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An ultrastructural study of the development of oligodendrocytes in the midbrain of the lizard*

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INTRODUCTION

It has been widely recognised that mature mammalian oligodendrocytes vary in size and in the electron density of their cytoplasm (Kruger & Maxwell, 1966; Mori & Leblond, 1970; Peters, Palay & Webster, 1970; Ling *et al.* 1973; Phillips, 1973; Sturrock, 1974, 1976). The ultrastructural changes in oligodendrocytes during development have also been the subject of a number of investigations (Caley & Maxwell, 1968; Vaughn, 1969; Phillips, 1973; Ling & Leblond, 1973; Sturrock, 1974, 1976; Skoff, Price & Stocks 1976*a*, *b*; Imamoto, Paterson & Leblond, 1978; Parnavelas *et al.* 1983; Lord & Duncan, 1987). It is now generally accepted that oligodendrocytes are responsible for myelination in the central nervous system and it is therefore not surprising that the rapid increase in glial cell number which precedes the onset of myelination and continues during the phase of rapid myelination (Roback & Scherer, 1935) is mainly due to proliferation and differentiation of oligodendrocytes (Vaughn, 1969; Sturrock, 1974, 1982*a*, *b*; Skoff *et al.* 1976*a*, *b*; Lord & Duncan, 1987).

Whilst most ultrastructural studies of mature oligodendrocytes have been carried out in mammals, the ultrastructure of glial cells has also been investigated in amphibia (Stensaas & Stensaas, 1968; Schonbach, 1969; Stensaas, 1977), in teleosts and reptiles (Kruger & Maxwell, 1967) and in the chicken (Lyser, 1972) but oligodendrocyte development in the reptile appears to have been largely neglected apart from an immunohistochemical study of gliogenesis in the lizard *Gallotia galloti* using myelin basic protein (MBP) as a marker (Monzon-Mayor *et al.* in preparation). This study set out to investigate the ultrastructural changes occurring in oligodendrocytes in pre- and postnatal lizard midbrain as an adjunct to the immunohistochemical study.

MATERIALS AND METHODS

The material consisted of the midbrains of twenty embryo lizards, eight postnatal and ten adult lizards of the species *Gallotia galloti*. These were the same animals used for the companion paper on astrocyte development in which the methods by which these were obtained and the subsequent processing of the brain are described (Monzon-Mayor, Yanes, James & Sturrock, 1990).

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RESULTS

Oligodendroblasts

Oligodendroblasts were present from E34 to the early postnatal stages. They differed from glioblasts (Monzon Mayor *et al.* 1990), in having a paler cytoplasm with an extensive Golgi complex, rosettes of free ribosomes, short cisternae of rough endoplasmic reticulum and numerous microtubules in the perikaryon and processes (Fig. 1).

Active oligodendrocytes

These cells were most numerous from E37 to hatching which coincides with the period of rapid myelination. Active oligodendrocytes had a moderately dark to dark cytoplasm containing an extensive Golgi complex, numerous mitochondria, microtubules and rough endoplasmic reticulum often arranged in long parallel stacks (Fig. 2). The nucleus, which was usually irregularly shaped, contained a nucleolus (Fig. 3) and euchromatin well dispersed throughout the nucleus with only a very thin rim attached to the nuclear envelope.

Light oligodendrocytes

Light oligodendrocytes were present at all stages from E37 onwards. The palely staining cytoplasm contained free ribosomes, numerous mitochondria, a Golgi complex and abundant microtubules. A centriole or pair of centrioles (Fig. 4) were sometimes found. The nucleus was often indented and was filled with coarsely scattered euchromatin.

Medium oligodendrocytes

Medium oligodendrocytes could be found at all ages from E40 onwards. The cytoplasm of medium oligodendrocytes was more electron-dense than the surrounding neuropil and the nuclear chromatin tended to be arranged in clumps, particularly around the cell membrane (Figs. 5–7). There was a wide spectrum of cytoplasmic density (contrast Figs. 5–7) and classification of borderline cells as either light, medium or dark was somewhat subjective. Medium oligodendrocytes contained fewer organelles than active oligodendrocytes; in particular the number of mitochondria and the quantity of rough endoplasmic reticulum appeared to be much reduced. A Golgi complex could usually be identified and centrioles were often observed (Fig. 6).

Dark oligodendrocytes

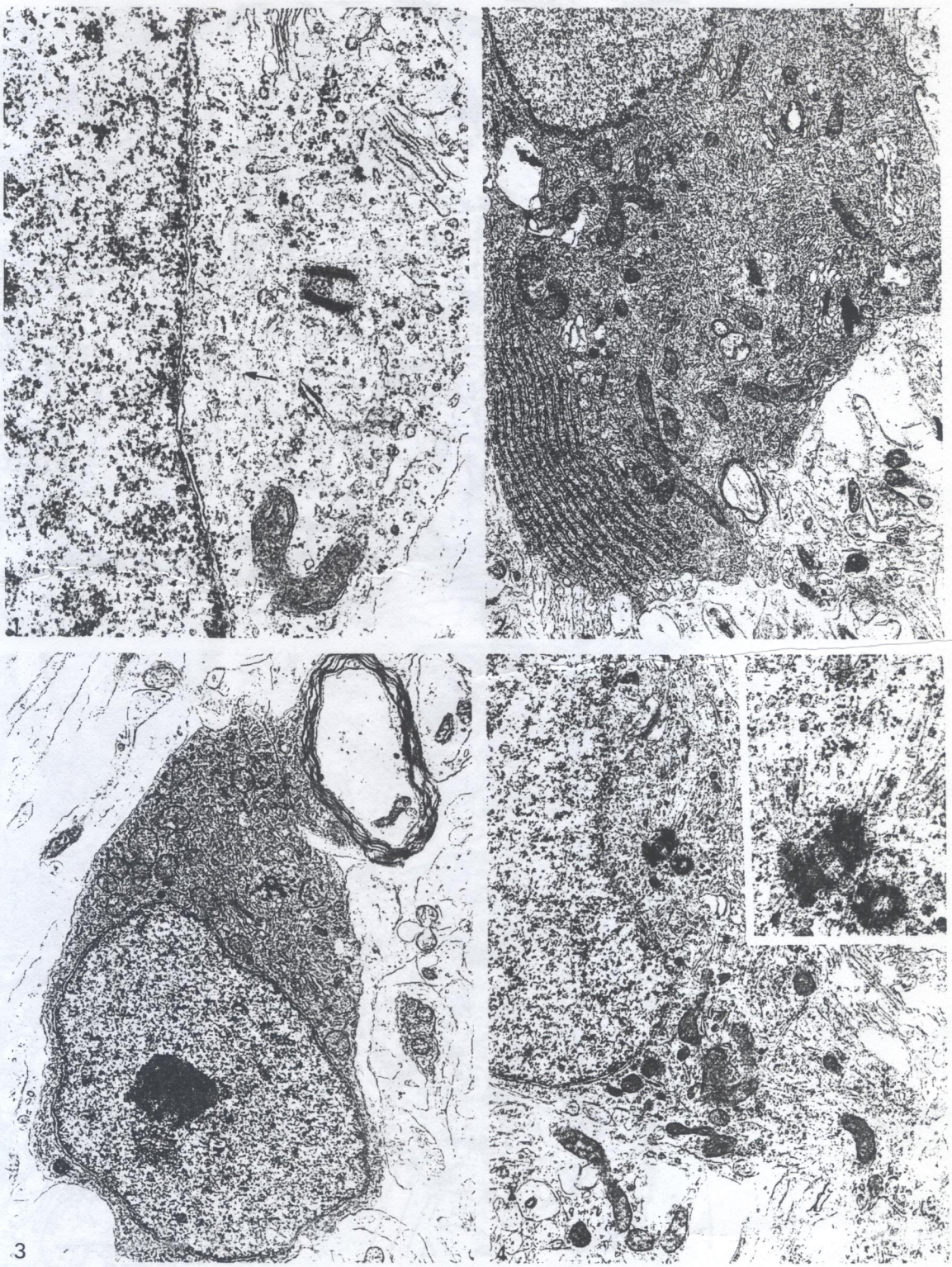
Dark oligodendrocytes were present at all stages from hatching onwards. Their main characteristic was a very electron-dense cytoplasm as shown in Figure 8 in which the dark oligodendrocyte is lying adjacent to a mature microglial cell and a protoplasmic astrocyte.

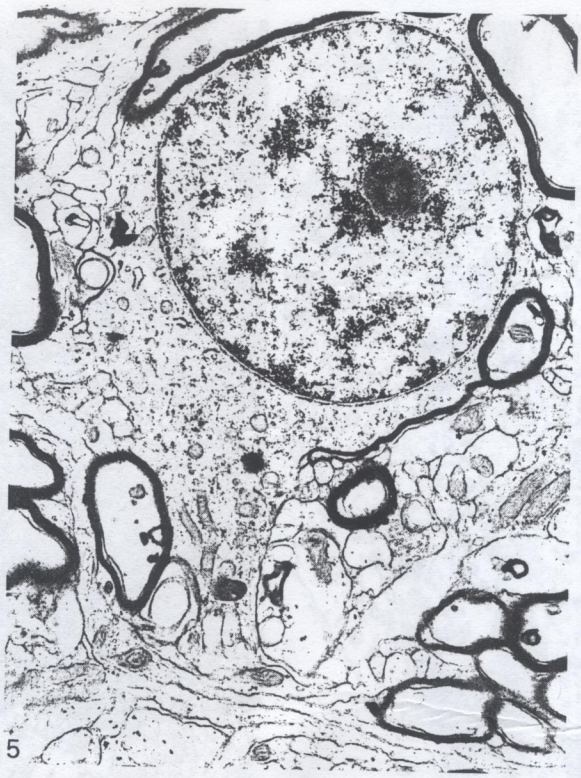
Fig. 1. Oligodendroblast with a pale cytoplasm containing numerous microtubules (arrow). E35. ×31000.

Fig. 2. Active oligodendrocyte with an electron-dense cytoplasm. Note the long parallel stacks of rough endoplasmic reticulum. Hatching. ×12000.

Fig. 3. Active oligodendrocyte with an electron-dense cytoplasm and a pale nucleus containing a nucleolus. E39. ×12000.

Fig. 4. Light oligodendrocyte with a pale cytoplasm. Note the pair of centrioles. E40. ×12000; inset ×31000.





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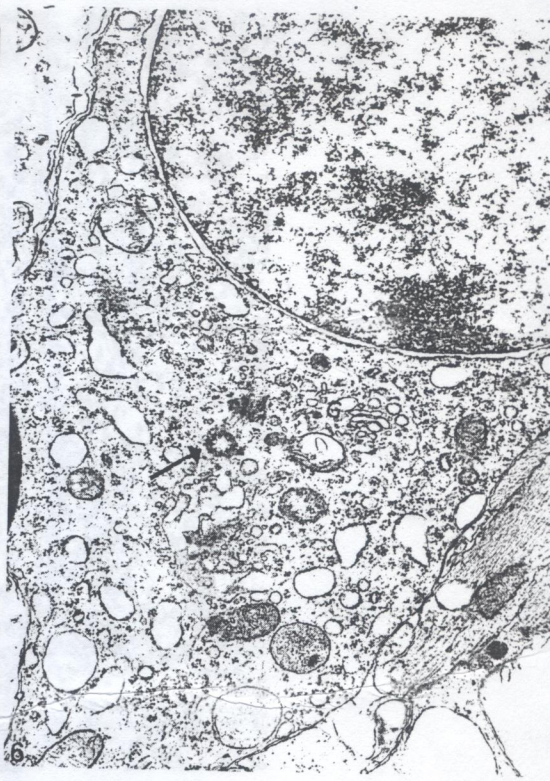

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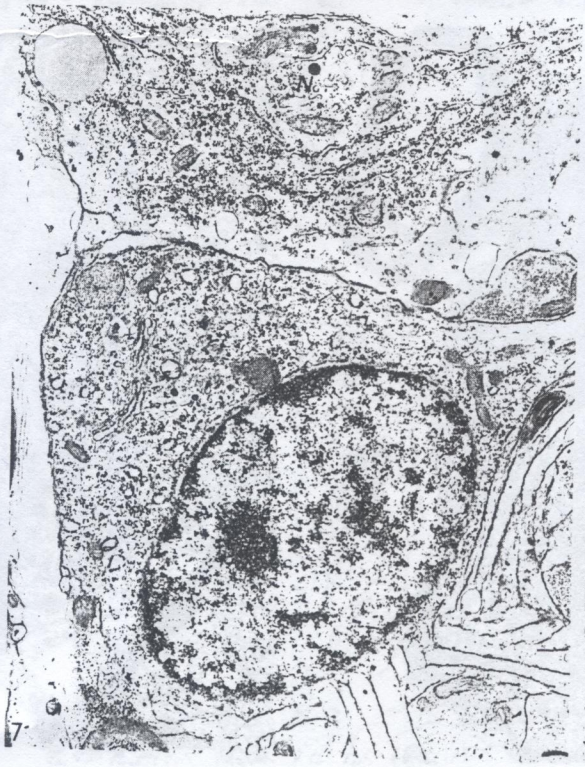

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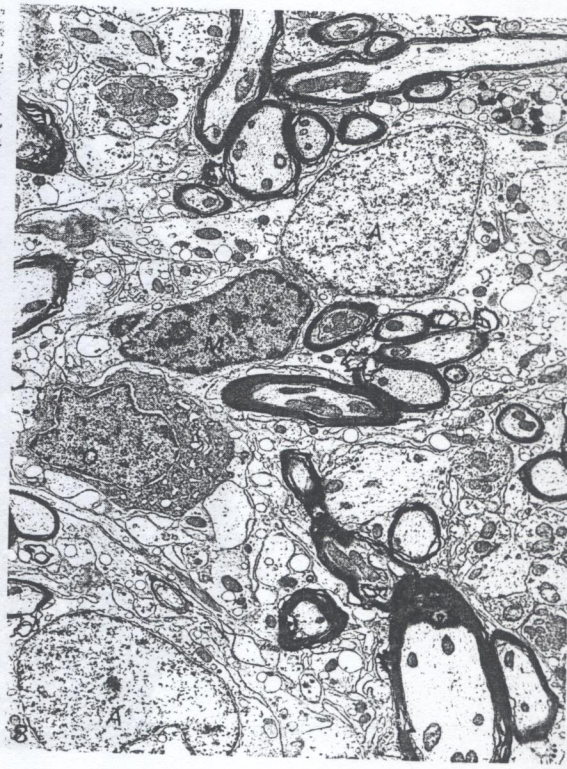

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DISCUSSION

Electron micrographic studies of the mammalian central nervous system have shown that oligodendrocytes exhibit a wide spectrum of electron density (Kruger & Maxwell, 1966; Mori & Leblond, 1970; Ling *et al.* 1973; Sturrock, 1974; Vaughan, 1984) although most authors agree that, in the adult, dark oligodendrocytes form the major part of the oligodendrocyte population. The results of Mori & Leblond (1970) led to further autoradiographic investigations into the developmental significance of the three main shades (light, medium and dark) of oligodendrocytes by Paterson, Privat, Ling & Leblond (1973) and Imamoto *et al.* (1978) and led them to conclude that the three types represented different stages in oligodendrocyte development with light oligodendrocytes differentiating first into medium and finally into dark oligodendrocytes. This conclusion was supported by the electron microscopic study of gliogenesis by Parnavelas *et al.* (1983).

Blakemore (1982), however, stated that the electron density of oligodendrocyte cytoplasm was directly related to the size of the oligodendrocyte which in turn depended on how metabolically active the oligodendrocyte was. As a consequence of this hypothesis he proposed that oligodendrocytes which myelinated large axons with thick myelin sheaths had a paler cytoplasm that those which myelinated small ones with thin sheaths and this seems to be the case in the adult lizard midbrain. In the lizard midbrain the oligodendrocytes present during the most rapid phase of myelination have a number of features in common with medium oligodendrocytes as described by Mori & Leblond (1970) and Parnavelas *et al.* (1983), in particular the long parallel stacks of rough endoplasmic reticulum and the nucleus with dispersed chromatin and a prominent nucleolus. Structurally similar cells, called active oligodendrocytes by the authors, have been described during myelination in the rat optic nerve (Vaughn, 1969; Vaughn & Peters, 1971) and dorsal funiculus (Matthews & Duncan, 1971) and in the mouse anterior commissure (Sturrock, 1974). In the young pup corticospinal tract Lord & Duncan (1987) illustrated similar myelin-forming cells but named them dark oligodendrocytes. In the lizard the cells described by us as active oligodendrocytes often have a moderate to dark cytoplasm and are not always medium oligodendrocytes as judged by their electron density. The nucleus with its prominent nucleolus and dispersed chromatin and the stacked arrays of rough endoplasmic reticulum are indicative of a cell which is engaged in very active biosynthesis. We therefore suggest that the term 'active oligodendrocyte' is more appropriate than 'medium oligodendrocytes' since it reflects the structural and functional characteristics of this stage of oligodendrocyte development.

Our active oligodendrocytes are similar to the active oligodendrocytes described by Vaughn (1969), Vaughn & Peters (1971), Matthews & Duncan (1971) and Sturrock (1974) and also to those described as medium oligodendrocytes by Mori & Leblond (1970) and Parnavelas (1983). These cells are structurally different from the medium oligodendrocytes found in the adult lizard midbrain. The nuclear chromatin of medium oligodendrocytes is much more clumped and the nucleus is more often

Fig. 5. Medium oligodendrocyte with a moderately electron-dense cytoplasm closely associated with three myelinated axons. Adult. × 12000.

Fig. 6. Medium oligodendrocyte containing a Golgi complex (*G*) and a centriole (arrow). Adult. × 12000.

Fig. 7. Medium oligodendrocyte in a satellite position to a neuron (*N*). Adult. × 12000.

Fig. 8. Dark oligodendrocyte (*O*), microglial cell (*M*) and astrocytes (*A*) in the white matter of the mesencephalon. Adult. × 2600.

spherical or elliptical, unlike the irregularly shaped nuclei of active oligodendrocytes. There are fewer organelles in medium oligodendrocytes than in active oligodendrocytes and, in particular, rough endoplasmic reticulum is greatly reduced in quantity. In the lizard, therefore, active oligodendrocytes and adult medium oligodendrocytes are quite different cells.

Most investigations into mammalian gliogenesis have been carried out in the corpus callosum (Mori & Leblond, 1970; Ling *et al.* 1973; Paterson *et al.* 1973; Imamoto *et al.* 1978), optic nerve (Vaughn 1969; Vaughn & Peters, 1971; Skoff *et al.* 1976*a*) or anterior commissure (Sturrock, 1974) all of which are tracts containing small axons. Blakemore (1982) examined cat spinal cord in which tracts with large diameter axons are common. Large diameter axons are also present in the midbrain, for example in the reticular formation, and it is possible that the medium oligodendrocytes found in the lizard midbrain are cells responsible for maintaining thick myelin sheaths surrounding medium to large diameter axons. In an immunohistochemical study of lizard midbrain (Monzon-Mayor *et al.* in preparation) myelin was first detected with the MBP marker at E37 which coincides with the first appearance of active oligodendrocytes in the ultrastructural study. This would seem to confirm the validity of the ultrastructural criteria for identification of myelin producing oligodendrocytes.

SUMMARY

Oligodendrocyte development was investigated in the midbrain of the lizard *Gallotia galloti* using the electron microscope. Oligodendroblasts, which had a pale cytoplasm containing numerous microtubules in the perikaryon and processes, were present from E35.

Active oligodendrocytes had a pale nucleus, usually containing a nucleolus, and an electron-dense cytoplasm with long parallel stacks of rough endoplasmic reticulum. These were present from E37 to hatching which coincides with the period of rapid myelination.

The three types of oligodendrocyte (light, medium and dark) first classified by Mori & Leblond (1970) in the rat could be identified in the lizard. Light oligodendrocytes were present at all ages from E37 to adult. Medium oligodendrocytes first appeared at E40 and dark oligodendrocytes were present at all ages from hatching onwards.

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