Development of the ventral striatum in the lizard
Gallotia galloti

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INTRODUCTION

The ventral striatum (VS) is one of the basal nuclei of the reptile telencephalon which is phylogenetically more uniform than other areas such as the anterior dorsal ventricular ridge (ADVR) (Ulinski, 1983). It is situated ventral to the anterior dorsal ventricular ridge (ADVR) and lateral to the septum occupying the rostral levels of the telencephalon. In the adult it is made up of three cellular groups: the ventral group (v_1s), the ventral medial group (v_2s) and the dorsolateral group (v_3s) (Yanes, Martín-Trujillo & López, 1977). Various morphological and topographical studies (Johnston, 1923; Crosby, De Jonge & Schneider, 1967) as well as histochemical studies (Parent & Oliver, 1970; Parent, 1986) have suggested that these three cellular populations of the reptile VS are homologous with regions of the caudate nucleus, putamen and globus pallidus of mammals. Functionally, VS has been related to somatic coordination and olfactory sensation (Goldby & Gamble, 1957; Powell & Kruger, 1960). Its connections are not well known; however, the latter authors also point out that some of its afferents proceed from the thalamus (rotundus and dorsomedial nuclei) via the dorsal peduncle of the lateral forebrain bundle. On the other hand, Hoogland (1977) indicates that its afferents are sent particularly through the ventral peduncle of the lateral bundle, especially toward the tegmentum of the midbrain. Little information exists on its development (Källen, 1951; Hetzel, 1974) in different reptiles. In this paper a structural and ultrastructural study is described of the development of the ventral striatum of the lizard *Gallotia galloti*.

MATERIALS AND METHODS

We used 30 embryos, postnatal specimens and adults of the lacertidian *Gallotia galloti* (Reptilia: Lacertidae). These were obtained from female lizards captured in Tenerife (Canary Islands) during May and June, and from eggs dug from the ground in identified laying areas in North Tenerife from July to September. The embryos were staged according to the Table of Development of *Gallotia galloti* in which stages of development are equated with the Development Table of *Lacerta vivípara* by Dufaure & Hubert (1961). Postnatal classification was made according to size (length head–cloaca), criteria used by Rose (1957) and Pleticha (1968). In our study we have used examples of 3·6 and 4·5 cm. The embryos with an encephalic length greater than 2 mm – Stage 30 (E. 30) onwards – were decapitated and immediately fixed by immersion. The specimens near hatching, postnatal specimens and adults were

anaesthetised with sodium pentobarbitone (Nembutal) and perfused with Bouin's solution through the heart ventricle while others, similarly anaesthetised, were perfused with glutaraldehyde.

For light microscopic studies the specimens were fixed in Bouin's solution and Lillie's formalin, embedded in paraffin wax, sectioned at 7–10 μm and stained with haematoxylin and eosin or toluidine blue and orange-G (Mann–Dominici). For electron microscopy they were fixed with glutaraldehyde in 2·5% phosphate buffer (0·1 M and pH 7.2), postfixed with 2% osmium tetroxide in Millonig buffer at 4 °C for 2 hours, dehydrated with acetone and embedded in Araldite. The semithin sections were stained with 1% toluidine blue.

RESULTS

The VS develops in the ventral wall of the telencephalic vesicle at Stage E. 31. The main part of the VS is made up of cells from the intermediate zone adjacent to the ventricular zone, which contains the first migratory neuroblasts. At E. 32, a modification of these characteristics has taken place, since a growth in the cellular population is observed, which coincides with intense proliferation in the sulci terminalis and ventralis (Fig. 1). As a result of these proliferations numerous neuroblasts are incorporated into the intermediate zone, giving rise to nucleus formation (Fig. 1). In this first developmental stage it is common to find various neuroblasts adjacent to thick cellular processes in the VS (Fig. 5A). During the first developmental stages the delimitation of this nucleus (VS) with respect to other adjacent structures (ADVR and septum) is poorly defined (Figs. 1, 2), but as from E. 37 a zone with few cells appears which permits the establishment of dorsal limits with the ADVR and lateral limits with the septum (Fig. 4). At E. 35, the cellular population begins to show its distribution into the three groups (v_1s, v_2s, v_3s) (Fig. 2), that show up clearly at E. 40 (Fig. 4). At the same time the sulcus proliferations descend (Fig. 3) and neuroblasts in the course of migration are not observed (Fig. 6A). At the ultrastructural level, neuroblasts at Stage E. 32 show a cytoplasm with few membranous organelles but with abundant ribosomes. They are disposed radially to the ventricular surface of their respective sulci, leaving ample intercellular spaces between them (Fig. 5A). As from E. 35 the cells show a cytoplasm rich in organelles and the intercellular spaces diminish in size. At E. 38, neurons appear with a degree of development analogous to the adult while intercellular spaces have practically disappeared (Fig. 6A). The first indices of degeneration and cell death appear in E. 34; these cells show a strongly pycnotic nucleus (Fig. 7A, B), and their frequency gradually increases till hatching; however, they have not been seen at the postnatal or adult stages. As from E. 40, we can find a second type of degeneration, characterised by a dense condensation of the cytoplasm and a marked dilatation of the Golgi cisterns. This type of death is present postnatally and in the adult (Fig. 8).

The first synaptic contacts are observed from stage E. 38 onwards and are of the axodendritic type with sparse clear vesicles (Fig. 9). As from E. 40, an increase of clear vesicles is observed (Fig. 10), and some mixed synapses with clear vesicles and dense cores begin to appear (Fig. 10). This last type of cell increases from postnatal stages up till adulthood (Fig. 11).

The ventral striatum is one of the most vascular nuclei, and appears to be invaded by numerous ramifications of the archistriatal artery as from E. 35, and this increases progressively until E. 40.
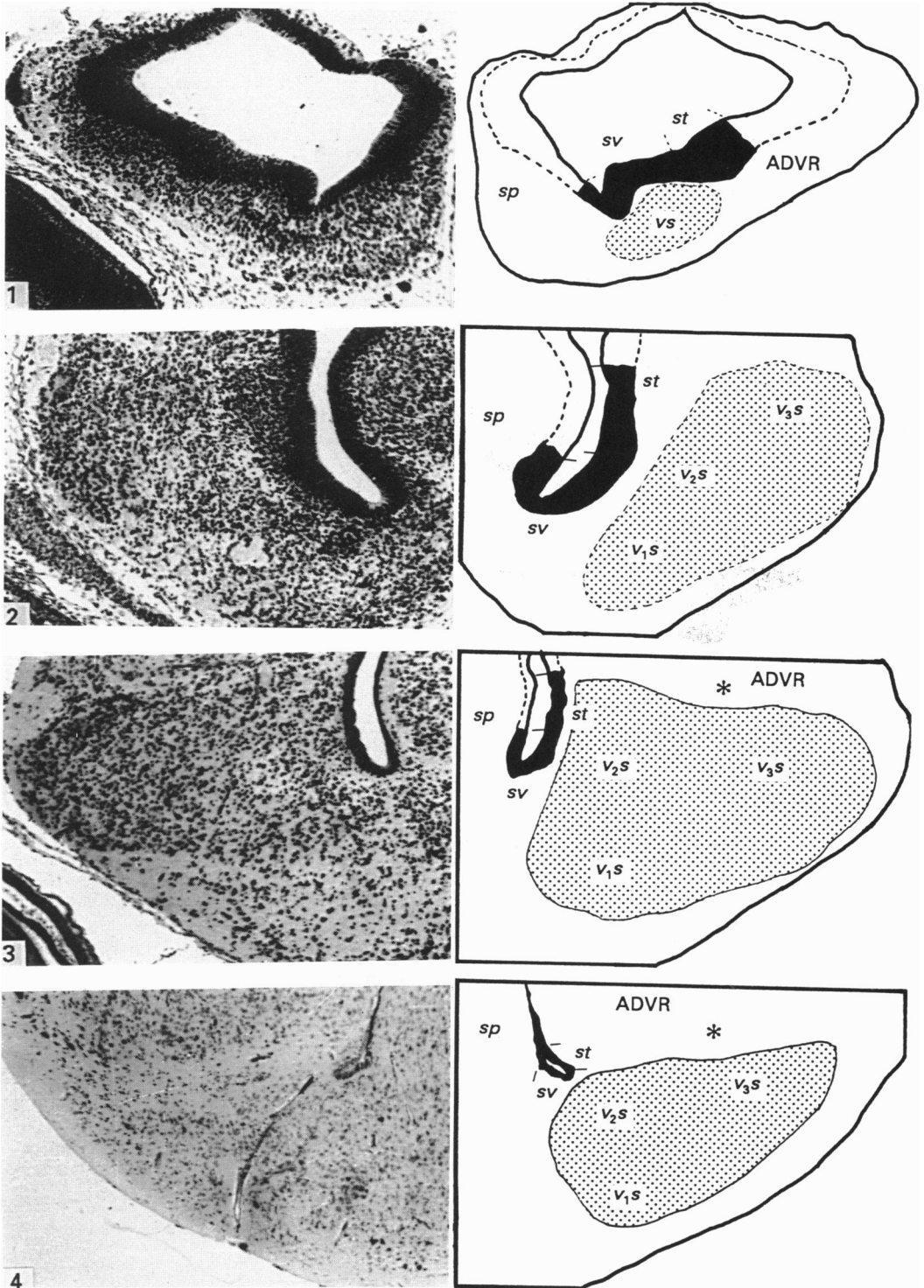
Figs. 1–4. Drawings and sections of the telencephalic location of the ventral striatum (*vs*) in transverse sections of an anterior level of the telencephalon at Stage E. 32 (1), E. 35 (2), hatching (3) and adulthood (4). Haematoxylin–eosin (1–3) and Mann–Dominici (4). v_1s, ventral group; v_2s, ventromedial group; v_3s, latero-dorsal group; *sv*, sulcus ventralis; *st*, sulcus terminalis; *sp*, septum. (1–2) ×40; (3) ×32; (4) ×10.
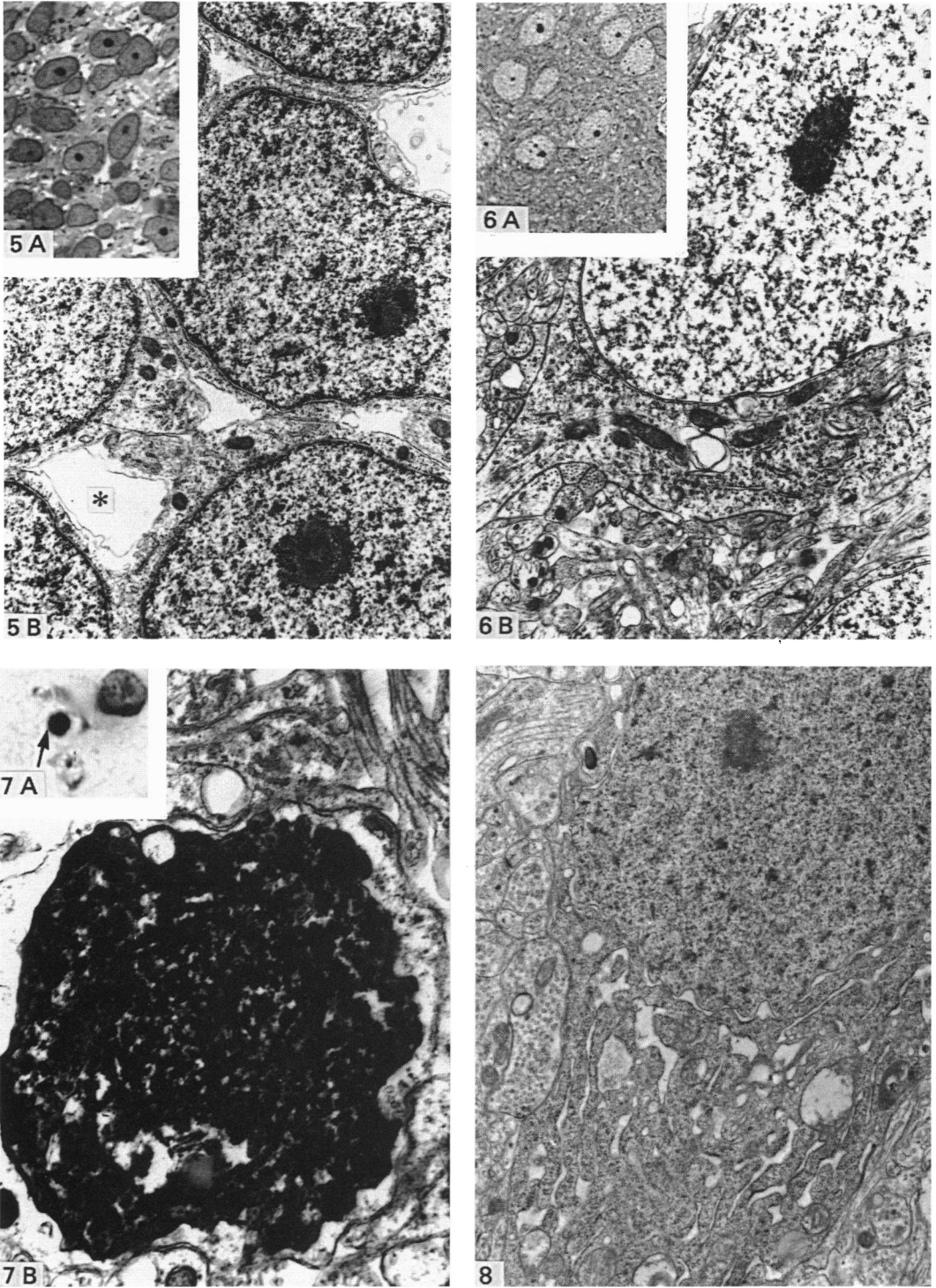
Fig. 5(A–B). (A) Observe the neuroblast grouping in the intermediate zone and cellular processes. Semithin section at E. 32. × 640. (B) Ultrastructural detail of these neuroblasts showing scanty cytoplasm and diffuse chromatin. The intercellular spaces are prominent (asterisk). × 14000.
Fig. 6(A–B). (A) Neuron and neuropil in a semithin section at E. 40. × 221. (B) Observe the differentiated cytoplasm of a neuron. × 5001.
Fig. 7(A–B). (A) A cell with a pycnotic nucleus (arrow). Haematoxylin–eosin. × 1280. (B) Ultrastructural characteristics of advanced nuclear degeneration (E. 40). × 15000.
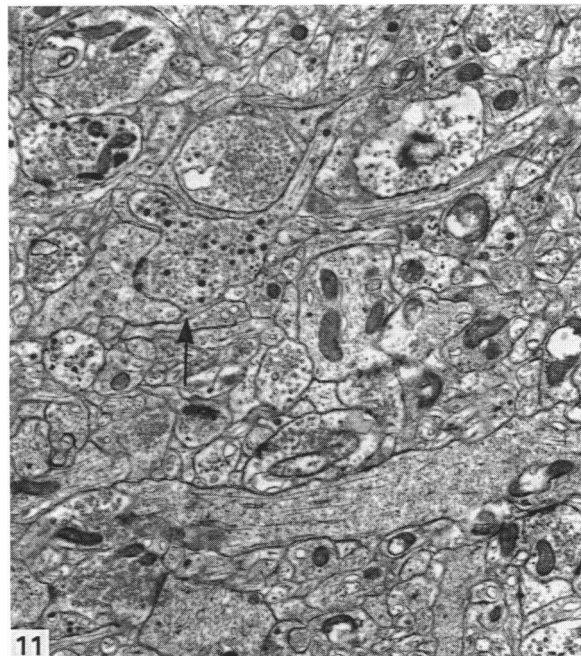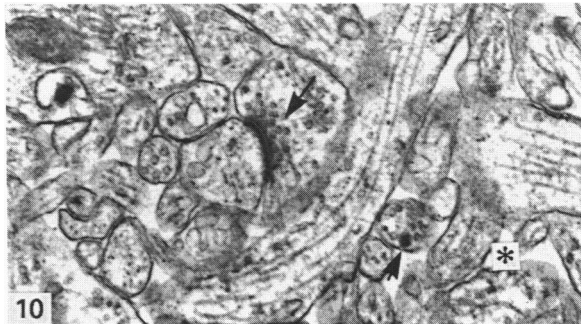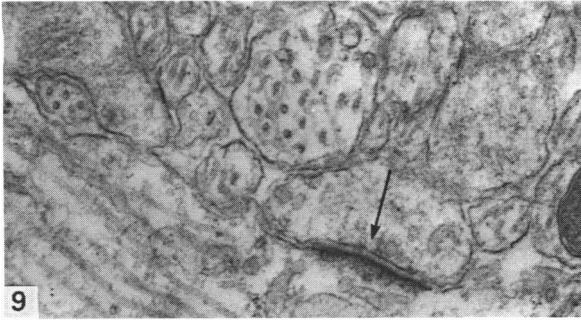Fig. 8. A neuron which shows cytoplasmic degeneration affecting especially cistern dilatation in the adult. × 12000.

Fig. 9 Initial axodendritic synapse (arrow) at E. 38. × 30000.
Fig. 10. Axodendritic synapse with a slight concentration of synaptic vesicles (arrow) at E. 40. Note the presence of some synapses with dense-core vesicles (arrowhead). The asterisk marks remaining intercellular spaces. × 15000.
Fig. 11. Adult neuropil. Note the accumulation of different synaptic types, a few containing dense and clear vesicles (arrow). × 9100.

DISCUSSION

In a previous work (Yanes *et al.* 1977) we have characterised three cellular populations (v_1s, v_2s, v_3s) in the ventral striatum. The dorsolateral group (v_3s) corresponds with the Källen (1951) cll area, the ventral (v_1s) and the ventromedial (v_2s) groups with Källen's (1951) c_vl area and the region (v_1 and v_2) of Kirsche (1972).

Warner (1946) in *Natrix sipedon*, Kirsche (1972) in *Testudo hermani*, Hetzel (1974) in *Lacerta sicula* and Huei-Met Tsai, Barber & Larramendi (1981) in the chick suggest that this nuclear condensation originates from the ventral proliferative neuro-epithelium of the telencephalic vesicle. Our observations agree with these authors and furthermore, as we have described in other works (Yanes *et al.* 1988*a*, *b*), a clear correlation exists between the thickness of the proliferative matrix and the growth of the cellular population, confirming that the cellular populations v_1s and v_2s proceed from the sulcus ventralis while the cellular population v_3s fundamentally originates from the sulcus terminalis. With respect to the possible forms of neuroblast migration, the presence of radial glia cells in the sulcus and also the radial disposition of the neuroblasts have been observed (unpublished results) which suggest that displacement takes place through the radial glia as described in the monkey cortex (Levitt & Rakic, 1980).

The ventral striatum neuroblast ultrastructural characteristics in the first development stages (E. 31 and E. 35) are analogous to those mentioned by Sturrock (1980) in the mouse neostriatum, Goffinet (1983) in the cortex of *Emys* and *Lacerta*, Brand & Rakic (1984) in the neostriatum of the monkey, Trujillo, Pérez, Fernández & Martín (1985) in the thalamus and Yanes *et al.* (1987) in the ADVR of *Gallotia galloti*.

The degree of maturation of the VS cells proceeds gradually from Stage E. 38 to Stage E. 40 while in other areas such as the ADVR it is a rapid process (Yanes *et al.* 1987). This gradual attainment of the mature stage is a characteristic of the ventral nuclei such as the basal DVR and amygdaline structure of *G. galloti* (Yanes, 1985). On the other hand, neurons with different degrees of maturation can be observed in perinatal specimens of this lizard, while in the mouse neostriatum a mixture of all stages of neuron differentiation can be found throughout the developing postnatal stage (Sturrock, 1980). We think that these differences can be explained by the more simple structure of the reptile striatum compared with the higher degree of complexity in mammals.

Synaptogenesis begins at stage E. 38 with morphological characteristics analogous to those described by Sturrock (1980), Goffinet (1983), Brand & Rakic (1984) and Yanes *et al.* (1987). The axo-dendritic synaptic morphology is established perinatally from Stage E. 40; however, these synapses are more numerous in postnatal specimens. Brand & Rakic (1984) and Marín-Padilla (1986) in mammals have reported that afferents can influence the development of a nucleus or an area of the nervous tissue. It is now been shown by means of Golgi techniques that the efferent axons of ADVR passing towards the ventral striatum develop from Stage E. 35 onwards (Díaz, unpublished results) and in addition, the dorsal peduncle of the lateral forebrain bundle also develops early (E. 32–E 35). Therefore, this synaptic development from Stage E. 40 can be fundamentally explained by the fact that the afferents to the nucleus from ADVR and from the thalamus arrive through the dorsal peduncle of the lateral forebrain bundle. Another explanation would be that the olfactory sensations acquired at the postnatal stage are essential for synaptic development at this critical period (Rogowski & Feng, 1981).

With regard to cellular death, we have identified two types. Nuclear degeneration (Type I), and cytoplasmic (Type II). The characteristics are those described for other zones of the brain of *Gallotia galloti* (Trujillo *et al.* 1987). The first stage, the occurrence of pycnotic cells (Type I), fundamentally affects the neuroblasts, since if the number of cells increases at perinatal stages (Monzón, Yanes, Trujillo & Marrero, 1986), it also coincides with the beginning of gliogenesis (Yanes, 1985). Cytoplasmic death (Type II) is characteristic of perinatal stages (Trujillo *et al.* 1987). It is specific for neurons that have already reached a certain degree of differentiation, a stage which coincides with the establishment of synapses probably originating in those neurons that may receive adequate initial contacts, but the balance of inputs and target contacts is not suitable (Cunningham, 1982). Finally, this degenerative process leads to the presence of electrodense masses – 'debris' – which will be phagocytosed by the cells of the microglial type (Trujillo *et al.* 1987; Yanes *et al.* 1987).

SUMMARY

The ventral striatum nucleus (VS) begins development at Stage 31 (E. 31) from the neuroblasts which proceed from the cellular proliferation of both the ventral and terminal sulci. The ultrastructural features of the neuroblasts of VS between E. 31 and E. 34 have the aspect of immature cells, but as from E. 38 neuronal maturity is gradual until hatching. At E. 34 cellular death occurs. The first degenerated cells belong to Type I (nuclear degeneration) of the pycnotic cells; as from E. 40 cytoplasmic degeneration appears. Vascularisation starts at E. 35 and from E. 38 the first synaptic contacts are observed, especially those of the axodendritic type.

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