	6.77 ± 0.62	7.93 ± 0.90	NS
	Beta	9.34 ± 1.44	10.32 ± 2.04	8.88 ± 0.96	11.11 ± 1.84	10.90 ± 1.51	14.18 ± 1.32	14.88 ± 2.66	NS

0.05) higher than in both occipital electrodes and frontal electrodes. P_{tot} in the O1 electrode was nonsignificantly higher than in the F4 electrode but significantly (P < 0.05) higher than in the F3 electrode. The P_{tot} activity was higher in all right electrodes except in the case of the frontal, where it was dominant in the left frontal, even though the differences were nonsignificant. P_{tot} in the right frontal and parietal electrodes was nonsignificantly higher than in the left electrodes though total power in O1 was nonsignificantly higher than in the O2 electrode.

The relative power (Table 3) depicted that δ waves were dominant in all electrodes with nonsignificant differences amongst them. The frontal electrodes were found to be exhibiting higher delta bands compared to the other electrodes. Further, the θ band was exhibiting significantly (P < 0.05) higher activity in F3 and Cz electrodes from the rest of the electrodes except the left frontal. The θ band in the left frontal electrode was significantly (P < 0.05) higher as compared to the occipital electrodes but nonsignificantly from the others, thereby indicating desynchronized activity in these regions of the brain. The left occipital exhibited the lowest θ band strength followed closely by the right parietal and frontal, in that order. The dominance of the θ band was also evident in the vertex electrode contributing maximum power after the delta band. The alpha band was the lowest contributor towards relative power and differed nonsignificantly between the electrodes. The beta band differed nonsignificantly between different electrodes, and was highest amongst the occipital electrodes followed by the parietal electrodes. The right frontal electrode exhibited higher activity than the left frontal and the lowest beta activity was exhibited in the vertex electrode.

4. Discussion
The length of the brain observed in the present investigation was slightly greater than the length of the brain observed

in West African Dwarf goats (19) but less than that of Red Skoto goats (20) and West African Dwarf sheep (14). However, the length of the cerebrum and cerebellum was longer in the local × Jamunapari breed than those reported for West African Dwarf goats (19), Red Skoto goats (20), and West African Dwarf sheep (14) though it was closer to that of West African Dwarf sheep. The probable reason could be species or breed difference or morphological adaptation of the skull due to agroclimatic conditions or methodology of fixation of the head wherein unfixed, frozen heads have been used (14) while in our study fixation was done with formalin–phenol solution followed by freezing. The results indicated that the cerebrum was longer in the crossbred Jamunapari breed as compared to African breeds even though the total length of the brain and the length of the cerebellum are more closely related to them.

The width of the brain was found to be 53.78% of the distance between the bases of the two ears and as this mass is equally divided between both sides of the midline of the head, the edge of the brain tissue at this level occupied 26.89% (½ of 53.78) of the total distance between the bases of the two ears. The point of placement of the occipital electrodes (O_1 and O_2) was decided to be 75% of this distance from the midline. Hence it came out to be (75% of 26.89) 20.16%. Two imaginary straight lines (left and right) were drawn, from locations finalized for the two occipital electrodes, caudorostrally parallel to the midline and the point where they cut the line joining the two lateral canthi of the eye; just behind this point placement of the frontal electrodes (F_3-left and F_4-right) was done. The midpoint between the left frontal and left occipital electrode posterior to the horn was taken as the site for the left parietal (P_3) electrode and similarly on the right side (P_4). A line joining the F_3 with P_4 was drawn and another line joining the F_4 with P_3 was drawn. At the point of their intersection the Cz (vertex) electrode position was finalized.

Radiological confirmation revealed that occipital electrodes were placed lateral to the occipital bone overlying the occipital lobes of the brain (marks A and B, Figures 6 and 7). The frontal electrodes were placed on the cribriform plate at the bilateral orientation overlying the frontal lobes of the brain (marks C and D, Figures 6 and 7). The parietal electrodes were placed on the domes of the parietal bones overlying the parietal lobes of the brain (marks E and F, Figures 6 and 7). The vertex electrode was placed on the frontoparietal area of the skull overlying the rostral part of the parietal lobe (mark H, Figures 6 and 7). The reference electrode was on the incisive bone (I, Figures 6 and 7) while the ground electrode was placed posterior to the inion (G, Figures 6 and 7). The radiographs depicted that the electrode positions were in consonance with anatomical findings in live animals.

P_{tot} was significantly ($P < 0.05$) higher in the vertex electrode as compared to the occipital and frontal electrodes. Similarly, P_{tot} in the P4 electrode was significantly ($P < 0.05$) higher than in both the occipital and frontal electrodes. The data suggested higher activity in the right parietal and vertex areas. Dominance of δ waves in all electrodes with nonsignificant differences amongst them demonstrates that animals are in a state of comfort. The frontal electrodes were found to be exhibiting higher delta bands as compared to the other electrodes. The θ power was significantly ($P < 0.05$) higher in the vertex electrode as compared to the rest of the electrodes except the left frontal. The θ band in the left frontal electrode was significantly ($P < 0.05$) higher as compared to the occipital electrodes but nonsignificantly from the others, thereby indicating desynchronization in these regions of the brain. The alpha band was the lowest contributor towards relative power and differed nonsignificantly between the electrodes. The beta band was highest amongst the occipital electrodes, though nonsignificantly differing from the other electrodes.

EEG power indicates the number of neurons discharging synchronously and it could be assumed that EEG power is a measure that reflected the capacity or performance of cortical processing information. Relative power tends to give higher estimates for dominant frequency range and lower estimates for frequencies, which fall outside this range (6). It has been shown that theta power is an oscillatory component of hippocampal EEG, which is related to the memory process (21). From the anatomical point of view, the hippocampus belongs to the limbic system, which is related to emotions. Theta frequency ranges from about 3 to 12 Hz and its great power makes it easy to observe frequency and power changes in animals. The values decided in this study for theta and alpha bandwidth were slight modifications of those reported (9). Theta and alpha oscillation defined in narrow frequency bands are regarded as reflecting the activity of multifunctional neuronal networks, differentially associated with sensory, cognitive, and affective processing (22). Conversely, alpha and theta respond in different and opposite ways. According to the literature, emotional/affective processing seems to be frequency dependent, revealing that among the four frequency bands analyzed, theta oscillating networks could be the fastest in discrimination. EEG power in a resting condition depicts a decrease in alpha power (desynchronizes) and an increase in theta power (synchronizes). Many results suggest that higher theta activity is better interpreted as an electrophysiological manifestation of higher activation, related to orienting, attention, memory, and affective and cognitive processing (23). Orienting is a coordinated response that indicates arousal or readiness to process information and it is manifested in cats during exploration, searching, and motor behavior (24). Hence, orienting is closely linked to attentive state and learning. Desynchronization in the lower and medium alpha bands is associated with the process of external attention, such as alertness/vigilance and expectancy, whereas desynchronization of upper alpha reflects enhanced cognitive processing (25).

Overall, it became clear that the parietal and vertex areas of the brain are most active during the resting phase in goats. Moreover, the right hemisphere of the brain is more active as compared to the left hemisphere in the resting phase. Studies on cerebral lateralization in humans have shown that the right cerebral hemisphere is more involved in spatial and attentional processes (26). This has also led to the theory of right hemisphere conservatism (27). Dominance of the θ band with maximum power in the vertex electrode followed closely by the right parietal region indicated a higher activity in the right hemisphere, suggesting an attentional process during the resting phase.

In conclusion, this study provided a standardized method for placement of electrodes for electroencephalography that can be utilized for the development of a suitable montage for application in goats. Further, through this particular lead placement protocol, coverage of the brain area has been maximized, thereby giving a better and clearer picture of activity occurring in various areas of the brain during the resting physiological (diestrous phase) state. The method developed can be extrapolated to other small ruminants and the montage designed can be used as such or with some changes depending upon the type of study as its application in physiological conditions, clinical, or behavioral studies may vary. Further, the increase in θ band strength in the parietal and vertex areas of the brain exhibited that these areas are most active with the right hemisphere more active than the left during the resting phase and higher activity in the right hemisphere suggests an attentional process during the resting phase, which could have been due to training imparted to goats during the pre-experimental phase.

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