A Study of Spontaneous Electrical Activity in the Brain of *Caiman sclerops*.* (30192)

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Most of the electroencephalographic studies made previously on cold-blooded vertebrates have been acute, bipolar recordings, often from isolated or exposed brains and from selected aspects of the brain. Sometimes they have been involved with rather exotic experimental procedures. The attempt here was to make a systematic and broad survey of the brain under normal conditions.

For this study a South American Caiman, *Caiman sclerops*, was selected because as a crocodilian it represents a "main stream" reptile, and also this particular species is easily obtained and kept in captivity. Handling the Caiman presents few problems; it is resistant to infection and its flat, firm skull lends itself well to implantation of permanent electrodes.

Materials and methods. Eight animals were used, 5 females and 3 males, ranging in size from 450 to 1050 g and measuring 56-66 cm in length. The supplier of these specimens stated that they were 18-24 months old.

An acrylic plastic (plexiglass) plate served as the matrix for the 3 pairs of stainless steel electrodes. The electrodes were 0-80 stainless steel screws with fillister heads. The tips of the screws were filed to a 45° angle, and after threading through the plate to the desired depth of 6-12 mm they were secured in the plate with Dupont's Duco cement. The shafts were insulated with polyethylene jackets to within 0.5 to 1 mm of the tip. The electrodes were designated as follows: 1 and 2 were anterior and in contact with the right and left cerebral hemispheres, respectively; 3 and 4 were intermediate and adjacent to the right and left optic lobes; 5 and 6 were posterior and over the right and left sides of the cerebellum. The distances between the electrodes were constant; the depth varied according to the size of the animal. Choice of positions for the electrodes was based upon knowledge gained from brain dissections, serial sections, and skull studies. Actual positions were confirmed by X-ray and terminal serial sections. Implantation was carried out under aseptic conditions and no post-operative infection was encountered. Stabilizing screws were used on the 4 corners of the plexiglass plate; the size of the screw used here was also 0-80, those at the anterior corners being 6 mm long and those at the posterior 9 mm. The posterior stabilizing screws were left exposed to be used as inert electrodes for monopolar recording. A stainless steel template was used in determining electrode positions exactly, and the sites were drilled with No. 49 drill tips with a bar-stop pre-set for the desired depth. Animals were released and observed for 24-48 hours after implantation.

Recordings of electroencephalograms were made with a 3-channel Physiograph (E. and M. Instrument Co.) with 3 high gain pre-amplifiers grounded in series and to the animal. The lead wires from the pre-amplifiers, which were resting on a shelf within a Faraday cage, were clamped to the electrodes and suspended so as to permit free movement by the unrestrained animal within the cage. After adjustments were made, animals were left undisturbed for 30 minutes before recording was begun.

A second series of recordings was made with the animals restrained; restraint included taping the jaws closed, taping the legs to the sides, and fastening the animal firmly to a plexiglass plate; again, 30 minutes was allowed to elapse before recording was begun. Each animal was allowed to rest 24 hours between the unrestrained recording and the beginning of the recording under restraint.

Because it was possible to make 3 recordings simultaneously with either monopolar or bipolar leads, the order in which these were to be taken was prescheduled and rotated to

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eliminate constant errors. The overall results for each electrode or bipolar electrode pair were treated as a unit. Bipolar electrode combinations were 1 and 2, 1 and 3, 2 and 4, 3 and 4, 3 and 5, 4 and 6, and 5 and 6. Both bipolar and monopolar studies were deemed necessary to provide data comparable to previous investigations.

From each individual minute of recording 2 randomly selected 5-second intervals were analyzed in detail. Frequencies and amplitudes were classified according to the mammalian standards outlined in the *Handbook of Physiology*(1).

Results and discussion. The results of the analyses are given in Table I. In recordings made in the unrestrained condition, a fast (β) frequency of 18-24 cycles with an amplitude range of 10-20 μv was seen superimposed on a slow (α) frequency of 7-12 cycles per second with an amplitude of 20-40 μv. Under restraint there was a definite decrease in the amplitude of the fast frequency and a lesser decrease in the amplitude of the α frequency. Bipolar recordings, made across the cerebral hemispheres, were the same for both restrained and unrestrained animals and not essentially different from the monopolar records, particularly those taken under restraint.

These frequencies are similar to bipolar recordings from fish as reported by Enger(2) and Schade and Weiler(3), but the amplitudes for the fish were double those of the reptiles. The caiman records were similar in both frequency and amplitude to those reported for amphibians by Peters and Vonderahe(4) and others. However, the "silent" periods reported for frog brain by Smolin(5) were not observed in the alligator. Bremer *et al*(6) working on turtles reported a faster frequency, 40-50 cycles per second, whereas Hunsaker and Lansing(7) reported frequencies in the lizard more like those of the caiman but also incorporating a very prominent ultra-slow 3-5 cycle wave.

The electroencephalographic records obtained from the brains of birds such as those done by Tokaji and Gerard(8) and others, bear a strong resemblance to those obtained from the caiman. Upon analysis, however, they reveal an ultra-slow wave in some cases and also a very high frequency wave in some cases.

In monopolar recordings from electrodes 3 and 4, those in the area of the optic lobes, both unrestrained and restrained animals showed a well-defined dominant frequency of 6-8 cycles per second with an amplitude of 20-25 μv. Superimposed on the slow wave is an α wave of 14-18 cycles per second with

TABLE I. Analyses of Electroencephalographs of *Caiman sclerops.*

Condition	No. of animals	Electrode #	Dominant frequency	Beta frequency, c/sec	Amplitude, μv	Alpha frequency, c/sec	Amplitude, μv	Slow frequency	Amplitude, μv
			A. Monopolar recordings						
Unrestrained	8	1 & 2	Mixed	18–24	10–20	7–12	20–40		
Restrained	8	1 & 2	"	18–25	5–10	8–11	15–30		
Unrestrained	8	3 & 4	Slow			14–18	5–10	6–8	20–25
Restrained	8	3 & 4	"			14–18	5–10	6–8	20–25
Unrestrained	8	5 & 6	"	14–26	4			6–8	4–7
Restrained	8	5 & 6	"	14–20	4			6–8	4–7
			B. Bipolar recordings						
Unrestrained	8	1 & 2	Alpha to mixed	18–25	10–20	8–17	15–30		
Restrained	8	1 & 2	" " "	18–25	10–15	8–17	15–30		
Unrestrained	8	3 & 4	Slow					5–8	3–8
Restrained	8	3 & 4	"	12–25	5			5–8	3–8
Unrestrained	8	5 & 6	Blocked					5–12	3–6
Restrained	8	5 & 6	"						

an amplitude of 5-10 μv. In bipolar recordings the principal wave is a slow one of 5-8 cycles per second. The frequencies are similar to those found in fishes by Schade and Weiler(3) and Enger(2). However, in both of these cases the amplitude of the waves was considerably greater than that found in the caiman.

Gerard and Young(9), recording from the optic lobes of isolated frog brains, found 2 distinct groups of animals, those with a slow frequency wave, 4-5 cycles, and those with a very fast frequency of 100-150 cycles.

Heric and Kruger(10), using microelectrodes on different areas of the exposed optic tectum of alligators, recorded a variation in electrical responses at different depths. This suggests a possible explanation for failure to record high amplitude waves in the caiman: that is, that the varied waves from different levels of optic cortex cancelled each other out in recordings from the general area or cortical surface.

In monopolar recordings from the cerebellar area in the caiman, a slow wave, 6-8 cycles per second, with an amplitude of 4-7 μv was observed. Superimposed upon this wave a faster frequency wave, 14-20 cycles per second, was sometimes seen, but this was of a very low aplitude, less than 4 μv, and may have been noise. There was little or no difference between the restrained and unrestrained animals except when the latter were actually moving, at which times both amplitude and frequency of waves were enhanced. In all bipolar recordings, both restrained and unrestrained, the spontaneous electrical activity appears to be blocked out with the exception of an occasional slow wave of 6-8 cycles per second in the unrestrained animals.

Schade and Weiler(3) report that in the goldfish a constant freqqency wave of 25-35 cycles per second with an amplitude of 20-50 μv is present, and also a very fast wave of 120-180 cycles per second with an amplitude of 5-15 μv. Failure to demonstrate the ultrafast wave in the caiman may have been an instrumental limitation.

In the analysis of all records, a certain amount of asymmetry and asynchrony be-

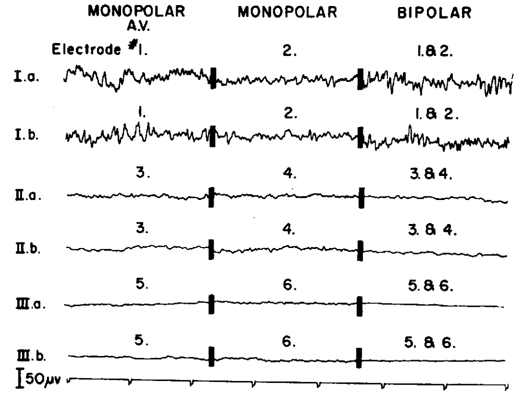

FIG. 1. Typical recordings from monopolar and bipolar electrodes in region of cerebrum (Ia unrestrained, Ib restrained); optic lobes (IIa unrestrained, IIb restrained); and cerebellum (IIIa unrestrained, IIIb restrained) of *Caiman sclerops.*

tween the two sides of the brain became apparent (Fig. 1). For example, the left cerebral hemisphere has a slightly faster average frequency than the right, amounting to 2-6 cycles per second, and in addition to this an average depression in amplitude of 5-10 μv is seen. It is believed that all possible sources of instrumental error in this regard were eliminated. There remains the possibility that consistent orientation of the caiman with the left side toward the recording instrument might explain observed differences. The fact that asynchrony is seldom recorded in normal mammals had led to the suggestion that a deep pacemaker (or pacemakers) may exist (Garoutte and Aird(11)). Perhaps, then, the pacemaker in crocodilians is not as highly developed and influential as in mammals.

Summary. A method was developed for recording electroencephalograms of *Caiman sclerops* using monopolar and bipolar electrodes. Both monopolar and bipolar recordings from the telencephalon revealed a dominant alpha frequency of 7-12 cycles per second with an amplitude of 20-40 μv. A fast frequency of 18-24 cycles per second with an amplitude of 10-20 μv was sometimes seen superimposed on the alpha wave and sometimes appeared as an independent rhythm. Restraint appears to have little effect on the frequency; however, some depression in amplitude may be seen. Monopolar electroencephalographs from the optic lobes demonstrated a dominant slow wave of 6-8 cycles

per second with an amplitude of 5-10 μv, with a superimposed α wave 14-18 cycles per second. Bipolar recordings show only a slow 5-8 cycle wave. In monopolar cerebellum recordings a slow wave of 6-8 cycles per second with an amplitude of 4-7 μv is seen as the dominant frequency. Most electrical activity appears to be blocked in bipolar recordings. Bilateral asynchrony and asymmetry were observed when monopolar recordings were made from contralateral areas of the brain. Possible causes of asynchrony are discussed.

1. Walter, W. G., in Handbook of Physiology, Neurophysiology, Am. Physiol. Soc., Washington, D. C., 1959, v1, 280.

2. Enger, S., Acta Physiol. Scand., 1957, v39, 55.

3. Schade, J. P., Weiler, I. I., J. Exp. Biol., 1959, v36, 435.

4. Peters, J. J., Vonderahe, A. R., Electroenceph-alog. & Clin. Neurophysiol., 1954, v6, 253.

5. Smolin, L. N., Byull Eksptl. Biol. Med., 1961, v52, 17.

6. Bremer, F., Dow, R. S., Moruzzi, G., J. Neurophysiol., 1939, v2, 473.

7. Hunsaker, D., Lansing, R. W., J. Exp. Zool., 1962, v149, 21.

8. Tokaji, E., Gerard, R. W., Proc. Soc. Exp. Biol. & Med., 1939, v41, 653.

9. Gerard, R. W., Young, J. Z., Proc. Roy. Soc., Series B., 1937, v122, 343.

10. Heric, T., Kruger, L., Anat. Rec., 1963, v145, 238.

11. Garoutte, B., Aird, R. B., Diamond, M. C., Trans. Am. Neurol. Assn., 1961, v86, 153.

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Effect of Alloxan on Feed Consumption in Rats.* (30193)

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The variation in the voluntary feed consumption of normal mature female rats of the Sprague-Dawley-Rolfsmeyer strain has been observed(1). The mean feed intake of 265 g rats was 5.26 g/100 g body weight (bw) with a range from 2.9 to 7.6 g/100 g bw/day. While the neural regulation of feed intake has been studied extensively(2) the possible role of the endocrine glands and their hormones has been neglected. It is interesting to speculate as to the cause of the difference in feed consumption of individual animals. May the difference be due, in part, to variation in the secretion rate of the various hormones? In initiating experiments to determine the effect of various hormones on feed consumption, the effect of endocrine gland removal on feed consumption has been undertaken.

In our initial study, the effect of thyroparathyroidectomy and of adrenalectomy on feed consumption has been reported(3). It was shown that feed consumption gradually declined 24% about 60 days after thyroparathyroidectomy and 27% after adrenalectomy. Removal of the 2 glands reduced feed intake 30%.

The present paper reports upon the effect of alloxan at a level sufficient to induce a chronic state of diabetes(4) on feed consumption.

Materials and methods. Twenty-five nulliparous mature female rats of the Sprague-Dawley-Rolfsmeyer strain weighing a mean of 223 g were placed in metabolism cages constructed so as to prevent coprophagy and feed wastage. Temperature was maintained at 78 $\pm$ 1°F and the rats were artificially illuminated during daylight hours. Purina Lab Chow with an energy value of 4.41 calories per gram and 23.4% total protein was fed during a preliminary period of 2 weeks to determine the mean daily feed consumption of each animal in the group. While the normal feed consumption of the animals prior to alloxan administration is considered the preferable control, the feed consumption of a

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