

# Neurons of the Medial Cortex Outer Plexiform Layer of the Lizard *Podarcis hispanica*: Golgi and Immunocytochemical Studies

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## ABSTRACT

The study of Golgi-impregnated lizard brains has revealed a scarce but heterogeneous neuronal population in the outer plexiform layer of the medial cortex. Some of the neuronal types detected here resemble the neurons of the dentate molecular layer of the mammalian hippocampus.

According to their morphology, five intrinsic neuronal types have been clearly identified: short axon aspiny bipolar neuron (type 1, or sarmentous neuron), short axon aspiny juxtasonic neuron (type 2, or coral neuron), short axon sparsely spinous multipolar neuron (type 3, or stellate neuron), short axon sparsely spinous juxtasonic multipolar neuron (type 4, or deep stellate neuron), and sparsely spinous juxtasonic horizontal neuron (type 5, or couchant neuron).

Most neuronal types were identified as  $\gamma$ -aminobutyric acid (GABA) and parvalbumin immunoreactive, and are thus probably involved in medial cortex inhibition. Moreover, a small fraction of them displayed  $\beta$ -endorphin immunoreactivity.

The distribution of these neuronal types is not uniform in the laminae of the outer plexiform layer. Type 1 (sarmentous) and type 3 (stellate) neurons overlap the axonal field projection coming from the dorsal cortex and the thalamus, whereas types 4 (deep stellate) and 5 (couchant) neurons overlap ipsi- and contralateral dorsomedial projection fields as well as raphe serotonergic and opioid immunoreactive axonal plexi. Thus, these neuronal types may be involved in the control of specific inputs to the medial cortex by presumably feed-forward inhibition; nevertheless, feed-back inhibition may also occur regarding type 4 (deep stellate) neurons that extend deep dendrites to the zinc-rich bouton field. © 1994 Wiley-Liss, Inc.

**Key words:** interneurons, hippocampus, GABA, parvalbumin, opioid peptides

The cerebral cortex of the Squamata reptiles consists of four areas: the medial, dorsomedial, dorsal, and lateral cortices. They all show a simple three-layered organization: somata are highly packed in a central stratum, which is the cell layer and which is in turn flanked by two plexiform layers (inner and outer) constituting the neuropil.

The medial cortex can be divided into three subregions according to the orientation of the cell layer: a dorsal horizontal limb, a medial vertical limb, and a ventral subventricular limb which appears only at caudal levels. The outer plexiform layer of the squamate medial cortex is mainly composed of the dendritic trees of principal neurons and their incoming axonal input; furthermore, a glial network primarily consisting of vertical ependymocytic shafts and occasional glial and neuronal somata forms the parenchyma of this layer.

The neuronal population of the cerebral cortex of reptiles has been studied with the Golgi method (Ramón y Cajal, 1911, 1917; Crosby, 1917; Curwen, 1937; Goldby and Gamble, 1957; Goldby, 1964; Minelli, 1966; Northcutt, 1967; Ebbesson and Voneida, 1969; Regidor et al., 1974; Regidor, 1977; Ulinski, 1974, 1977; Lacey, 1978; Wouterlood, 1981; Berbel et al., 1987; Guirado et al., 1984; Berbel, 1988; Ulinski, 1990), as well as with the electron microscope (Abraham, 1972; Ebner, 1975; Ulinski, 1977; Ebner and Colonnier, 1978; Wouterlood et al., 1981; Garcia-Verdugo et al., 1984; Davila et al., 1985). A few immunocytochemical studies with antibodies against gamma-amino-

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butyric acid (GABA; Schwerdtfeger and Lopez-Garcia, 1986; Blanton et al., 1987; Schwerdtfeger and Lorente, 1987, 1988), neuropeptides, somatostatin, and neuropeptide Y (Davila et al., 1988, 1991), as well as the calcium-binding proteins parvalbumin, calbindin, and calretinin (Martinez-Guijarro et al., 1991; Martinez-Guijarro and Freund, 1992), provide fragmentary descriptions of the outer plexiform layer neurons.

The aim of the present work is to describe the morphology of the neurons in the outer plexiform layer of the lizard medial cortex as seen in a large sample of Golgi-impregnated brains as a first step to characterising this neuronal population. Immunocytochemical data are a valuable complement to assess this tentative typology.

## MATERIALS AND METHODS

### Golgi study

Sixty lizards (*Podarcis hispanica*) of both sexes, ranging from 23 to 55 mm in head-cloaca sizes (representing individuals from perinatal to adult ages), were used in this study. Animals were captured in the environs of Burjassot (Valencia, Spain) and were kept in terraria until sacrifice. Under ether anaesthesia, animals were transcardially perfused with 30–50 ml of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.12 M phosphate buffer, pH 7.2–7.4. The brains were then removed from the skulls and were kept overnight (sometimes longer) in the same fixative at 4°C. For staining we used the Golgi-Colonnier method (Colonnier, 1964) with some improvements adapted to our material (i.e., 2–7 days of induration at 4°C in a mixture of 2.4% potassium dichromate and 5% glutaraldehyde followed by 1–4 days of impregnation in 0.75% silver nitrate). After impregnation, 125–200 µm thick transverse sections were obtained with a tissue chopper and embedded in Araldite between two cover glasses. Selected cells were photographed and drawn with the aid of a Zeiss photomicroscope equipped with a camera lucida.

Of 1,263 well-impregnated neurons studied in the medial cortex, 88 neurons had their somata and dendrites in the outer plexiform layer, 44 of these were intact (i.e., no significant part of the dendritic or axonal arbors were cut), and they were all drawn with the camera lucida. This sample was classified according to the following criteria: 1) axonal trajectory and morphology; 2) morphology of the soma and the dendritic tree; 3) occurrence and frequency of dendritic spines; and 4) position of the soma in the outer plexiform layer.

We apply two names to every interneuronal type: “long names” (e.g., short axon, aspinous bipolar neuron) refer to the general morphology, whereas the “short names” (e.g., sarmentous, coral, stellate, deep stellate, and couchant) were inspired by the appearance of the neurons and facilitate the identification of neurons with similar long names.

### Immunocytochemical study

Two lizards, under ether anaesthesia, were transcardially perfused first with saline (0.9% NaCl) for 1 minute and then with 2% paraformaldehyde and 2% glutaraldehyde in phosphate buffer. Their brains were extracted and immersed in the same fixative for 4 hours at 4°C and then transversely cut at 100 µm on a vibratome. These slices were osmium postfixated and flat embedded in Araldite. Semithin sections were obtained and immunostained using a polyclonal antibody for GABA (Clonotec, Marseille), ac-

ording to a procedure described elsewhere (Somogyi and Freund, 1989).

Four lizards, under ether anaesthesia, were transcardially perfused first with saline (0.9% NaCl) and then with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Their brains were extracted and immersed in the same fixative for 4 hours at 4°C and then transversely cut at 60 µm on a vibratome. These slices were processed for immunodetection of parvalbumin by overnight incubation in 1:5,000 diluted mouse antiparvalbumin (clone McAB; Celio et al., 1988) and opioid peptides by overnight incubation in 1:100 diluted mouse antibody against the peptide sequence Tyr-Gly-Gly-Phe of human β-endorphin (Boehringer Mannheim); this sequence is also present in Met- and Leu-enkephalin and in dynorphin. Antigen-antibody complexes were visualised using biotinylated anti-mouse IgG (Boehringer) and the avidin-biotin-peroxidase complex (ABC; Vector Labs.). All the immunoreagents were diluted in phosphate buffered-saline containing 5% normal goat serum, 0.2% gelatine, and 0.1% Triton X-100. The immunoperoxidase reaction was developed by using 3,3'-diaminobenzidine as a chromagen. Thereafter, the sections were washed, mounted on gelatine-coated slides, and coverslipped in Eukitt. Control sections were processed in the same way but the primary antibodies were omitted; no immunostaining was observed under these conditions.

## RESULTS

### Golgi study

Although the outer plexiform layer has fewer cells than other layers of the medial cortex, a large sample of impregnated neurons allowed the identification of the following types: 1) short axon, aspinous bipolar neuron (type 1 or sarmentous neuron); 2) short axon, aspinous juxtacortical neuron (type 2 or coral neuron); 3) short axon, sparsely spinous multipolar neuron (type 3 or stellate neuron); 4) short axon, sparsely spinous juxtacortical multipolar neuron (type 4 or deep stellate neuron); 5) sparsely spinous juxtacortical horizontal neuron (type 5 or couchant neuron); 6) bitufted neuron of the outer plexiform layer (type 6 or ectopic bitufted neuron); and 7) long-spined polymorphic neuron of the outer plexiform layer (type 7 or ectopic long-spined neuron). Types 1 to 5 are intrinsic to the outer plexiform layer of the medial cortex, while the other two occur unevenly and may be considered ectopic neurons in this layer.

The type 1 or sarmentous neurons are preferentially distributed in the medial vertical aspect of the outer plexiform layer, whereas the type 3 stellate neurons are distributed in the dorsal horizontal aspect of the outer plexiform layer. No preferential distribution was observed for types 2, 4, and 5.

**Type 1: short axon, aspinous bipolar neurons (sarmentous neurons).** Type 1 neurons (Figs. 1, 2) have oval to triangular, medium-sized (10 × 15 µm) somata that are smooth or with few intermittent somatic spines (Figs. 1a,d, 2d). Two or three thick primary dendrites arise from the soma and branch poorly, always parallel to the cell layer (Fig. 1a–e), giving the neurons a horizontal bipolar orientation. At each branch point, thinner daughter dendrites diverge, forming open angles. This gives the dendritic field (Fig. 2a) the aspect of a vine shoot. The dendrites never reach the pial surface (Figs. 1b,c, 2a) but curve in their proximity. Occasionally a dendrite (Figs. 1b,f, 2e) enters the

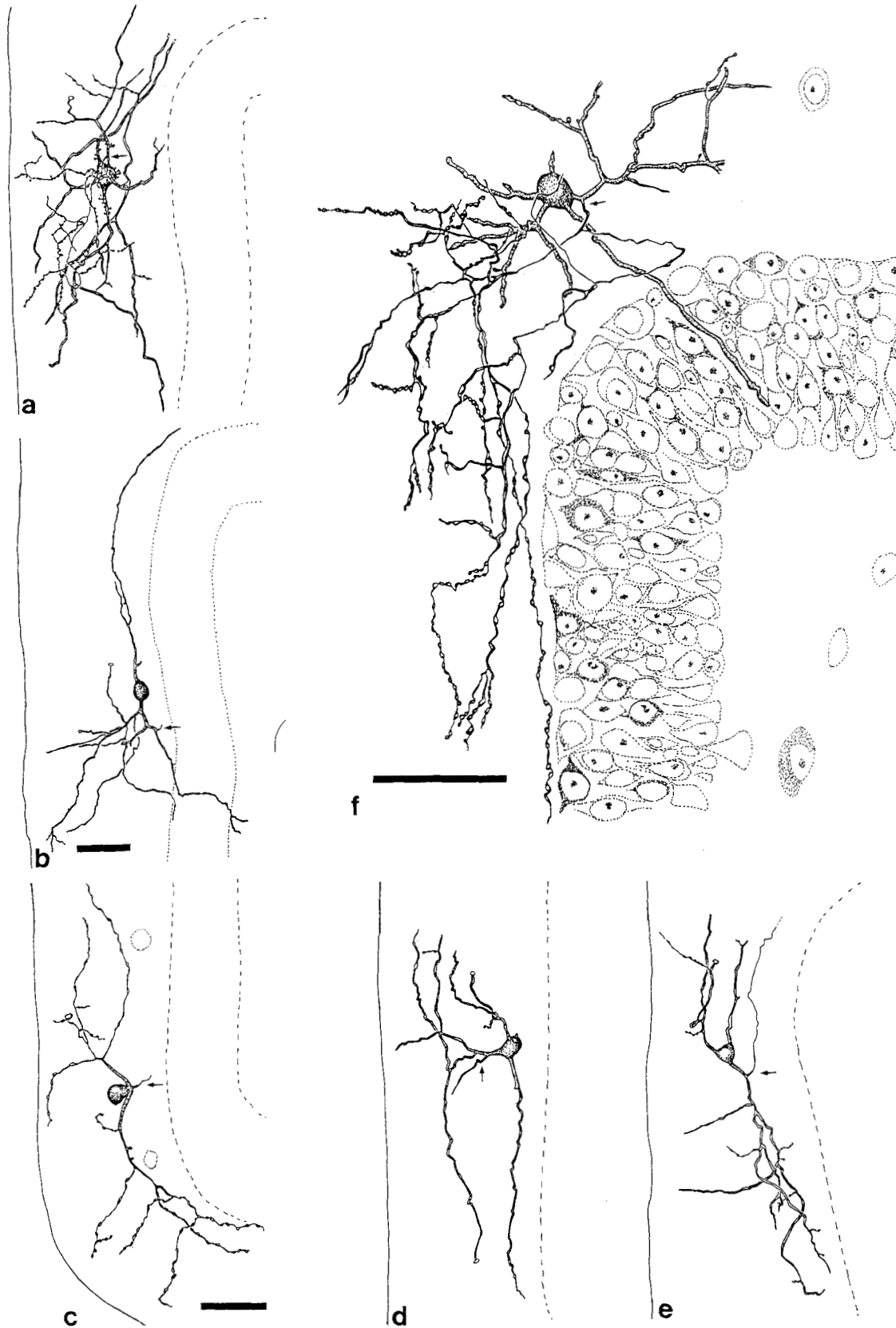


Fig. 1. Camera lucida drawings of type 1 sarmentous neurons. **a-e**: Five typical sarmentous neurons showing open branching dendritic trees; the neuron in **a** has dendritic and somatic spines. **f**: Sarmentous

neuron with axonal arbor overlapping the dendritic field. Small arrows indicate the origin of the axon; the encircled tips represent sectioned dendrites or axons. Scale bars = 20  $\mu\text{m}$ .

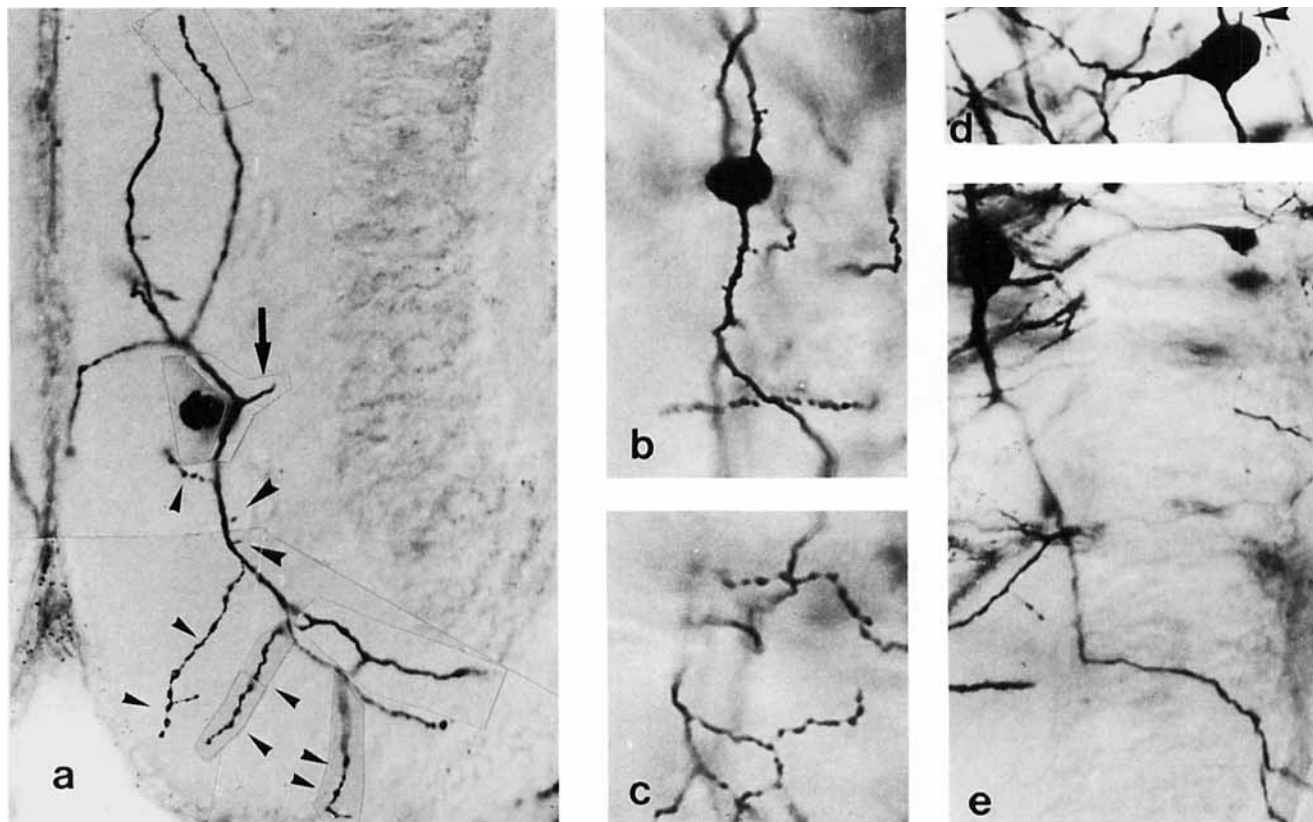


Fig. 2. Photomicrographs of type 1 sarmentous neurons. **a:** Photomontage of the neuron shown in Figure 1c, showing some conspicuous axon-like branchlets (small arrowheads) and a few isolated dendritic spines (arrowheads); the axonal hillock is seen near the soma (arrow).  $\times 275$ . **b:** Photomicrograph from the neuron seen in Figure 1a showing

dendritic and somatic spines.  $\times 440$ . **c:** Part of the axonal plexus of the neuron in Figure 1f, displaying terminal branches with boutons.  $\times 490$ . **d:** A somatic spine on the soma (arrowhead) seen in Figure 1d.  $\times 475$ . **e:** The large sarmentous neuron seen in Figure 1b, displaying a dendrite that traverses the cell layer.  $\times 575$ .

cell layer, reaching the upper inner plexiform layer. Dendritic spines (Fig. 2a) are scarce but, when present (Figs. 1a, 2b), appear in both proximal and medial dendritic segments. The dendrites show varicose, variable length, thread-like lateral outgrowths—axon-like branchlets—(Fig. 2a), arising from secondary or higher order dendrites. The axon starts from a short curved axonal hillock located on the cell body (Fig. 2a) or on a proximal dendrite (Fig. 1a,b,e,f). Axonal branches appear initially smooth and thin, giving off some thicker collaterals that form a loose plexus in the outer plexiform layer that overlaps the dendritic field (Fig. 1a,f). Distal collaterals have abundant boutons (Figs. 1f, 2c), giving them a beaded appearance. The majority of axonal collaterals are parallel to the cell layer, sometimes running above it. These neurons occur preferentially in the middle and deep levels of the outer plexiform layer at the vertical limb of the medial cortex.

**Type 2: short axon, aspinous juxtasonic neurons (coral neurons).** These neurons (Figs. 3, 4) have large round to oval somata ( $14\text{--}16\ \mu\text{m}$ ) which lie mainly in the medial and ventral aspects of the medial cortex. Their major axis is parallel to the cell layer. Very thick primary dendritic stalks ( $2.5\ \mu\text{m}$  or thicker) emerge laterally from the soma (Fig. 4a) and branch in open angles near the cell body, giving rise to thinner daughter dendrites. Occasionally, a short, thick proximal dendrite (Figs. 3d, 4c) enters or

traverses the cell layer (Fig. 3b). Distal thin dendritic segments run toward the pial surface (Fig. 3b–d) but curve back without contacting it. Dendritic (Fig. 4d–f) and somatic (Fig. 4b) spines are scarce and show pedicelated morphology. In addition, thin and varicose lateral outgrowths (Fig. 3b–d, 4c,d,f) may arise from secondary or higher order dendrites and (rarely) from the somata (Fig. 4h). The axon originates from the soma (Fig. 4a) or from a proximal dendrite and engenders thin axonal collaterals (Fig. 3a) that ramify overlapping the dendritic field. Reconstructions of axonal plexuses from several sections show discrete thickenings but no boutons. These neurons resemble sarmentous neurons but differ from them in three aspects. First, their somata are located at the border between the outer plexiform layer and the cell layer (Fig. 3a–d), frequently intermingled with the upper row of somata of this layer. Second, their dendritic tree (Figs. 3a–d, 4c) spreads into the outer plexiform layer like a fan, without the typical bipolar appearance of the sarmentous neuron. Third, their axons are distributed in a reduced field and show thin varicosities (Fig. 3a) but no boutons. This aspect resembles that of common corals, hence the short name of this type.

**Type 3: short axon, sparsely spinous multipolar neurons (stellate neurons).** Type 3 neurons (Figs. 5, 6) have polygonal or pyriform cell bodies ( $12\text{--}18\ \mu\text{m}$ ) with three to

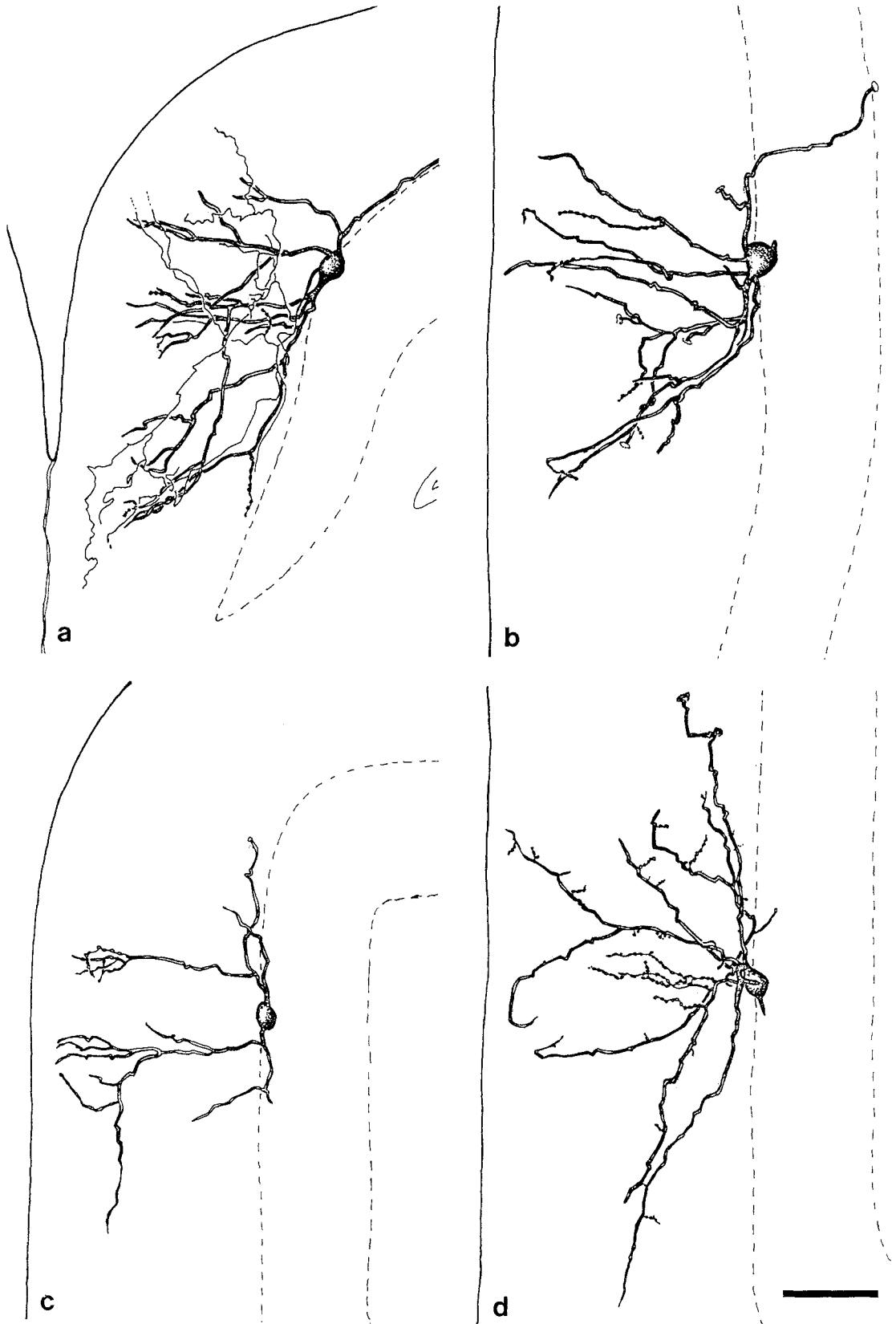


Fig. 3. Drawings of type 2 coral neurons. **a:** Drawing of a coral neuron exhibiting the axonal arbor overlapping the dendritic tree. **b:** Large coral neuron with a secondary dendrite crossing the cell layer. **c:** Small coral neuron displaying some distal axon-like branchlets. **d:** Coral neuron with dendrites rising in an open fan and bearing long and short

varicose branchlets; note that the dendrites curve back before reaching the upper limiting border, avoiding contact with it. Small arrows indicate the origin of the axon; the encircled tips represent sectioned dendrites or axons. Scale bar = 20  $\mu$ m.

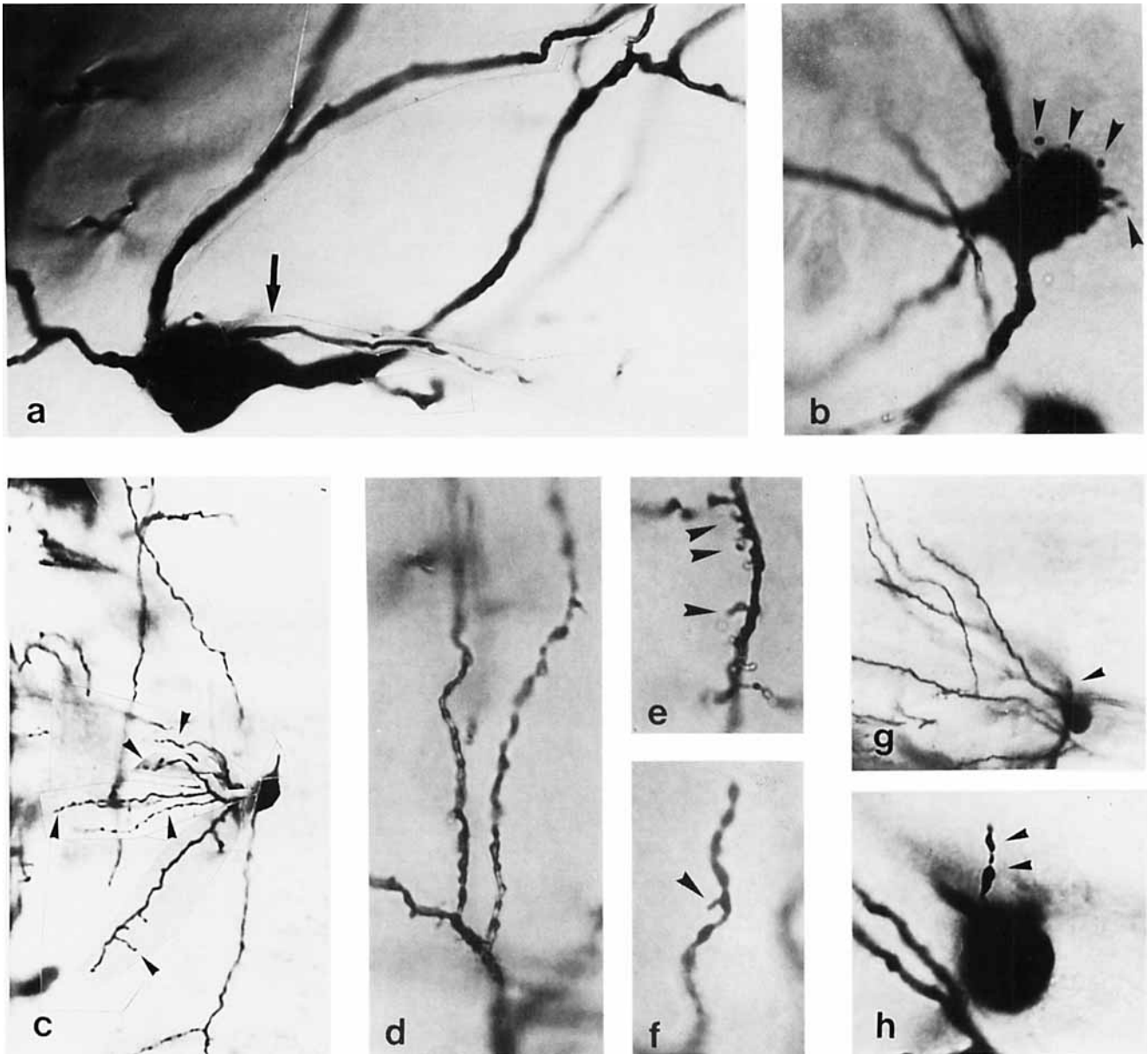


Fig. 4. Photomicrographs of type 2 coral neurons. **a**: Photomontage of the same neuron as in Figure 4a showing the axonal hillock (arrow) arising from the top of the soma.  $\times 1,160$ . **b**: Neuronal soma with spines (arrowheads) located at the basal pole.  $\times 1,160$ . **c**: Photomontage of the neuron seen in Figure 4d showing prominent axon-like branchlets (arrowheads).  $\times 315$ . **d**: Two long varicose processes arising from the

same parent dendrite.  $\times 1,160$ . **e**: Intermediate segment of a dendrite bearing dendritic spines (arrowheads).  $\times 1,160$ . **f**: Medium-sized varicose process with a small spine on one knot (arrowhead).  $\times 1,300$ . **g**: Coral neuron with a short varicose somatic outgrowth (arrowhead).  $\times 250$ . **h**: The round soma shown in g and the varicose process (arrowheads) at a higher magnification.  $\times 975$ .

five dendrites that ramify near the soma with acute angles. Horizontally oriented dendrites (Fig. 5b,c) are long (up to  $250 \mu\text{m}$ ). There is usually a thicker and profusely ramified primary dendrite (Fig. 6a). Pedunculated spines may be seen on dendrites (Fig. 5b) and cell bodies (Fig. 6b), but they are sparsely distributed. Distally, dendrites may present some irregular large varicosities (Fig. 5b,c). The axon is thin and arises from a thin axonal hillock located either on the cell body (Fig. 5b) or on a proximal dendrite (Figs. 5a, 6b). The axonal plexus (Fig. 5a) coincides with the dendritic tree, showing small boutons in the distal collaterals. These

neurons are exclusively located in the dorsal limb of the medial cortex. Frequently, both soma and dendrites (Figs. 5c, 6c) show some degree of polarisation as though adapting to the thickness of the outer plexiform layer in this zone.

**Type 4: short axon, sparsely spinous juxtасomatic multipolar neurons (deep stellate neurons).** Type 4 neurons (Figs. 7, 8) have somata located close to the cell layer. The dendritic tree of these neurons is similar to that of multipolar type 3 neurons but also has one to three long descending dendrites that cross the cell layer and reach the deepest strata of the inner plexiform layer (Fig. 8b–e), where they

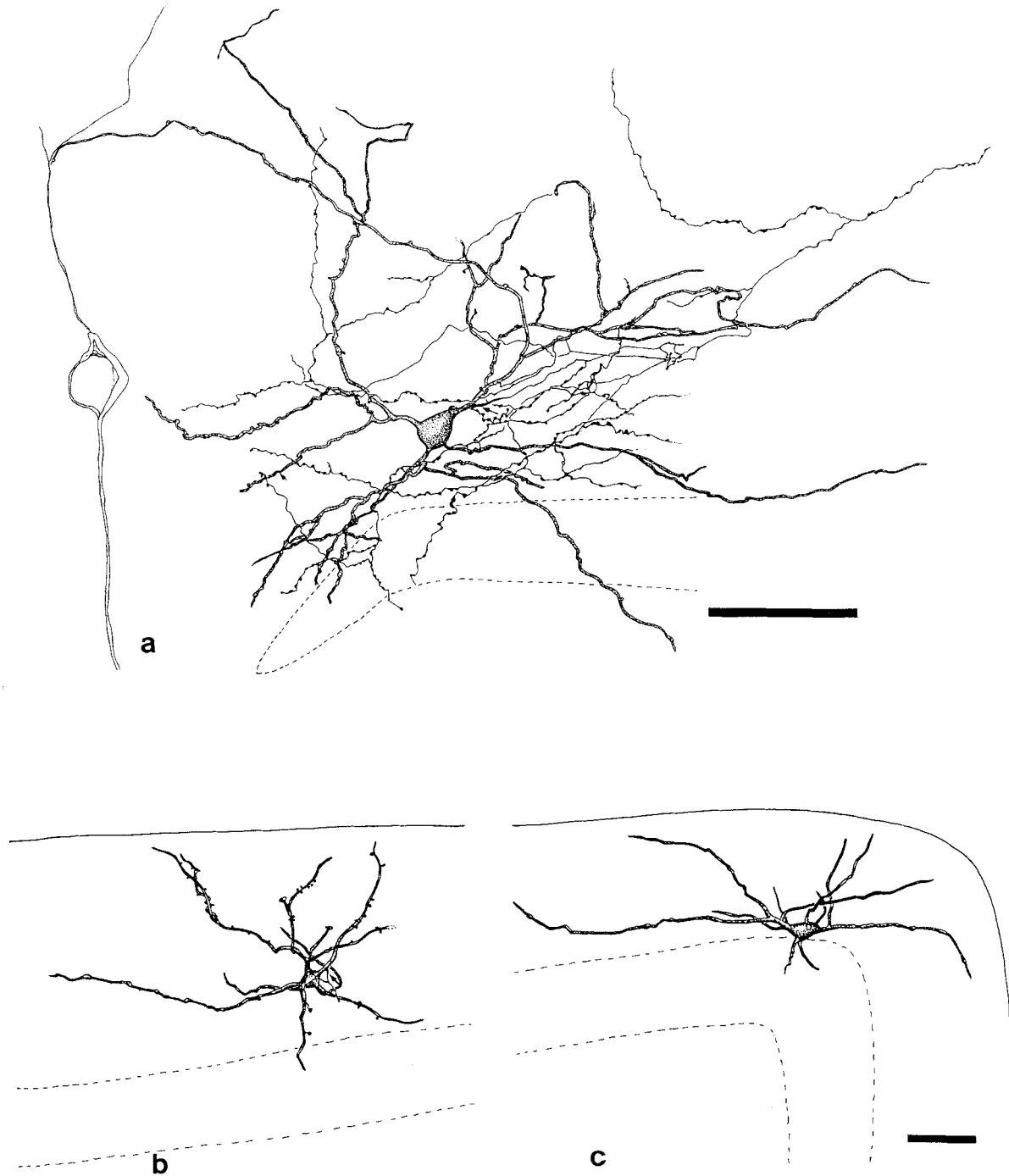


Fig. 5. Drawings of type 3 stellate neurons. **a:** Stellate neuron from a young lizard at a rostral level; the large span of this neuron was captured by the tangential orientation of the section; the axon starts at a primary dendrite near the soma (arrow) and gives rise to a plexus of fine, parallel collaterals full of boutons; the position of the cell layer is orientative; in fact, no dendrite from this neuron traversed it. **b:**

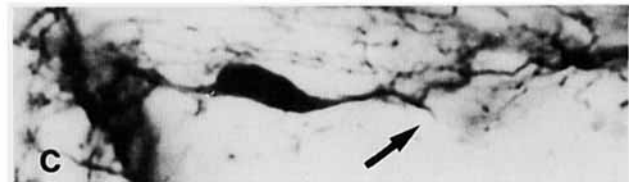
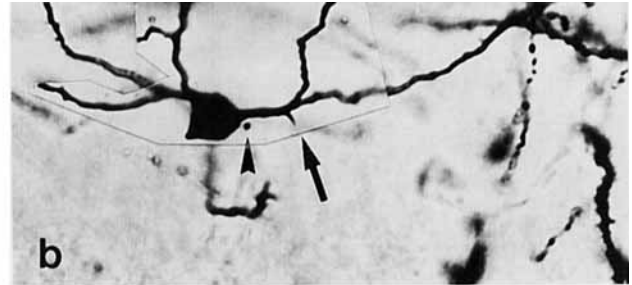
Stellate neuron showing sparsely spinous dendrites. **c:** Stellate neuron occupying a deeper position on the cell layer; the long dendrites show some distal knotty varicosities. Small arrows indicate the origin of the axon; the encircled tips represent sectioned dendrites or axons. Scale bars = 50  $\mu\text{m}$  in a, 20  $\mu\text{m}$  in b and c.

sometimes ramify (Figs. 7b, 8f). These descending dendrites show different morphology while crossing the cell layer before reaching the limits of the ependyma. The segments in the cell layer (Fig. 8c) and upper half of the inner plexiform layer (Fig. 8b,d) are smooth or bear isolated

spines. Those running in the deep half of the inner plexiform layer (Fig. 7a,c) bear frequent small spines and some complex spine-like appendages (Fig. 8e). The most distal segments sometimes show varicosities and/or long spines. The axon (Fig. 7a) emerges from the soma or from a



Fig. 6. Photomicrographs of type 3 stellate neurons. **a:** Photomicrograph of the same neuron represented in Figure 5a; the arrow points to the axonal hillock.  $\times 750$ . **b:** Cell body displaying a somatic spine (arrowhead) and the axonal hillock (arrow) arising from a dendrite.  $\times 750$ .



$\times 400$ . **c:** The somata of stellate neurons are highly variable in shape; this one has a pronounced fusiform horizontal appearance with the axonal hillock (arrow) arising from a dendrite.  $\times 725$ .

primary dendrite. It is very thin and gives off many distal collaterals which are studded with abundant small boutons, and run along distances over the cell layer of the medial and dorsomedial cortices. Sometimes thin axonal filaments with a few boutons appear surrounding a soma of the upper rim of the cell layer in a basket-like fashion.

**Type 5: sparsely spinous juxtasonic horizontal neurons (couchant neurons).** These neurons (Figs. 9a,b, 10a,b) have discoidal somata (from 12–15 to 7–10  $\mu\text{m}$ ) with a smooth surface (Fig. 10a) located superficial to the cell layer (Fig. 9a,b). Three to five thin dendrites radiate laterally from the soma (Figs. 9a,b, 10b) and run close to the cell layer (this gives the appearance of a supine or couchant dendritic tree). Secondary or higher order dendrites are filiform and run along anteroposterior distances. Dendritic spines are moderately scarce and pedunculated (Fig. 9b); they appear in medial and distal dendritic segments. A very thin axon (Fig. 10a) emerges from the basal part of the cell body and penetrates the cell layer, describing a wide arc. It is very fine and becomes indistinguishable soon after intermingling with the somata of the cell layer.

**Type 6: ectopic bitufted neurons of the outer plexiform layer (ectopic bitufted neurons).** Such neurons are rare (Figs. 9c, 10e) and display morphological features similar to those of the principal neurons of the cell layer. Bitufted dendritic trees, ranging from sparsely to heavily spinous, arise from small (Fig. 9a) to medium-sized (Fig. 10c) somata. An apical tuft ramifies in the outer plexiform layer, and a basal less developed tuft penetrates the inner plexiform layer. The axon arises from the basal pole of the soma, traverses the cell layer, and runs directly towards the alveus; along its trajectory in the deep inner plexiform layer some thin beady collaterals with thick boutons are frequently seen.

**Type 7: long-spined polymorphic neurons of the outer plexiform layer (ectopic long-spined neurons).** Type 7 neurons (Figs. 9c, 10c,d) have large cell bodies giving off several thick primary dendrites. At least one of them runs parallel to the cell layer, but the others cross the cell layer and reach the inner plexiform layer. The dendrites are

profusely covered with characteristic complex long spines and branchlets (Fig. 10c,d). Only the initial portion of the axon was impregnated.

### Immunocytochemical study

Most of the somata located in the outer plexiform layer displayed GABA immunoreactivity (Fig. 11). In semithin sections they appeared round, oval, or elongated, varying in size from  $6 \times 5 \mu\text{m}$  to  $17 \times 9 \mu\text{m}$  and were distributed throughout the outer plexiform layer, occupying its deep half. GABA immunoreactive puncta were distributed throughout the outer plexiform layer (Fig. 11a,b,f) but were more abundant in the superficial half of this layer. Often, GABA immunoreactive puncta contacted neuronal somata of the outer plexiform layer and the first row of somata of the cell layer (Fig. 11d). A few GABA-negative somata (Fig. 11a,b,e–g) were seen in the outer plexiform layer. They were identical in size and shape to the GABA immunoreactive somata.

The immunostaining with antibodies against parvalbumin (Fig. 12) yielded Golgi-like labelled neurons. Their somata had diameters ranging from 4.5 to 10  $\mu\text{m}$  and were stained with diverse intensity from very weak to strong immunostaining (Fig. 12a,c,d). They were located preferentially at deep levels in the outer plexiform layer. The morphology of parvalbumin-immunoreactive neurons resembled that of the coral (Fig. 12b), stellate (Fig. 12d,g), deep stellate (Fig. 12e,h), and couchant (Fig. 12c,f) neurons observed in Golgi material.

Somata stained with antibodies against opioid peptides were seen in the superficial half of the outer plexiform layer (Fig. 13). Occasionally, proximal dendritic segments were also labelled (Fig. 13d,e). Opioid immunoreactive puncta were seen dispersed throughout the outer plexiform layer except in a narrow juxtasonic lamina, where they appeared highly abundant (Fig. 13c,e). They often formed basket-like arrangements around granule cell somata in the cell layer and around cell bodies in the outer plexiform layer (Fig. 13c).



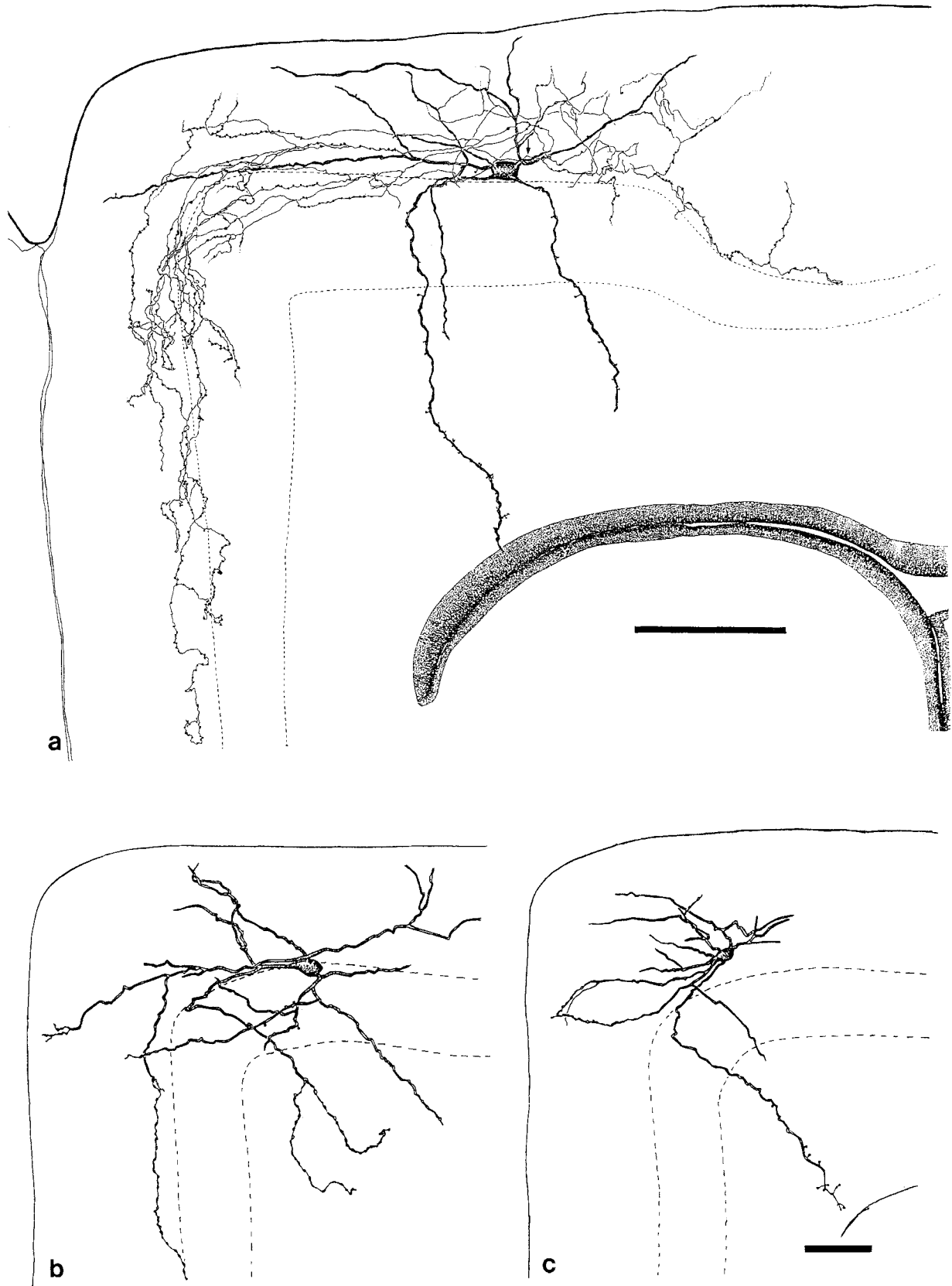


Figure 7

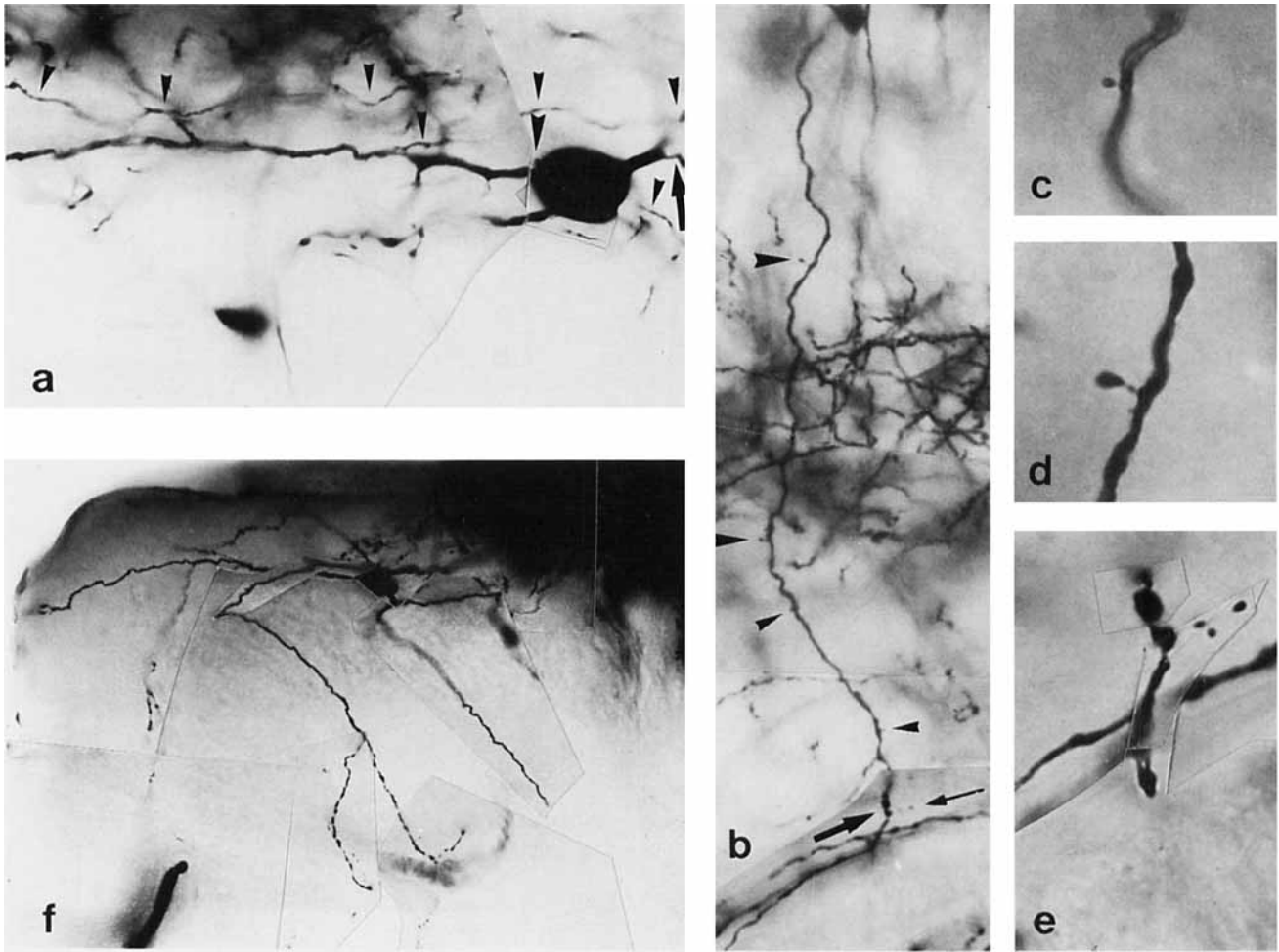


Fig. 8. Photomicrographs of type 4 deep stellate neurons. **a:** Photomontage illustrating the soma of the neuron shown in Figure 7a, abundant fine axonal branches (arrowheads), and a horizontal dendrite with irregular contours and some thickenings; the axonal hillock (arrow) arises from a dendrite.  $\times 885$ . **b:** The deep dendrite of the same neuron traversing the whole of the inner plexiform layer and bearing some spines (arrowheads), thickenings (thick arrow), and a long complex spine (thin arrow); the ependymal layer is at the bottom of the

figure.  $\times 410$ . **c-e:** Dendritic spines of the deep dendrite as seen in dendritic segments traversing the cell layer (c), the upper inner plexiform layer (d), and the deep inner plexiform layer (e); note the terminal dendritic segment, displaying large thickenings and a long complex three-headed dendritic spine in e.  $\times 1,515$ . **f:** Photomontage of the neuron shown in Figure 7b: observe a dendrite that curves and traverses the cell layer.  $\times 315$ .

## DISCUSSION

### The neuronal population in the outer plexiform layer is scarce but heterogeneous

In spite of the low number of cells present in the outer plexiform layer of the lizard medial cortex (about 1,500/hemisphere in *Podarcis hispanica*) (Lopez-Garcia et al.,

1984), this neuronal population shows a heterogeneity that has so far been overlooked. Occasional brief mentions (Crosby, 1917; Northcutt, 1967), but no detailed description has been made of outer plexiform layer neurons. As a result, from a typological point of view, the outer plexiform layer has been considered a very simple area. This idea is supported by electron microscope studies that describe a single class of soma with similar features: large cell body, abundant perinuclear cytoplasm with many organelles, and an oval nucleus with one or two deep invaginations and a central nucleolus (Ulinski, 1977; Wouterlood et al., 1981; Garcia-Verdugo et al., 1984; Davila et al., 1985; Schwerdtfeger and Lorente, 1987, 1988). Golgi studies carried out in other squamate reptiles described between none (Ebbesson and Voneida, 1969; Regidor et al., 1974) and three neuronal types (Table 1); no mention of the number of Golgi-impregnated neurons is made in these studies; it could be that they were performed on small samples of Golgi-impregnated brains, leading to a partial view of the neuronal population of the outer plexiform layer.

Fig. 7. Drawings of type 4 deep stellate neurons. **a:** Deep stellate neuron displaying an arborised axonal plexus occupying the whole upper rim over the cell layer of the medial cortex; distal collaterals bear many small boutons; note that three dendrites traverse the cell layer and the inner plexiform layer, one of them reaching the ependyma. Small arrow indicates the origin of the axon. **b:** Deep stellate neuron with two deep dendrites, one of them bifurcates into varicose daughter branches after entering the inner plexiform layer. **c:** Small deep stellate neuron giving off a dendrite that reaches deep levels of the inner plexiform layer; this dendrite exhibits some long complex spines on the inner distal segment. Scale bars = 100  $\mu\text{m}$  in a, 20  $\mu\text{m}$  in b and c.

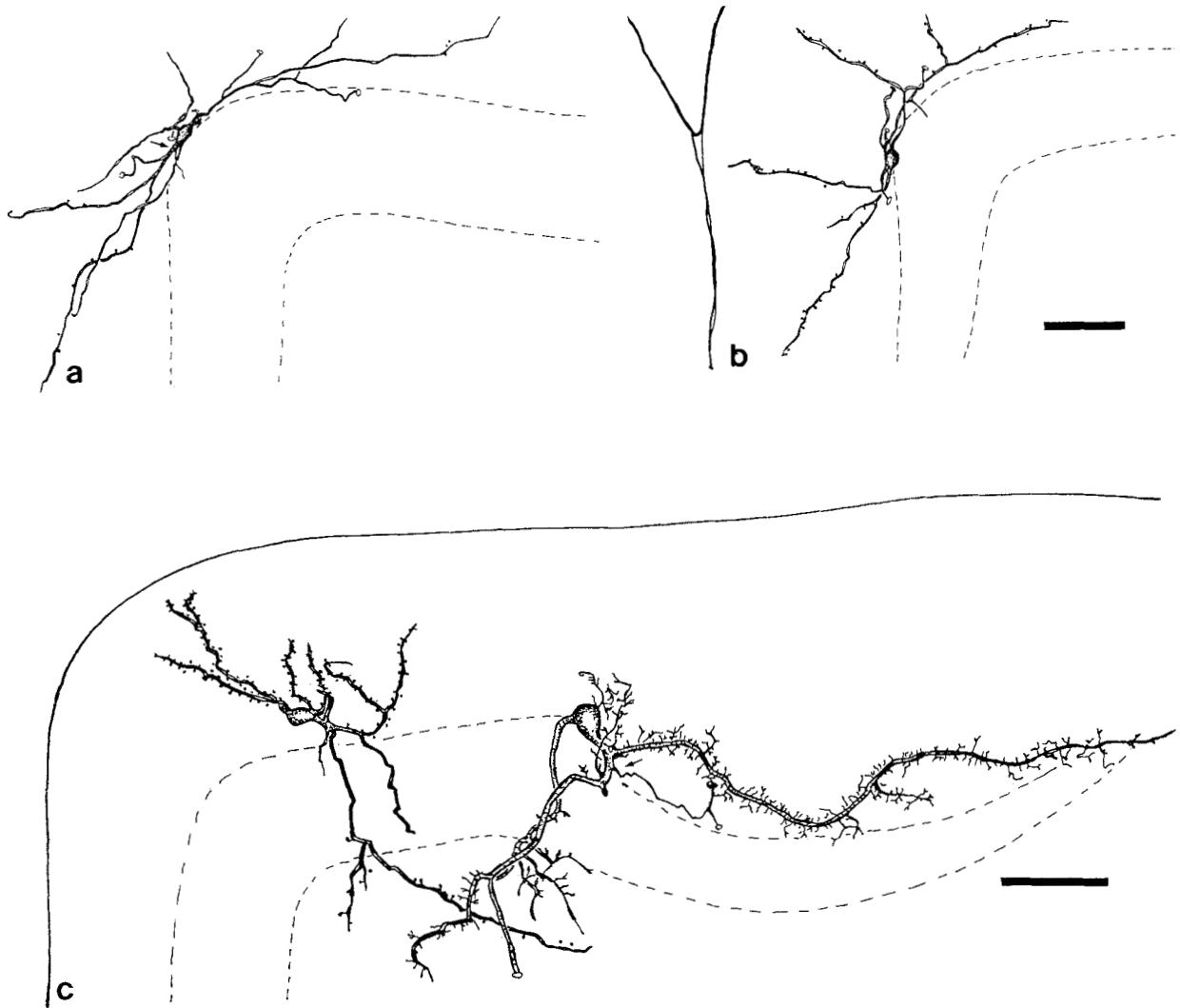


Fig. 9. Drawings of type 5 couchant neurons and types 6 and 7 ectopic neurons. **a:** Couchant neuron with fine dendrites above the cell layer and a slender axon (arrow) directed toward the cell layer. **b:** Couchant neuron displaying sparsely spinous dendrites; the axon originates at a primary dendrite near the soma, and enters the cell layer. **c:** Camera lucida composition showing two ectopic neurons: one spinous bitufted neuron (left) and one long-spined polymorphic neuron

(right); in the former, the axon emerges from the origin of the basal plume and enters the cell layer and in the latter it starts from a thick dendritic stalk and is directed to the outer plexiform layer of the dorsomedial cortex, where it changes plane. Small arrows indicate the origin of the axon; the encircled tips represent sectioned dendrites or axons. Scale bars = 20  $\mu\text{m}$  in a and b, 25  $\mu\text{m}$  in c.

In this study we have described five neuronal types in the outer plexiform layer of the medial cortex and two types of ectopic neurons. According to their dendritic morphology, three major different groups can be established within the neuronal types of the outer plexiform layer. These are the aspiny neurons (types 1, sarmentous, and 2, coral), the multipolar neurons (types 3, stellate, and 4, deep stellate), and the sparsely spinous horizontal neurons (type 5, couchant).

The two aspiny neurons (types 1 and 2) have similar dendritic patterns but differ in axonal morphology. Likewise, the multipolar neurons (types 3 and 4) show a similar dendritic morphology, but differ by the presence of descending dendrites in the deep stellate neurons and by differing axonal arborisation. The type 5 sparsely spinous horizontal neuron is a conspicuous neuronal type both in regard to its dendritic and axonal morphology; it has a very thin axon

that initially enters the cell layer. Although the target region of the axon is not known, its orientation suggests that it could reach zones other than the outer plexiform layer.

### Comparative aspects

**Other reptiles.** The five intrinsic neuronal types described in the present study may be recognised in previous Golgi studies for other Squamata species (Table 1). Both the ectopic neurons have been reported in the literature for the outer plexiform layer of the medial cortex (Ramón y Cajal, 1917) and the outer plexiform layer of the dorsal cortex (Desfilis, '89) of lizards. In addition, there is another cell type described in the literature, the small stellate corpuscle (Ramón y Cajal, 1917), which could not be identified in our material. From its marked neurogliform

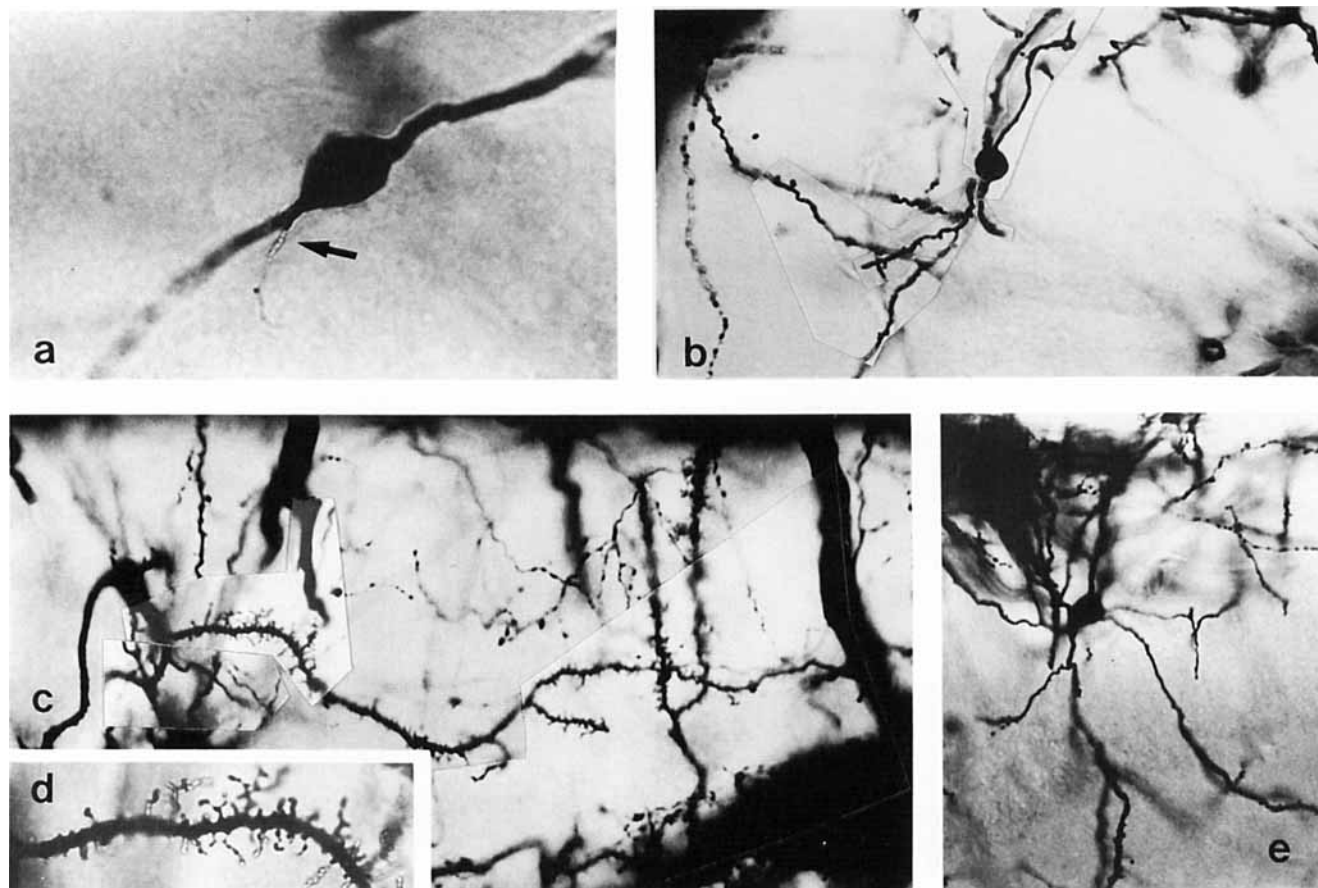


Fig. 10. Photomicrographs of type 5 couchant neurons, and types 6 and 7 ectopic neurons. **a:** Photomicrograph of the soma of the couchant neuron given in Figure 9a showing the axonal hillock (arrow) and the fine axon entering the cell layer.  $\times 850$ . **b:** Photomontage of the same cell as in Figure 9b.  $\times 330$ . **c:** Photomontage of the ectopic long-spined

polymorphic neuron shown in Figure 9c.  $\times 350$ . **d:** Detail of a proximal dendritic segment at a higher magnification; note the presence of conspicuous long spines or microdendrites.  $\times 825$ . **e:** Ectopic heavily spinous bitufted neuron.  $\times 250$ .

appearance, its description resembles that of the stellate cell reported in *Psammodromus algirus* (Guirado et al., 1984), and that of the fibrous astrocyte reported in *Agama* (Wouterlood, 1981). In fact, we have found a spectrum of small stellate cells in *Podarcis* that we identified as glial in nature, mostly resting or activated microglia (Berbel et al., 1981; Castellano et al., 1991). First, because they are morphologically identical to the resting microglial cells described both in light and electron microscopy in the toad (Stensaas and Stensaas, 1968a,b) and in the lizard *Podarcis pityusensis* (Berbel et al., 1981), a species close to *Podarcis hispanica*, and second and more important, due to the histochemical demonstration of nucleoside diphosphatase, a specific microglial marker, labelling an abundant population of this glial cell type in healthy individuals of *Podarcis hispanica* and *Podarcis muralis* (Castellano et al., 1991).

Studies with techniques other than the Golgi method performed in *Podarcis* yield results congruent with our data. The spherical multipolar neuron (Schwerdtfeger and Lopez-Garcia, 1986), the medium-sized, multipolar neuron, and the small multipolar neuron (Schwerdtfeger and Lorente, 1987, 1988) resemble the stellate and sarmentous neurons, whereas the horizontal neuron of the outer rim of layer 2 (Schwerdtfeger and Lorente, 1987, 1988) and the horizontal fusiform neuron (Olucha et al., 1988) should

have its correspondent in the couchant and/or deep stellate neurons.

Some features characteristic of some neuronal types of the outer plexiform layer of the medial cortex of *Podarcis*, i.e., dendritic appendages, were observed in previous studies. The axon-like branchlets, characteristic of sarmentous and coral dendrites, have been previously reported both in the medial cortex of other Squamata reptiles (Ramón y Cajal, 1917; Ulinski, 1977; Wouterlood et al., 1981; Wouterlood, 1981) and in the dorsomedial cortex (Martinez-Guijarro, 1985) and dorsal cortex (Desfilis, 1989) of *Podarcis hispanica*. With the exception of this latter work, axon-like branchlets have been almost completely ignored before in reptiles. Likewise, the long spines typical of the long-spined polymorphic neuron, have been described in detail even at ultrastructural level (Lopez-Garcia et al., 1988).

**Mammals.** The homology between the mammalian hippocampal fascia dentata and the Squamata medial cortex has been generally admitted for a century (Etinger, 1896; Ramón y Cajal, 1917). There is a growing body of evidence supporting this hypothesis: the cytoarchitecture (trilaminar organisation, similar neuronal types), connectivity (commissural, association, and extrinsic afferents ending in a laminar pattern), histochemistry (the Timm-positive

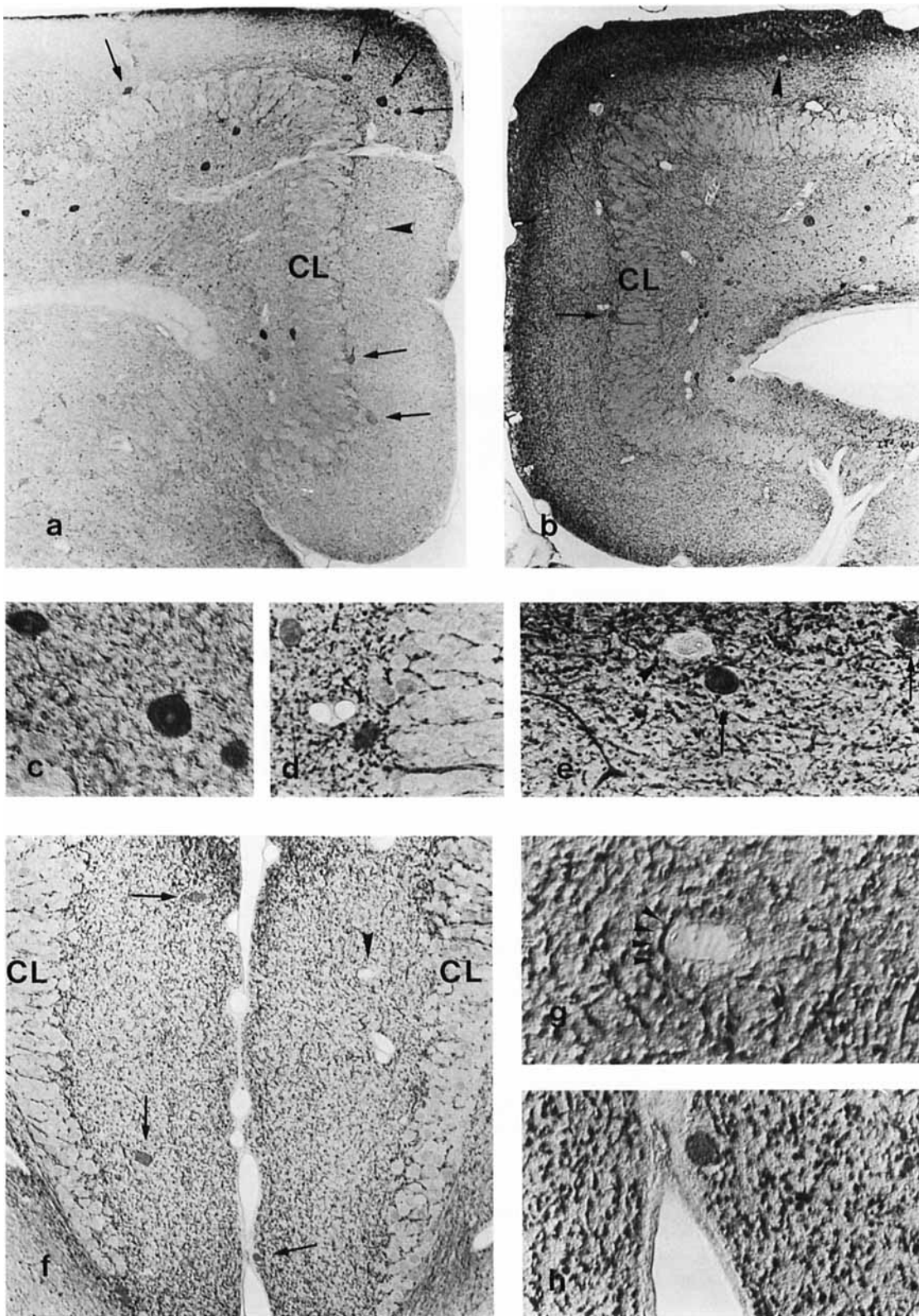


Fig. 11. GABA immunostaining. **a,b:** GABA-immunostained transverse semithin sections at postcommissural (a) and caudal (b) levels; note the presence of GABA immunoreactive (arrows) and nonstained (arrowheads) cell somata.  $\times 145$  in a;  $\times 115$  in b. **c:** Detail of three immunostained cell bodies located in the outer plexiform layer (shown in a, upper right corner).  $\times 600$ . **d:** Two GABA immunoreactive cell bodies in the outer plexiform layer showing different intensity of staining; note the presence of paired capillaries; somata located in the cell layer are not GABA immunoreactive. **e:** GABA immunoreactive

(arrows) and GABA-negative (arrowhead) somata in the outer plexiform layer. **f:** Commissural section showing GABA-positive (arrows) and -negative (arrowhead) somata in the outer plexiform layer as well as a dense plexus of immunostained puncta; note that these puncta intermingle with granule somata of the cell layer (CL).  $\times 210$ . **g:** Detail of a GABA-negative cell body and GABA immunoreactive puncta (arrowheads) that appear surrounding it.  $\times 1,075$ . **h:** Small GABA immunoreactive cell body juxtaposed to the glial limiting membrane between the two hemispheres.  $\times 865$ .

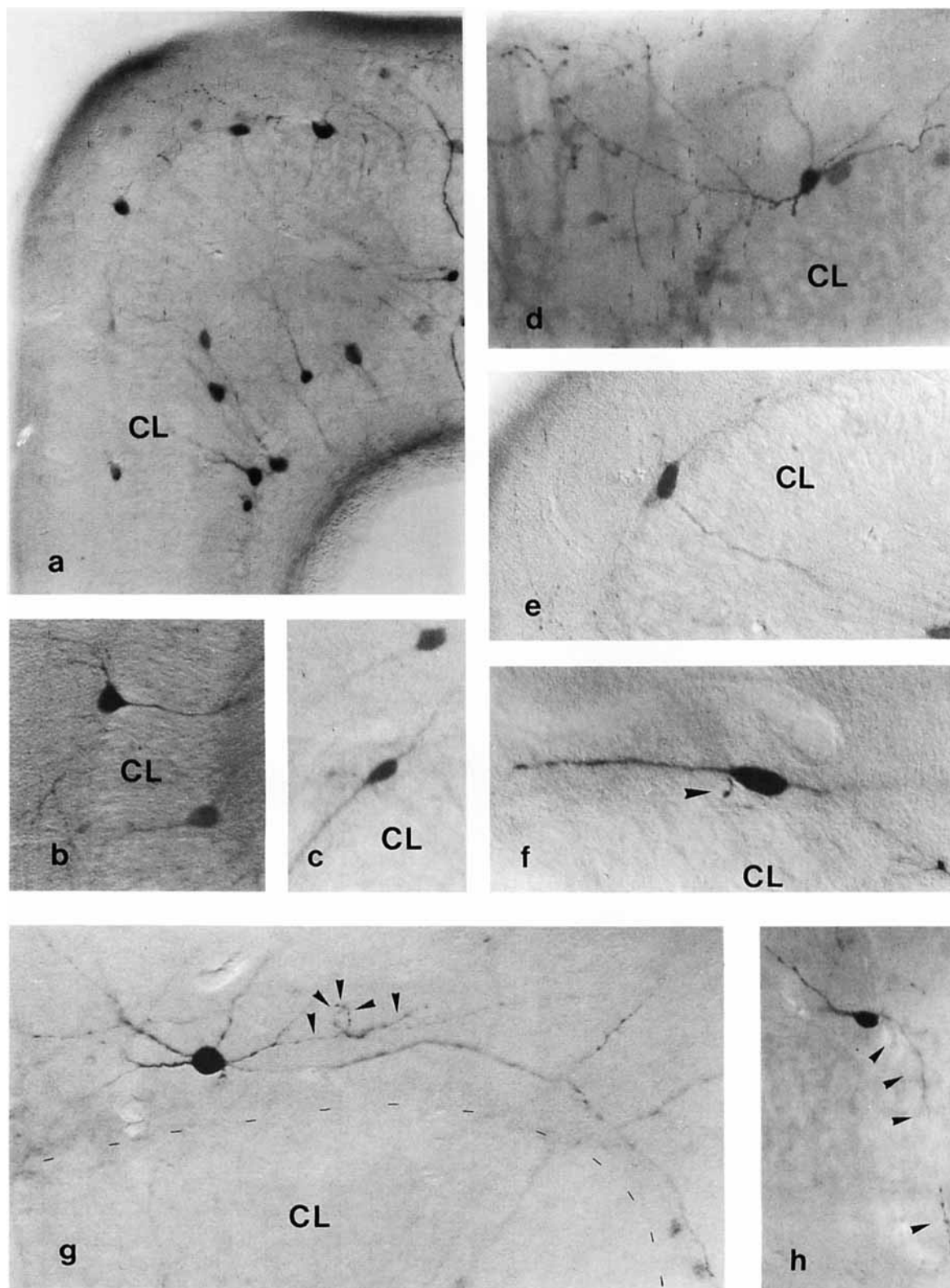


Fig. 12. Parvalbumin immunostaining. **a:** Parvalbumin-immunostained 60  $\mu\text{m}$  thick transverse section of the medial cortex at a caudal level; note the Golgi-like appearance of the immunostained cells; even, short axonal segments appear immunostained.  $\times 185$ . CL, cell layer. **b:** Juxtasonic parvalbumin-immunoreactive neuron (left) with dendrites traversing the cell layer and bifurcating in the inner plexiform layer; this is probably a deep stellate neuron.  $\times 320$ . **c:** Two parvalbumin immunoreactive cells horizontally arranged; the lower one closely resembles a couchant neuron.  $\times 410$ . **d:** Parvalbumin immunoreactive neuron resembling the coral neuron as seen in Golgi material.  $\times 410$ . **e:** Parvalbumin immunoreactive cell displaying a deep dendrite reaching

the inner plexiform layer; it resembles a deep stellate neuron archetype.  $\times 320$ . **f:** The couchant-like parvalbumin immunoreactive cell shown in **c**; the axon starts from the soma and shows a minute bouton near it (arrowhead)  $\times 715$ . **g:** Parvalbumin immunoreactive cell resembling a stellate neuron; note the presence of long distally varicose dendrites and the axon making a loop in the centre of the image (arrowheads); the broken line marks the borderline with the cell layer (CL).  $\times 450$ . **h:** Deep stellate-like parvalbumin immunoreactive cell displaying a deep dendrite (arrowheads) that traverses the cell layer and enters deeply in the inner plexiform layer.  $\times 310$ .

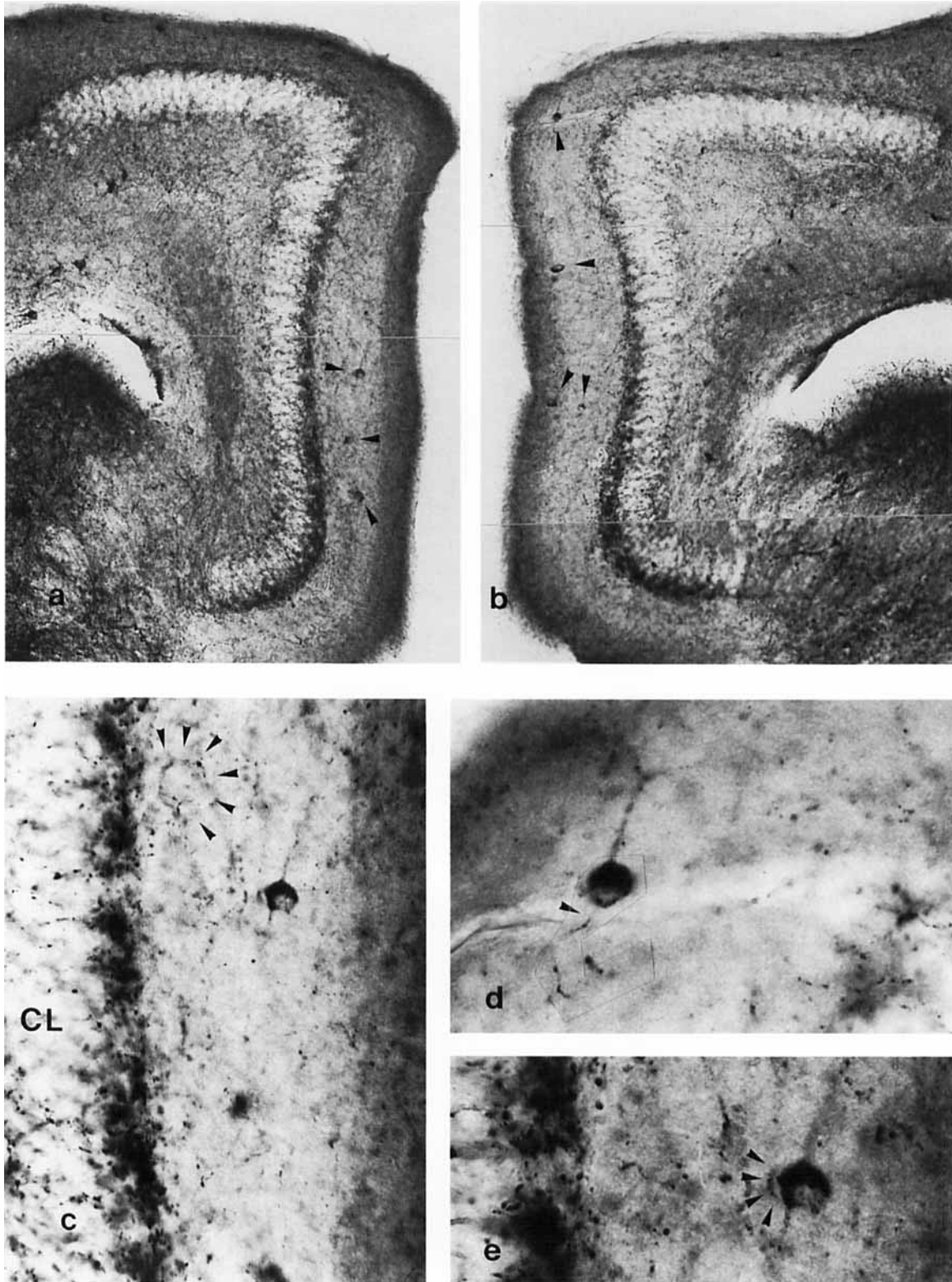


Fig. 13. Opioid immunostaining. **a,b**: Opioid immunostaining of 60  $\mu\text{m}$  thick transverse sections at the postcommissural level; note the presence of immunostained somata (arrowheads) in the outer plexiform layer and a narrow but densely immunostained juxtasonic strip made by a plexus of immunostained axons.  $\times 150$ . **c**: Three immunostained somata in the outer plexiform layer; note that only short dendritic

segments appear immunostained. At the top of the image an opioid negative soma appears surrounded by immunostained boutons (arrowheads).  $\times 445$ . **d**: Detail of the soma of the upper left in b, close to a blood vessel; its axon (arrowhead) starts from the soma.  $\times 825$ . **e**: Detail of the upper soma in c; note that some immunostained puncta appear to make contact with it (arrowheads).  $\times 825$ .

TABLE 1. Comparison of the Neuronal Types Described for the Squamata Medial Cortex Outer Plexiform Layer

Author, year, species	Type 1: sarmentous	Type 2: coral	Type 3: stellate	Type 4: deep stellate	Type 5: couchant	Type 6: ectopic bitufted	Type 7: ectopic long spined
Present study, '93; <i>Podarcis hispanica</i>	Fusiform spherical	—	Fusiform spherical	—	—	—	—
Ramón y Cajal, 1891; <i>Lacerta agilis</i>	Fusiform superficial	—	—	—	—	—	—
Ramón, 1891; <i>Lacerta agilis</i> , <i>Lacerta viridis</i>	—	Fusiform horizontal	Small stellate cell (without equivalent)	—	—	Long axon triangular	—
Ramón y Cajal, '17; <i>Lacerta agilis</i> , <i>Iguana iguana</i>	Round-oval cell	—	Small stellate cell (without equivalent)	—	—	Triangular soma cell	—
Minelli, '66; <i>Lacerta viridis</i> , <i>Lacerta muralis</i>	—	—	Sparsely branched, multipolar	—	—	—	—
Ebesson and Voneida, '69; <i>Tupinambis nigropunctatus</i>	—	Cell layer large fusiform	—	Cell layer large fusiform	—	—	—
Regidor, '77; <i>Lacerta galloti</i>	—	Type m <sub>1</sub> A (cell layer)	—	—	—	—	—
Ulinski, '77; <i>Boa constrictor</i> , <i>Natrix sipedon</i>	Stellate cell	—	Stellate cell	—	—	—	—
Lacey, '78; <i>Sceloporus undulatus</i>	Golgi II class cell	Class No. 1 (cell layer)	Golgi II class cell	—	—	—	—
Wouterlood, '81; <i>Agama agama</i>	Type S <sub>1</sub> (solitary)	—	Type S <sub>1</sub> (solitary)	—	—	—	—
Guirado et al., '84; <i>Psammotromus algerus</i>	—	—	Stellate cell	—	—	—	—
Berbel et al., '87; <i>Lacerta pityusensis</i>	A: Smooth bipolar	—	—	—	—	—	—

TABLE 2. Neuronal Types Reported for the Molecular Layer of the Mammalian Fascia Dentata

Author, year, species, procedure	Cell type
Ramón y Cajal; rabbit; Golgi study	Short axon superficial neurons Molecular layer small neuron Short axon deep neuron Triangular displaced cell (displaced grain)
Lorente de Nó, '34; rabbit; Golgi study	Superficial neuron Deep neuron Ectopic granule cell
Stensaas, '67; foetal rabbit; Golgi study	Small bipolar neuron of the marginal lamina Large Cajal-Retzius cell of the marginal lamina
Hazlett and Farkas, '78; opossum; Golgi study	Short axon molecular layer neuron
Seress and Pokorny, '81; rat; Golgi study	Molecular layer basket cell
Seress and Ribak, '83; rat; Golgi study and GAD immunocytochemistry	Small superficial cell Large multipolar juxtasonic cell
Ribak and Seress, '83; rat; Golgi-EM study	Molecular layer basket cell
Marti-Subirana et al., '86; rat; Golgi study	Molecular layer ectopic granule neuron
Soriano et al., '89; rat; <sup>3</sup> HT autoradiography and GAD immunocytochemistry	Spheroidal fusiform small neuron Large deep neuron
Soriano and Frotscher, '89; rat; Golgi-EM study and GABA immunocytochemistry	Molecular layer axoaxonic deep neuron
Soriano et al., '90; rat; Golgi-EM study and GABA and parvalbumin immunocytochemistry	Molecular layer axoaxonic neuron

projecting system), histogenesis (postnatal neurogenesis), and immunocytochemistry (the glutamatergic system) of these two centres are so close that they strongly corroborate this view (Lopez-Garcia, 1993; Lopez-Garcia et al., 1992). However, a striking difference is that the extremely developed zinc-containing axonal projection from the medial cortex to the lizard bilateral septum (Molowny and Lopez-Garcia, 1978) seems to be missing in mammals. Moreover, subpopulations of cells displaying parvalbumin, calretinin, and calbindin immunoreactivities show different distributions in squamates and mammals (Martínez-Guijarro et al., 1992).

Scarce neuronal types (Table 2) have been reported in the mammalian fascia dentata molecular layer. Ramón y Cajal (1911) reported two types in the molecular layer and an additional ectopic type (displaced grains). Nevertheless, he represented in his figures three neuronal types specific to the molecular layer and an additional ectopic type. Other authors (Lorente de Nó, 1934; Stensaas, 1967; Seress and

Ribak, 1983; Soriano et al., 1989) have reported two types specific to the molecular layer: 1) a small superficial neuron, spherical or fusiform in shape; and 2) a deep or juxtasonic, large multipolar neuron.

Immunocytochemical studies reveal that, as in the outer plexiform layer of the Squamata medial cortex, the molecular layer of the mammalian fascia dentata displays a heterogeneous neuronal population; somata immunolabelled with antibodies against parvalbumin (Katsumaru et al., 1988; Nitsch et al., 1990), calbindin (Celio, 1990), cholecystokinin, vasoactive intestinal peptide, somatostatin, corticotropin-related factor, neuropeptide Y, enkephalins, and choline-acetyltransferase (Swanson et al., 1987) have been detected. A number of them are also glutamic acid decarboxylase immunoreactive or GABA immunoreactive (Sloviter and Nilaver, 1987).

There is a strong similarity between the neurons of these two homologous centres. The descriptions for certain neurons of the fascia dentata molecular layer fit surprisingly well with those of their counterparts in the Squamata outer plexiform layer. The molecular layer small superficial neuron (Ramón y Cajal, 1911; Lorente de Nó, 1934; Stensaas, 1967; Hazlett and Farkas, 1978; Seress and Ribak, 1983; Soriano et al., 1989) is quite similar to the outer plexiform layer sarmentous neuron. The axon-like branchlets typical of the sarmentous dendrites have also been described in rabbits (Ramón y Cajal, 1911). The short axon molecular layer neuron of the opossum fascia dentata (Hazlett and Farkas, 1978) is also very similar; the detailed description that the authors give for this neuronal type matches fully with the sarmentous neuron, especially with regard to what they called "axon-like processes." The limitations of the Golgi procedure do not allow speculation on the role and synaptic relationship of these dendritic specialisations. However, their existence in such remotely related groups of animals would suggest a significant importance.

Two types of deep or juxtasonic, large multipolar neurons (Ramón y Cajal, 1911; Lorente de Nó, 1934; Seress and Pokorny, 1981; Seress and Ribak, 1983; Ribak and Seress, 1983; Soriano et al., 1989, 1990; Soriano and Frotscher, 1989) have been reported. The first is the molecular layer basket neuron (Seress and Pokorny, '81)



which shows morphological, ultrastructural, and immunocytochemical features identical to the deep stellate neuron, including one or two dendrites that traverse the cell layer and reach the deep hilus. Its axon gives rise to pericellular baskets in the stratum granulare. The other type is the so-called chandelier or axoaxonic neuron (Soriano and Frotscher, 1989). It has a large soma, and its dendrites seldom reach the hilus or only penetrate it superficially. These neurons are GABA and parvalbumin immunoreactive, their axons spread along the granular and infragranular layers, making synapses on the initial axonal segments. There is no report about the existence of axoaxonic neurons in the lizard cortex so far, but axoaxonic contacts have been described (Martinez-Guijarro et al., 1990). Axoaxonic interneurons in the lizard cortex remain, therefore, an expected possibility.

Finally, some authors have reported the presence of displaced grains (Ramón y Cajal, 1917) or ectopic granular neurons (Lorente de Nó, 1934; Marti-Subirana et al., 1986) identical to the ectopic bitufted neurons of the medial cortex outer plexiform layer of *Podarcis hispanica*. However, long-spined polymorphic neurons have never been seen ectopically located outside of the hilus in the mammal hippocampus (Amaral, 1978).

### Most intrinsic neurons of the outer plexiform layer have short axons and are presumably GABAergic inhibitory interneurons

Both the aspinous (types 1 and 2, sarmentous and coral) and multipolar (types 3 and 4, stellate and deep stellate) neurons are clear short axon neurons that spread their axonal arbors in the outer plexiform layer. It is not known whether the sparsely spinous horizontal neuron (type 5, couchant neuron) is a short axon neuron or a projecting neuron. In horseradish peroxidase (HRP) transport studies performed in *Podarcis*, horizontal-fusiform somata located in the outer rim of the cell layer were labelled after retrograde HRP transport when injections were placed in the medial cortex and dorsomedial cortex (Olucha et al., 1988). These neurons had a descending recurrent axon that crossed the cell layer and returned to the outer plexiform layer, giving off some short ramifications along their trajectory. This could well be the case for the couchant neuron, which would then behave like a short axon neuron; further investigation is needed to confirm this point.

The intrinsic neurons (types 1–5) of the outer plexiform layer are presumably GABAergic short axon neurons. In this work we have found both GABA-negative and GABA immunoreactive cell bodies. Most of the GABA-positive somata are similar to those previously described (Schwerdtfeger and Lopez-Garcia, 1986; Schwerdtfeger and Lorente, 1987, 1988), and fit the morphological patterns of Golgi-impregnated neurons. However, some GABA-negative cell bodies have been observed to display identical shape and size to those of GABA immunoreactive somata. The presence of GABA-negative cell bodies may imply: 1) that they are true GABAergic neurons with a long axon projecting outside the outer plexiform layer and thus not expressing GABA immunoreactivity in their somata, as occurs in mammals (Onteniente et al., 1986); or 2) that they are non-GABAergic cells either projecting (i.e., ectopic granule neurons) or interneuronal (e.g., opioid immunoreactive neurons) in nature.

Some neurons of the GABA immunoreactive population are also parvalbumin immunoreactive (Martinez-Guijarro et al., 1991). The parvalbumin immunostaining labels the

TABLE 3. Correlation Between the Golgi Neuronal Types and the Immunoreactivity to the GABA, Parvalbumin, and Opioid Peptides<sup>1</sup>

Golgi neuronal types	GABA immunoreactive neurons	Parvalbumin immunoreactive neurons	Opioid immunoreactive neurons
1, sarmentous	±	?	±
2, coral	+	+	–
3, stellate	±	+	±
4, deep stellate	+	+	–
5, couchant	+	+	–

<sup>1</sup>Several groups of neurons may be differentiated: GABA immunoreactive neurons, which may be parvalbumin immunoreactive and parvalbumin negative and are represented by Golgi types 1–5; GABA-negative neurons (Golgi types 1 and 3); opioid immunoreactive neurons (Golgi types 1 and 3) and opioid negative neurons (Golgi types 2, 4, and 5).

neuron in a Golgi-like way. This permits comparison of the morphology of the Golgi-impregnated and parvalbumin-immunolabelled neurons. Most Golgi neuronal types in the outer plexiform layer were well represented in the parvalbumin material. The opioid immunostaining did not permit precise comparison of the scarce opioid immunoreactive somata with Golgi neuronal types; nevertheless, opioid immunoreactive somata were found in the outer and intermediate laminae that were only populated by sarmentous and stellate neurons. Thus the only candidates for opioid immunoreactivity are type 1 (sarmentous) and type 3 (stellate) neurons. Finally, those somata which are neither parvalbumin nor opioid immunoreactive have no clear counterpart in any Golgi type; perhaps the lack of immunoreactivity in these neurons is related to their physiological state and is not an anatomically determinative feature. (Likely assignation of immunocytochemical reactivities to the Golgi types is summarised in Table 3.)

As in mammals, GABA has been described to be inhibitory in reptiles (Connors and Kriegstein, 1986; Kriegstein and Connors, 1986; Blanton et al., 1987). Consequently, the short axon GABA immunoreactive neurons of the outer plexiform layer of the lizard medial cortex are, presumably, inhibitory. The axons of these cells arborise in the neuropil of the outer plexiform layer (types 1, 2, and 3: sarmentous, coral and, stellate) and over the cell layer (type 4: deep stellate), where they may exert their inhibitory function. Although speculative, outer plexiform layer neurons displaying parvalbumin immunoreactivity are likely to be fast spiking cells such as those which occur in the mammalian hippocampus (Kawaguchi et al., 1987); on the contrary, a second subset of outer plexiform layer neurons (i.e., the opioid-containing cells) may act in a different, slow manner (Neumaier et al., 1988; Wimpey and Chavkin, 1991).

### Intrinsic neurons overlap the laminated input synaptic fields in the outer plexiform layer of the medial cortex

Neuronal types in the outer plexiform layer show a nonhomogeneous distribution with respect to the laminar organisation of the outer plexiform layer itself. In Squamata reptiles, the outer plexiform layer of the medial cortex may be divided into three conspicuous laminae according to their axonal afferents: external lamina, intermediate lamina, and juxtasonic lamina. Briefly, axons coming from the lateral cortex (olfactory) end in the external lamina; those coming from the dorsal cortex end in the intermediate lamina, and those coming from the dorsomedial cortex end in the juxtasonic lamina (Voneida and Ebbesson, 1969; Lohman and Mentink, 1972; Ulinski, 1975, 1976, 1981; Butler, 1976; Lohman and Van Woerden Verkley, 1976; Bruce and Butler, 1984; Martinez-Garcia et al., 1986;

Hoogland and Vermeulen Van der Zee, 1988; Ulinski, 1990). In addition, there are somatostatin, neuropeptide Y, and cholecystokinin immunoreactive projections that form plexuses in the external lamina and cholecystokinin and opioid immunoreactive projections in the deep juxtasonic lamina (Lopez-Garcia et al., 1992). Extracortical afferents also end in a laminar fashion; thalamic afferents end in the intermediate lamina, whereas raphe mesencephalic nuclei send serotonergic axons which form dense plexuses in the deep juxtasonic lamina (Lopez-Garcia et al., 1992).

Due to the laminar distribution of afferents in the outer plexiform layer, and the laminar arrangement of dendritic and axonal trees of the outer plexiform layer interneurons, these neurons could exert a feed-forward control of the input to the different laminae. Type 1 (sarmentous) neurons extend their dendrites and axonal arbour into the intermediate lamina which is the terminal field of the dorsal cortex and thalamic axonal projection. Types 4 and 5 (deep stellate and couchant) neurons are located in the deep juxtasonic lamina which is the terminal field of ipsi- and contralateral dorsomedial cortex projection and that of the raphe serotonergic axonal input and opioid immunoreactive axonal plexus.

One can, therefore, speculate that specific neuronal types may be related to the control of specific inputs to the medial cortex: olfactory input—type 3 (stellate) neuron; thalamic and dorsal cortex input—type 1 (sarmentous) neuron; ipsi- and contralateral input of dorsomedial cortex—types 4 and 5 (deep stellate and couchant) neurons. A recent study has shown that raphe serotonergic axons terminate on opioid somata of the outer plexiform layer of the medial cortex of lizards (Martinez-Guijarro et al., in preparation).

Although feed-forward inhibition may be the main role of outer plexiform layer interneurons, feed-back inhibition may also occur in deep stellate neurons (type 4) that extend deep dendrites to the zinc-rich bouton field or "hilus" which is the first axonal projection field of the medial cortex neurons. This neuronal type presumably receives a significant additional synaptic input from the zinc-rich, main axonal projection system of the medial cortex (Lopez-Garcia et al., 1983a,b; Molowny et al., et al., 1987). Their deep dendrites that reach the deep Timm-positive inner plexiform layer are likely targets for zinc-positive boutons. About 97% of synapses in that lamina are made by zinc-positive boutons (Martinez-Guijarro et al., 1987) coming from axonal collaterals of the principal granular neurons of the medial cortex (Lopez-Garcia and Martinez-Guijarro, 1988); the few zinc-negative boutons in this zone usually make axosomatic or occasionally axodendritic synapses on long spined neurons (Lopez-Garcia et al., 1988). It is likely that the deep dendritic segments of deep stellate neurons receive zinc-rich synaptic input from principal granular neurons of the medial cortex. If this is true, deep stellate neurons may exert an extra inhibitory feed-back effect driven by principal granular neuron output. Furthermore, type 4 (deep stellate) neurons may also participate in the general feed-forward effect driven by the axonal input to the outer plexiform layer.

### **Intrinsic neurons in the outer plexiform layer also locate preferentially in medial cortex subareas with different output**

There are two main parts to the lizard medial cortex based on its outgoing axonal projections: 1) the vertical-medial-anterior part of the medial cortex projecting to the dorsomedial cortex, the medial part of the dorsal cortex

(which projects contralaterally), and both ipsi- and contralateral septa; and 2) the dorso-caudal-ventral part of the medial cortex projecting to the dorsal cortex and ipsilateral septum (Lopez-Garcia and Martinez-Guijarro, 1988; Olucha et al., 1988).

Type 1 (sarmentous) neurons populate the intermediate lamina of the outer plexiform layer (dorsal cortex and thalamic inputs) of the vertical-medial part of the medial cortex, thereby influencing its output to the dorsomedial cortex and other zones implicated in contralateral projections. On the other hand, type 3 (stellate) neurons populate the outermost lamina of the outer plexiform layer (olfactory cortex input) of the dorsal-caudal part of the medial cortex, thus influencing its output to the dorsal cortex which is the only cortical area emitting extratelencephalic projection.

The remaining neuronal types in the outer plexiform layer do not have such a clearly segregated position in the medial cortex wiring; they are, perhaps, engaged in control of the various input sources to the medial cortex and in the corticocortical, contralateral, and extracortical flows influenced by it.

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