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BIOELECTRIC ACTIVITY FROM ISOLATED BRAIN OF *LACERTA ATLANTICA*

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Abstract—1. A method of maintaining the isolated lizard brain in a Ringer solution is described.
2. Microelectrodes and EEG recordings from different areas of telencephalon and optical tectum were made.
3. The prolonged cells survival under the experimental conditions described has been demonstrated for several hours.
4. The isolated brain of *Lacerta* makes it easy to reach anatomical pathways which otherwise, in the whole animal, would be more difficult to reach.

INTRODUCTION

Much knowledge has accumulated on the neurophysiology of two animal groups which are located at extremes of the animal kingdom: the molluscs and the mammals. Surprisingly few data have been published on the neurophysiology of lower vertebrates, particularly reptiles. Very little is known about the central or peripheral natural neurotransmitters, their agonists and antagonists. Nevertheless the brain of reptiles has many advantages for neurophysiological research since one can find a high level of functional specialization with a corresponding and prominent anatomical development of the brain centres, which in turn are located in separate areas.

For instance, some anatomical features are unique to the reptile brain: the pallium of the telencephalon is where histological organization into five layers appears for the first time in vertebrates (Ramon y Cajal, 1911; Pedro Ramón, 1917, 1918). The mesencephalic and diencephalic centres show a great advance in comparison with those of amphibia; the different areas being more or less complex depending on the corresponding sensory systems development, which in turn changes with species (Kappers *et al.*, 1936). Many sensory non-specific projections to the telencephalon cortex have been shown, although they cannot be considered as homologous with the mammal neocortex and its projections. EEG recordings have been made on the telencephalic cortex of *Lacerta galloti*, obtaining evoked potentials following optic, acoustic and somatosensory stimulation (Gonzalez and Rial, 1977). Reptiles, being poikilotherms, have stimulated interest about the study of temperature effects and behaviour (Gonzalez *et al.*, 1978; Patterson and Davies, 1978).

A "simple preparation" is needed for the application of microelectrodes in order to elucidate the natural neurotransmitters, ionic behaviour and agonists and antagonists in the central nervous system of reptiles. The great success achieved with the snail brain (Walker, 1968) and more recently with the *in vitro* preparations from mammal brain (Kerkut and Wheal, 1981) prompted us to keep the lizard brain alive and isolated in a bath. Since lizards are poikilotherms it might be expected to be easier to keep the isolated reptile brain alive under the experimental conditions than the mammal brain.

MATERIALS AND METHODS

The experiments were performed on eleven specimens of the lizard *Lacerta atlantica*. They were obtained from Xenopus Ltd (Surrey, U.K.) and housed in a vivarium at room temperature. Seven specimens were used for the experiments on isolated brain. Once anaesthetized with chloroform the animal was decapitated. The head was stored in reptile Ringer solution (NaCl 155 mM; KCl 5.5 mM; $CaCl_2$ 2.2 mM; $MgCl_2$ 2.0 mM; Tris–HCl buffer 3.0 mM; final pH 7.4), oxygenated and maintained at 0°C. In the medium the skull was removed and the brain cleaned from the enveloping spongy tissue and meninges. The head with the exposed brain was then placed in an experimental bath which was fitted with inlet and outlet tubes to allow washing with Ringer. The bath solution was continuously oxygenated and kept at room temperature. The isolated brain was left for 1 hr before making any recording in order to allow the cells to recover. The recordings were made using fine glass microelectrodes (pulled on a Narishige vertical electrode puller). These electrodes had a tip of approximately 0.5 μm and an electrical resistance of 10–50 MΩ when filled with the chosen saline solution. The tips of the microelectrodes were filled by capillarity with agar at 1% concentration in the saline solution used to fill up the microelectrodes: 4 M NaCl for extracellular recordings. Electrical activity was amplified with a Medistor negative capacitance cathode follower and displayed on a Tektronix 502 A oscilloscope. Permanent recordings were made with a pen recorder. Electrical stimulation was applied with a Grass S44 stimulator. For the EEG recordings four specimens were used. The electrodes were fine metal needles isolated all over their length except for the tip and soldered to a coaxial cable. All the experiments performed were made with the entire animal, anaesthetized with ether. The localization of the different areas to be explored was made taking as reference the cranial plates (Fig. 1). On the basis of the (0,0) coordinates we could locate the different areas moving

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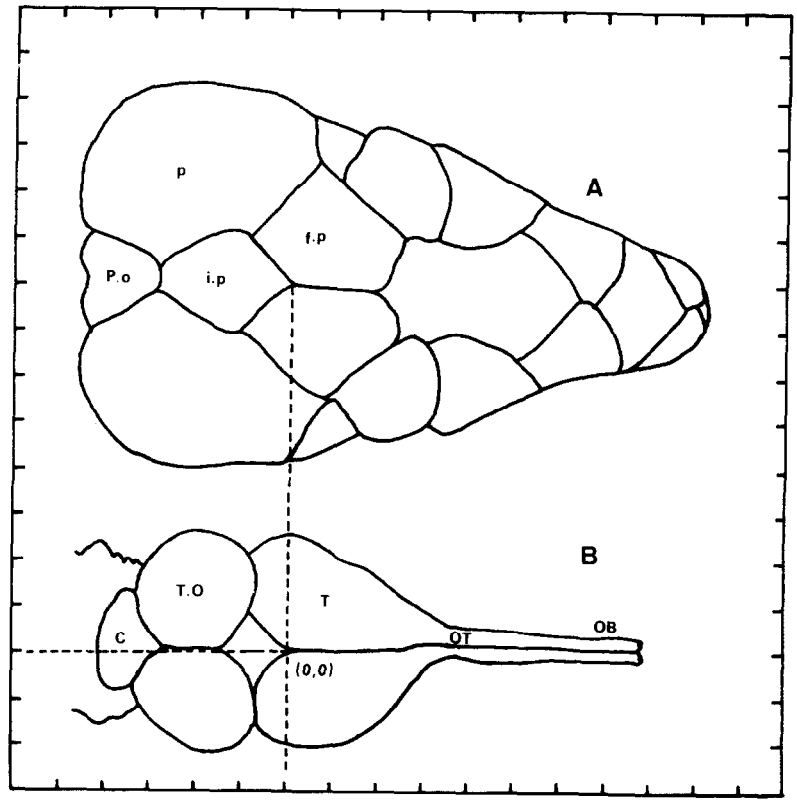

Fig. 1. A, Cranial plates of Lacertidae: P.o.: occipital plate: p.: parietal; i.p.: interparietal; f.p.: frontoparietal. B, areas of the Lacertidae brain, dorsal view: C: cerebellum; T.O.: tectum opticum; T: telencephalic vesicles; O.T.: olfactory tract; O.B.: olfactory bulb. Brain average size, 7.7 mm. Scale 10:1. The (0,0) coordinates is the intersection point between the interparietal and frontoparietal plates.

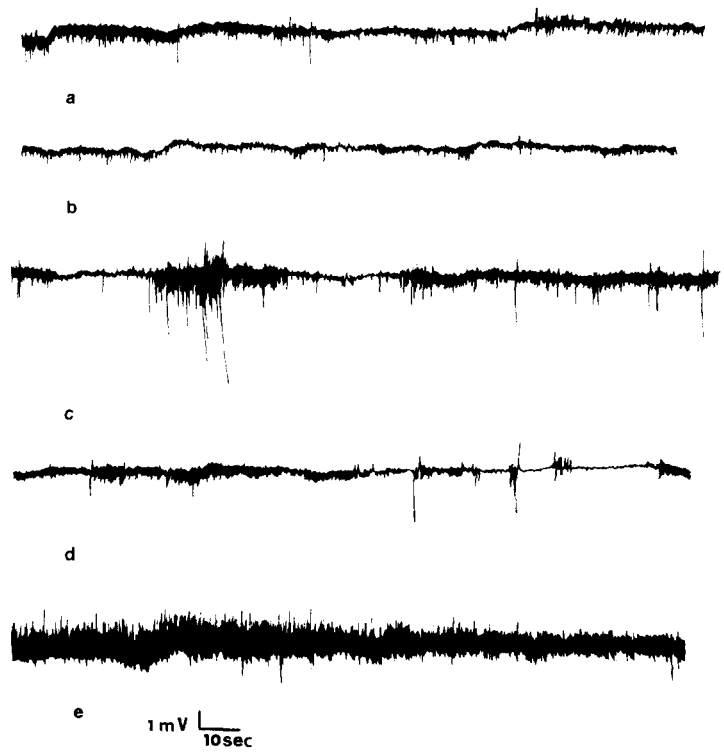

Fig. 2. Bioelectric activity from the left telencephalic vesicle (R = 18 MΩ). (a–c) Different locations at the medial area. (d) Rostral area. (e) Caudal area.

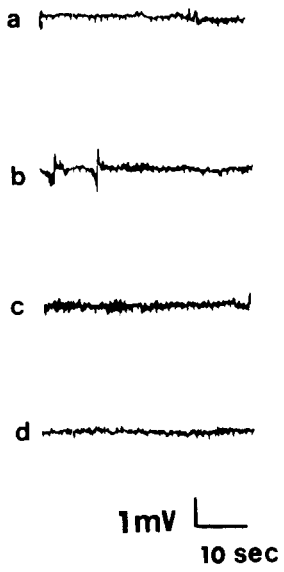

Fig. 3. Bioelectric activity from the left tectum opticum rostral area (R = 18 MΩ). (a) Spikes from the surface. In (b–d) the tip of the microelectrode has been introduced 0.4–1.1 and 1.5 mm respectively from the (a) location.

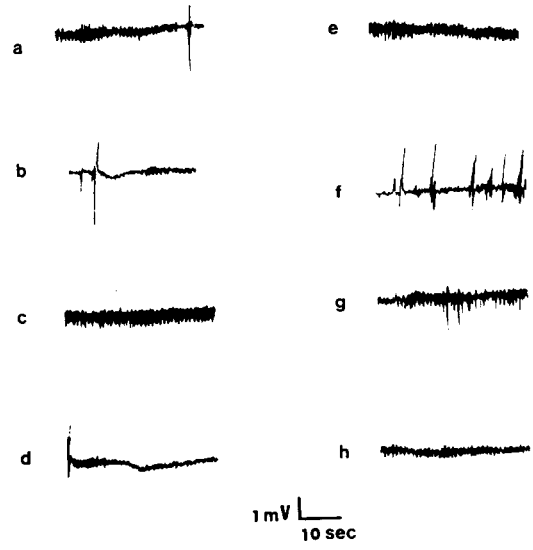

Fig. 4. Bioelectric activity from the left tectum opticum posterior area (R = 18 MΩ). (a) Spikes from the surface. In (b–e) the tip of the microelectrode has been introduced 0.4–0.8–1.2 and 1.5 mm respectively from the (a) location. In (f–h) the microelectrodes are coming out and the tip is situated at the corresponding locations shown in (b–c) and (d) respectively.

the electrode by means of a micromanipulator (Prior) along the antero posterior, horizontal and vertical axis. For the implantation of the electrodes the skull was locally perforated. Electrical activity was recorded on a Polygraph.

RESULTS AND DISCUSSION

The results obtained from the isolated brain of *Lacerta* show that the method tried here is suitable for neurophysiological research. The bioelectric activity recorded with microelectrodes changes in amplitude in the different brain areas. The basal activity recorded from the telencephalic vesicles is characterized by spikes of rather large amplitude (1–5 mV) in the different areas: medial, rostral and caudal (Fig. 2). In comparison the registered basal activity from any of the tectum areas is contained in a narrower range (0.2–0.4 mV) (Fig. 3).

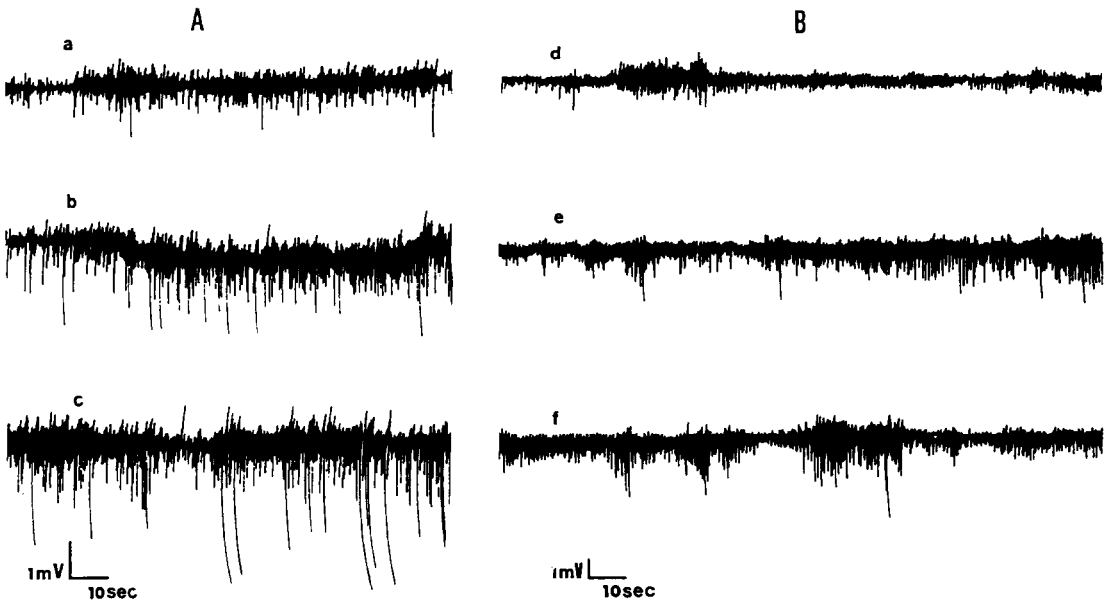

Fig. 5. Evoked potentials registered after stimulation. A, Piriform cortex (R = 15 MΩ). (a) Basal activity. (b,c) Stimulus of 1 V (10 nA) and 10 V (100 nA) have respectively been applied at the level of olfactory tract. B, Tectum opticum cortex, right vesicule (R = 15 MΩ). (d) Basal activity. (e,f) Stimuli of 1 V and 10 V have respectively been applied at the level of the optic tract.

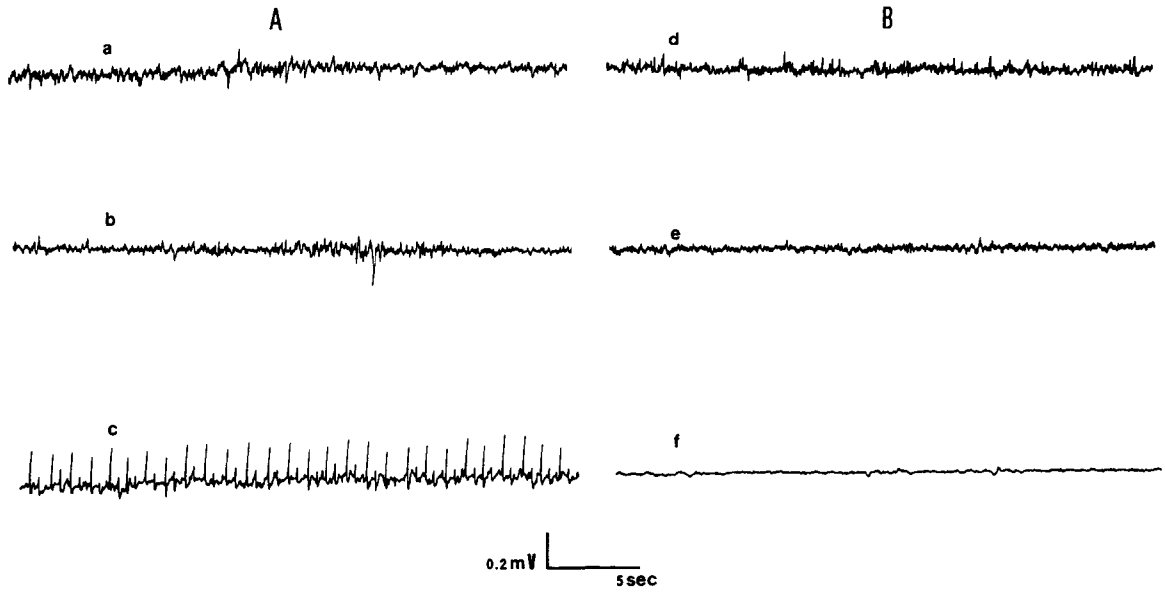

Fig. 6. EEG recordings. A, (a) From the olfactory tract, (b) from the telencephalic cortex, (c) ECG. B, In a different specimen the morphology of the different areas is maintained: (a) olfactory tract, (b) telencephalic cortex, (c) tectum opticum.

For a given anatomical area the morphology of the spikes also changes with the depth as shown in Figs 3 and 4. The microelectrodes scanned the different histological layers of the tectum opticum: stratum opticum, stratum griseum superficiale, stratum griseum centrale, stratum fibrosum, stratum griseum periventriculare and stratum fibrosum periventriculare (Kappers *et al.*, 1936).

The isolated brain is a preparation which makes anatomical pathways accessible, which otherwise (in the entire animal) would be difficult. Figure 5A shows the evoked potentials in the telencephalic cortex (piriform cortex) recorded after electrical stimulation of the olfactory tract. The amplitude of the spikes increases (from 2.5 to 4.5 mV) with increasing intensity of the applied stimulus (1–10 V, lc.p.s.). A similar result happens at the level of the tectum opticum when the stimulation is applied on the optic chiasma (Fig. 5B). In order to impale the stimulation electrode into the optic tract an ablation of the telencephalic vesicles was made to exhibit the optic pathway. The bioelectric activities shown in Fig 5 were made after 3 (Fig. 5A) and 7 (Fig. 5B) hr respectively after decapitation of the animal. The brain shows a good bioelectric activity which is in favour of the viability of this experimental method. All the recordings made were extracellular.

The EEG recordings (Fig. 6) equally show that the pattern is specific for each area and it is maintained for different specimens at the level of telencephalic vesicle, olfactory pathway and tectum opticum.

CONCLUSIONS

It can be concluded that the isolated brain of *Lacerta* is suitable for neurophysiological research. Prolonged survival of electrical activity has been demonstrated. It allows the ablation of parts of the brain to exhibit deep pathways as well as scanning of selected brain areas on surface and depth. Its accessibility to microelectrodes will contribute to the clarification of many problems concerning the nature of neurotransmitters, agonists and antagonists for the different pathways, aspects which are little known in reptiles.

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