

Ependymogenesis of the Lizard Basal Areas

II. Sulcus

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With 8 Figures

Summary

A structural and ultrastructural study has been carried out, of the four telencephalic sulcus (sulcus lateralis, terminalis, ventralis and septomedialis), during embryonic development, postnatal and adult of *Gallotia galloti*. The proliferation grade shown by the number of cellular layers and mitotic activity from E-32 to E-34, is quite intensive at all levels of the sulcus and coincides with a high cellular homogeneity and numerous intercellular spaces. As from E-38, the beginning of ependymogenesis in the sulcus is detected, but at the same time mitotic activity is not lost, it lasts until postnatal and adult stages, specially in the sulcus terminalis. It is concluded that these sulcus represent matricial rests in the adult and are made up of different types of cells with different grades of differentiation.

Key words: Sulcus – Development – Telencephalon – Matricial Rest – Lizard.

Introduction

The telencephalic sulcus represents embryonic unities that follow a specific way of differentiation in relation to ependymal zones or areas. The concept of sulcus has presented different versions within the literature, as far as KÄLLEN (1951) is concerned, sulcus represent proliferative regions whose variations are reflected by their own proliferative intensity. FLEISCHHAUER (1957), certain regions of the ventricular wall formed by an ependyme with little differentiation and that even in the adult has matricial functions. KIRSCH (1983), areas of great importance in the brains regeneration process that can extrapolate that observed in mammals. Along this line DEL GRANDE and MINELLI (1980), have investigated the regeneration of the telencephalon applying the growth factor (NGF) with positive results for these proliferative zones. Until SCHULZ (1967) study there was enormous confusion about the denomination of the same in the field, in this work an exhaustive recopilation is carried out of the different names and they are simplified by

denominating them, sulcus lateralis, terminalis, ventralis and septo medialis, this terminology will be followed in this study.

The works carried out up to now have fundamentally versed on partial studies (OM), during embryonic development, such as those of KÄLLEN (1951), FLEISCHHAUER (1957) and KIRSCH (1967, 1972), in different reptiles and SCHULZ (1969), in *Lacerta agilis*, studies the localization of these proliferative areas during posnatal development.

In this work following the same line as the anterior one we intend to carry out a structural and ultrastructural study of the four telencephalic sulcus during the embryonic, postnatal period and in the adult *Gallotia galloti*.

Material and Method

The same material and methology described in the first part was used.

Results

Four sulcus have been identified, the sulcus lateralis (SL), sulcus terminalis (ST), sulcus ventralis (sv) and sulcus septo medialis (SSM).

At ultrastructural level all sulcus from E-32 to E-35, have the same matricial characteristics as those described for ependymal zones (Fig. 5a).

Sulcus Lateralis. The primordium of the SL begins to develop at E-32 (Fig. 1a), morphologically it has a semicircular aspect which gradually stretches until reaching stage, where it shows a very narrow parable form, a form which characterizes the adult (Fig. 1e).

At the mean level of E-32, it is formed by approximately 9–10 layers (Fig. 1b), at E-35, the number of layers reduce slightly at all levels. As from E-37, the presence of two zones in this sulcus are observed, taking into account the cytological and topographic characteristics, we have termed them as cortical and angular. Together these two zones represent variations in a rostro-caudal sense, in the number of cellular layers and the morphology of the nuclei, although we will centre on the angular zone, as we consider it representative of the sulcus. In E-37, the number of layers is variable in the rostro-caudal sense (Fig. 1d). At hatching stage all levels are formed by 3 layers (Fig. 1f) with the exception of the rostral ones that have 4–5. In the postnatal stage these characteristics are maintained. The morphology of cellular nucleus varies in a caudal-rostro sense (E-37) (Fig. 1d). At hatching stage and at all levels the nuclei are fusiform except at rostral levels where they are round and dented (Fig. 1f).

At the ultrastructural level in E-40 this sulcus stil has the classic characteristics of the matricial cells, the nuclei are fusiform and in the cytoplasm the majority of organules appear concentrated in the internal processes that reach the ventricular light. These processes continue to make contact with adjacent cells by means of tight adherent bindings. The presence of clear intercellular spaces is notorious and futhermore many of them contain prolongations to other cellular processes (Fig. 2a).

From E-32 to E-35, numerous mitotic figures are seen (Fig. 1c), these begin to descend abruptly as from E-35, and their number is stabilized at the hatching stage (Fig. 8).

The cilia are constant as from the first embryonic stages to the postnatal one, but with scarce representation (Figs. 2a, 8). Also scarce and small lipidic droplets were detected as from E-38 to the postnatal stage (Fig. 8).

In the adult it is dorsally limited by the dorsal cortex, laterally by the lateral cortex and ventrally by the anterior ventricular ridge (ADVR) and occupies the whole caudal-

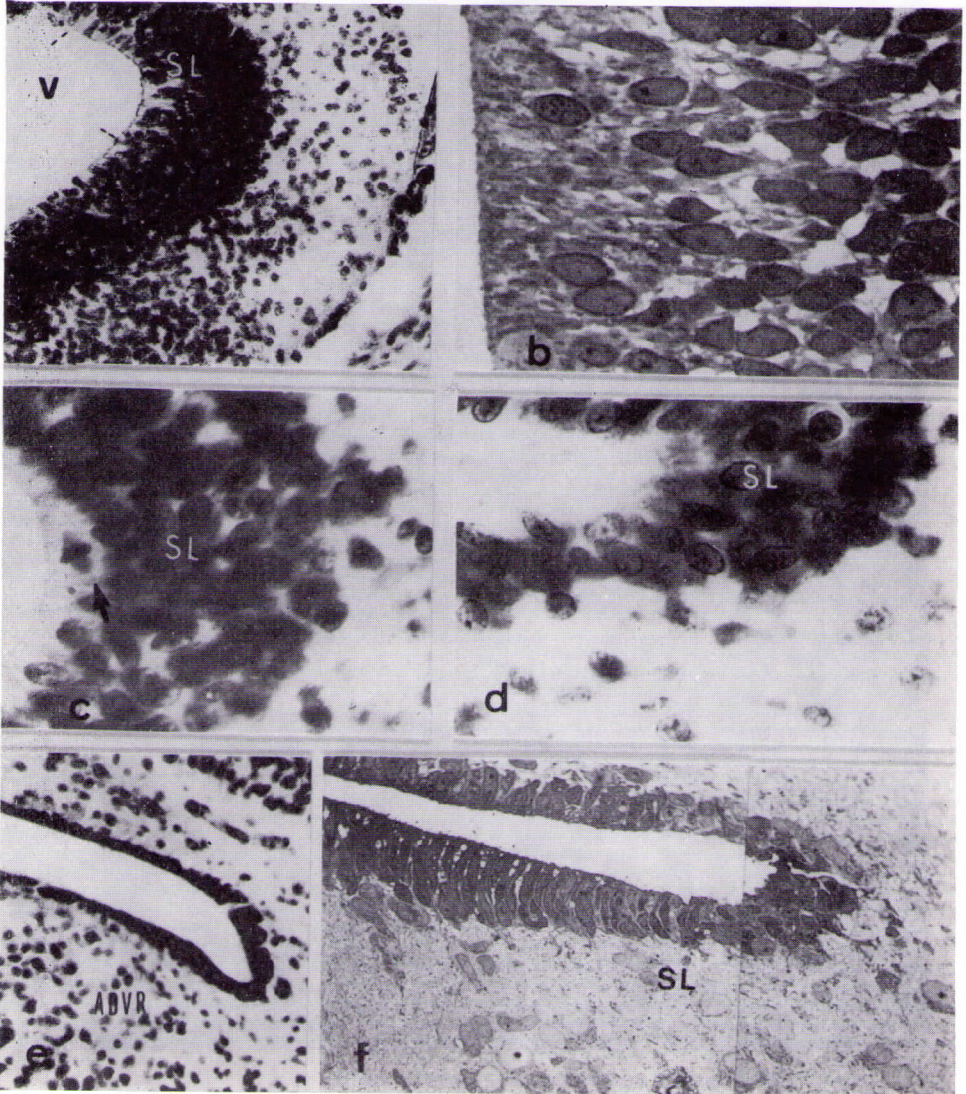


Fig. 1. Sulcus Lateralis (SL), topographic and cytological characteristics

- a) SL anterior level in E-32. $\times 67$; b) semithin section in E-32, observe the cellular homogeneity. $\times 1920$; c) numerous cellular layers and the presence of mitotic figures stand out (arrow) $\times 640$; d) medial level in E-37, note the reduction in the number of layers. $\times 640$; e) medial level at E-40. $\times 256$; f) semithin section of an anterior level in E-40. $\times 960$

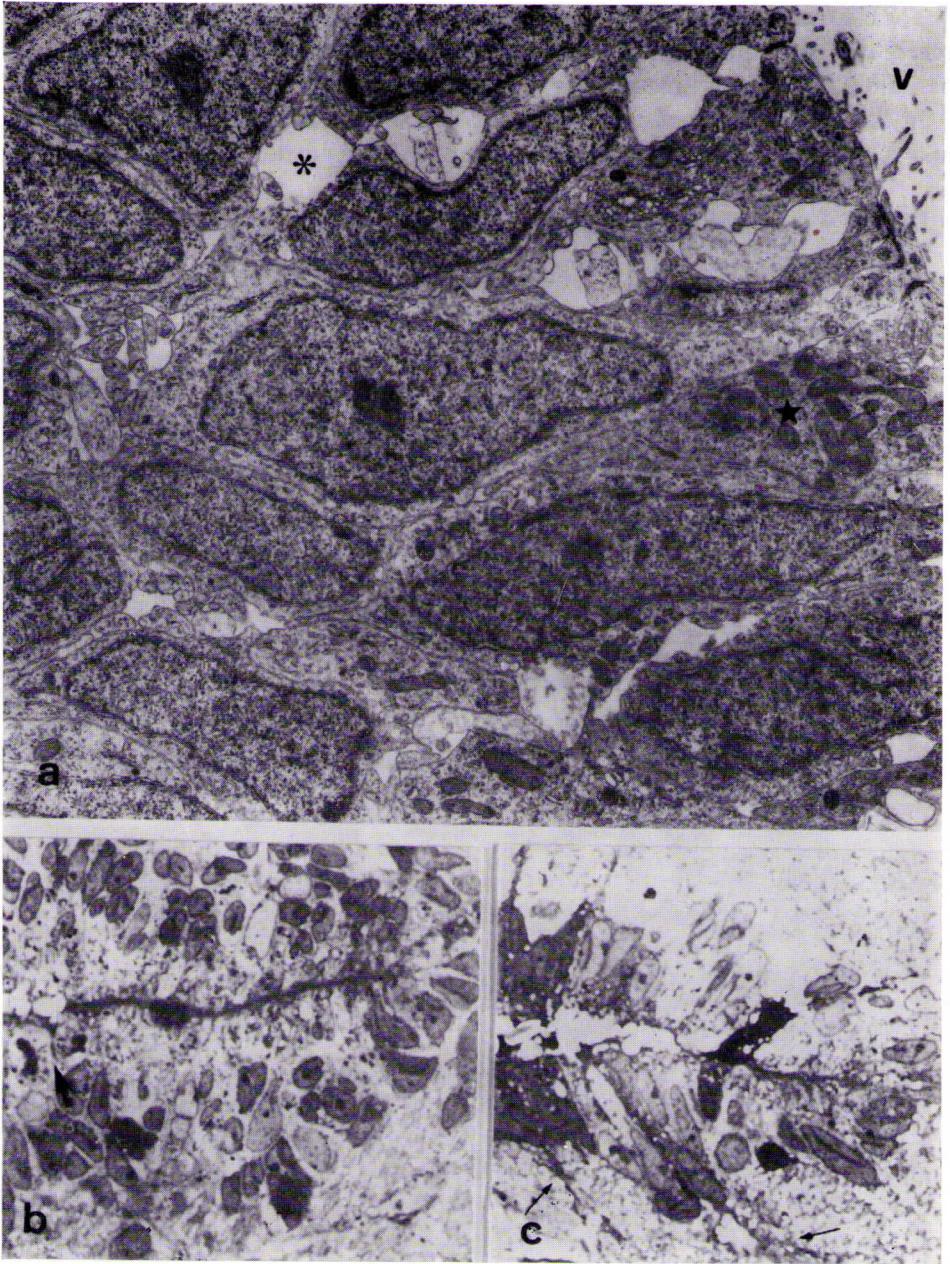


Fig. 2. Ultrastructural and cytological aspects of the SL

a) electron micrography of the SL at E-40, observe the mitochondrial density of the internal cellular processes (star) and the constant extracellular spaces (asterix). $\times 12000$; b) anterior level in the adult, the presence of mitosis is shown (arrow). $\times 640$; c) medial level in the adult, see the presence of clear and dark cells and basal processes (small arrow). $\times 640$

rosto extension of the telecephalon. This sulcus presents a different width in the caudal-rostro sense, being the rostral levels the more proliferative (8–10 cellular layers).

The morphology of the cellular nuclei are fusiform and dented and among them we often find cells with clear and dark nuclei with long prolongations (anterior and medial levels) (Fig. 2c). In the cortical zone it is only formed by cells whose nuclei are fusiform and in the angular one by oval and fusiform cells (Fig. 2b, c). We have also found neurons very close to the cellular components of this sulcus, above all in the limiting zone in ADVR angular position. The cilia are numerous in the uncoortion part of the ventricle. Another characteristic which stands out is the presence of mitosis (Fig. 2b).

Sulcus Terminalis. The sulcus begins to anlage as from E-32, between the ventricular zone b [vz(b)] and sv with diffuse (Fig. 3a) limits which gradually define until E-37. At middle and caudal levels of E-32, it is formed by 5–6 nuclear layers, in the anterior ones by 10–12 and in rostral ones by 12. In E-35 the anterior characteristics are constant at all levels except the caudal ones. As from E-37, two zones in this sulcus are differentiated, one which contacts with the vz(b) and the other would be the apice of the sulcus (more active zone). At medial levels the first zone is formed by 5 layers while the second has three. The anterior levels have 6 layers in the whole area and in the caudal zone 3 and 2 in the proximity of vz(b). In E-35, the triangular aspect of the sulcus appears almost defined (Fig. 3a). The anterior levels maintain their original thickness. The medial levels have 3–4 layers and the caudal ones 2–3. These characteristics remain constant until the postnatal state except in anterior levels which have 3–4 layers (Fig. 4b).

In the first stages (E-32, E-33) the cell nuclei are oval and round in shape. At E-35, some fusiform nuclei appear (Fig. 3c), and at ultrastructural level the ventricular cells are seen notably longer in respect to anterior stages (Fig. 3b). As from E-37, the nuclear diversity of the cells that form this sulcus begins to show itself (Fig. 3d), this diversity extends gradually (Fig. 4a) throughout the whole cellular population until the postnatal ones (Fig. 4b). In the hatching stage can be seen, for the first time, the dark nuclei in cells with radial processes (Fig. 3f).

Its proliferative activity in relation to the number of mitosis, is high as from the first stages (Fig. 8), at all levels until the middle stages (E-37). In E-39, its mitotic activity slows down slightly and is then maintained until the postnatal stage (Fig. 8). The proportion of cilia is scarce from the first stages up to E-35 (Fig. 3b, 8), and gradually increases from this stage to hatching. The lipidic droplets are rather scarce throughout all development levels.

In the adult the width of this sulcus in the rostral caudal sense is not uniform. The medial and anterior levels are formed by 3 layers and the caudal by only one layer (Fig. 4c, d, e).

This sulcus is characterised by the presence of great morphological nuclear diversity in its cells (Fig. 4c). The predominant ones are round and oval and the lesser stick-like and fusiform. Numerous cells with clear and dark nuclei are also observed, alternatively positioned (Fig. 4e). Others can present dents, though these are very scarce (Fig. 4d). The presence of mitotic figures is stil constant but less so than in the postnatal period

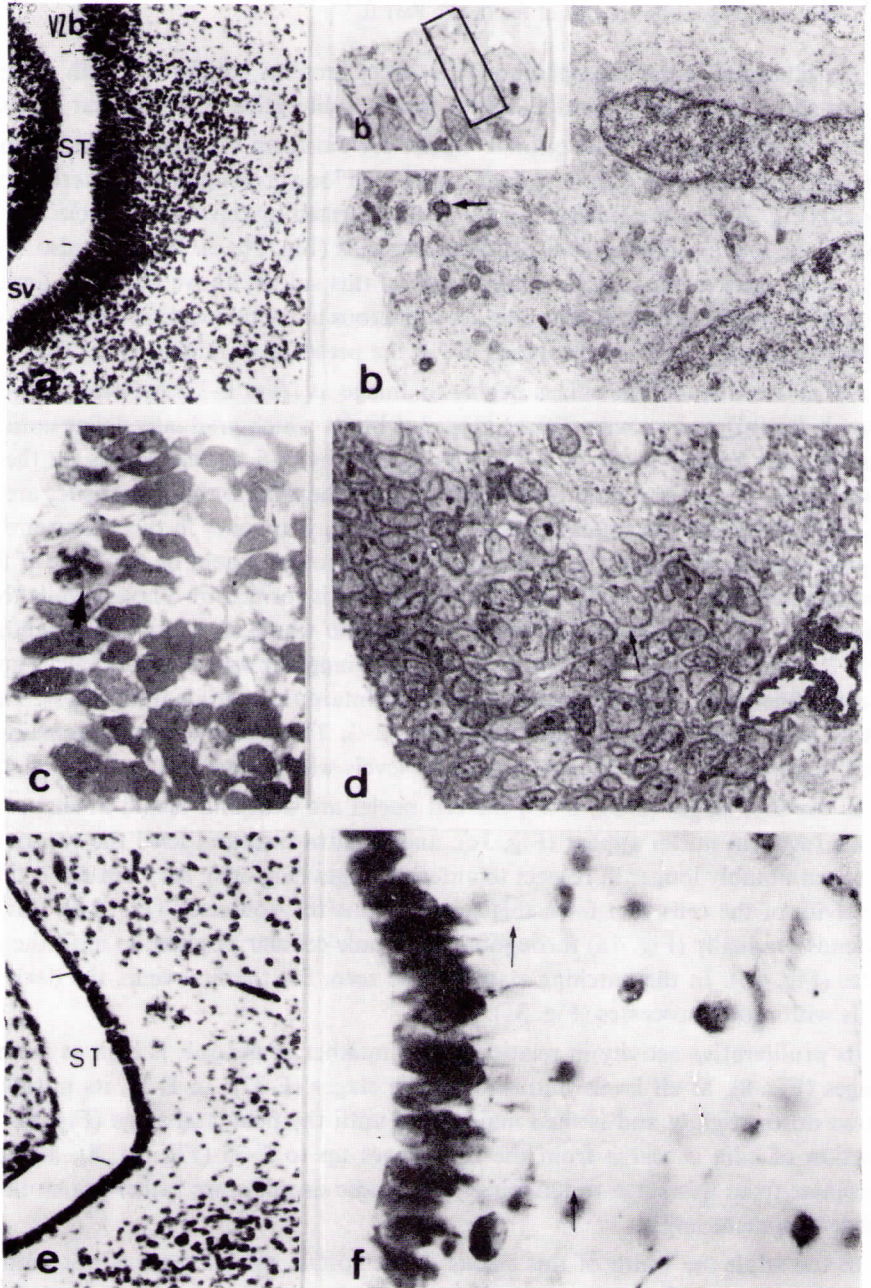


Fig. 3. Topographic and cytological characteristics and electron micrography of the Sulcus Terminalis (ST)

a) anterior level at E-35. $\times 160$; b) ultrastructural aspects in E-35, observe the presence of a basal body (arrow). $\times 5000$; b') semithin section in E-35, the marked area is represented in b). $\times 960$; c) an anterior level in E-35, the constant cellular homogeneity and presence of mitotic figures is shown (arrow). $\times 640$; d) an anterior level in E-38, observe the beginning of diverse nuclear morphology (small arrow), $\times 840$; e) medial level at hatching stage. $\times 1601$; f) medial level at hatching stage, the radial disposition of the basal processes is shown (small arrow). $\times 1600$

(Fig. 4d). The ciliar density is high at anterior levels and medial levels and regularly distributed. The lipidic droplets are constant in the whole extension of the sulcus (Fig. 8).

Sulcus Ventralis. In the sv anlage begins at E-32, at ventricular apice levels (PLATEL, 1971) (Fig. 5b). It limits with vz (e) and ST, are diffuse. In E-39, these limits are defined. On the other hand, this sulcus remains almost bound to the intermediate zone from E-32 to E-37, at this stage a slight wall can be identified which separates it from the ventral striatum in formation (Fig. 5e). At hatching stage some neurons bound to their cellular components appear delimited by a clear ample zone that at the same time separates them from the ventral striatum (Fig. 6b).

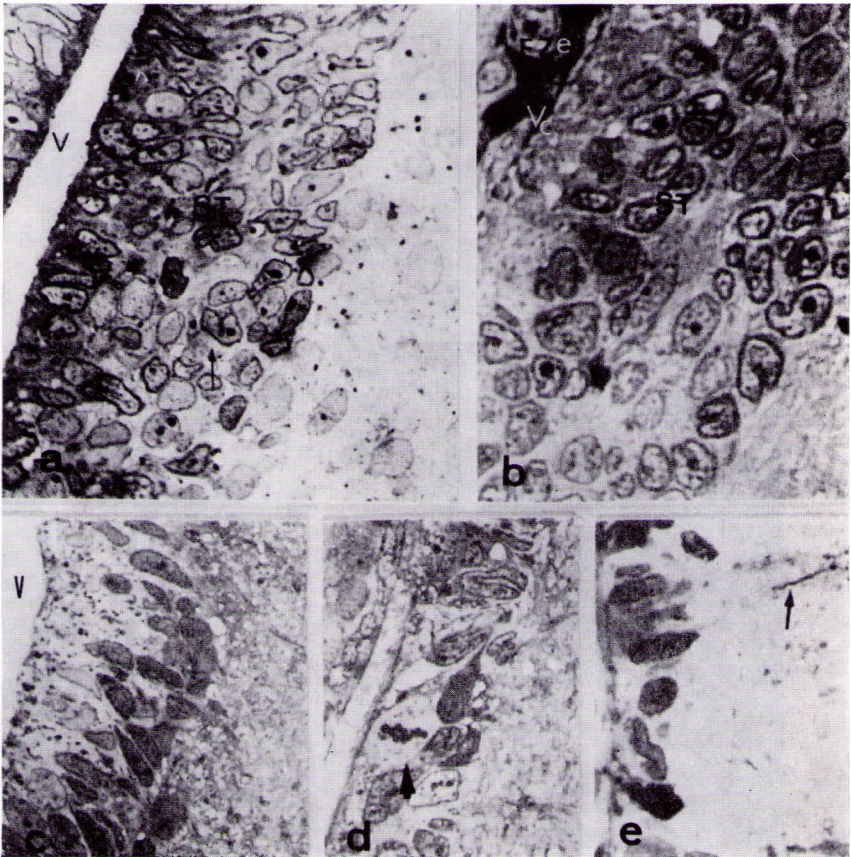


Fig. 4. Cytological characteristics of the ST

a) an anterior level in E-40, morphological diversity of the nuclei is accentuated (small arrow). $\times 840$; b) semithin section of an anterior level in postnatal. $\times 1280$; c) semithin section of an anterior level in the adult, numerous cellular layers are shown. $\times 640$; d) a medial level, the presence of mitotic figures is shown (point and arrow). $\times 640$; e) a middle level, observe basal processes (small arrow). $\times 640$. Vc, Coartion of the Ventricle; V, Ventricular Lumen; Eze, Ependymal Zone (e)

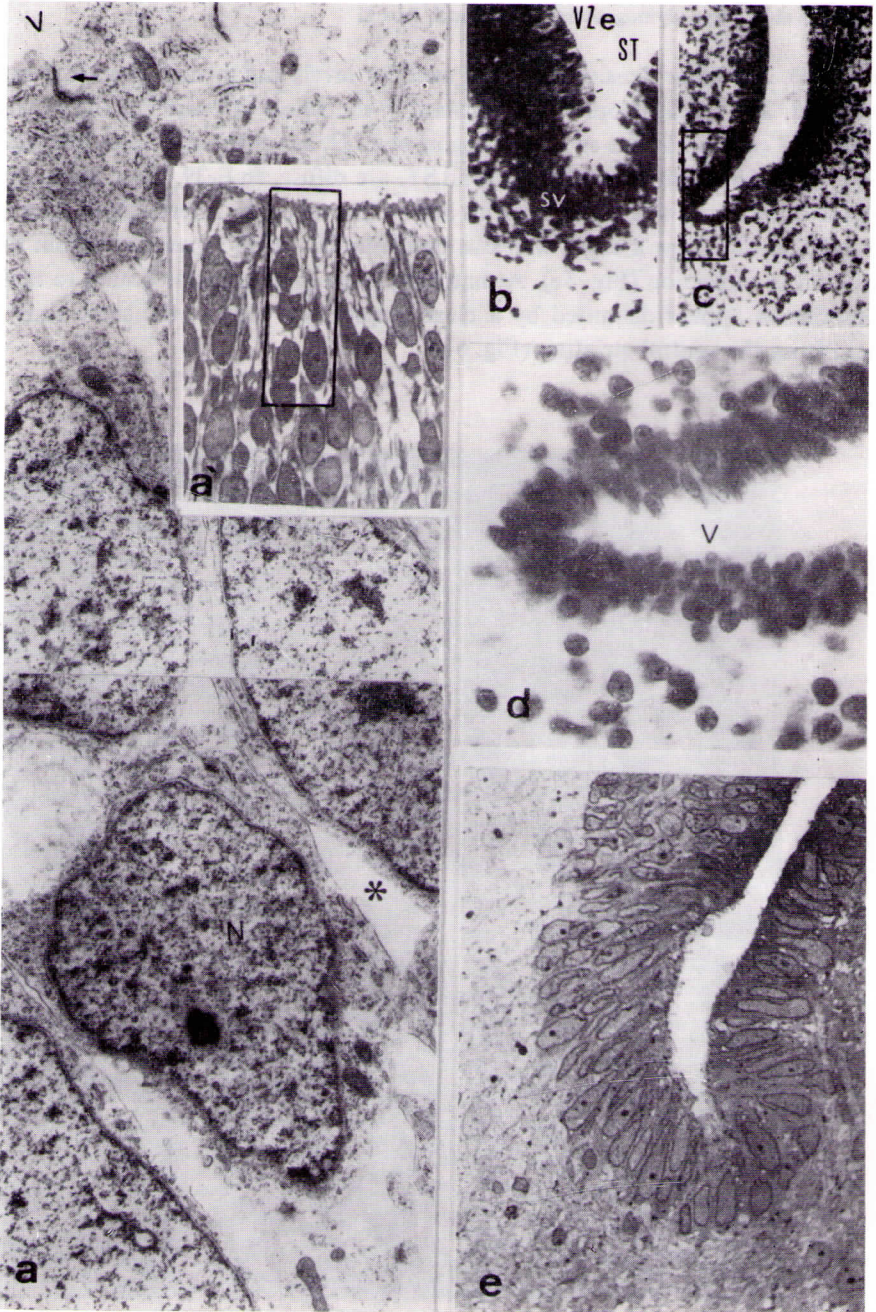


Fig. 5. Topography, cytological and ultrastructural characteristics of the sulcus ventralis (sv) a) electron micrography of the sv at E-32, note the long morphology of the (N) cells, among them numerous extracellular spaces can be seen (asterix). $\times 10500$, a') semithin section of an anterior level in E-32, the marked area is represented in a). $\times 640$; b) anterior level in E-32. $\times 66$; c) anterior level in E-35; d) this corresponds to the marked areas in c), observe in the angular zone nuclei with a uniform aspect and the reduction in the number of layers. $\times 620$; e) semithin section of a middle level in E-38, note a clearer manifestation of nuclei with fusiform aspect. $\times 420$

Mitotic figures are frequent as from the first stages to E-37 and gradually increase until the postnatal stage and then notably decrease (Fig. 8). The cilia are scarce from E-32 until E-37, their increase is gradual up to the postnatal period (Fig. 6c, 8), and they show an analogue density as the adult.

At E-37 the basal processes in some cells are observed, the aspect is similar to that of the adult.

The lipidic droplets show up as from E-37, above all in the angular zone. Their presence is constant at all stages (Fig. 6b).

At E-32 and at all levels the nuclei are round and oval in shape (Fig. 5a'). In E-35 nearly all are oval, round and some fusiform (Fig. 5d). In E-37, at half levels the nuclei are fusiform, and some oval and round (Fig. 5e). At rostral levels they are oval. At caudal levels, oval and fusiform. This model continues up to the postnatal stage. In E-32 all levels are made up by 6-7 layers. In the medial and caudal levels of E-35, 3-4 layers (Fig. 5d); the anterior levels are formed by 5-6 nuclear layers and the rostral ones by 7-8. From E-37 to E-39 variations are not seen (Fig. 5e). At the hatching stage the rostral and anterior levels are reduced to 3 layers (Fig. 6b). At postnatal stage the medial and caudal levels are monostratificated. In E-37 all levels show fusiform nuclei with the exception of the anterior levels, where some rounded and oval nuclei appear (Fig. 5e). In E-39 the oval aspect predominates at caudal levels. At hatching and postnatal stages all levels have fusiform nuclei and in some isolated cases dented nuclei (Figs. 6b, c).

At ultrastructural level this sulcus as from E-38 is found covered by cells with an ependyme aspect. In these cells the nucleus is long and the cytoplasm is rich in organules with abundant oval and round shaped mitochondrias. In the cells apical surface, towards the ventricular light, cilia and microvillae appear in more profusion. These differentiations increase in number and development, close to the hatching stage. In E-40, the cytoplasmatic organules increase and some lipidic droplets and small dense bodies with a lysosomal appearance are seen (Fig. 6a).

At adult stage it is dorsally limited by the ST and ventrolaterally by the ventral striatum (sv_2) and laterally by the cellular grouping of the ventral layer (sv_1) and the nucleus septilateralis pars inferior. It is constituted at medial and caudal levels by one only layer and increases a little to two at the anterior levels and to 7-8 at rostral levels.

In the angular zone the nuclei of the cells are fusiform and oval, and furthermore, some dented nuclei can be seen, at rostral levels they are usually oval and round (Fig. 6d). The joined neurons are another of its characteristics (Fig. 6d). Also dark and long prolongation cell nuclei, are numerous (Fig. 6d). On the other hand, the presence of mitosis is observed but with scarce representation. Numerous cilia with uniform spacing over the whole surface of the sulcus and abundant lipidic droplets, principally concentrated in the apical portions of the cells, have been seen.

Sulcus Septo Medialis. The anlage of this sulcus appears diffusely in E-33 between the $vz(f)$ and the $vz(a)$ (Fig. 7a). It is observed from the initial stage of anlage to E-39, and is defined gradually, in E-39, its limits are similar to those found in the adult.

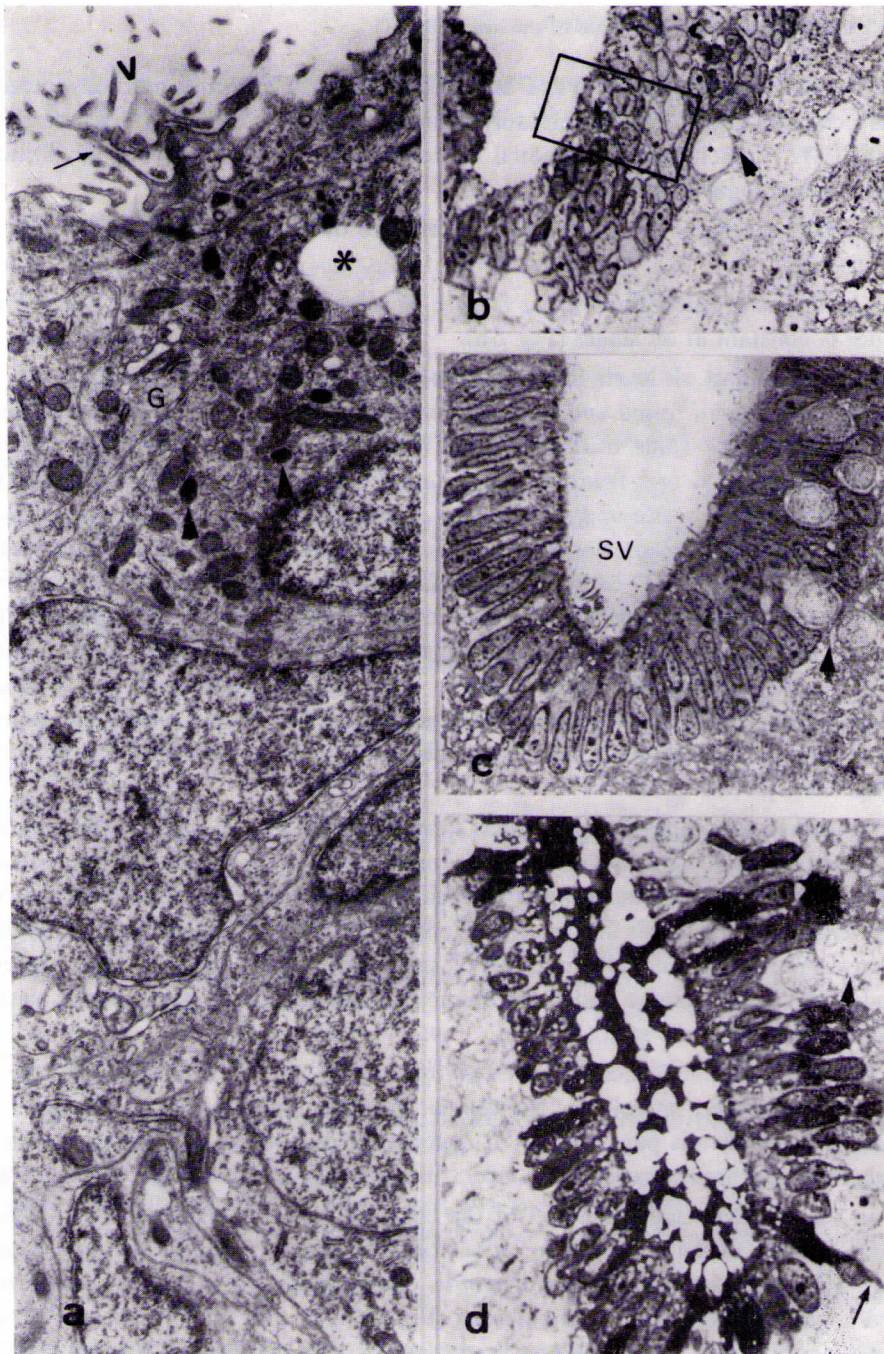


Fig. 6. Ultrastructural and cytological aspects of the sv
 a) ependymary-like cells in E-40, note an enriching of the cellular organules and the presence of lipidic-like droplets (asterix) and dense bodies (point and arrow). $\times 10000$; b) semi-thin section in E-40, the marked area is shown in a). $\times 630$; c) an anterior level in postnatal lizards, the closeness of neurons in the lateral region of the sulcus is shown (point and arrow). $\times 640$; d) an anterior level in the adult, note the presence of basal processes (small arrow) and the closeness of neurons (point and arrow). $\times 640$

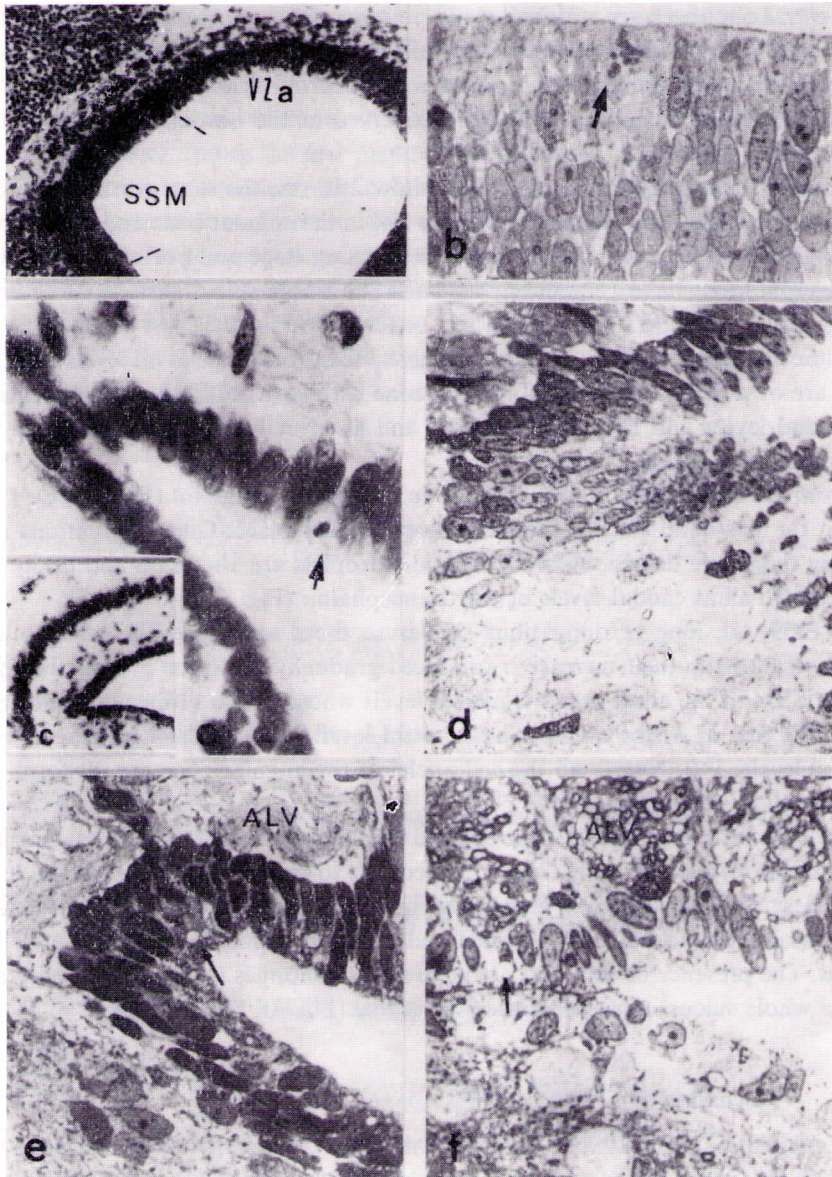


Fig. 7. Topography and cytological characteristics of the sulcus septomedialis (SSM)

a) anterior level in E-33. $\times 33$; b) an anterior level in E-33, observe the presence of mitotic figures (arrow). $\times 640$; c) anterior level in E-38, note the reduction in the number of layers and the presence of mitotic figures (arrow). $\times 1280$; c') anterior level in E-38. $\times 40$; d) semi-thin section in E-38. $\times 930$; e) a medium level at hatching stage, note the lipidic droplets (small arrow). $\times 775$; f) an anterior level in the adult, constant lipidic droplets are shown (small arrow). Alveus (al). $\times 930$

In E-32 it is constituted by a width of 8–10 nuclear layers. In E-35, at all levels except the rostral one there are still 4–5.

From E-32 to E-35, the shape of the cells nuclei is oval at all levels (Fig. 7b). In E-37, at anterior and caudal levels the round and fusiform nuclei appear (Fig. 7c, d).

In E-39, slight variations have been observed in the bordering zones of this sulcus with vz (a) and vz (f).

In E-39, the medial and caudal levels show 2 layers, the same in the angular zone as in the limitrophes; the anterior levels have 3–4 in the angular zone and in the limitrophes and 3–4 nuclear layers in all zones. At the hatching stage and postnatal ones, the medial and caudal levels have 1–2 nuclear layers in the whole sulcus surface, at anterior levels 2 (Fig. 7e).-

In the angular zone of E-37, the cells have a fusiform shape at all levels and the rostral ones are oval (Fig. 7c). In the bordering zone with vz (a) of E-39, the cell nuclei are oval at medial levels and fusiform at caudal and anterior level. At hatching and postnatal stages the nuclei are fusiform.

From the first stages up to E-35 mitotic figures are abundant (Fig. 7b, c). From E-37 up to the postnatal stage a gradual decrease takes place. Ciliary formations are scarce during the whole development while lipidic droplets are abundant and usually of a large size, above all at caudal levels of the telencephalon (Fig. 7e, 8).

In E-39, the long prolongations similar to those already explained in other sulcus begin to show up their increase is produced gradually up to the postnatal stage.

The SSM in the adult presents medial levels whose whole surface is monostratificated (Fig. 7f), just as in the anterior and rostral levels there are 3–4 nuclear layers and at caudal levels, 1–3. Nearly all the cell nuclei of the angular zone are fusiform and some of them show dents (Fig. 7f).

The cells near the cortical zone (a) and ependymal zone septal (f) have fusiform and oval nuclei respectively (Fig. 7f). It is frequent to find, above all at caudal levels, cells with clear and dark nuclei, distributed in an intercalated way, the last usually have long basal processes radially disposed and are more numerous than those seen in other sulcus. The presence of cilia and lipidic droplets continues to be another of its constants in the whole sulcus. However, mitosis is scarce (Fig. 7f, 8).

Discussion

In general KÄLLEN (1951), FLEISCHHAUER (1957), KIRSCH (1967) and SCHULZ (1969), suppose that the sulcus are made up by the matricial rests in adult Reptiles. From our observations it is deduced that the four studied sulcus really represent embryonic rest that stay during a long period of the lizards life but with a different proliferation grade and cell differentiation. The proliferation grade reflected by the number of cellular layer from E-32 to E-35, is very intense at all levels of the sulcus. Observations which agree with those of HETZEL (1974), in *Lacerta sicula*.

During embryonic development the SL also forms a part of the column (d) of KÄLLEN (1951c) and could fit into the alar plate SCHULZ (1969). The ST or sulcus limit between

the alar plate and basal (KÄLLEN 1951) also forms part of the generative ventral zone, if we compare their cellular and topographic characteristics described with those of KIRSCHKE (1972), in the tortoise. At this level the so called linea terminalis has been established, the cytoarchitectonic limit between both ventralis germinative and dorsalis zones (KIRSCHKE 1972). Our observations in *Gallotia galloti*, also confirm this fact. Also, due to its characteristics during the first phases of development with those indicated by KÄLLEN (1951), it could represent the column (C_d), and therefore in the postnatal stages it effectively constitutes a part of this column as supposed by SCHULZ (1969).

The sv corresponds to the lateral portion of the germinative ventral zone (KIRSCHKE 1967). Its intense proliferative activity in E-32 and E-35, coincide to the intense migrations for the formation of the ventral striatum and the amygdaline structure (unpublished observations). These proliferations could correspond to the second wave of column proliferation (c) (KÄLLEN 1951c).

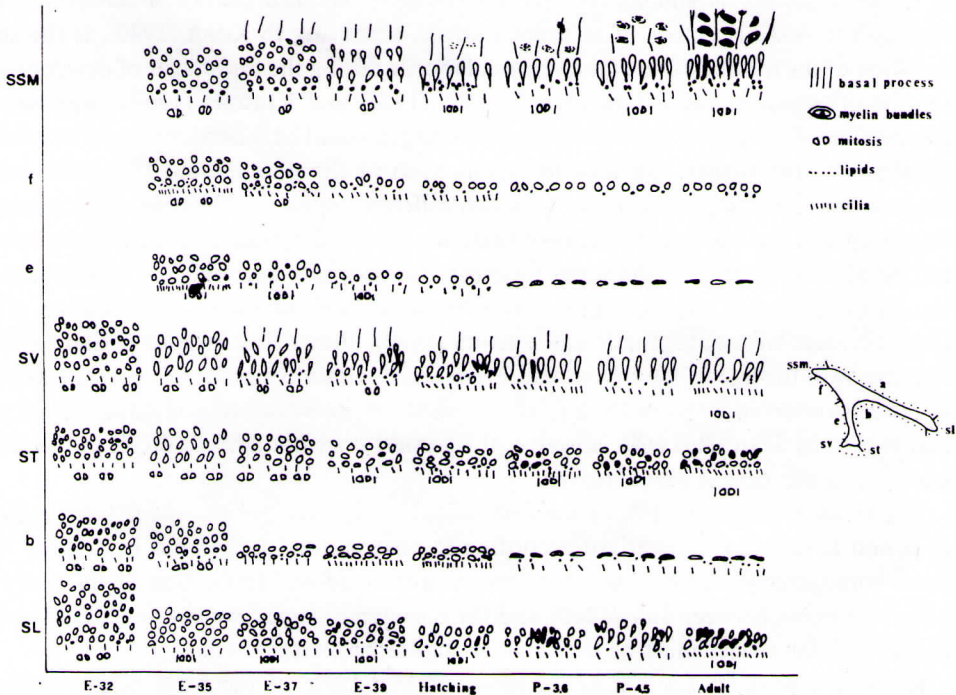


Fig. 8. Graphic representation of the evolution of the ventricular zone and sulcus of *Gallotia galloti*

The SSM, begins its intense proliferative activity late when compared to other sulcus (E-34), this is possibly related to the late medial cortex and medial dorsal formation.

At later embryonic development stages (E-35, E-37) and even at E-39, in contraoposition to future ependymal zones, the proliferative activity of the sulcus is stil very intense, above all in the SSM and ST, we have found that during this phase the mentioned

cortical zones and amygdaline structures are still forming, the largest part of the neuronal population of these groups probably proceeds from these proliferative zones (unpublished data).

One of the sulcus whose proliferative activity is seen during more time in the postnatal and adult is the ST, and according to KAHLE (1951) is interpreted as, an advanced matricial waste. It must be pointed out that the activity of this sulcus in the adult decreases notably and above all at caudal levels. An observation which is in agreement with that of SCHULZ (1969). Another proof which shows the sulcus activity in the adult is that of DEL GRANDE and MINELLI (1980), in *Lacerta viridis*, where a large increase in the germinative ventral zone is observed (sv and ST) by application of the nervous growth factor (NGF). Mitosis frequency found, above all, during embryonic development (early stages), was as high as in the ependymals. From mid stages (E-37, E-38) to the postnatal ones and in the adult it has been constantly observed, above all in the anterior levels of the sulcus, presenting the ST the most mitosis. The proportion of mitosis found by us in *Gallotia galloti*, is similar to that described by HETZEL (1974), in *Lacerta sicula*. Among the possible causes of so much mitosis, according to KORR (1980), is the short duration of the mitotic cycle of the ventricular cells during the first stages of development. On the other hand, authors such as ALTMAN (1966) and KIRSCH (1967), suppose that the rhythm of mitosis at postnatal and adult stages, could be related, not only to intrinsic problems of the tissues, but also to factors such as the photoperiod that exist during the year and latency period, those to which different reptiles are submitted, it is known that sleep and rest increase the mitotic rate. We do not deny that mitotic divisions could be conditioned by those factors but we must also take into account, the temporal duration of the period G_1 , KÄLLEN (1961) and ALTMAN (1966) said that "the duration of mitosis increases with ontogenetic age", KORR (1980), shows that in mice period G_1 does increase with the age of the individual. From the quantitative point of view, the number of mitosis observed by us in the ST, sv and SSM, in the postnatal and adult agrees with that shown by SCHULZ (1969), however, this author does not detect mitosis in the sulcus lateralis, in our case it was observed.

The cellular characteristics of the four sulcus during the period understood between E-32 and E-35, are characterized according to observations carried out by OM by their great homogeneity, these data are proved at ultrastructural level. Due to the homogeneity that exists between sulcus cells and the ependymal zones, we consider that the role of intercellular spaces must be the same as that already explained.

KIRSCH (1967), in *Lacerta agilis*, indicates that "one of the ways of knowing the exact time when the matricial cells differentiate into ependyme, would be carrying out an ultrastructural study", HETZEL (1978), continues in the same line as the first author. From our results it can be seen that the sulcus like the ependymal zones begin to take on the ependymary structure as from E-38, without losing their mitotic capacity, as in the case of sv; however, the SL continues to show matricial characteristics in E-40. These facts confirm that in *Gallotia galloti*, the sulcus represents embryonic rests.

Other cellular characteristics that coincide with the differentiation towards ependyme, are the presence of cilia and lipidic droplets. The cilia density increases as from this

development phase, above all in the sv and ST. SCHULZ (1969), in *Lacerta agilis*, points out the presence of cilia in the ventral germinative zone. HETZEL (1977), also supposes that "the great ciliar diversity of these proliferative zones, seem to be related to the nutritive properties of the cerebrospinal fluid". As support of this hypothesis, we could cite other data such as the positivity of these sulcus for GFA protein (unpublished data) in the so called ependymary astrocytes. Lipidic droplets appear in all the sulcus, but with a distinct representation, the sv and SSM are the ones which showed more density of the same, their presence has been seen at ME level. Similar to that explained in the ventricular zones the increase of these lipidic droplets seem to be related with the transfer of the radial glia towards mature glia (ependymal astrocytes).

Another of the problems that caught our attention, is the constance of the morphological cellular variety, which according to OM and EM data, is different in each sulcus. This fact could be related to that described by FLEISCHHAUER (1957). The coexistence of diverse cellular forms in Reptiles persists during their whole life, while in mammals it transcurrs at the same time. The model observed in *Gallotia galloti*, would constitute another confirmation of the slow growth and differentiation of the same.

Following this line of thought, we can deduce from our results, that in the four sulcus different cells are present with distinct grades of differentiation, the mitotic capacity is prolonged until adulthood. Among the different types of ependymal aspects, cubic and prismatic (EM) appear others (OM) with long periventricular somas, with long radially disposed processes. Similar cell characteristics have been observed by SCHULZ (1969) and HETZEL (1977), and they are termed tanocytes. In literature we have found two terms of the same, on one side HORSTMAN (1954), FLEISCHHAUER (1957), CIANI and FRANCESCHINE (1981), term them tanocytes, on the other side SCHEMECHEL and RAKIC (1979), STEVENSON and YOON (1981), describe them as ependymal astrocytes and KRIGSTEIN et al. (1986), name them ependymal glial cells. We have observed that during embryonic development and postnatal development, using as a marker GFA and glutamine synthetase (unpublished work), sulcus in studied cells that show the same characteristics as those described by the last authors and we are inclined to term them "ependymal astrocytes".

From the functional point of view HETZEL (1977), attributes to the sulcus septo medialis, the transport of substances that come from cerebrospinal fluid and the striatum would be particularly substituted by dense innervation. In other unpublished works (OM, EM and astrocytes markers), it is also observed that in the ventral striatum and in the septum, the irrigation is, in fact, abundant but, also the astrocytes marker expression is high, in this case we presume that nutrition is carried out through the ependymary astrocytes and the strong irrigation.

Other factors that could indicate the indifferiated character of some sulcus cells, as in the case of SL and sv, would be the binding of neurons to these zones, it is possible, that these cells have only recently differentiated as indicated by KIRSCH (1967); however, cells with identical topographic situation observed in Golgi preparations present a mature differentiated aspect and the relation of these neurons to sulcus cells is unknown,

they are probably in direct contact with the ependymal astrocytes, or it is also possible a typical SL disposition and structures that are phylogenetically recent (ADVR) according to SENN (1968).

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