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Interhemispheric Connections Through the Pallial Commissures in the Brain of *Podarcis hispanica* and *Gallotia stehlinii* (Reptilia, Lacertidae)

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ABSTRACT The cells-of-origin and the mode and site of termination of the interhemispheric connections passing through the anterior and posterior pallial commissures in the telencephalon of two lizards (*Podarcis hispanica* and *Gallotia stehlinii*) were investigated by studying the anterograde and retrograde transport of unilaterally injected horseradish peroxidase. The commissural projections arise mainly from pyramidal cells in the medial, dorsomedial, and dorsal cortices (medial subfield). Additionally some non-pyramidal neurons in the medial and dorsal cortices contribute to the commissural system. Medial cortex neurons project to the contralateral anterior septum through the anterior pallial commissure. The dorsomedial cortex projects contralaterally via the anterior pallial commissure to the dorsolateral septum and to the medial, dorsomedial, and dorsal cortices. The projection to the medial cortex terminates in two bands at the inner and outer border, respectively, of the cell layer; the projection to the dorsomedial and dorsal cortex ends in a zone in layer 1 which previously has been described to be Timm-negative, and in a diffuse band in the inner half of layer 3. The medial subfield of the dorsal cortex projects through the anterior pallial commissure to the dorsomedial and dorsal cortices with a similar pattern of termination to that found for the dorsomedial cortex. The posterior pallial commissure contains only the projections from the ventral cortex to its contralateral counterpart and to the ventral part of the caudal medial cortex. The similarities found between this commissural system and the mammalian hippocampal interhemispheric connections are discussed.

In the telencephalon of reptiles, the two major commissures are the anterior pallial commissure and the anterior commissure. In *Squamata* and *Sphenodon*, a third commissure is found, namely, the posterior pallial commissure or "commissura aberrans" (Elliot-Smith, '03). Finally, a fourth commissure with extratelencephalic location, the habenular commissure, is composed by fibers that also arise from telencephalic structures such as the main olfactory bulbs (e.g., Halpern, '76, '80; Heimer, '69; Reiner and Karten, '85; Scalia et al., '69; Ulinski and Peterson, '81) and the lateral cortex (Martínez-García et al., '86).

Data on these commissural systems have been the basis for comparisons between reptilian and mammalian cortices (Butler, '76; Eliott-Smith, '10; Ramón y Cajal, '18; Voneida and Ebbesson, '69). Hence the knowledge of the complete pattern of interhemispheric connections may be helpful in better understanding the functional and comparative significance of the cortical and subcortical areas in the telencephalon of reptiles.

Whereas the number of studies on the connectivity of the reptilian telencephalon has considerably increased during the past 20 years, only a few of them have paid special attention to the two pallial commissures (Butler, '76; Voneida and Ebbesson, '69). Furthermore, most of them employed anterograde degeneration techniques, by which the precise origin of the commissural connections cannot be determined with certainty. In fact, although the name "pallial commissure" suggests an exclusive relationship with the cerebral cortex, there is inadequate evidence to support this view.

Therefore, anterograde and retrograde transport of injected horseradish peroxidase (HRP) have been employed in this study to determine the telencephalic areas that contribute to the anterior and posterior pallial commissures, the

course of their contralateral projections and their way of termination in the opposite hemisphere in two species of lizards, *Podarcis hispanica* and *Gallotia stehlinii* (*Reptilia, Squamata, Lacertidae*).

MATERIALS AND METHODS

Seventy-four adult specimens of *Podarcis hispanica* (head-cloaca length 45–55 mm) and 24 specimens of *Gallotia stehlinii* (head cloaca length 140–250 mm), both sexes, were employed in the present work. They were collected in Valencia (*P. hispanica*) and Las Palmas (Canary Islands, *G. stehlinii*) and maintained in the laboratory at ambient temperature in terraria for less than 1 month with food and water ad libitum.

Either injections of 10–25% HRP in 2% dimethyl sulfoxide or 10% saponin, or deposits of HRP-saponin crystals which were obtained by desiccation from a 25% HRP 5% saponin solution, were used for administration of the tracer. Two injection strategies were used; first, large injections (about 50–100 nl) or depositions were made to label completely the contralateral component of the commissural system; in other animals, small injections (10–20 nl) were placed into distinct cortical areas at different rostrocaudal levels (sometimes including the dorsal septum) in an attempt to study the interhemispheric connections of each cortical area separately.

Surgical operations were carried out under ether anesthesia, followed in some cases by intraperitoneal injections of 2.5 mg/g body weight of sodium pentobarbital in saline. HRP solution was injected using glass micropipettes (tip diameter: 10 μm) attached to a 1 μl Hamilton syringe, the piston of which was connected to a Narishige hydraulic micromanipulator of 2 μm movement precision (MO 103). This injection system permitted injection volumes as small as 5 nl. In order to accurately place injections in the desired cortical area, a stereotaxic device was used in which the head was firmly held and stereotaxic coordinates were measured from the caudal tip of the interfrontoparietal suture.

After 5–8 days of survival at room temperature (18–22°C), animals were anesthetized and transcardially perfused with 40 ml of cold fixative containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were extracted, immersed in the same fixative for 4 h, and rinsed in 30% phosphate-buffered sucrose overnight in the refrigerator (4°C). Fifty or 75 μm-thick transverse sections were cut on a freezing microtome. Serial order was maintained throughout the experimental procedure.

For histochemical detection of peroxidase activity, sections were treated according to either the tetramethyl benzidine ammonium heptamolybdate method (Olucha et al., '85) (TMB-AHM), with incubation times of at least 2 h, or the cobalt-nickel intensified diaminobenzidine (DAB) technique (Adams, '81). Finally sections were mounted on chrome-alum-coated slides and the TMB-AHM reacted series were counterstained with neutral red.

RESULTS

Terminology

In the telencephalon of lizards, a true cerebral cortex is found with a simple laminar organization (e.g., Curwen, '37; Northcutt, '67; Ebbesson and Voneida, '69; Lohman and Mentink, '72; Smeets et al., '86) (Fig. 1A): a cell layer (layer 2) that contains most of the neuronal somata, an outer plexiform layer (layer 1), and an inner plexiform layer (layer 3) that is adjacent to the ependyma. In juxtaependymal position, a partially myelinated tract is found, which is usually called "alveus," as is the white matter of the mammalian hippocampus. In the order *Squamata,* four different cortical fields are distinguished (e.g., Curwen, '37; Goldby and Gamble, '57; Northcutt, '67; Ebbesson and Voneida, '69; Lohman and Mentink, '72): the medial cortex (or small-celled medio-dorsal cortex), the dorsomedial cortex (or large-celled medio-dorsal cortex), the dorsal cortex, and the lateral cortex. Additionally, a ventral cortex has been defined in a caudo-ventral position (Ebbesson and Voneida, '69). In *P. hispanica,* the ventral cortex is a caudal area, the cell layer of which displays continuity with those of the medial and dorsomedial cortices (Fig. 7D,E). However, as we will see below, the ventral cortex can be clearly distinguished from its adjacent cortical areas on the basis of its commissural connections.

Methodology

As described above, the tracer was administered in two different ways. In some cases injections of a HRP solution were stereotaxically placed. In other lizards HRP was placed directly on the cortical surface in solid form (HRP-saponin crystals) under visual guidance. Stereotaxic injections have been found to be more accurate and repeatable than visually guided depositions.

Applications of HRP in solid form rendered an intense and complete labeling of cell bodies and neuronal processes, usually with a Golgi-like appearance (Fig. 1A,B) (even dendritic spines and axonic enlargements resembling "boutons

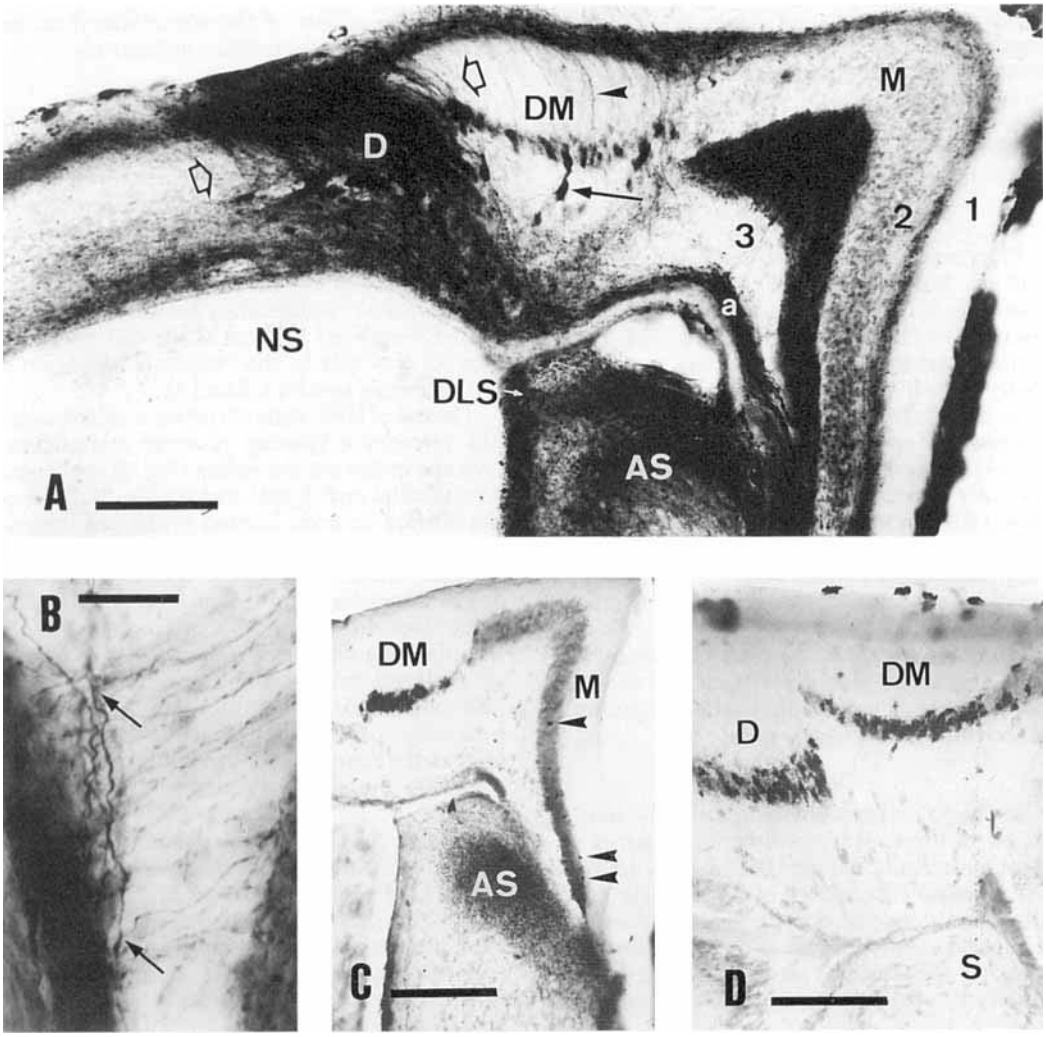


Fig. 1. **A:** Contralateral labeling in the medial (M), dorsomedial (DM), and part of the dorsal cortex (D) and septum following a HRP deposit in cortex and septum (case P2214). Large arrows mark apical dendritic trees of labeled neurons in the medial part of the dorsal cortex. Fine arrow indicates a displaced pyramidal neuron in the dorsomedial cortex (see also Fig. 3b). Arrowhead shows a spiny dendritic branch in layer 1 of the dorsomedial cortex belonging to a bipyramidal neuron. a, alveus; AS, anterior septal nucleus; DLS, dorsolateral septum; NS, nucleus sphericus; 1, outer plexiform layer; 2, cell layer; 3, inner plexiform layer. Bar = 80 μm. **B:** Labeled axons in the alveus giving off collaterals (arrows) that cross the inner half of layer 3 of the medial cortex. Bar = 20 μm. **C:** Labeled neurons in the cell layer of the dorsomedial (DM) and medial (M) (arrowheads) cortices after injection of HRP in the contralateral medial cortex and part of the anterior septum (see also Fig. 5). Additional anterograde labeling is seen in the anterior septum (AS). Bar = 150 μm. **D:** Retrograde labeling in the contralateral dorsomedial cortex (DM) and medial part of the dorsal cortex (D), after injection of HRP into the dorsal cortex (lateral aspect). S, septum. Bar = 80 μm.

en passant" were observed). However, when HRP was injected in solution, retrogradely labeled cells usually showed discrete granules of reaction product within their perikarya and proximal main dendrites (Fig. 1C,D), while fibers appeared very often incompletely labeled and devoid of details (such as varicosities).

Another variable in our work has been the use of two methods for histochemical detection of HRP. The TMB-AHM method (Olucha et al., '85) resulted in a bright green crystalline product, the size of which depended on the reaction time employed. On the other hand, the heavy metal enhanced DAB technique (Adams, '81),

rendered a black amorphous product. Both methods produced a conspicuous contrast between labeled and unlabeled structures. However, some background became apparent when the reaction time surpassed 30 min with the DAB method, and after $2\frac{1}{2}$ h with the TMB-AHM technique.

Injection sites

Our previous studies (Martínez-García and Olucha, '88; Olucha et al., '88) revealed no remarkable differences between the cortical connections of *Podarcis* and *Gallotia*. This finding is not surprising in view of their taxonomic proximity (both species belong to the family *Lacertidae* and they had been included in the old genus *Lacerta*). Therefore, we used indiscriminately either one or the other species depending on their experimental advantages. The larger size of the brain of *Gallotia* (about 35 × 10 mm) as compared to that of *Podarcis* (about 15 × 4 mm) facilitated placing restricted injections in small areas such as the medial and dorsomedial cortices.

Table 1 shows the number of specimens of each species employed for injections into each cortical area, as well as the mode of application of the tracer.

Large injections

In the following we will describe the labeling found in the contralateral cerebral cortex in a representative specimen (P2214; Figs. 1A,B and 4) that received a deposit of a large HRP crystal affecting the medial, dorsomedial and dorsal cortical areas, as well as part of the dorsolateral and anterior nuclei of the septum. In this case, intensely labeled fibers were observed entering or leaving the alveus of the contralateral cortex, from/to the anterior pallial commissure.

TABLE 1. Injection sites

Extent of the injection site[1]	Mode of application	No. of specimens	
		G. stehlinii	*P. hispanica*
Large injections			
D + DM + L	Injection	—	1
D + DM + M + S	Injection	—	2
	Deposit	—	2
Restricted injections			
M + S	Injection	—	4
M	Injection	12	9
DM	Injection	9	9
D	Injection	3	36
L	Injection	—	11
Total		24	74

[1]D, dorsal cortex; DM, dorsomedial cortex; L, lateral cortex; M, medial cortex; S, septum.

Retrograde labeling

Some of the alvear labeled fibers could be traced up to retrogradely labeled neurons in layer 2 of the dorsomedial cortex and of the medial part of the dorsal cortex. A few labeled somata were also found in the cell layer of the medial cortex, the axons of which were not observed. Most of the medial cortex labeled cells were found at the boundary between the medial and dorsomedial cortices while the rest were located ventrally in the "vertical" subregion of medial cortex (see Figs. 2 and 4).

The use of HRP-saponin crystals caused Golgi-like retrograde labeling of some contralateral neurons in the medial cortex (Fig. 2) and in the dorsomedial and dorsal cortex (Fig. 3). Most of the labeled neurons located in the cell layer of the *medial cortex* were pyramidal neurons, with ventrally descending axons. One to three main apical dendrites ramified in layer 1 giving off spiny dendritic branches that seemed to reach the pia, whereas the basal dendritic tree was poorly developed (see Fig. 2a,b). However, a few non-pyramidal, apparently aspiny stellate neurons were observed, the soma of which was situated at the margins of layer 2 (Fig. 2c,d). Their axon left the cell body ventrally towards the alveus.

Labeled cells of the *dorsomedial cortex* could be clearly classified as bipyramidal neurons (Ramón y Cajal, 1896; Northcutt, '67; Berbel et al., '87) (Fig. 3a), although their basal dendritic trees were inconspicuous, especially at caudal levels (possibly due to incomplete labeling). The arrangement of the apical dendritic tree depended on the location of the neuronal body; lateral neurons showed shorter dendritic branches than more medial neurons did. Moreover, some basally displaced pyramidal neurons (small arrow in Fig. 1A; Fig. 3b,c) were found; these neurons showed a single aspiny apical dendritic branch that, after passing layer 2, ramified into a tuft of dendritic branches densely covered with spines (Fig. 3b,c).

Finally, the medial part of the dorsal cortex showed a large number of labeled neurons distributed all along the cell layer. However, since layer 2 lacks distinct borders in this part of the dorsal cortex, in some cases it is difficult to decide whether a particular neuronal body is placed in the cell layer or not. The morphology of labeled neurons of this cortical subfield was similar to that found for displaced pyramidal cells of the dorsomedial cortex (compare Fig.

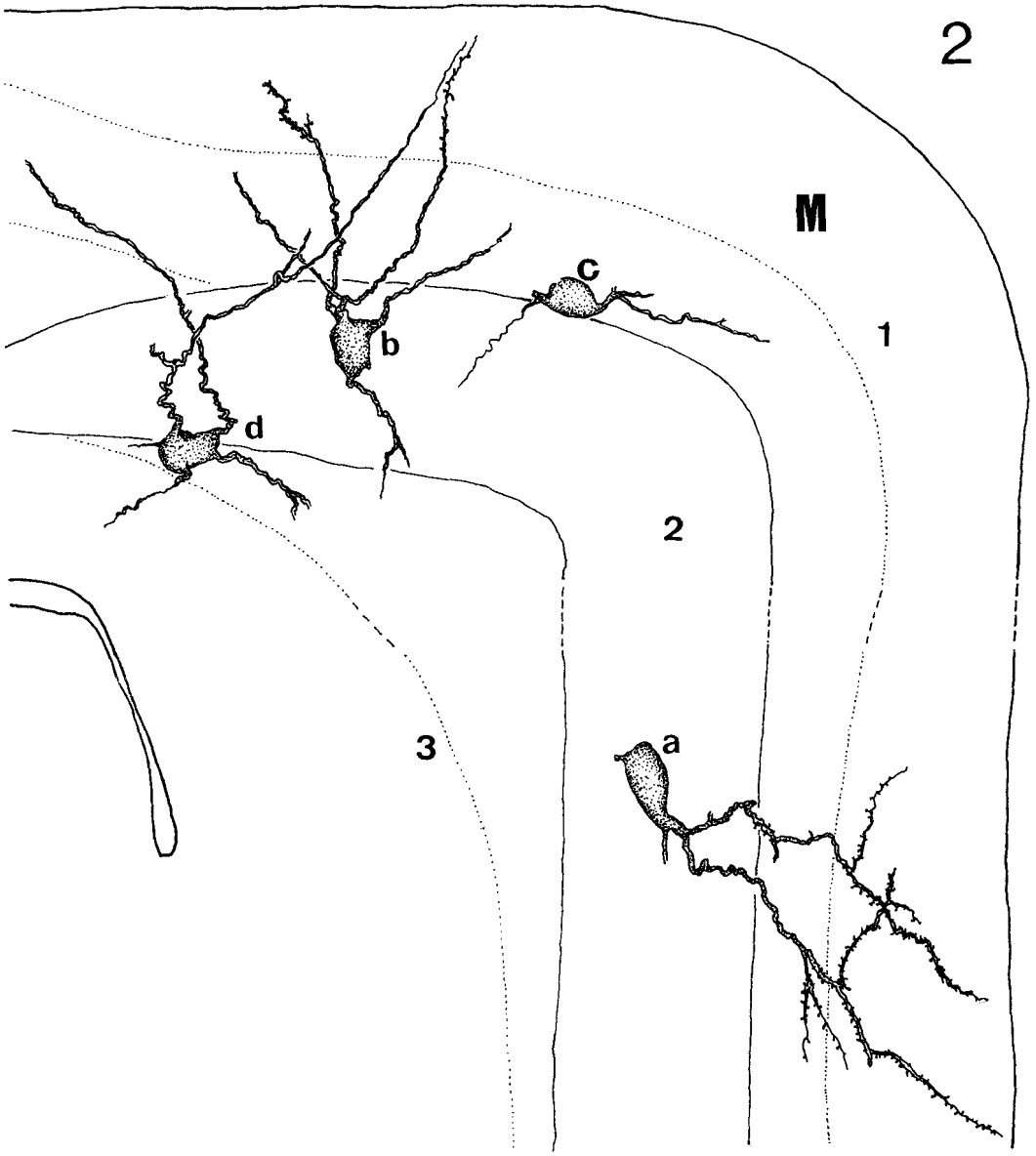


Fig. 2. Camera-lucida drawing of labeled contralateral neurons in the medial cortex (M) after a large HRP-saponin deposition into the cortex and anterior septum: Pyramidal neuron (a), superficial pyramidal neuron (b), and aspiny non-pyramidal neurons (c and d). Dotted lines in layers 1 and 3 delimit the outer and inner borders, respectively, of the commissural termination in the two layers. Layers 1–3 are also indicated.

3b,c with e,f). Moreover, a few non-pyramidal neurons were labeled at different rostro-caudal levels of the dorsal cortex. They were mainly found near the boundary between layers 2 and 3 and showed a stellate aspect, either aspiny (Fig. 3h) or spiny (Fig. 3g). Some juxtaependymal neurons (Fig. 3i,j) with poorly developed dendritic trees were also observed.

Terminal-like and fiber labeling

Within the medial cortex, terminal-like labeling displayed a conspicuous lamination (Figs. 1A

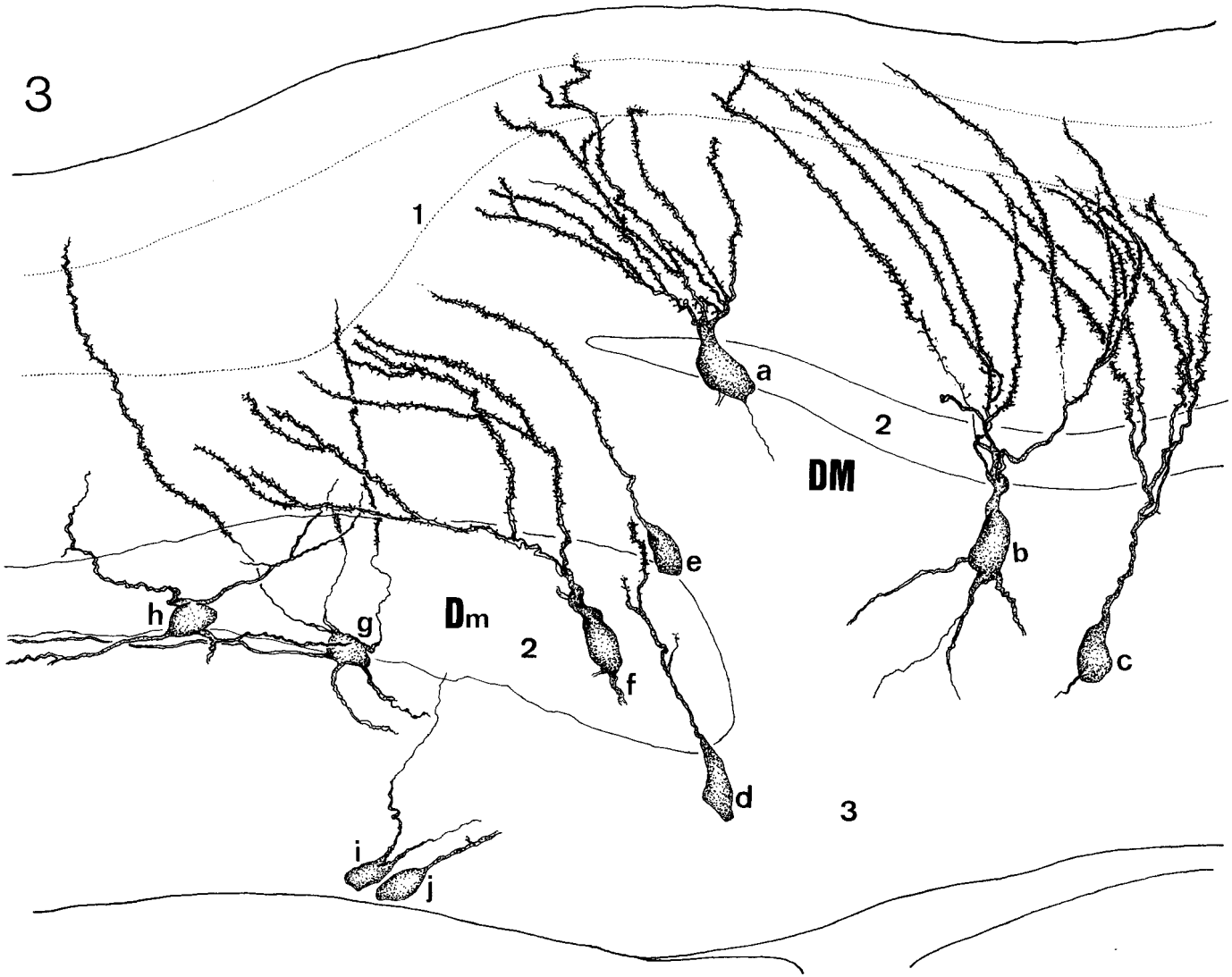


Figure 3

and 4); dense terminal-like labeling was observed in the inner third of layer 1 and in the outer half of layer 3. Sparse labeling was found in the cell layer, mainly due to fine fibers crossing the cell layer and joining in this way both main terminal fields. Some of the labeled axons in the alveus ramified as they crossed the medial cortex, giving off ascending collaterals that entered the terminal field of layer 3 (Fig. 1B).

A labeled terminal field was observed in the dorsomedial and dorsal cortices, where it defined an intermediate band in layer 1 (Figs. 1A and 4). This outer terminal field showed topographic continuity with that of the medial cortex. Many labeled fibers of the alveus crossed the cell layer of the dorsomedial and dorsal cortices and entered this band, where they arborized. Moreover, some fibers of the alveus gave rise to a terminal field in layer 3 of both, the dorsomedial and dorsal cortical areas (Figs. 1A and 4). Finally, dense terminal-like labeling was observed in the dorsolateral septum and in the dorsal part of the anterior septal nucleus (Figs. 1A and 4).

Restricted injections

Injections into the medial cortex

Injections of HRP that were restricted to the medial cortex at any rostro-caudal level, labeled the anterior pallial commissure. In the contralateral cortex, the cell layer of the dorsomedial field showed retrogradely labeled somata at the injection level and caudal to it (see P854 Figs. 1C and 5).

Terminal-like contralateral labeling in the anterior septum was found only in those cases in which the injection involved the rostral medial cortex. These injections included also the ipsilateral dorsal septum, but since labeled contralateral somata were found only in the cortex and not in the septum, we suppose that the origin of the terminal-like labeling was in the cortex (Figs. 1C and 5).

Injections into the dorsomedial cortex

Injections into any antero-posterior level of the dorsomedial cortex (as an example see G847, Fig. 6) labeled contralateral somata in the medial part of the dorsal cortex and in the dorsomedial cortex. In the dorsomedial cortex, nearly all of these somata were seen at the injection level and caudal to it, while those in the dorsal cortex were mainly observed at levels slightly rostral to the injection site.

Contralateral terminal-like labeling was observed 1) in the dorsolateral septum; 2) in the inner third of layer 1 and outer half of layer 3 of the medial cortex, where the labeling was especially intense at caudal levels; 3) in layer 3 of the dorsomedial cortex and of the medial aspect of the dorsal cortex, where the labeling was rather diffuse; and 4) in a narrow band of layer 1 in the dorsal and dorsomedial cortices at the injection level and rostral to it (Fig. 6A–C).

In those cases in which the caudal pole of the dorsomedial cortex was included in the injection site (e.g., case P2132, Fig. 7), labeled fibers were observed not only in the anterior pallial commissure but also in the posterior pallial commissure. Labeled fibers of both commissures intermingled in the contralateral postcommissural septum (Fig. 7B). Contralateral retrograde and anterograde labeling in this case generally showed the same distribution described above for more rostral injections in the dorsomedial cortex. Additionally, the ventral cortex showed retrogradely labeled somata and intense terminal-like labeling in the outer half of layer 1. The medial cortex showed terminal-like labeling even in its ventral portion (Fig. 7C–E).

Injections into the dorsal cortex

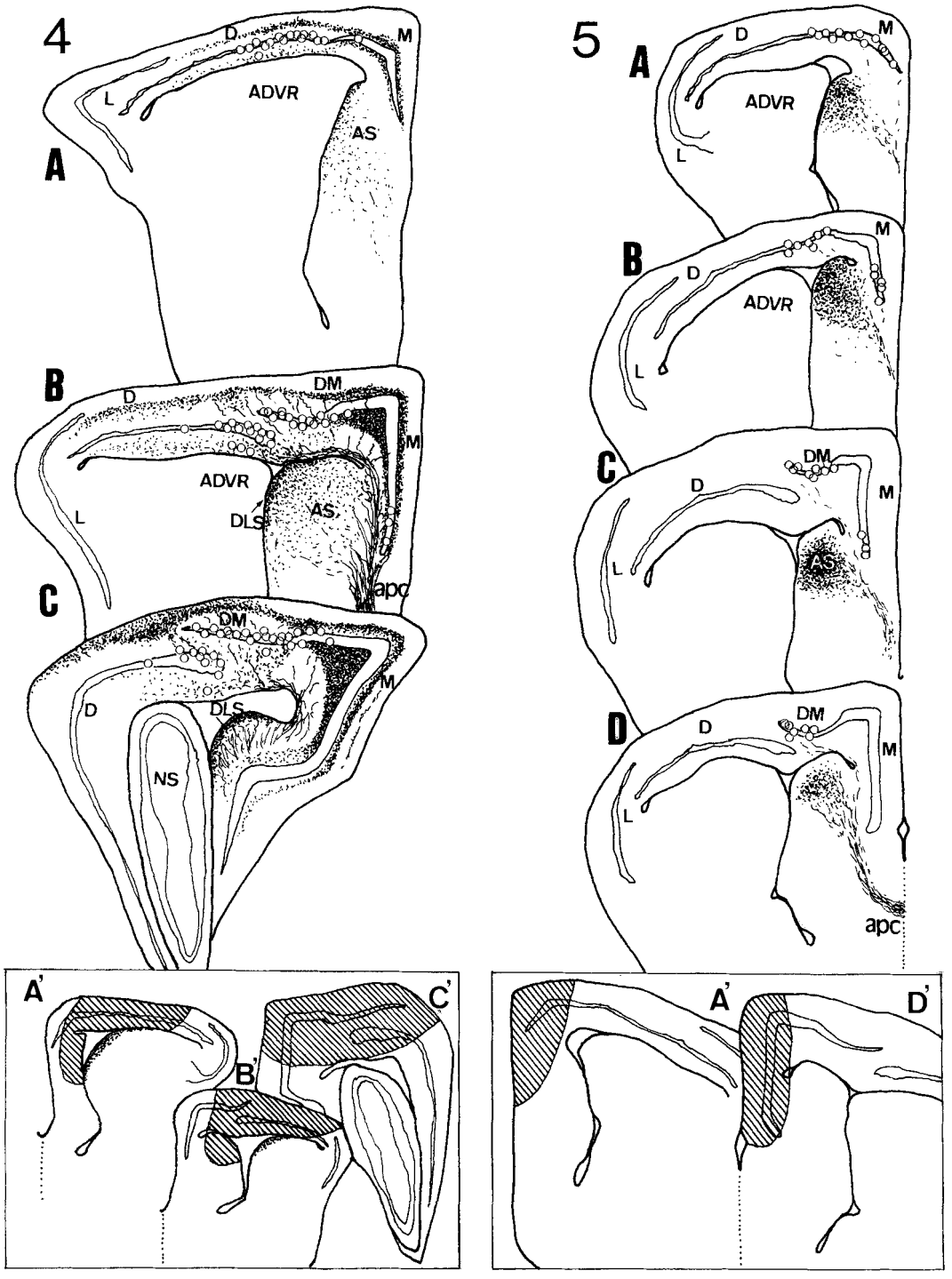
The anterior pallial commissure contained labeled fibers whenever the dorsal cortex was included in the injection site. Concomitantly, retrograde labeling of neurons was found in the contralateral cortex: in the cell layer of the dorsomedial cortex and the medial portion of the dorsal cortex (Fig. 1D). No terminal-like labeling was found in those cases in which the injection site was restricted to the lateral portion of the dorsal cortex.

However, when the medial subfield of the dorsal cortex received HRP injection (Fig. 8), diffuse terminal-like labeling was observed in layer 3 of the dorsal cortex and a narrow band of labeling was found in the outer half of layer 1 in both the dorsomedial and dorsal cortices. This band was distally adjacent to the termination zone of the projection arising in the dorsomedial cortex.

Injections in other areas of the telencephalon

No labeling was found in the pallial commissures after HRP injections or deposits in other telencephalic areas such as the lateral cortex, the

Fig. 3. Camera-lucida drawing of labeled neurons in the dorsomedial (a–c) and dorsal cortex (d–j) in the case shown in Figure 4: Bipyramidal neurons (a,e, and f), displaced pyramidal neurons (b–d), spiny stellate neuron (g), aspiny stellate neuron (h), and juxtaependymal neurons (i and j). Dotted lines in layer 1 delimit the termination field of the commissural system. DM, dorsomedial cortex; Dm; medial subfield of the dorsal cortex; 1, 2 and 3 indicate the three layers of the cortex.



Figures 4-5

anterior dorsal ventricular ridge, the amygdala (nucleus sphericus and adjacent regions) and the striatum. Additionally, several specimens of *Podarcis* received deposits of HRP-saponin crystals in different levels of the septum, and the results of these deposits are consistent with the results described above. In fact, the only areas of the contralateral hemisphere showing retrograde labeling in these cases were the medial, dorsomedial and dorsal cortices.

DISCUSSION

Methodological considerations

The use of combined anterograde and retrograde transport of HRP in the same material has proved to be very useful in determining the connections of a commissural system, but an important question is raised concerning interpretation of the terminal-like labeling. This labeling can result from two different mechanisms: a) anterograde transport from neuronal bodies within the injection site; and b) anterograde transport from retrogradely labeled neurons, through axonic collaterals. Three reasons lead us to think that the terminal-like labeling seen in this study is due, at least in part, to the first-mentioned mechanism.

1. Similar, but sometimes less extensive, patterns of termination of the commissural system have been described in other squamate reptiles using different anterograde tracing techniques: anterograde degeneration (Voneida and Ebbesson, '69; Lohman and Mentink, '72; Lohman and Van Woerden-Verkley, '76; Ulinski, '75, '76) and anterograde transport of *Phaseolus vulgaris* leucoagglutinin (Hoogland and Vermeulen Van der Zee, '89).

2. Anterograde transport from retrogradely labeled cells through axonic collaterals have been observed in the thalamo-cortical system of *Podarcis* (Martínez-García and Lorente, '90), but only when survival time was as long as 11–12 days. In the present study, terminal-like labeling appeared in the contralateral hemisphere even at day 5 of survival. It is important to note that, even in the specimens employed in this work undergoing the longest survival time (8 days), no anterograde transport through axonic collaterals in the thalamo-cortical system was observed.

3. A complete agreement is found between the patterns of retrograde transport and terminal-like labeling, if one considers the latter due to anterograde transport from neurons within the injection site.

Therefore, in the following we will consider terminal-like labeling as representative of anterograde transport from the injection site. However, the possibility cannot be ruled out that the second mechanism contributes also to the presence of terminal-like labeling in the hemisphere contralateral to the injection or deposition.

The administration of the tracer in the form of HRP-saponin crystals was more effective than its injection since the former greatly enhanced transport of the tracer and its resolution. This is especially evident in the appearance of a higher number of Golgi-like figures produced by retrograde labeling; this approach has allowed us to even study the neuronal typology of the neurons contributing to the pallial commissures.

Concerning the chromogen employed, TMB has been reported to be more sensitive than DAB (e.g., Mesulam and Rosene, '79; Morrell et al., '81). However, the DAB reaction, especially after intensification with heavy metal salts (Adams, '81) renders more detailed images of labeled axons and dendrites, revealing as well dendritic spines and axonic enlargements that can be interpreted as boutons "en passant."

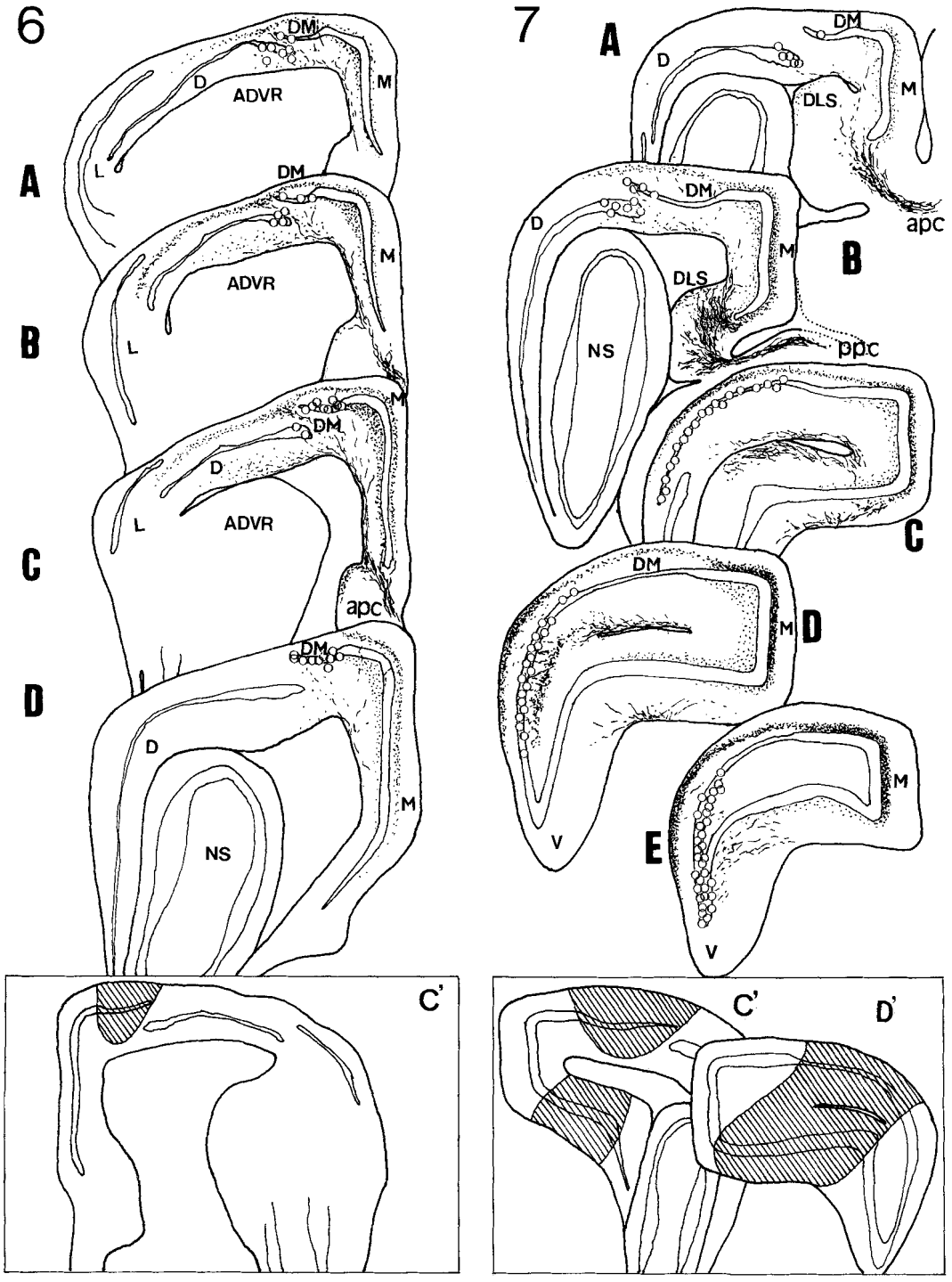
Anterior pallial commissure

On the basis of our results, a general pattern of interhemispheric connections passing through the anterior pallial commissure, can be outlined.

1. The *medial cortex* projects to the contralateral dorsal part of the anterior septum (Fig. 9A). This projection, which is also found in snakes (Ulinski, '75), forms part of a system which is characteristically positive to the sulfide silver reaction (Martínez-García and Olucha, '88; Olucha et al., '88). Similarly, a bilateral projection from the medial cortex to the septum has been described in *Gekko* and *Iguana* (Bruce and Butler, '84). The presence in *Gekko* and *Iguana*

Fig. 4. Terminal-like (dots) and retrograde (small circles) labeling found in three transverse sections of the left telencephalon (**A–C** from rostral to caudal) of a specimen of *Podarcis* that received a large HRP-saponin deposit in the contralateral cerebral cortex and part of the septum (A'–C'). ADVR, anterior dorsal ventricular ridge; apc, anterior pallial commissure; AS, anterior septum; D, dorsal cortex; DLS, dorsolateral septum; DM, dorsomedial cortex; L, lateral cortex; M, medial cortex; NS, nucleus sphericus.

Fig. 5. Semischematic camera-lucida drawing of four transverse sections of the left telencephalic hemisphere (**A–D** from rostral to caudal) of a specimen of *Podarcis* that received a restricted HRP injection into the medial cortex and part of the dorsal septum (**A'** and **D'**) of the right hemisphere. Dots, terminal-like labeling; open circles, retrogradely labeled somata. ADVR, anterior dorsal ventricular ridge; apc, anterior pallial commissure; AS, anterior septal nucleus; D, dorsal cortex; DM, dorsomedial cortex; L, lateral cortex; M, medial cortex.



Figures 6-7

(Bruce and Butler, '84) of a commissural projection connecting the two medial cortices and terminating in the outer margin of layer 1 probably reflects interspecific variation. In fact, it has been found neither in snakes nor in other lizards irrespective of the applied tracing technique. The contralateral projection of the medial cortex in *Podarcis* arises mainly from pyramidal spiny cells (Berbel et al., '87) also named "candelabra cells" (Ulinski, '77) (see Fig. 2a), as well as from other partially spiny neurons whose cell bodies are located at the border between layers 1 and 2 (Fig. 2b) that could be classified as "superficial pyramidal neurons." However, some non-pyramidal aspiny neurons give rise also to contralateral projections (Fig. 2c,d).

2. The *dorsomedial cortex* is the main source of projections to the contralateral cortex (Fig. 9B). Neurons in the cell layer or near the cell layer, project 1) to the opposite medial cortex (inner third of layer 1 and outer half of layer 3); 2) to the dorsomedial and dorsal cortices (to a narrow band of layer 1 and, faintly, to layer 3); 3) to the dorsolateral septum. Thus, the contralateral projections of the dorsomedial cortex of *Podarcis* and *Gallotia* coincide closely with their ipsilateral efferents (Martínez-García and Olucha, '88; Olucha et al., '88). The cells-of-origin of these commissural projections can be classified as bipyramidal neurons on the basis of the morphological features of their dendritic tree (Ramón y Cajal, '17; Northcutt, '67; Berbel et al., '87).

Although our results coincide more or less with previous reports in other lizards (Voneida and Ebbesson, '69; Lohman and Mentink, '72; Ulinski, '75, '76; Butler, '76; Bruce and Butler, '84) some differences are found that are probably due to the tracing techniques employed. The bilateral projection from the dorsomedial cortex to the dorsolateral septum has not been detected previously, perhaps a result of the low sensitivity of the lesion-degeneration technique as compared to the intraaxonic transport methods. Likewise the termination zones in layers 1 and 3 of the dorsal cortex have been observed only in some of the species studied (Voneida and Ebbesson, '69; Butler, '76).

3. The *medial part of the dorsal cortex* shows a pattern of projections to the contralateral dorsomedial and dorsal cortex similar to that found for the dorsomedial cortex (Fig. 9C). However, the fibers to layer 1 terminate more superficially than the projection from the dorsomedial cortex. The cells-of-origin of this projection are mainly found in the cell layer and display morphological features of pyramidal neurons (Guirado et al., '87), although their basal dendritic tree was only partially filled by the tracer. Some non-pyramidal neurons were also found to project contralaterally. Following Guirado et al. ('87) we have classified them as multipolar cells (highly spiny—Fig. 3g—and scarcely spiny subtypes—Fig. 3h) and juxtaependymal neurons (Fig. 3i,j).

The proximity of the dorsomedial cortex and the medial subfield of the dorsal cortex (which together form the "superpositio medialis" of Ramón y Cajal, 1896) makes it difficult to restrict lesions and injections to one of the two fields. This technical problem, as well as the general similarity between the commissural projections of both areas, may explain why the contralateral projection of the medial subfield of the dorsal cortex has not been found by the use of anterograde degeneration techniques in other squamate reptiles (Halpern, '80; Lohman and Mentink, '72; Lohman and Van Woerden-Verkley, '76; Ulinski, '76; Voneida and Ebbesson, '69). Recently, Hoogland and Vermeulen Van der Zee ('89) have described in *Gekko gecko* a commissural projection from the medial subfield of the dorsal cortex to its contralateral counterpart. Moreover, according to their study, no commissural projections arise from any other dorsal cortex subfield. However, some differences have been observed between their results and ours on the extent of this commissural projection. Provided that differences are not caused by some unknown technical problem, it appears that species differences exist in the cortical connectivity in lizards; interspecific differences have also been found in the mammalian brain (Van Groen and Wyss, '88).

Fig. 6. Contralateral terminal-like labeling (dots) and retrograde (open circles) labeling in four transverse sections (**A–D** from rostral to caudal) of the telencephalon of *Gallotia* after injection of HRP restricted to the dorsomedial cortex (**C'**). ADVR, anterior dorsal ventricular ridge; apc, anterior pallial commissure; D, dorsal cortex; DM, dorsomedial cortex; L, lateral cortex; M, medial cortex; NS, nucleus sphericus.

Fig. 7. Semischematic camera-lucida drawing of 5 transverse sections through the caudal telencephalon of *Podarcis* (**A–E** from rostral to caudal) showing the terminal-like (dots) and retrograde (open circles) labeling observed in the contralateral hemisphere. The injection was placed into the caudal pole of the dorsomedial cortex, the ventral medial cortex and part of the ventral cortex (**C'** and **D'**). apc, anterior pallial commissure; D, dorsal cortex; DLS, dorsolateral septum; DM, dorsomedial cortex; L, lateral cortex; M, medial cortex; NS, nucleus sphericus; ppc, posterior pallial commissure; V, ventral cortex.

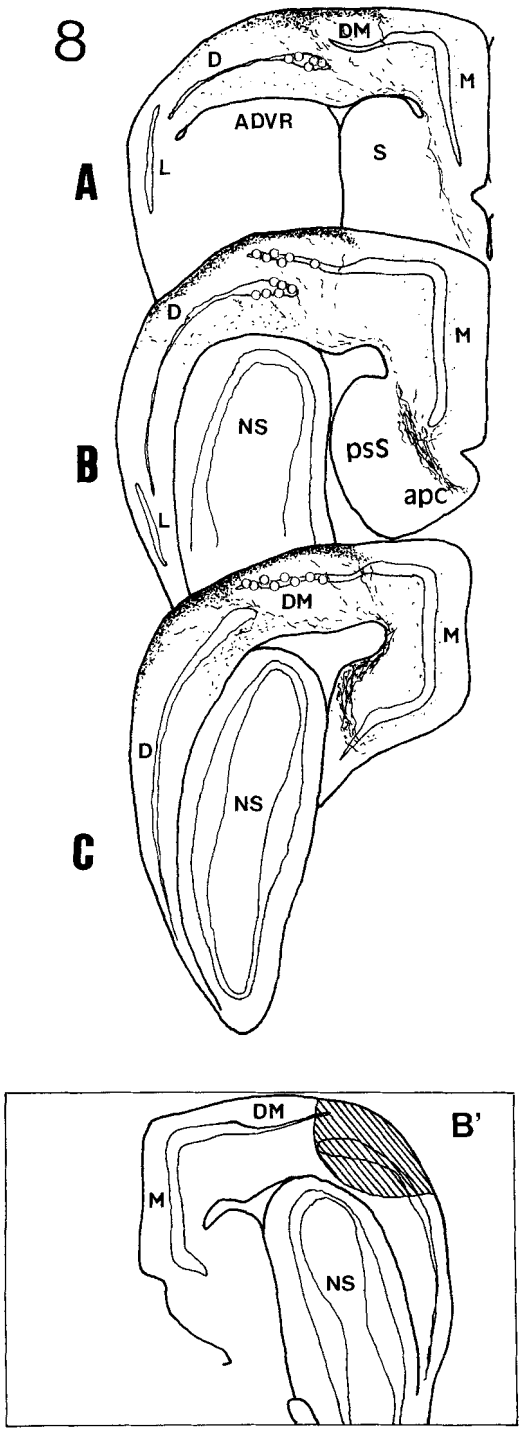


Fig. 8. Terminal-like (dots) and retrograde labeling (open circles) in three transverse sections (**A**, rostral; **C**, caudal) of the left telencephalon of *Podarcis* after an injection of HRP into the medial subfield of the dorsal cortex, and a small portion of the dorsomedial cortex (**B'**). ADVR, anterior dorsal ventricular ridge; apc, anterior pallial commissure; D, dorsal cortex; DM, dorsomedial cortex; L, lateral cortex; M, medial cortex; NS, nucleus sphericus; psS, postcommissural septum; S, septum.

Posterior pallial commissure

This commissure showed fiber labeling in those cases in which the injection site included the caudal pole of the dorsomedial cortex and/or the ventral part of the caudal medial cortex; in these cases, the anterior pallial commissure was also labeled. Labeling of the posterior pallial commissure was paralleled by the appearance of retrogradely labeled somata in the ventral cortex and in the caudal pole of the dorsomedial cortex. In the anterograde direction, labeled posterior pallial commissure fibers could be traced up to terminal-like endings in the ventral part of the caudal medial cortex and in the caudal dorsomedial cortex. Thus, it is likely that the posterior pallial commissure carries fibers which originate in the ventral cortex and caudal dorsomedial cortex, and end in the caudo-ventral portion of the medial cortex and in the caudal dorsomedial cortex itself (Fig. 9D). This projection pattern supports results reported by Lohman and Van Woerden-Verkley ('76) for *Tupinambis*.

The ipsilateral and commissural projection systems of the cerebral cortex of lizards

We found that all of the commissural projections described here have an ipsilateral counterpart terminating in the same target zones. Most of these pathways have also been found in other lizards and snakes by Lohman and Mentink ('72), Lohman and Van Woerden-Verkley ('76), Ulinski ('76), Halpern ('80), Bruce and Butler ('84), and Martínez-García and Olucha ('88).

Comparison with mammals

A number of studies report similarities of the medial and dorsomedial cortices of reptiles with the mammalian area dentata and Ammon's horn (Ariens Kappers et al., '36; Curwen, '37; Elliot Smith, '10; Goldby and Gamble, '57; Lacey, '78; Lohman and Van Woerden-Verkley, '76; Northcutt, '67; Olucha et al., '88; Shen and Kriegstein, '86). One of the arguments supporting similarity is that these cortical areas are at the origin of the pallial commissure which has been considered by some authors as homologous to the hippocampal commissure (Crosby, '17; Goldby and Gamble, '57; Johnston, '13; Voneida and Ebbesson, '69).

9

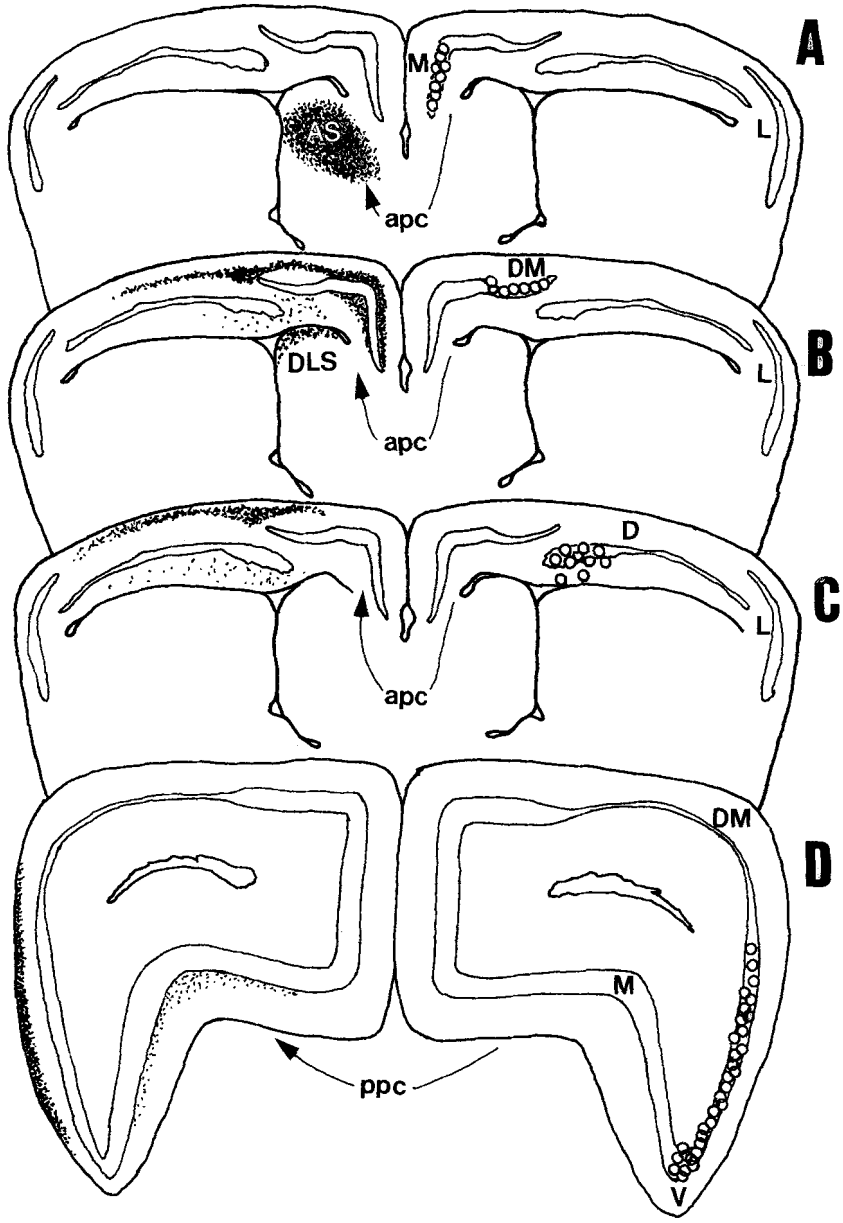


Fig. 9. Schematic diagram of the interhemispherical connections through the anterior pallial commissure (**A–C**) and through the posterior pallial commissure (**D**) based on our results. In the right hemisphere small circles indicate the location of the cells-of-origin of each projection, the termination of which is depicted in the left hemisphere (dots). AS, anterior septum; apc, anterior pallial commissure; D, dorsal cortex; DLS, dorsolateral septum; DM, dorsomedial cortex; L, lateral cortex; M, medial cortex; ppc, posterior pallial commissure; V, ventral cortex.

The results of this study present some additional similarities: 1) As in the mammalian hippocampus (Amaral et al., '84; Gottlieb and Cowan, '73; Laurberg and Sorensen, '81), there exists an ipsilateral analogue for each contralateral projection. 2) Comparing the results of Timm staining (mammals: Zimmer and Haug, '78; reptiles: Pérez-Clausell, '88) with the termination pattern of the contralateral projection (mammals: Blackstad, '56; Swanson et al., '78; Van Groen

and Wyss, '88), one finds that the commissural projection ends in Timm-negative zones. 3) Part of the commissural projection arises from non-pyramidal neurons (mammals: Seress and Ribak, '84; Schwerdtfeger and Buhl, '86). It is likely that some of these non-pyramidal neurons projecting contralaterally are GABAergic, since the morphological features of the horizontal and stellate aspiny neurons in the medial cortex and the stellate and juxta-ependymal neurons of the dorsal cortex, coincide with those of GABA-like immunoreactive neurons in the medial, dorsomedial (Schwerdtfeger and Lorente, '88) and dorsal (Teruel et al., '90) cortices. The presence of a GABAergic component within the commissural system of the hippocampus has been demonstrated (Ribak et al., '86). Further GABA immunoreactivity-HRP tracing double-labeling experiments are needed to test this possibility.

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