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0014-4754/87/080925-03$1.50 + 0.20/0

Presence of giant mitochondria during cerebellar ontogenesis in reptiles[1]

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Summary. Giant mitochondria were observed in the perikarya and dendrites of Purkinje cells in the developing cerebellar cortex of the lizard *Gallotia galloti* at several stages previous to hatching. Such mitochondria are absent form the adult cerebellum.
Key words. Purkinje cells; cerebellar ontogeny; giant mitochondria; reptiles.

The ultrastructural embryonic development of the cerebellum has scarcely been studied in lower vertebrates. However, this subject has been approached in teleostei[2, 3], in the frog[4], in the lizard[5], in the chick[6, 7], in the rat[8–11], in the mouse[12], in the hamster[13] and in man[14].

The development of the cerebellar cortex of *Gallotia galloti* occurs during the embryonic period. At hatching the cerebellar cortex is morphologically nearly mature; most of the proliferative external granular layer, of which only a few cellular clusters remain, has disappeared. At this stage the cortex shows its three characteristic layers; molecular, Purkinje cell and inner granular layer. In the depth of the granular layer there is a non-stratified ependyma in continuity with the rhombencephalic fourth ventricle. The adult cerebellum of *Gallotia* is anatomically a single plate, without apparent subdivisions, and is joined to the brainstem by the basal pedunculi.

In this work we describe the presence, distribution and morphology of giant mitochondria in the developing reptilian cerebellum.

Materials and methods. The embryos of *Gallotia galloti* (Reptilia: Lacertidae) were collected in the field and classified according to the developmental table of *Lacerta vivipara*[15] and were sacrificed immediately. They were fixed by immersion or by cardiac perfusion, in 5% glutaraldehyde buffered with Millonig solution at pH 7.3. They were postfixed in 2% osmium tetroxide buffered in the same solution, dehydrated in acetone and embedded in araldite. Ultrathin sections were stained with lead citrate and examined in a Hitachi H-300 electron microscope.

Results and discussion. In the embryonic stages 39, 40 and hatching of *Gallotia galloti,* the Purkinje cells are aligned in a single layer. The giant mitochondria have been observed in the perikarya and in the main stem dendrites (in the molecular layer neuropil) in all the Purkinje cells. In these stages they are not observed in any other cerebellar cell type. In earlier embryonic stages or in postnatal or adult lizards giant mitochondria are not detectable (figs 1 and 2).

The giant mitochondria are always observed in smaller numbers than the normal-sized mitochondria.

The morphological characteristics of the giant mitochondria are as follows. They are five to seven times larger than normal mitochondria; their shape is spherical, oval or occasionally almost triangular in the dendritic branches; they show abundant cristae which are sometimes radially arranged. Electron dense granules are observed in the mitochondrial matrix, as in normal mitochondria (fig. 1).

In the same embryonic stages in which giant mitochondria appear, we have observed small protrusions in the Purkinje cell body. They have a thin pedicula and their distal portion is thicker and piriform or ovally shaped. They are longer than the somatic spines which are typical in the mature cell.

In the later embryonic stages, near the hatching stage, the progressive disappearance of the external granular layer in the *Gallotia* cerebellum occurs. This disappearance coincides with an increase in the development of the distal dendritic tree of the Purkinje cells. Moreover, this period of development shows a progressive increase in the number of synapses, mostly in the superficial half of the molecular layer. This corresponds to the strata where dendritic spines and also dendritic arborizations are most abundant. A great number of these synapses are established between the en passant and terminal boutons of the parallel fibers of the granular cells and the dentritic spines of the Purkinje cells.

The large mitochondria are present only in the Purkinje cells of the lizard and the chick[7]. Its transitory presence coincides with the establishment of a great number of synapses by the Purkinje cell and the start of an accelerated myelination in the lizard's cerebellar cortex. Although this temporal coincidence does not offer conclusive evidence, we think that there is a relation between the presence of these mitochondria and the physiological maturation of the cerebellar cortex. But we have no evidence of its exact role in cellular metabolism, and more data are necessary to elucidate this event.

1 The authors would like to thank Prof. L. Puelles (Murcia University) for his critical assistance and the Edafology Department of La Laguna University for facilities offered, specially for the use of the electron microscope. This work has been partially supported by a CAICYT research grant No 909-2.
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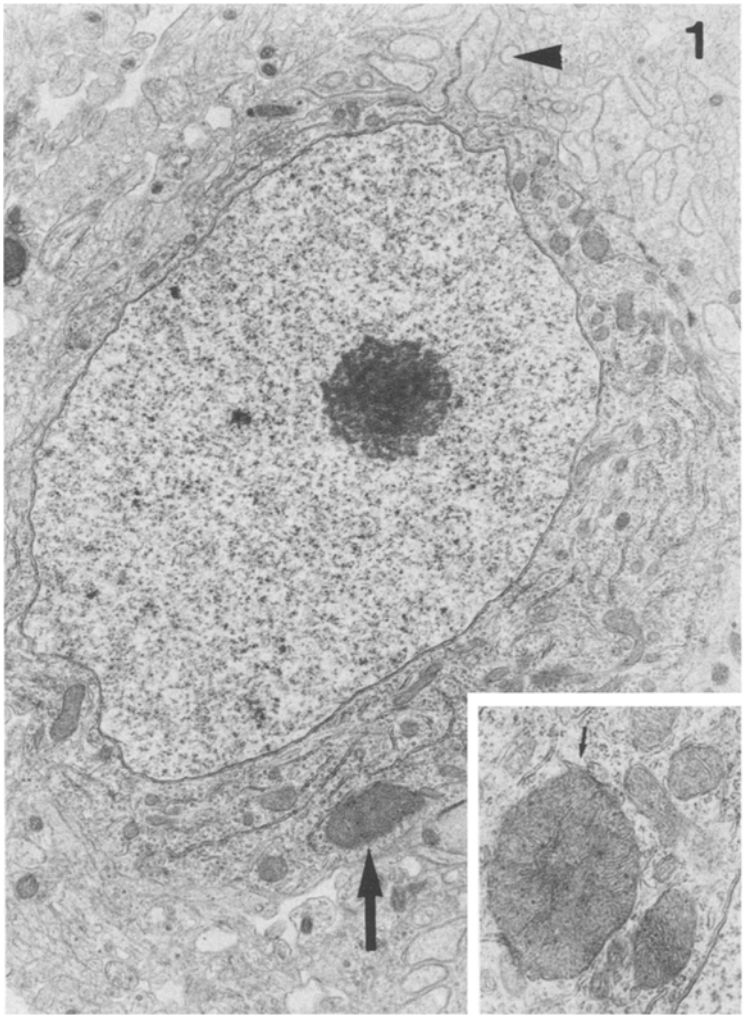

Figure 1. Purkinje cell at stage E-40. In the perikarya there is a giant mitochondrion (arrow). The arrowhead marks a somatic protrusion. × 8300. The inset shows a giant mitochondrion (arrow). × 14500.

Figure 2. Sagittal section through the lizard's cerebellum in late embryonic stages. Arrows: external granular layer; arrowheads: Purkinje cell layer. (H-E stain. Bar: 100 μm.).

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