Original Paper

Brain Behav Evol 1992;40:157–181

Loreta Medina[a]
Wilhelmus J.A.J. Smeets[b]

[a] Departamento de Biología Celular, Facultad de Biología, Universidad de La Laguna, Tenerife, Spain;
[b] Department of Anatomy and Embryology, Faculty of Medicine, Vrije Universiteit, Amsterdam, The Netherlands

Cholinergic, Monoaminergic and Peptidergic Innervation of the Primary Visual Centers in the Brain of the Lizards *Gekko gecko* and *Gallotia galloti*

Key Words
Tyrosine hydroxylase
Serotonin
Choline acetyltransferase
Substance P
Enkephalin
HRP
Tracing
Immunohistochemistry

Abstract

In order to study the relationship between retinal projections and immunohistochemically identified neurotransmitter systems in the primary visual centers of the brain in lizards, intraocular injections of horseradish peroxidase were combined with immunohistochemistry. Antibodies raised against six substances were applied: choline acetyltransferase (ChAT), serotonin (5-HT), tyrosine hydroxylase (TH), dopamine (DA), substance P (SP), and leu-enkephalin (LENK). In the primary visual centers of the lizards *Gekko gecko* and *Gallotia galloti,* notable overlap was observed between retinofugal fibers with: 1) ChAT-immunoreactive fibers in almost all primary visual centers; 2) 5-HT-immunoreactive fibers in the ventral lateral geniculate body and the basal optic nucleus; 3) TH-immunoreactive fibers in the nucleus ovalis and the dorsal lateral geniculate body; 4) SP- and LENK-immunoreactive fibers in the perirotundal belt; and 5) TH- and SP-immunoreactive fibers in the pretectal posterodorsal nucleus. The latter nucleus also contains dopaminergic cell bodies that lie outside the retinal target area but have dendrites extending into it.

Several differences were noted in the distribution of 5-HT-, TH-, DA-, and LENK-immunoreactive fibers in the tectum of the midbrain in the two species studied. Distinct laminae of 5-HT-immunoreactive fibers (layer 9) and TH- and DA-immunoreactive fibers (layers 9 and 11) are present in *G. gecko* but absent or, at least, less distinct in *G. galloti*. On the contrary, the optic layers in the tectum of *G. galloti* show a rather dense plexus of LENK immunoreactive fibers, whereas the corresponding layers in *G. gecko* are devoid of LENK-immunoreactivity.

Since only a very few ChAT immunoreactive fibers were observed in the optic nerve of *G. galloti,* most of the observed immunoreactive fibers in the primary visual centers are considered to have an extraretinal origin. Putative sources of the cholinergic, the monoaminergic, and the peptidergic innervation of the primary visual centers in reptiles include the isthmic nucleus, the raphe nuclei, the substantia nigra and the nucleus of the posterior commissure, as reported in other amniotes.

Wilhelmus J.A.J. Smeets
Department of Anatomy & Embryology
Faculty of Medicine, Vrije Universiteit
van der Boechorststraat 7
NL-1081 BT Amsterdam (The Netherlands)

0006–8977/92/0404–0157
$ 2.75/0

Introduction

Recently, the retinal projections were studied in the developing and adult lizards of the species *Gallotia galloti* [Medina, 1991]. Simultaneously, immunohistochemical studies were carried out on the brains of several reptilian species by means of antibodies against choline acetyltransferase, serotonin, dopamine, noradrenaline, tyrosine hydroxylase, substance P, and Leu-enkephalin [see e.g. Smeets et al., 1986a, 1987; Smeets and Steinbusch, 1988, 1989, 1990; Hoogland and Vermeulen-van der Zee, 1990; Medina and Smeets, 1991]. It was found that the primary visual centers, i.e. the lateral geniculate body, the pretectum, the basal optic nucleus, and, in particular, the midbrain tectum, are well innervated by some of these neurotransmitter systems.

In reptiles, as in other vertebrates [see e.g. Kása, 1986; Ekström, 1987; Zottoli et al., 1987; Ciani et al., 1988; Schmidt et al., 1989; Vecino and Ekström, 1990], the tectum displays a laminar organization. Moreover, in some tectal layers there seems to be considerable overlap between the termination sites of the retinofugal fibers and fibers that are immunoreactive for antibodies against monoamines, peptides, and choline acetyltransferase. This implies either that the retinal projection fibers contain these neurotransmitters or that the neurotransmitters are present in nonretinal projection fibers that modulate visual information.

The aim of the present study was to establish the relationship between the retinal projection fibers and some immunohistochemically-identified neurotransmitter systems in the primary visual centers of the brain in lizards. For this purpose, double-labeling procedures involved intraocular injections of horseradish peroxidase combined with immunohistochemistry. Since a previous study had revealed remarkable differences in basal ganglia-tectal pathways in the nocturnal *Gekko gecko* and the diurnal *Gallotia galloti* [Medina and Smeets, '91], the same two species were chosen. By comparing the results of the present study with those obtained in other reptilian species, insight might be gained into primitive and derived conditions of neurotransmitters involved in visual information processing in reptiles.

Abbreviations

AT	area triangularis
BG	basal ganglia
Cgl	corpus geniculatum laterale
Cgld	corpus geniculatum laterale, pars dorsalis
Cglv	corpus geniculatum laterale, pars ventralis
d	deep tectal zone
Dll	nucleus dorsolateralis thalami, large-celled part
DM	nucleus dorsomedialis thalami
GP	nucleus geniculatum pretectale
GT	griseum tectale
hypoth	hypothalamus
i	intermediate tectal zone
Ic	nucleus intercalatus
Is	isthmic region
Ism	nucleus isthmi, pars magnocellularis
Isp	nucleus isthmi, pars parvocellularis
LM	nucleus lentiformis mesencephali
Ltp	nucleus lentiformis thalami, pars plicata
Ncp	nucleus of the posterior commissure
nIII	nervus oculomotorius
Ov	nucleus ovalis
Pd	nucleus posterodorsalis
pRot	perirotundal belt
Ra	raphe nuclei
Rot	nucleus rotundus
s	superficial tetal zone
SN	substantia nigra
tect	tectum
to	tractus opticus
tom	tractus opticus medialis
v	ventricle
Vld	nucleus ventrolateralis thalami, pars dorsalis
Vlv	nucleus ventrolateralis thalami, pars ventralis

Material and Methods

The brains of 16 lizards (10 *Gallotia galloti*, 6 *Gekko gecko*) were used. Eleven animals (7 *G. galloti*, 4 *G. gecko*) were anesthetized with Halothane and injected intraocularly (right eye) with 10–20 μl of a solution containing 20% horseradish peroxidase (HRP, Boehringer-Mannheim) in 10% nonidet by means of a Hamilton syringe. After a survival time of 3–7 days, the animals were reanesthetized by cold or by Nembutal and transcardially perfused with saline followed by a fixative solution. Four injected lizards (3 *G. galloti*, 1 *G. gecko*) were fixed with 2% glutaraldehyde in 0.1 *M*-phosphate buffer (pH 7.4). The brains were dissected and immersed overnight in 30% sucrose in phosphate buffer. The next day, 30–40 μm thick sections were cut on a freezing microtome in a transverse or corrected horizontal (parallel to the optic tract) plane. In order to reveal the HRP, the sections were processed following the procedure described by Adams [1981]. Some sections were counterstained with cresyl violet to facilitate interpretation of the results. The other seven injected lizards were fixed with 4% paraformaldehyde, 5% picric acid, and 0.1% glutaraldehyde in 0.1 *M*-phosphate buffer (pH 7.4). This fixative is suitable for both HRP histochemistry and immunohistochemistry.

The five lizards that did not receive an intraocular injection of HRP were anesthetized by cold and perfused according to a procedure that is optimal for choline acetyltransferase immunohistochemistry (see below).

Choline Acetyltransferase (ChAT)-Immunohistochemistry

The five lizards (3 *Gallotia galloti*, 2 *Gekko gecko*) used for ChAT immunohistochemistry were perfused with saline followed by 4% paraformaldehyde, 15% picric acid, and 0.1% glutaraldehyde in 0.1 *M*-phosphate buffer pH 7.4. The perfusion was continued with 4% paraformaldehyde in 0.1 *M*-phosphate buffer, pH 7.4, with increasing quantities of sucrose (5%, 10%, 15%) finishing with 20% sucrose for 60 min. The brains were dissected out and cut at 30–40 μm on a freezing microtome in the transverse or corrected horizontal (parallel to the optic tract) plane. Sections were collected serially in 0.1 *M*-phosphate buffer, pH 7.4. One series of sections were processed for ChAT immunohistochemistry, while adjacent sections were immunoreacted with other antisera as described below. Sections were rinsed three times in Tris-buffered saline (TBS, 0.05 *M*, pH 7.6) and incubated in a rat anti-ChAT antiserum (INCSTAR), diluted 1:100 in TBS containing 0.5% Triton X-100, for three days at 4 °C and under constant agitation. Then sections were rinsed three times in TBS and subsequently incubated in a rabbit anti-rat serum (CLB, Amsterdam), diluted 1:50 in TBS with 0.5% Triton X-100 during 1 day at 4 °C and under constant agitation. Following the secondary antibody incubation, sections were rinsed in TBS and immersed in a peroxidase, anti-peroxidase complex (rat PAP, Vector Laboratories, Burlingame, California, USA), diluted 1:400 in TBS with 0.5% Triton X-100, during 1 hour at room temperature under constant agitation. After the last incubation, sections were rinsed 2 times in TBS and 1 time in phosphate buffer and treated with 0.05% DAB and 0.01% H_2O_2 intensified with 0.04% ammonium nickel sulfate. Finally, sections were rinsed in Tris-HCl buffer (pH 7.6), mounted in a 0.3% gelatine solution in Tris-HCl buffer, dehydrated, and coverslipped.

Immunohistochemistry for Serotonin, Tyrosine Hydroxylase, Leu-Enkephalin and Substance P

Seven lizards (4 *Gallotia*, 3 *Gekko*) were anesthetized with Nembutal and perfused with saline followed by a solution of 4% paraformaldehyde, 5% picric acid, and 0.1% glutaraldehyde in phosphate buffer, pH 7.4 and, finally, with 5% sucrose in phosphate buffer to rinse the fixative. The brains were dissected and immersed overnight in phosphate buffer, pH 7.4, containing 30% sucrose. The brains were cut by means of a freezing microtome at 30–40 μm in transverse or corrected horizontal plane, and sections were collected in phosphate buffer. The sections were processed immunohistochemically with antisera against serotonin (5-HT), tyrosine hydroxylase (TH), leu-enkephalin (LENK) or substance P (SP). The primary antibodies were obtained in rabbit (TH: Eugene Tech. USA; LENK and SP: Cambridge Research Biology; 5–HT: provieded by Dr. H. Petter, Leipzig FRG). The dilutions used were 1:1000 for TH, and 1:1500 for LENK, SP, and 5-HT. The secondary antibody was swine antirabbit (Nordic, diluted 1:50), whereas the tertiary antibody was a rabbit PAP complex (Dakopatts, diluted 1:800). After the incubation in the secondary antibody, sections were rinsed and stained with DAB intensified with nickel (black reaction product) in order to reveal the HRP. The sections were incubated with the tertiary antibody and processed using the routine reaction with DAB (brown reaction product).

In addition to the material mentioned above, we had available brain material of a variety of reptiles stained immunohistochemically for catecholamines, leu-enkephalin and substance P [Smeets, 1988, 1991; Medina and Smeets, 1991]. The nomenclature in the present account is essentially that of Smeets et al., [1986b].

The original research reported herein was performed under guidelines established by the Committee for Experiments with Animals (The Hague).

Results

In this section, first the results of the hodological studies are dealt with. Subsequently, the immunohistochemical findings are reported.

HRP Tracing Experiments

After unilateral HRP injections in the eye, many fibers could be seen ascending in the optic tract in both *Gekko* and *Gallotia*. The optic tract issues fibers to several diencephalic centers, as well as to the midbrain tectum. In a plane parallel to the optic tract (fig. 1, 2), four primary visual centers are easily recognized rostrocaudally: 1) the ventrolateral thalamic nucleus, the ventral lateral geniculate nucleus, and the nucleus ovalis in the ventral thalamus; 2) the perirotundal belt, the dorsal lateral geniculate nucleus, and the nucleus intercalatus in the dorsal thalamus; 3) the pretectal geniculate nucleus, the mesencephalic lentiform nucleus, the posterodorsal nucleus, and the basal optic nucleus in the synencephalon, and 4) the griseum tectale and several of the superficial layers of the midbrain tectum.

As shown in figures 1, 2, the terminal fields of the retinofugal fibers in the thalamus, pretectum, and griseum tectale lie perpendicular to the optic tract. At two levels, the retinal fibers bend further inward and course to the medial neuropil of the ventral geniculate nucleus, the nucleus intercalatus, and the perirotundal belt, or to the medial neuropil of the pretectal geniculate nucleus and the posterodorsal nucleus. The retinal fibers enter the tectum through layers 9, 12, and 14, and terminate in layers 9–13.

The pattern described applies to both species studied, although the cellular plates of the ventral lateral geniculate and the pretectal geniculate nuclei are more prominent in *Gallotia* than in *Gekko*.

Immunohistochemical and Double-Labeling Experiments

ChAT-Immunohistochemistry. In the lizards *Gallotia galloti* and *Gekko gecko*, the diencephalic primary visual centers are densely innervated by ChAT immunoreactive (ChATi) fibers (fig. 3, 4). The griseum tectale and the

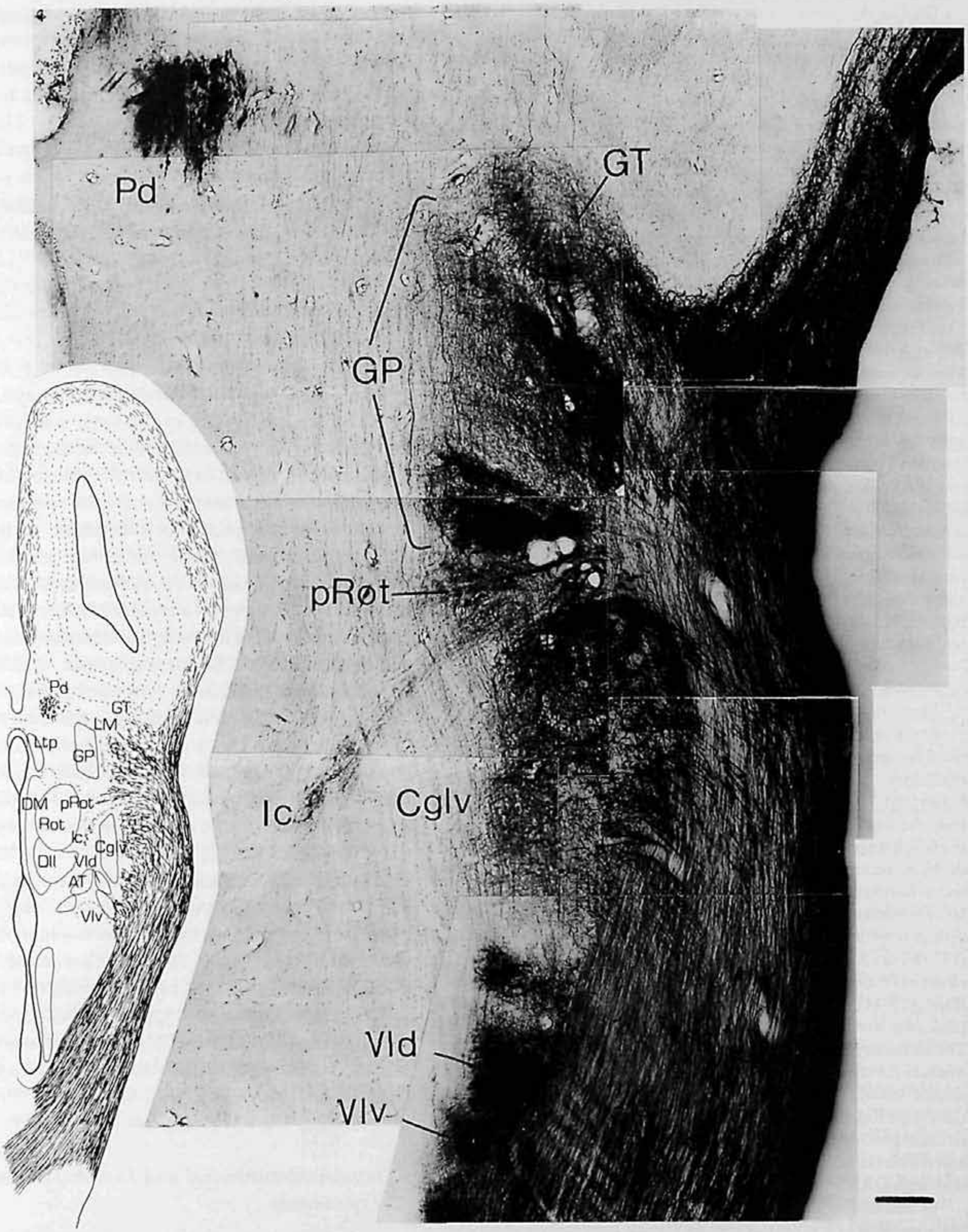


Fig. 1. Photomicrograph of a corrected horizontal section through the brain of *Gallotia galloti* showing the distribution of retinofugal fibers to the diencephalic and mesencephalic primary visual centers visualized after intraocular injection of HRP. For clarity, a camera lucida drawing of an adjacent section counterstained with cresyl violet is shown (inset). Bar = 100 µm.

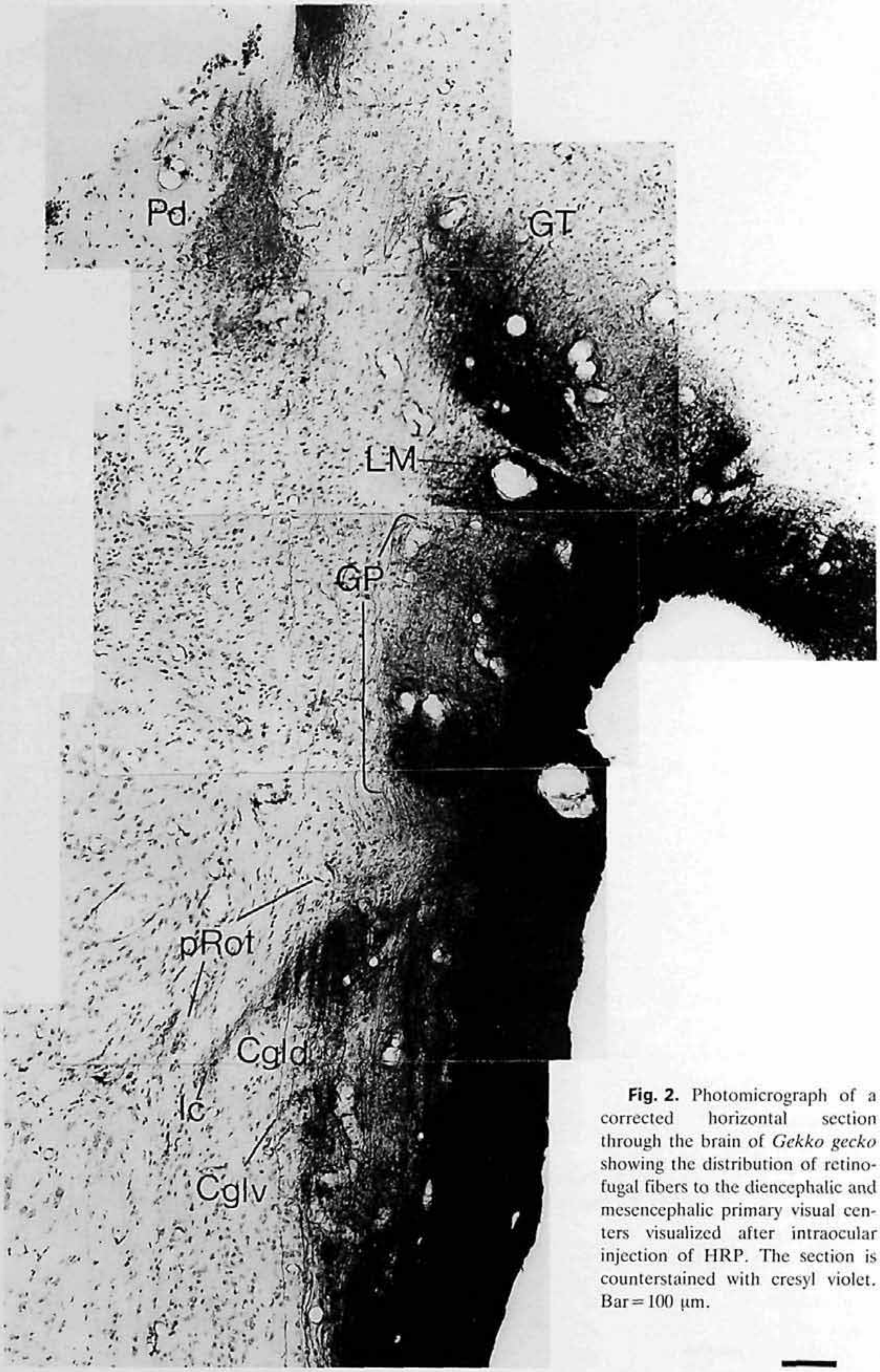


Fig. 2. Photomicrograph of a corrected horizontal section through the brain of *Gekko gecko* showing the distribution of retinofugal fibers to the diencephalic and mesencephalic primary visual centers visualized after intraocular injection of HRP. The section is counterstained with cresyl violet. Bar = 100 μm.

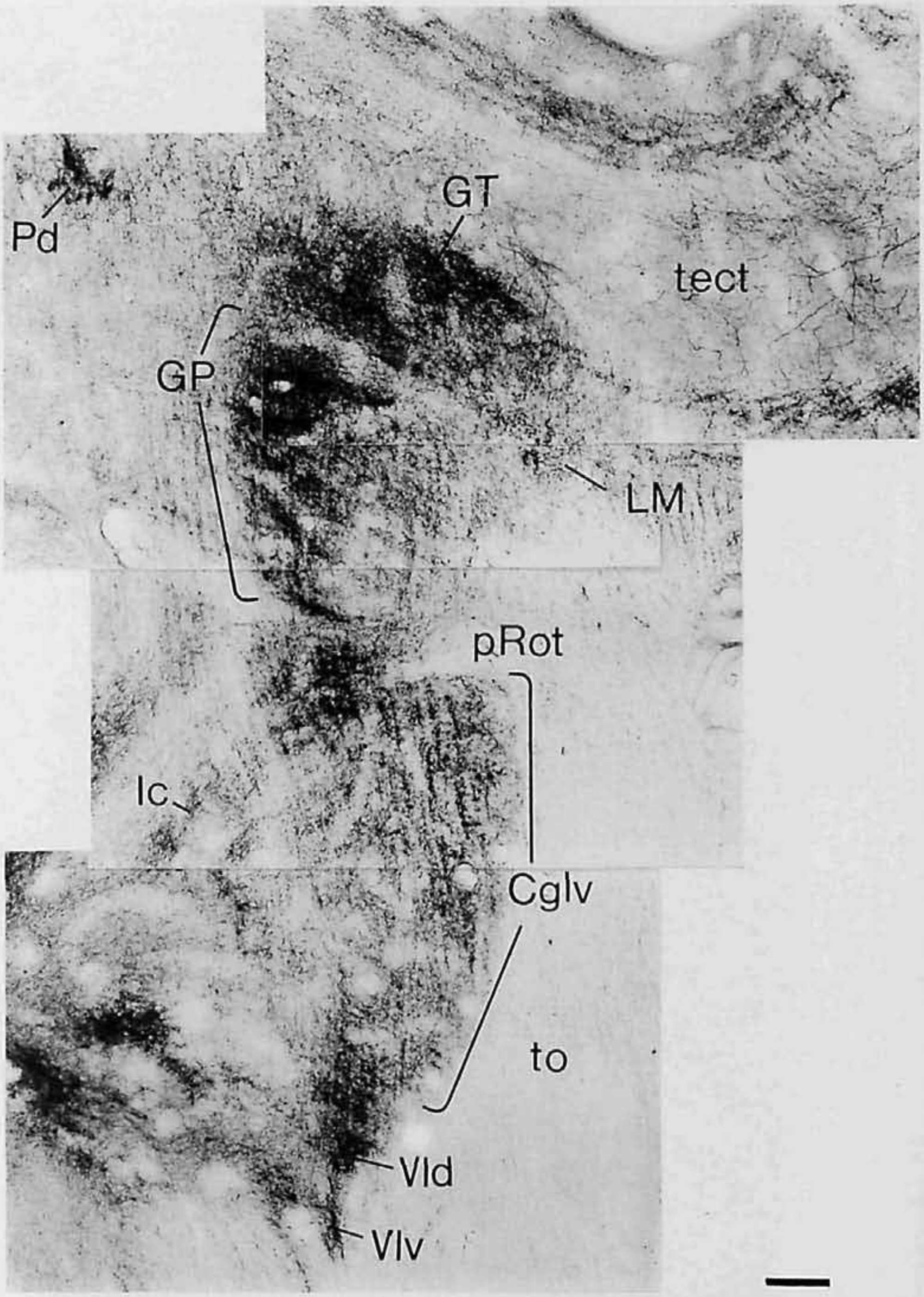


Fig. 3. Photomicrograph of a corrected horizontal section through the brain of *G. galloti* showing the distribution of ChAT immunoreactive fibers in the diencephalic and mesencephalic primary visual centers. Bar = 100 μm.

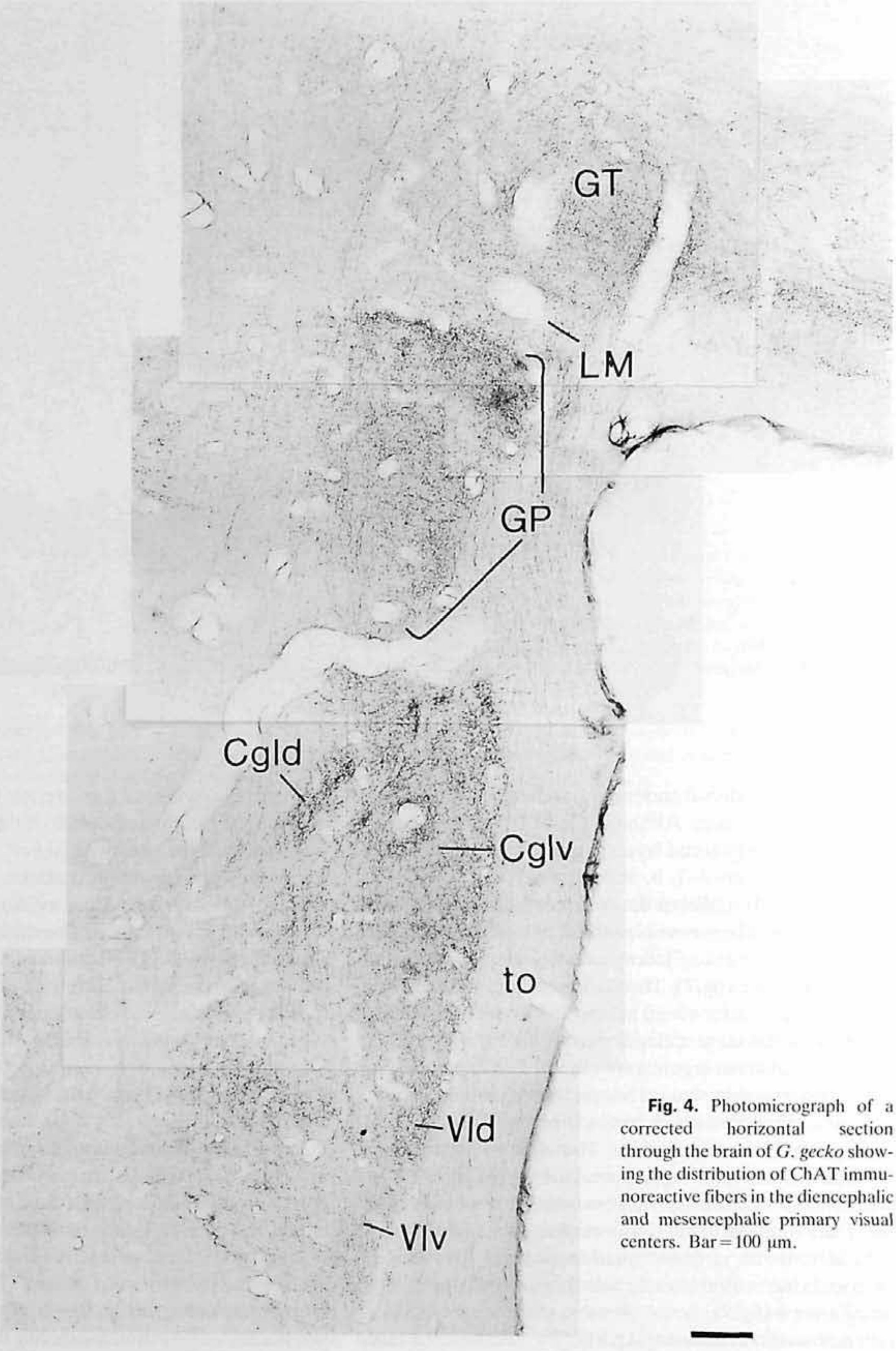


Fig. 4. Photomicrograph of a corrected horizontal section through the brain of *G. gecko* showing the distribution of ChAT immunoreactive fibers in the diencephalic and mesencephalic primary visual centers. Bar = 100 μm.

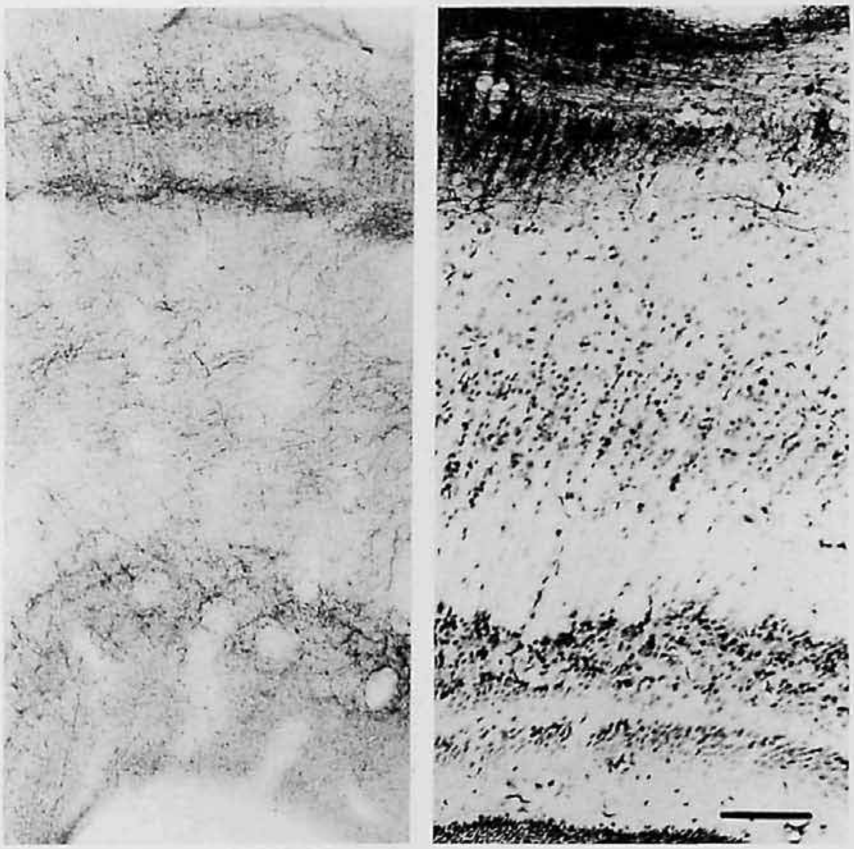


Fig. 5. Photomicrographs of the tectum mesencephali in *G. galloti* showing the distribution of ChATi fibers on the left and that of the retinofugal fibers on the right side. Note the ChAT immunoreactive plexuses in layers 9 and 11. Bar = 100 μm.

pretectal posterodorsal nucleus also show a dense plexus of ChATi varicose fibers. Although ChAT immunoreactivity was observed in all tectal layers, markedly higher densities are found in layers 3–5, 9, and 11 (fig.5, 6).

Internal and parallel to the optic tract, two distinct bundles of ChATi fibers course to the supraoptic commissure and, after decussation, follow a similar trajectory on the contralateral side (fig.7). The fiber bundles could be traced to the isthmic region where many ChATi cell bodies were observed in the magnocellular part of the isthmic nucleus and in the adjacent tegmentum (fig.7). In *Gallotia,* a few ChATi fibers are present in the optic nerve joining caudally the immunoreactive fibers in the supraoptic commissure.

Serotonin-Immunoreactivity. The optic nerve and tract do not show any 5-HT immunoreactivity (5-HTi) in *Gallotia* or *Gekko.* Some visual centers in the diencephalon and the midbrain show a 5-HTi innervation, which is considerably more dense in *Gekko* than in *Gallotia.* This is very obvious in the ventral lateral geniculate nucleus (fig.8), and tectal layer 9 (fig.9). A very dense serotoninergic innervation is observed in the ventral part of the ventrolateral thalamic nucleus, whereas a moderate to dense innervation occurs in the lateral neurophile of the ventral lateral geniculate nucleus, the perirotundal belt, the pretectal geniculate nucleus, the basal optic nucleus, and the griseum tectale (fig.8).

In the midbrain tectum, several layers of 5-HTi fibers can be recognized. The tectum of *Gekko,* in particular, shows a well-laminated pattern. Comparing the distribution of serotoninergic fibers and retinal projection fibers, it is obvious that there is an overlap of fiber systems in tectal layer 9 in both species studied (fig.9). In *Gekko,* an additional but less distinct band of 5-HTi fibers is found in tectal layer 12.

TH- and DA-Immunohistochemistry. With regard to the primary visual centers, there are no remarkable differences between sections stained with the TH-antiserum and those stained with the DA-antiserum. In *Gekko,* as well as in *Gallotia,* many immunoreactive cell bodies are present in the pretectal posterodorsal nucleus (fig.10A, B). The cells lie generally external to a dense plexus of THi and DAi varicose fibers. The double-labeling experiments by menas

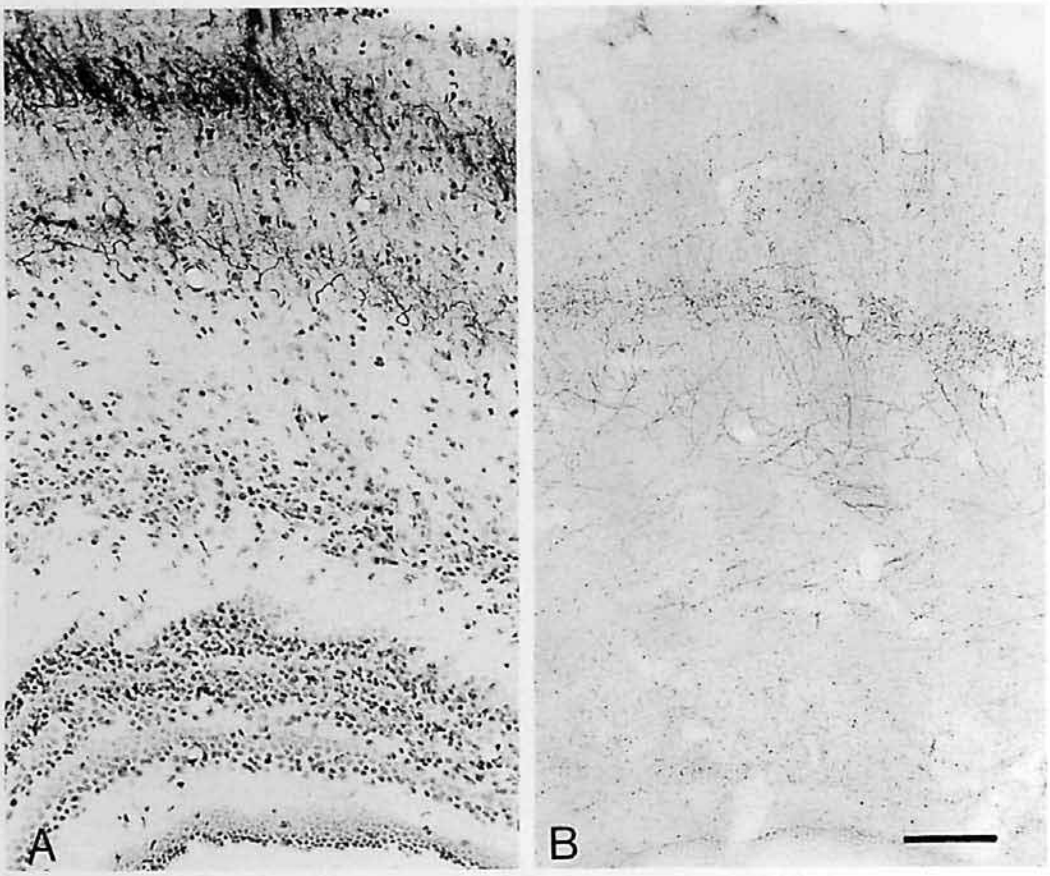


Fig. 6. Photomicrographs of the tectum mesencephali of *G. gecko* showing the distribution of retinofugal fibers on the left (section counterstained with cresyl violet) and that of ChATi fibers on the right side. Note that many retinal fibers occur in layers 9 and 12, whereas ChATi fibers are predominantly located in layer 9. Bar = 100 μm.

of intraocular HRP injections and TH immunohistochemistry reveal that in the posterodorsal nucleus the terminals of the retinal projection fibers largely overlap with the THi varicose fibers. The somata of the THi cell bodies, on the contrary, seem not to be contacted by retinofugal fibers, but they possess dendrites that extend into the retinal target area (fig. 10A, B).

Whereas the distribution of DA/TH-immunoreactivity in the posterodorsal nucleus is identidcal in both lizards studied, there are several differences in the other primary visual centers. In *Gallotia,* very dense TH- and DA-immunoreactive plexuses are present in the nucleus ovalis, the dorsal lateral geniculate nucleus, and, very medially, in the nucleus intercalatus (fig. 11B, D). In contrast, in *Gekko,* the dense plexuses of TH- and DA-immunoreactive fibers are confined to the lateral part of the thalamus and are absent in the nucleus intercalatus. the double-labeling experiments reveal that in both species a perfect overlap exists between the HRP labeled fibers and the THi fibers in the thalamic visual relay centers (fig. 11).

Also in the superficial layers of the midbrain tectum, there are notable differences in the distribution of THi and DAi fibers in the two lizards (fig. 12). In *Gekko,* two bands of immunoreactive fibers are located within tectal layers 9 and 11, whereas in *Gallotia* the superficial layers show only weak, if any, TH- and Da-immunoreactivity. The double-labeling experiments show that there is an overlap between retinal fibers and THi fibers in layer 11 and, particularly, layer 9. In both species, neither THi nor DAi fibers were ever observed in the optic nerve or in the optic tract.

Substance P-Immunohistochemistry. In the lizards *Gallotia* and *Gekko,* the most impressive substance P immunoreactive (SPi) plexus is found in the pretectal posterodorsal nucleus (fig. 10C). Coarse SPi fibers can be

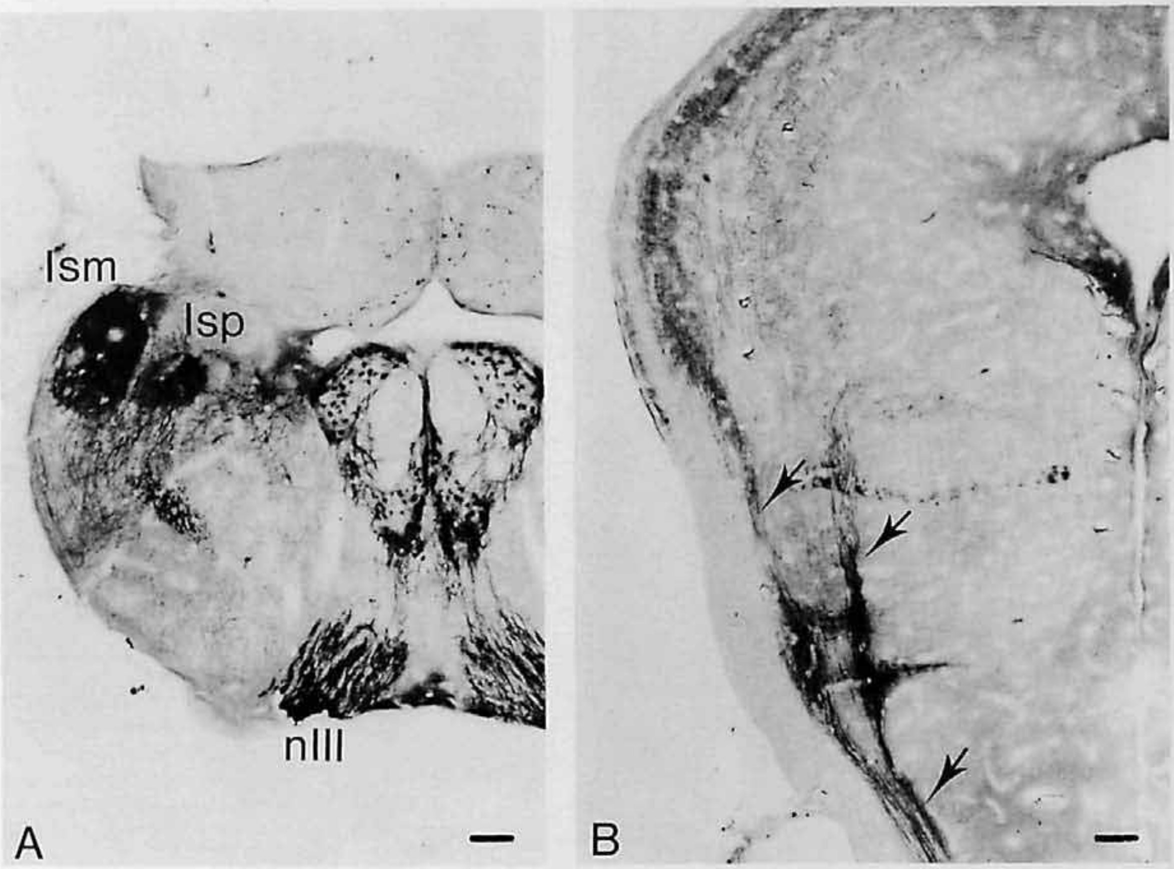


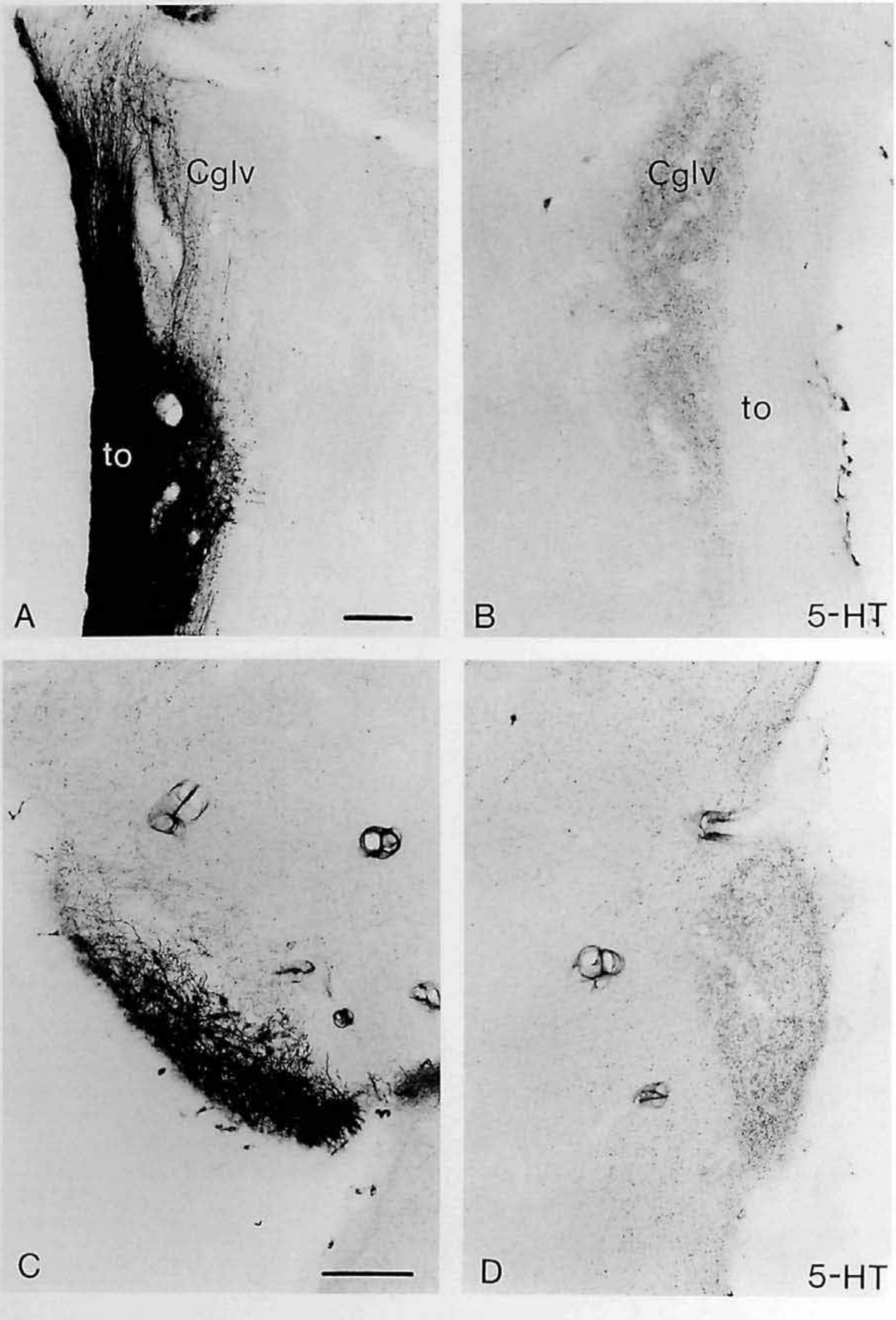
Fig. 7. Photomicrographs of corrected horizontal sections through the brain of *G. galloti* showing the ChATi cell bodies and fibers in the isthmic region and adjacent tegmentum on the left and the two distinct ChATi fiber bundles which constitute the cholinergic input to the primary visual centers on the right side. Bar = 200 µm.

traced to this nucleus from an area lateral to the lateral forebrain bundle. They traverse the laterodorsal part of the thalamus (fig. 10D). The double-labeling experiments show that there is an overlap of the retinal fibers and the coarse SPi fibers along this trajectory at the levels of the nucleus ovalis and the perirotundal belt in the thalamus (fig. 10C) as well as in the posterodorsal nucleus of the pretectum (fig. 10D). In some of the experiments, it was observed that a coarse SPi fiber encompasses several retinal fibers.

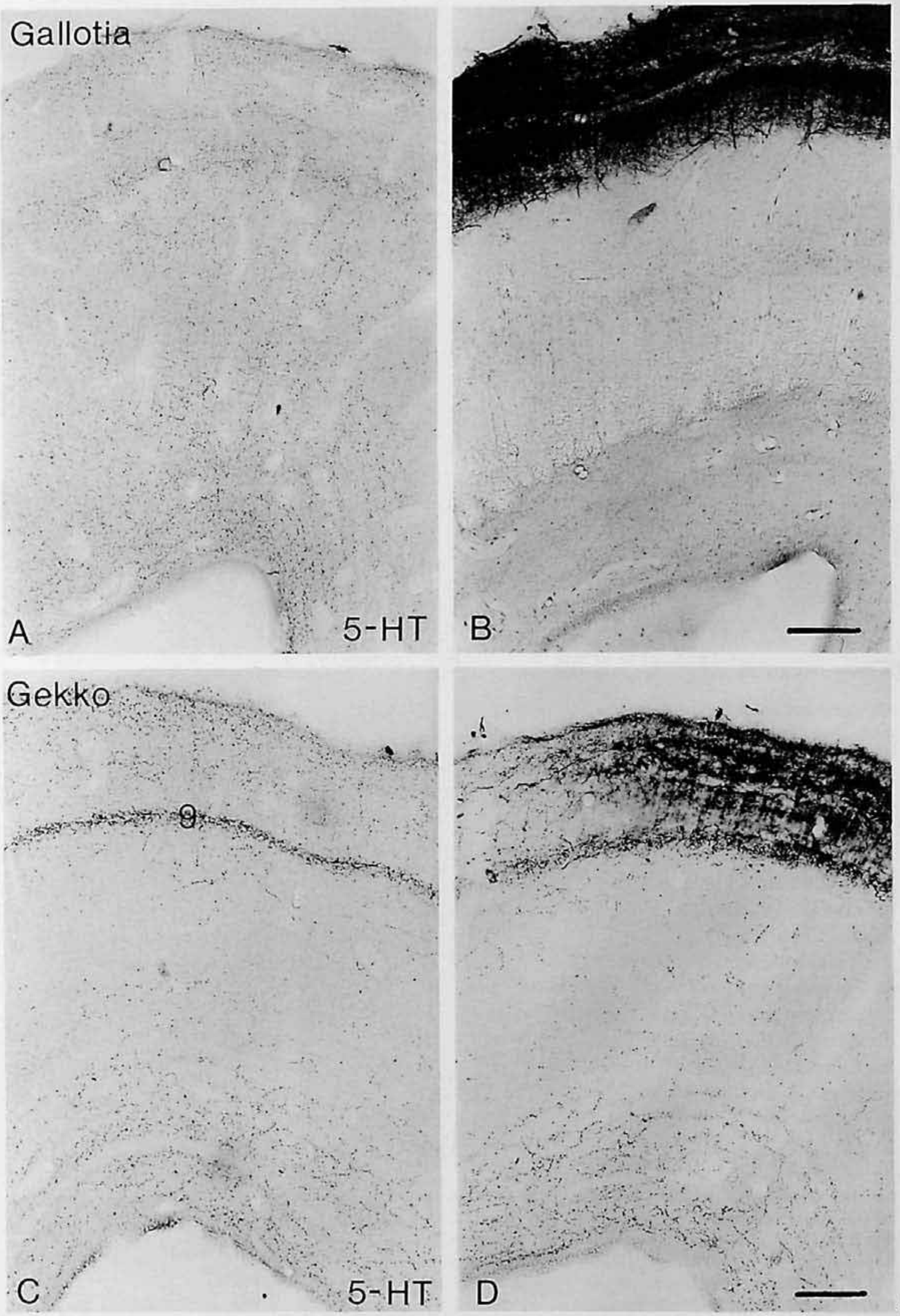
In the tectum of *Gekko* and *Gallotia*, several thin bands of SPi fibers were observed in the superficial layers (fig. 13). In *Gekko*, tectal layers 9, 11 and, particularly, 13 contain SPi fibers, whereas in *Gallotia* the immunoreactive fibers are confined to layers 9 and 13. The double-labeling experiments confirm the overlap between the immunoreactive fibers and the terminations of the retinal fibers (fig. 13). The deeper tectal layers also show SPi varicose fibers in both species studied. No SP immunoreactivity was found in the basal optic nucleus. In neither *Gekko* nor *Gallotia*, were SPi fibers observed in the optic nerve or optic tract.

Leu-Enkephalin-Immunohistochemistry. Compared to the distribution of substance P immunoreactivity, that of Leu-enkephalin immunoreactivity (LENKi) in the lizards *Gekko* and *Gallotia* is much more restricted. Almost all primary visual centers, except for the perirotundal belt and

Fig. 8. Photomicrographs of corrected horizontal sections through the brain of *G. gecko* showing the relationship between the retinofugal fibers and the serotoninergic innervation in the lateral neuropil of the ventral lateral geniculate nucleus (**A, B**) and the basal optic nucleus (**C, D**). **A** and **C** are double stained for HRP and 5-HT, whereas **B** and **D** are stained solely for 5-HT. Bar = 100 µm.



8



9

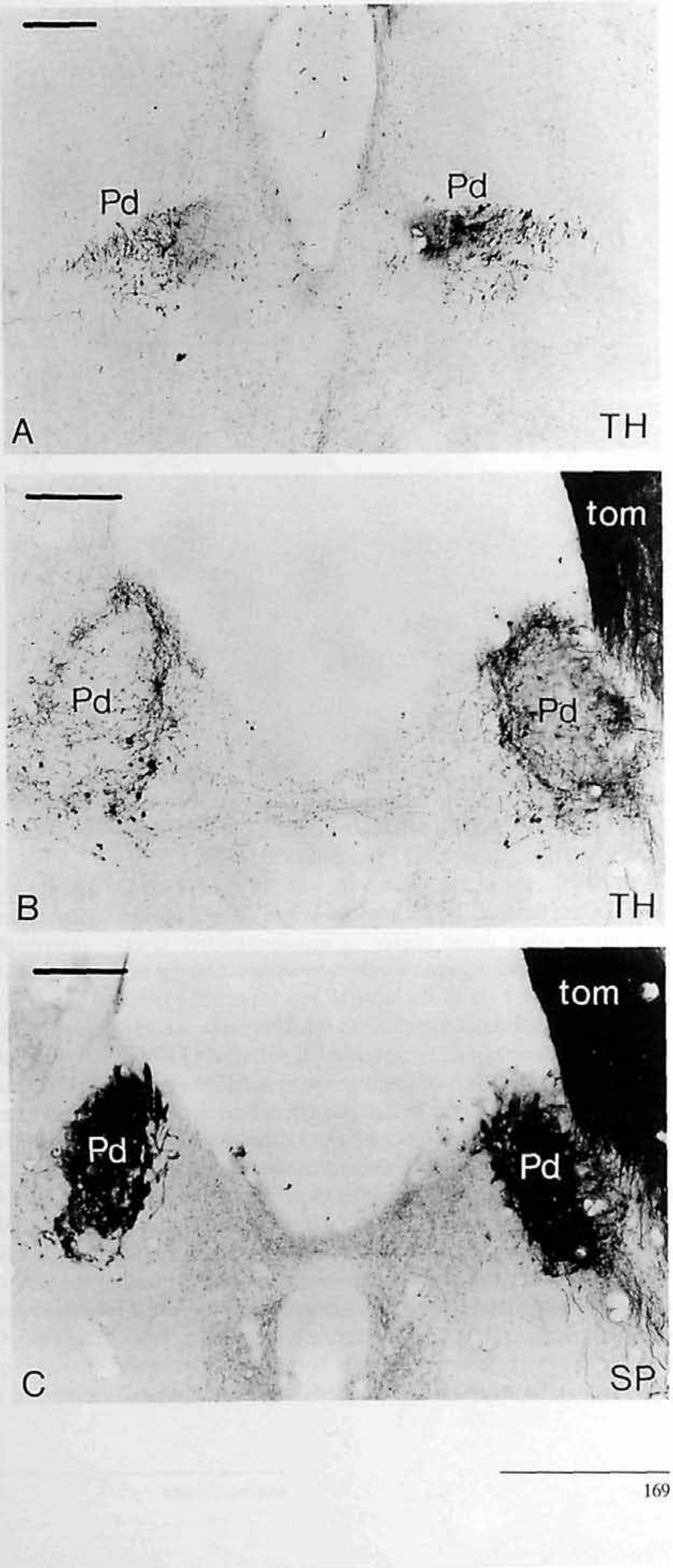
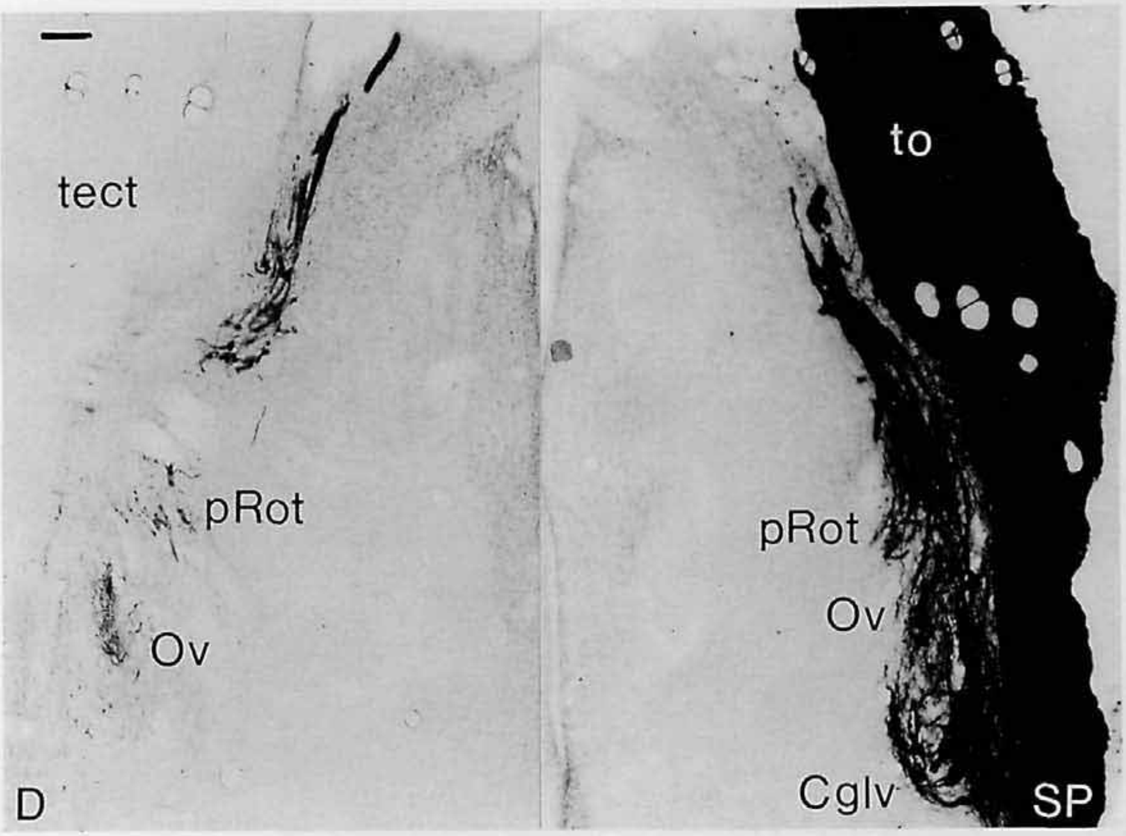


Fig. 9. Photomicrographs illustrating the relationship between the retinofugal fibers and serotoninergic fibers in the tectum of *G. galloti* (**A, B**) and *G. gecko* (**C, D**). **A** and **C** are stained for 5-HT immunoreactivity, and **B** for HRP, whereas **D** is double stained for 5-HT and HRP. Note the overlap in tectal layer 9. Bar = 100 μm.

Fig. 10. Photomicrographs illustrating the relationship between retinofugal fibers and either THi, putative dopaminergic, fibers (**A, B**) or SPi fibers in the posterodorsal pretectal nucleus (**C**) and the thalamus (**D**) in *G. galloti*, (**A**) and *G. gecko* (**B–D**). Bar = 100 μm.



the midbrain tectum, are devoid of LENKi fibers. In *Gallotia*, a few weakly stained cell bodies are seen in the perirotundal belt. Double labeling experiments in this area reveal an overlap of the retinal fibers with some of these LENKi cells. In the tectum, the deep tectal layers of both species contain a rather dense plexus of immunoreactive fibers (fig. 13). However, substantial differences between the two lizards are observed in the central and superficial layers (fig. 13). In *Gallotia*, the intermediate and superficial layers of the tectum show a very complicated pattern of LENK-immunoreactivity. Several laminae showing LENK-immunoreactivity are present in the superficial tectal layers. Numerous LENKi cell bodies are located in layer 7, whereas a few lie in layer 5. From these cells, long apical processes can be followed to the most superficial tectal lamina, which contains a very dense LENKi plexus (fig. 13). Occasionally, LENKi cell bodies were found in the deep tectal layers. The cells are larger in size than those of layers 5 and 7 and possess dendrites that ramify close to the somata.

The intermediate and superficial layers of the tectum in *Gekko* do not contain LENKi cell bodies or varicose fibers. However, in a previous study [Medina and Smeets, 1991] it was found that after colchicine treatment some cells in the intermediate layers stain weakly with the LENK antiserum, whereas in the deep tectal layers there are a few cells that stain darker and are larger in size than those in the intermediate layers. In neither species were LENK immunoreactive fibers observed in the optic nerve or optic tract.

Discussion

We have investigated the neurotransmitters that may be involved in visual information processing. In the following section, our results are compared with those obtained in other reptiles in order to infer primitive and derived conditions. Subsequently, the origins of the various immunoreactive fibers innervating the primary visual centers in reptiles are discussed and compared with those reported for other vertebrates.

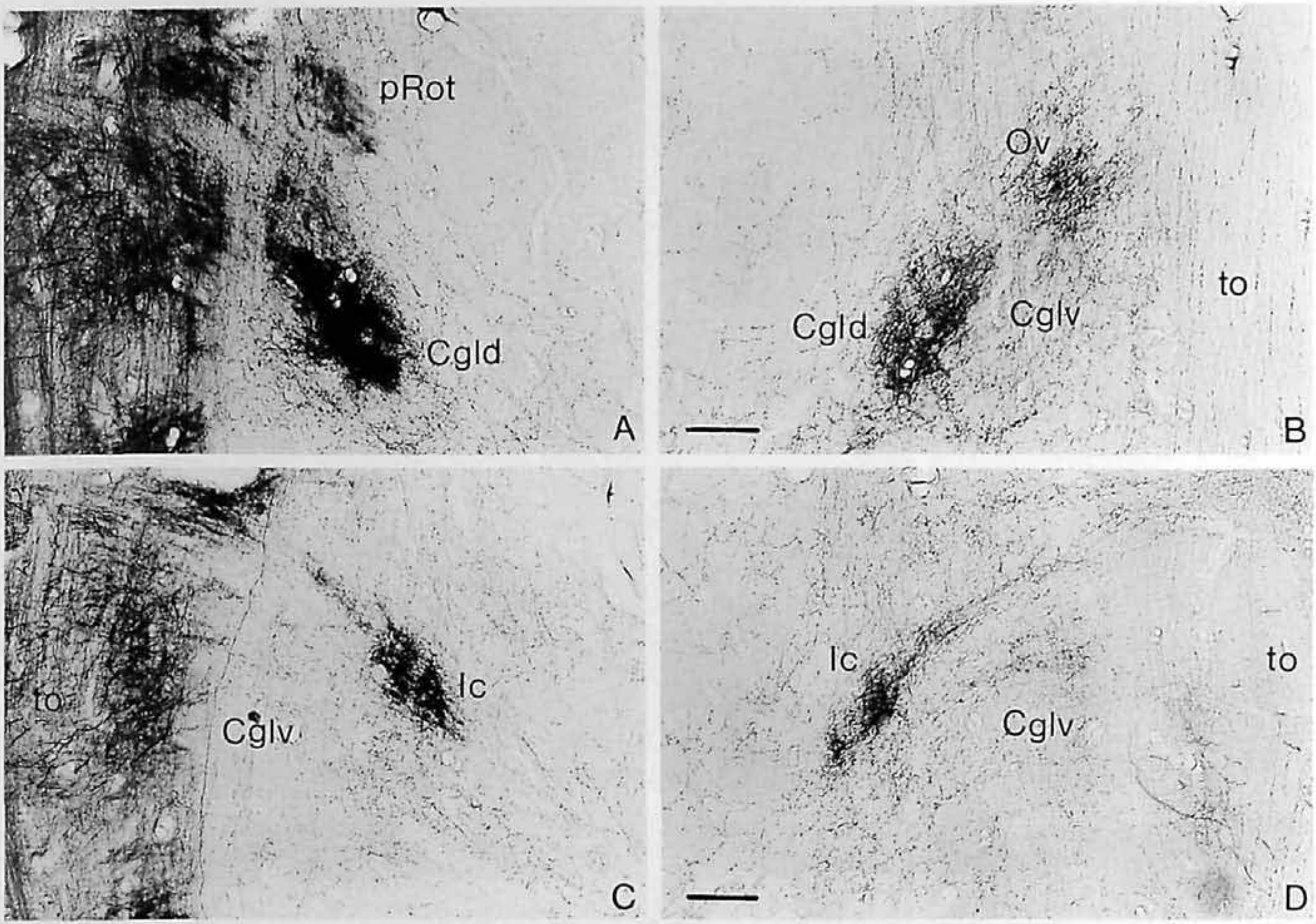


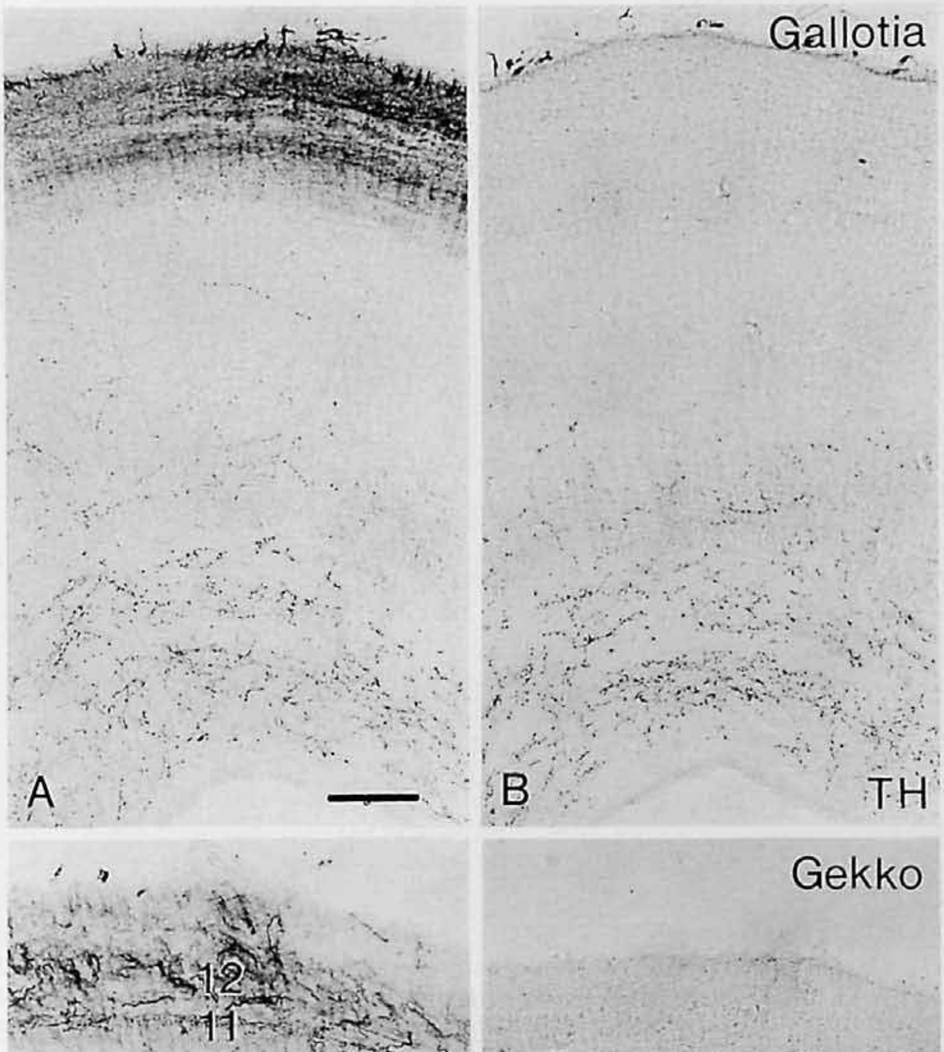
Fig. 11. Photomicrographs of corrected horizontal sections through the brain of *G. galloti* showing the relationship between the retinofugal fibers and the THi, putative dopaminergic, fibers in the nucleus ovalis and the dorsal lateral geniculate body (**A, B**) and the nucleus intercalatus (**C, D**). **A** and **C** are double stained for HRP and TH, whereas **B** and **D** are only stained with the TH antiserum. Bar = 100 μm.

Neurotransmitters in Primary Visual Centers of Reptiles

The present study has revealed several relationships between retinal projection fibers and neurotransmitter systems which appear to be widespread features of brain organization in extant reptiles (fig. 14). Both in *G. gecko* and *G. galloti,* a dense cholinergic innervation of all primary visual centers was observed. Studies in turtles and snakes, however, are needed to confirm whether this is a primitive character in reptiles.

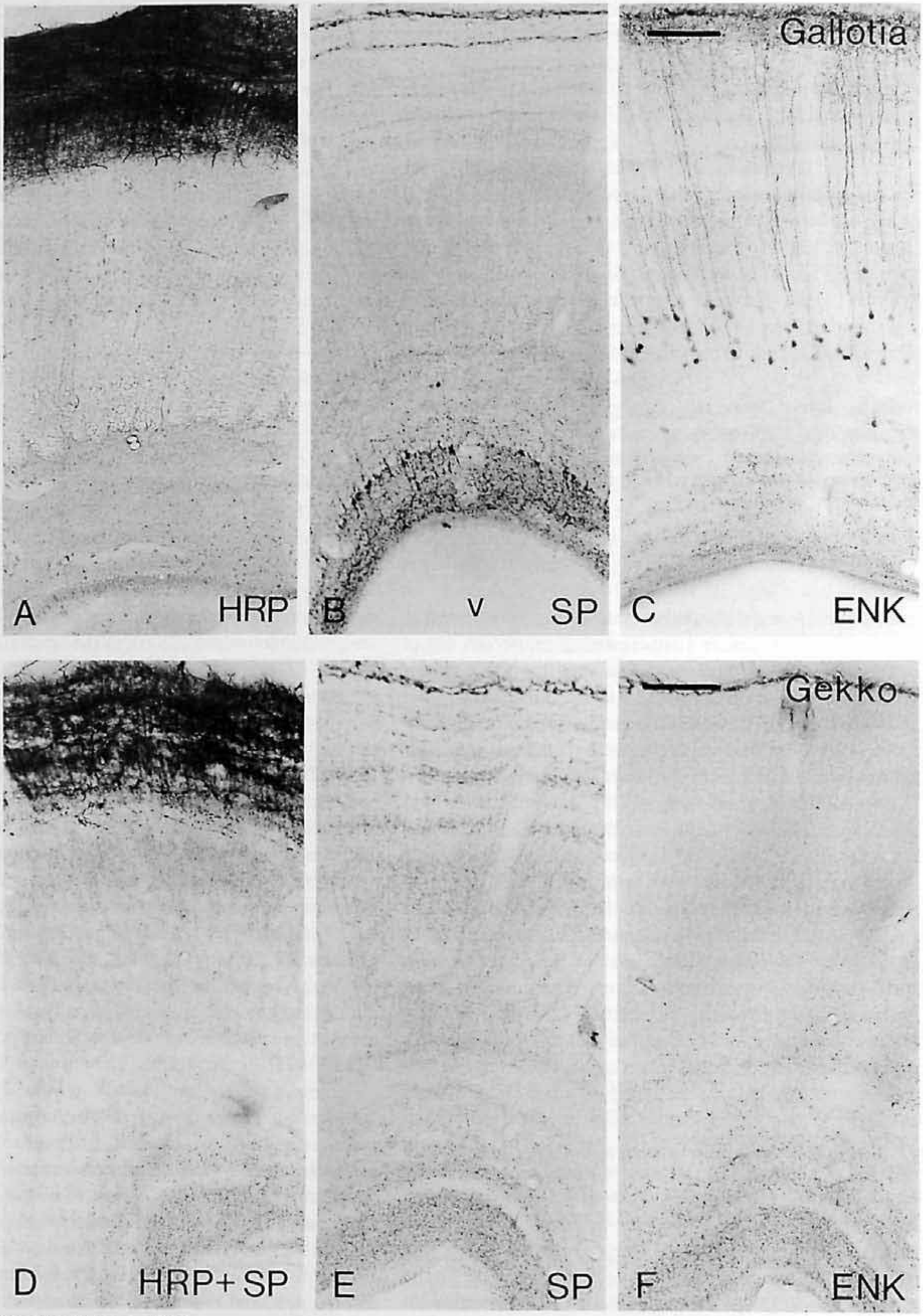
A remarkably constant feature of the visual system in reptiles is the serotoninergic innervation of the *basal optic nucleus.* A dense 5-HTi plexus is found not only in lizards [Wolters et al., 1985; present study] but also in turtles [Ueda et al., 1983] and snakes [Challet et al., 1991]. It should, therefore, be considered as a common feature of reptilian brains.

Another shared character of the two species studied is the chemoarchitecture of the *pretectal posterodorsal nucleus:* the nucleus contains cell bodies that are immunoreactive with TH- and DA-antisera, and a neuropil that stains densely for ChAT and SP antisera (fig. 14). In all species studied so far, putative dopaminergic cell bodies have been observed in the posterodorsal nucleus [Wolters et al., 1984, Smeets et al., 1986a, 1987; Brauth, 1988; Smeets, 1988], whereas a dense SP plexus in this brain structure has been reported also in turtles [Reiner et al., 1984]. Preliminary observations in the lizards *Podarcis sicula sicula* and



12

(For legend see p. 174.)



13

(For legend see p. 174.)

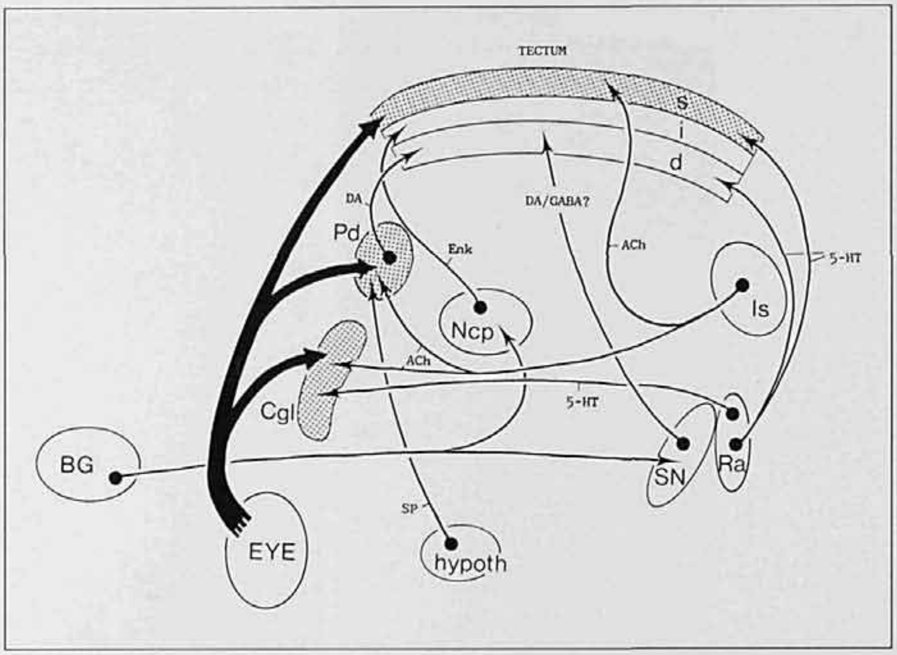


Fig. 14. Schematic drawing summarizing the putative sources of neurotransmitter systems that may be involved in visual information processing in the primary visual centers (shaded areas) in reptiles.

Eublepharis macularius and in the snake *Python regius* [Smeets, unpubl. observ.] also revealed coarse SPi fibers in the posterodorsal nucleus, suggesting that these chemical characteristics are common for reptiles.

Hodological studies have shown that retinal projections to the posterodorsal nucleus are present in other species of lizards [e.g. Butler and Northcutt, 1971; Northcutt and Butler, 1974; Reperant et al., 1978], in turtles [Bass and Northcutt, 1981], and in snakes [Schroeder, 1981; Reperant et al., 1987]. However, a projection from the posterodorsal nucleus to the tectum was observed only in lizards [ten Donkelaar et al., 1987; Medina and Smeets, 1991]. Recently, another difference was seen in the snake *Vipera aspis,* where the posterodorsal nucleus, in contrast to the corresponding structure in other reptiles, shows a dense serotoninergic innervation [Challet et al., 1991].

Considerable variation exists in the distribution of the neurotransmitters or their synthetic enzymes in the *tectum mesencephali* in various species of reptiles. The differences are predominantly confined to the central and superficial zones. In *Gekko*, two distinct bands of dopaminergic fibers are present in tectal layers 9 and 11 [Smeets et al., 1986a]. In other lizards *(Anolis, Eublepharis, Podarcis, Varanus)* similar but less distinct bands could be recognized [Smeets, 1988]. However, the superficical layers of the tectum in some lizards (*Gallotia*, present study), turtles [Smeets et al., 1987], crocodiles [Brauth, 1988] and snakes [Smeets, 1988] are almost devoid of dopaminergic fibers.

The serotoninergic innervation of the tectum also shows considerable variation between species. In *Gekko*, a distinct band of 5-HTi fibers is found in tectal layer 9 (fig.9) [Smeets and Steinbusch, 1988]. In other lizards, a similar but less distinct band seems to be present. Further, a laminar organization of serotoninergic fibers is found in the tectum in turtles [Ueda et al., 1983] but not in snakes [Challet et al., 1991].

Whereas the distribution of substance P in the tecta of the various reptiles studied is rather constant, notable differences have been observed in the distribution of Leu-enkephalin. The most striking difference is that in some species of lizards, i.e. *Anolis, Gallotia* and *Podarcis,* numerous LENKi cell bodies are present in tectal layer 7 and, to a lesser extent, layer 5 [Medina and Smeets, 1991]. Such cells were not observed in other lizards, e.g. *Gekko, Eublepharis,* and *Varanus,* or in turtles and snakes [Wolters

Fig. 12. Photomicrographs illustrating the relationship between the retinofugal fibers and the THi, putative dopaminergic, fibers in the tectum of *G. galloti* (**A, B**), and *G. gecko* (**C, D**). **A** and **C** are double stained for HRP and TH, whereas **B** and **D** are stained solely with the TH antiserum. Bar = 100 μ.

Fig. 13. Photomicrographs illustrating the relationship between retinofugal fibers and either SPi or LENKi fibers in the tectum of *G. galloti* (**A–C**) and *G. gecko* (**D–F**). Bar = 100 μm.

et al., 1986; Reiner, 1987; Medina and Smeets, 1991]. Because colchicine treatment in *Gekko* revealed weakly immunoreactive cell bodies in layers 7 and 5, we suggest that the presence of LENKi cell bodies in the midbrain tectum is the primitive condition in reptiles. Another difference in the distribution of LENK concerns the fibers and terminals. All species studied show a weakly to moderately immunoreactive plexus of fibers in the deep tectal zone, but in some species additional immunoreactive plexusses occur in the central and superficial tectal zones. This difference will be discussed in relation to the putative cells of orgin of the enkephalinergic innervation of the tectum.

Origin of Immunohistochemically Identified Afferents of Primary Visual Centers

Overlap in the distribution of neurotransmitters and the retinal projection fibers in the primary visual centers of reptiles suggests that the neurotransmitters are either confined within the retinofugal fibers or they modulate visual information processing at these sites. We will, therefore, first examine the possibility of a retinal origin of the observed immunoreactivity in the primary visual centers. Next, putative extraretinal sources as suggested by hodological studies will be discussed.

Neurochemistry of Retinal Ganglion Cells

The optic tract is composed of the axons of retinal ganglion cells, which receive visual information via a complex network of structures, including photoreceptors, amacrine cells, and horizontal cells. Whereas substantial information is available about the neurotransmitter content of amacrine and horizontal cells [e.g., Ehinger, 1982; Osborne et al., 1982; Brecha, 1983; Weiler and Schütte, 1985; Gläsener et al., 1988; Wulle and Wagner, 1990], little is known about that of the ganglion cells. Several candidate neurotransmitters have been proposed: acetylcholine, dopamine, serotonin, substance P, and several amino acids [Moffett et al., 1990, 1991].

From studies of the goldfish, it was concluded that a significant fraction of ganglion cells in the retina are cholinergic. Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE), the acetylcholine-synthesizing and -inactivating enzymes, respectively, were found in the optic nerve and in the layers of the midbrain tectum that receive retinal projection fibers [for review, see Tumosa and Stell, 1986]. Moreover, the concentrations of ChAT and AChE decrease following enucleation or optic nerve crush. However, these findings provide no direct evidence for the existence of cholinergic ganglion cells. In fact, the only cholinergic cells in the retinae of goldfish are the amacrine cells [Tumosa et al., 1984; Tumosa and Stell, 1986], as in other vertebrates [Hayden et al., 1980; Kása, 1986; Ekström and Korf, 1986]. A retinal origin of the cholinergic innervation of the visual centers in vertebrates seems, therefore, rather unlikely. Nevertheless, in the present study, some ChATi fibers were observed in the optic tract of a lacertid lizard, *Gallotia*, but not in a gekkonid lizard, *Gekko*. These cholinergic fibers may represent a retinopetal system, a notion that is supported by the finding that nicotinic cholinergic receptors are present on the surface of retinal bipolar cells in turtles [James and Klein, 1985].

There is growing evidence that some retinal ganglion cells use monoamines as a neurotransmitter. A serotonin-immunoreactive ganglion cell type has been detected in the retina of turtles [Weiler and Schütte, 1985; Weiler and Ammermüller, 1986]. Two distinct subpopulations of THi ganglion cells have recently been identified in pigeons: one population of displaced ganglion cells, which project to the accessory optic nucleus [Britto et al., 1988], and a second population located in the ganglion cell layer, which projects to the midbrain tectum [Keyser et al., 1987]. Recently, Dacey [1989] reported monoamine-accumulating ganglion cells in the retina in cats. Unfortunately, TH- or 5-HT-immunoreactive fibers are difficult to recognize in the optic nerves of the species studied, and we did not observe any fibers immunostained with TH- and 5HT-antisera in the optic tract in either of these lizards. Is should be noted, however, that in optic nerve of the turtle *Pseudemys scripta elegans*, Schütte and Weiler [1988] identified a single serotoninergic fiber that originated in the contralateral mesencephalon and arborized exclusively in the temporal hemisphere covering about one third of the total retinal surface. Although it can not be excluded that some of the TH- and 5-HT-immunoreactivity in the primary visual centers has a retinal origin, it is believed that the monoaminergic innervation of these centers is mainly derived from extraretinal sources.

Finally, it seems likely that substance P is a neurotransmitter substance in some retinal ganglion cells of vertebrates. In birds [Karten and Brecha, 1980, 1983; Ehrlich et al., 1987] and mammals [Brecha et al., 1982, 1984, 1987], retinal ganglion cells containing substance P-immunoreactivity have been described. In mammals, these cells probably project to several centers in the brain, e.g. the dorsal lateral geniculate body, the accessory optic nucleus, and the colliculus superior [Brecha et al., 1987]. Recently, substance P immunoreactive ganglion cells were identified in the retina in turtles [Cuenca and Kolb, 1989]. Immunoreactive fibers could be traced from the ganglion cell bodies to the optic nerve. However, in the lizards we studied, we were unable to identify substance P immunoreactive fibers in the optic tract.

Whereas substance P-containing ganglion cells appear to be a common feature of amniotes, their presence in anamniotes is less clear. For example, substance P containing ganglion cells in amphibians are found during developmental stages [Kuljis and Karten, 1986] but not in adults [Kuljis and Karten, 1982; Schmidt et al., 1989]. Also substance P-immunoreactive ganglion cells could not be identified in the retinae of developing and adult goldfish [Brecha et al., 1981].

Putative Extraretinal Sources of the Immunoreactive Fibers in the Primary Visual Centers

ChAT-Immunoreactivity. In order to determine possible sources of the cholinergic projections to the primary visual centers in reptiles, we will compare hodological data with immunohistochemical observations. In the present study, ChATi fiber bundles were traced from ChATi neurons in the isthmic region to the midbrain tectum and, further rostrally, to the supraoptic commissure where they decussate. These bundles may provide the cholinergic projections to the tectum and the diencephalic primary visual centers (fig. 14). This notion is supported by HRP studies of tectal afferents in reptiles, which revealed retrogradely labeled cells in the isthmic nucleus and the dorsolateral tegmentum [Foster and Hall, 1975; Gruberg et al., 1979; Welker et al., 1983; Dacey and Ulinski, 1986a; ten Donkelaar et al., 1987; Medina and Smeets, 1991].

Another putative source of cholinergic input to the primary visual centers, at least to the ventrolateral thalamic nucleus, is the basal forebrain. Projections from the basal forebrain where numerous cholinergic cells are present [Mufson et al., 1984; Brauth et al., 1985; Hoogland and Vermeulen-Van der Zee, 1990] to the ventrolateral nucleus have been reported [Brauth and Kitt, 1980; Russchen and Jonker, 1988; Gonzalez and Russchen, 1988].

TH-Immunoreactivity. Except for the tectum, almost nothing is known about the origin of TH-immunoreactive afferents to the primary visual centers in reptiles. The immunoreactivity we observed in the tectum probably represents dopaminergic fibers [Smeets and Steinbusch, 1990]. Hodological studies have established that the tectum in reptiles receives afferents from the hypothalamic periventricular nucleus, the pretectal posterodorsal nucleus, and the substantia nigra [Welker et al., 1983; ten Donkelaar et al., 1987]. Since all three nuclei contain dopaminergic cell bodies [Smeets, 1988], they are good candidates for the source of the dopaminergic innervation of the midbrain tectum.

5-HT-Immunoreactivity. Four groups of serotoninergic cell bodies are present in the brains of reptiles: the hypothalamic periventricular organ in the diencephalon, the nucleus raphes superior and the ventrolateral tegmentum in the midbrain, and the nucleus raphes inferior in the rhombencephalon [Ueda et al., 1983; Wolters et al., 1985; Smeets and Steinbusch, 1988]. On the basis of available data on tectal afferents [ten Donkelaar et al., 1987; unpubl. observ.], it is conceivable that the midbrain 5-HTi cell groups project to the tectum.

SP-Immunoreactivity. No SPi fibers were observed in the optic tract of the two lizard species studied, although SP might be considered as a putative neurotransmitter in retinal ganglion cells [Cuenca and Kolb, 1989]. A remarkable finding of the present study, however, is the presence of coarse SPi fibers in the pretectal region. In the double-labeling experiments, it was shown that these SPi fibers perfectly overlap the retinofugal fibers. Surprisingly, the coarse SPi fibers lie adjacent to, but nowhere, within the optic tract. They can be traced to the hypothalamus where numerous SPi cell bodies are present [Reiner et al., 1984; unpubl. observ.]. It is, therefore, very likely that the hypothalamic neurons provide the SPi innervation of the posterodorsal nucleus in reptiles.

LENK-Immunoreactivity. The search for the origin of LENKi fibers in the tectum of reptiles is complicated by the fact that in some species intrinsic LENKi cell bodies are present in layers 5 and 7, whereas in others these neurons are absent. Moreover, species differences have also been observed in the presence or absence of a tectopetal LENKi cell group in the pretectum [Medina and Smeets, 1991]. Taking these differences into account, the following preliminary conclusions can be reached. First, LENKi fibers in the superficial tectal zones *(Gallotia, Podarcis, Anolis)* are most likely derived from the intrinsic tectal LENKi cell bodies that have processes extending into the superficial zone where they ramify [Naik et al., 1981; Medina and Smeets, 1991]. Secondly, LENKi fibers in the central tectal zone are probably derived from the pretectal LENKi cell group, i.e. the nucleus of the posterior commissure, since species that do not possess such a cell group lack immunoreactive fibers in this zone [Medina and Smeets, 1991]. There must be a third source of LENKi fibers projecting primarily to the deep tectal layers, since species lacking both the LENKi pretectal cell group and the intrinsic tectal neurons show distinct immunoreactive plexusses in the deep tectal zone.

Comparative and Functional Considerations

Acetylcholine. A strong cholinergic innervation of the primary visual centers is not a feature that is unique to reptiles. Similar dense plexuses of ChATi fibers were found in fishes [Ekström, 1987; Zottoli et al., 1987], amphibians [Ciani et al., 1988], birds [Sorenson et al., 1989], and mammals [e.g., Kimura et al., 1981; Houser et al., 1983; Mesulam et al., 1984; Levey et al., 1987; Maley et al., 1988]. The cholinergic innervation of the midbrain tectum is derived partly from intrinsic tectal ChATi neurons in fishes [Tumosa et al., 1986; Ekström, 1987], birds [Sorenson et al., 1989], and mammals [Illing, 1990], and partly from cholinergic cells in the isthmic region [Ricciuti and Gruberg, 1985; Beninato and Spencer, 1986; Zottoli et al., 1988; Sorenson et al., 1989; Wallace et al., 1990]. The cholinergic projection from the isthmic regions to the retinorecipient layers of the tectum in lizards observed in the present study is comparable to the isthmotectal projections in other nonmammalian vertebrates and the parabigeminotectal projection in mammals. The cholinergic pathway from the isthmic tegmentum to the thalamus observed in our material (see fig. 7, 14) may be comparable to the projection from the pontomesencephalic reticular formation to the thalamus in mammals.

Data on the putative sources of the cholinergic projections to the thalamic visual centers are sparse. In mammals, the basal forebrain, the pontomesencephalic reticular formation (mainly the nucleus tegmenti pedunculopontinus and the laterodorsal tegmental nucleus), and the parabigeminal nucleus are the main contributors [De Lima and Singer, 1987a; Hallanger et al., 1987; Fitzpatrick et al., 1988; Parent et al., 1988]. In this regard, the putative projection from the cholinergic neurons in the basal forebrain to the ventrolateral thalamic nucleus in reptiles resembles the projection from the basal telencephalon to the thalamic reticular nuclei, part of which is visual, in mammals [Hallanger et al., 1987; Parent et al., 1988].

Dopamine. As in reptiles, much variation seems to exist in the dopaminergic innervation of the midbrain tectum in other vertebrates. In teleost fishes, there is weak immunoreactivity in the deep and intermediate layers but not in those superficial layers that receive visual information [Meek et al., 1989; Roberts et al., 1989]. The same pattern was observed in frogs [Gonzalez and Smeets, 1991] and is in sharp contrast with the pattern found in urodeles, where the optic layers of the tectum are densely innervated by DAi or THi fibers [Franzoni et al., 1986, Gonzalez and Smeets, 1991]. A dopaminergic innervation of the optic layers in the superior colliculus and of the dorsal lateral geniculate nucleus is also observed in mammals [Dacey, 1989, Papadopoulos and Parnavelas, 1990]. As putative sources of these fibers, retinal ganglion cells and the dopaminergic cells in the hypothalamus have been mentioned. Moreover, a dopaminergic projection from the substantia nigra to the superior colliculus has been demonstrated, and this may provide the morphological substrate for abnormal saccadic eye movements in Parkinsonism [Takada et al., 1988a, b; Campbell et al., 1990].

Serotonin. A strong serotoninergic innervation of structures related to the visual pathways has already been recognized by Braak et al. [1968] in several lizards. Subsequently, this relationship was confirmed in turtles [Ueda et al., 1983; Parent et al., 1984] and other lizards [Wolters et al., 1985; Smeets and Steinbusch, 1988]. A dense serotoninergic plexus in the lateral geniculate body and the pretectal area is also observed mammals [see e.g. Steinbusch, 1981; Cropper et al., 1984; Ueda and Sano, 1986; Morrison and Foote, 1986]. By contrast, the relay centers of the visual system in anamniotes generally do not show a particularly dense 5-HT innervation [see e.g. Parent and Northcutt, 1982; Kah and Chambolle, 1983; Ritchie et al., 1983; Ekström and van Veen, 1984; Meek and Joosten, 1989], although some anurans show a relatively dense plexus of serotoninergic fibers and terminals in the lateral geniculate body and the midbrain tectum [Parent et al., 1984; Ueda et al., 1984], which are known to recieve retinofugal fibers [Scalia, 1976; Lazar, 1978].

In mammals, the putative source of the serotoninergic innervation of the thalamic and mesencephalic visual centers is the midbrain raphe nuclei [Azmitia and Segal, 1978; Moore et al., 1978; Parent et al., 1981; De Lima and Singer, 1987b]. Hodological [ten Donkelaar et al., 1987] and immunohistochemical [Ueda et al., 1983; Wolters et al., 1985; Smeets and Steinbusch, 1988] data indicate that in reptiles also the midbrain raphe nuclei, together with the nucleus reticularis superior pars lateralis, constitute the major serotoninergic input to the primary visual centers.

Substance P. A close relationship between the distribution pattern of SPi fibers and that of retinofugal fibers is observed not only in reptiles (present study) but also in fishes [Vecino and Ekström, 1990], amphibians [Schmidt et al., 1989], birds [Ehrlich et al., 1987], and mammals [Cooper et al., 1981]. Such a relationship is probably a primitive trait for vertebrate brains. The possibility that substance P is contained within the axons of the retinal ganglion cells has already been discussed [see also Brecha et al., 1987; Nakagawa et al., 1988]. A remarkable finding of the present study is the intimate relationship between coarse SPi fibers and retinal projection fibers in the posterodorsal nucleus. Apparently, the SPi fibers course not within the optic tract but medial to the tract, suggesting that a retinal origin is unlikely. As pointed out earlier, the organization of the pretectal posterodorsal nucleus is rather similar in all reptiles studied. Recently, a dopaminergic cells group was identified in the pretectum of amphibians and, on the basis of its connections, is considered to be homologous to the posterodorsal nucleus in reptiles [González and Smeets, 1991]. Moreover, preliminary observations in the domestic chicken also revealed the existence of a pretectal dopaminergic cell group in a position resembling that of the area pretectalis dorsalis of Gamlin and Cohen [1988]. In an adjacent section stained with a SP antiserum, a rather dense plexus of immunoreactive fibers was found in a similar position [Smeets, unpubl. observ.]. These findings indicate that the observations made in reptiles may apply to other vertebrates as well. Although the functional significance of the posterodorsal nucleus has yet to be demonstrated [Fite, 1985], a putative hypothalamic SP and retinal input suggest that this nucleus integrates motivational and visual information.

Leu-Enkephalin. Several differences exist in the distribution of LENKi elements in the midbrain tectum of reptiles studied so far [Naik et al., 1981; Medina and Smeets, 1991; present study]. In some lizards, i.e. *Gallotia, Podarcis* and *Anolis*, numerous LENKi cell bodies are present in tectal layers 7 and 5, whereas such cells seem to be absent in other lizard species *(Gekko, Eublepharis, Varanus)*, in turtles *(Pseudemys)*, and in snakes *(Python, Thamnophis)*. In *Gekko*, some LENKi cell bodies could be revealed after colchicine treatment, and enkephalinergic cells were also demonstrated in the tecta of amphibians [Kuljis and Karten, 1982, 1983; Merchenthaler et al., 1989], birds [Reiner et al., 1982a], and mammals [Khachaturian et al., 1983; Graybiel et al., 1984]. It can, therefore, be assumed that their presence is the primitive condition. As previously reported for lizards [Naik et al., 1981; Medina and Smeets, 1991], the tectal LENKi cell bodies have processes that extend into the superficial zone where they ramify. The absence of LENK immunoreactivity in the superficial tectal layers in species that apparently lack LENKi tectal cell bodies supports the notion that the LENK immunoreactivity in the superficial tectal zone is largely accounted for by these cell bodies. By comparing the illlustrations of the intrinsic and efferent tectal neurons in the snake *Thamnophis* [Dacey and Ulinsli, 1986b, c] with our own results [Medina and Smeets, 1991], we could identify one kind of efferent neuron in *Thamnophis* that resembles the LENKi neurons in the tectum of *Podarcis* and *Gallotia.* These efferent neurons were described by Dacey and Ulinski [1986a] as tectal-isthmic cells with a long apical dendrite, which ramifies in the most superficial layer, and an axon which arises from the basal dendrite. Further support to the notion that the LENKi cells project to the isthmic nucleus is provided by immunohistochemical studies revealing a rather dense innervation of the parvocellular isthmic nucleus in reptiles possessing tectal LENKi cell bodies [Naik et al., 1981, unpubl. observ.] but not in reptiles lacking these cells [Wolters et al., 1985; unpubl. observ.]. Similar conditions are found in birds, where LENKi neurons are located in tectal layers 8–10 from which the projections to the isthmal-optic nucleus and parvocellular optic nucleus arise [Reiner and Karten, 1982; Reiner et al., 1982a], and in amphibians [see Lazar et al., 1983; Merchenthaler et al., 1989; Roth et al., 1990].

Another difference between the various reptiles studied concerns the distribution of LENKi fibers in layers 8–11 of the tectum. In a previous study [Medina and Smeets, 1991] it was suggested that this difference is likely due to the presence or absence of an LENKi cell group in the pretectum, viz. the nucleus of the posterior commissure, which, on the basis of its connections and chemoarchitecture, is considered to be homologous to the lateral spiriform nucleus of birds [Reiner et al., 1982a, b]. The nucleus of the posterior commissure in reptiles and its corresponding structure in birds constitute important relay centers within the basal ganglia-tectal connections and they may, therefore, be involved in transmitting basal ganglia influence on visuomotor behavior.

Acknowledgments

The authors thank Dr. A.H.M. Lohman and Dr. L. Puelles for critically rading the manuscript, Mr. A.J. Jonker for technical assistance, and Mr. D. de Jong for preparing the photographs. This study was supported by grants from the European Training Program (ETP) and the Caja Canarias-Gobierno Autonomo de Canarias (L.M.).

References

Adams, J.G. (1981) Heavy metal intensification of DAB-based HRP-reaction product. J. Histochem. Cytochem., *29:* 775.

Azmitia, E.C., and M. Segal (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J. Comp. Neurol., *179:* 641–668.

Bass, A.H., and R.G. Northcutt (1981) Retinal recipient nuclei in the painted turtle, *Chrysemys picta:* an autoradiographic and HRP study. J. Comp. Neurol., *199:* 97–112.

Beninato, M., and R.F. Spencer (1986) A cholinergic projection to the rat superior colliculus demonstrated by retrograde transport of horseradish peroxidase and choline acetyltransferase immunohistochemistry. J. Comp. Neurol., *253:* 525–538.

Braak, H., H.G. Baumgarten, and B. Falck (1968) 5-Hydroxytryptamin im Gehirn der Eidechse *(Lacerta viridis,* and *Lacerta muralis).* Z. Zellforsch. Mikrosk. Anat., *90:* 161–185.

Brauth, S.E. (1988) Cathecholamine neurons in the brainstem of the reptile *Caiman crocodilus.* J. Comp. Neurol., *270:* 313–326.

Brauth, S.E., and C.A. Kitt (1980) The paleostriatal system of *Caiman crocodilus.* J. Comp. Neurol., *189:* 437–465.

Brauth, S.E., C.A. Kitt, D.L. Price, and B.H. Wainer (1985) Cholinergic neurons in the telencephalon of the reptile *Caiman crocodilus.* Neurosci. Lett., 58: 235–240.

Brecha, N.C. (1983) Retinal neurotransmitters: histochemical and biochemical studies. *In* Chemical Neuroanatomy (ed. by P.C. Emson), Raven Press, New York, pp. 85–129.

Brecha, N.C., S.C. Sharma, and H.J. Karten (1981) Localization of substance P-like immunoreactivity in the adult and developing goldfish retina. Neuroscience *6:* 2737–2746.

Brecha, N.C., A. Hendrickson, I. Floren, and H.J. Karten (1982) Localization of substance P-like immunoreactivity within the monkey retina. Invest. Ophthalmol. Vis. Sci., *23:* 147–153.

Brecha, N.C., W. Eldred, R.O. Kuljis, and H.J. Karten (1984) Identification and localization of biologically active peptides in the vertebrate retina. In Progress in Retinal Research (ed. by N. Osborne and J. Chader), Vol. 3, pp. 185–226.

Brecha, N.C., D. Johnson, J. Bolz, S. Sharma, J.G. Parnavelas, and A.R. Lieberman (1987) Substance P immunoreactive retinal ganglion cells and their central axon terminals in the rabbit. Nature, *327:* 155–158.

Britto, L.R.G., K.T. Keyser, D.E. Hamassaki, and H.J. Karten (1988) Catecholaminergic subpopulation of retinal displaced ganglion cells projects to the accessory optic nucleus in the pigeon *(Columa livia).* J. Comp. Neurol., *269:* 109–117.

Butler, A.B., and R.G. Northcutt (1971) Retinal projections in *Iguana iguana* and *Anolis carolinensis.* Brain Res., *26:* 1–13.

Campbell, K., M. Takada, and T. Hattori (1990) A re-examination of dopamine and GABA localization in single cells of the substantia nigra. Eur. J. Neurosci. Suppl. *3:* 27.

Challet, E., J. Pierre, J. Reperant, R. Ward, and D. Miceli (1991) The serotoninergic system of the brain of the viper, *Vipera aspis.* An immunohistochemical study. J. Chem. Neuroanat., *4:* 233–248.

Ciani, F., V. Francechini, and P. Del Grande (1988) Histochemical and biochemical study on the acetylcholinesterase and choline acetyltransferase in the brain and spinal cord of frog, *Rana esculenta.* J. Hirnforsch., *29*: 157–163.

Cooper, P.E., M.H. Fernstrom, O.P. Rorstad, S.E. Leeman, and J.B. Martin (1981) The regional distribution of somatostatin, substance P and neurotensin in human brain. Brain Res., *218:* 219–232.

Cropper, E.C., J.S. Eisenman, and E.C. Azmitia (1984) An immunocytochemical study of the serotoninergic innervation of the thalamus of the rat. J. Comp. Neurol., *224:* 38–50.

Cuenca, N., and H. Kolb (1989) Morphology and distribution of neurons immunoreactive for substance P in the turtle retina. J. Comp. Neurol., *290:* 391–411.

Dacey, D.M. (1989) Monoamine-accumulating ganglion cell type of the cat's retina. J. Comp. Neurol., *288:* 59–80.

Dacey, D.M., and P.S. Ulinski (1986a) Optic tectum of the eastern garter snake, *Tamnophis sirtalis.* V. Morphology of brainstem afferents and general discussion. J. Comp. Neurol., *245:* 423–453.

Dacey, D.M., and P.S. Ulinski (1986b) Optic tectum of the eastern garter snake, *Thamnophis sirtalis.* II. Morphology of efferent cells. J. Comp. Neurol., *245:* 198–237.

Dacey, D.M., and P.S. Ulinski (1986c) Optic tectum of the eastern garter snake, *Thamnophis sirtalis.* III. Morphology of intrinsic neurons. J. Comp. Neurol., *245:* 283–300.

De Lima, D.A., and W. Singer (1987a) The brainstem projection to the lateral geniculate nucleus in the cat: identification of cholinergic and monoaminergic elements. J. Comp. Neurol., *259:* 92–121.

De Lima, D.A., and W. Singer (1987b) The serotoninergic fibers in the dorsal lateral geniculate nucleus of the cat: Distribution and synaptic connections demonstrated with immunohistochemistry. J. Comp. Neurol., *258:* 339–351.

Ehinger, B. (1982) Neurotransmitter systems in the retina. Retina, *2:* 305–321.

Ehrlich, D., K.T. Keyser, and H.J. Karten (1987) Distribution of substance P-like immunoreactive retinal ganglion cells and their pattern of termination in the optic tectum of the chick *Gallus gallus).* J. Comp. Neurol., *266:* 220–233.

Ekström, P. (1987) Distribution of choline acetyltransferase-immunoreactive neurons in the brain of a cyprinid teleost (*Phoxinus phoxinus* L.). J. Comp. Neurol., *256:* 494–515.

Ekström, P., and H.W. Korf (1986) Putative cholinergic elements in the photosensory pineal organ and retina of a teleost, *Phoxinus phoxinus* L. (Cyprinidae). Distribution of choline acetyltransferase immunoreactivity, acetylcholinesterase-positive elements, and pinealfugally projecting neurons. Cell Tissue Res., *246:* 321–329.

Ekström, P., and T. van Veen (1984) Distribution of 5-hydroxytryptamine (serotonin) in the brain of the teleost *Gasterosteus aculeatus* L. J. Comp. Neurol., *226:* 307–320.

Fite, K.V. (1985) Pretectal and accessory-optic nuclei of fish, amphibia and reptiles: theme and variations. Brain Behav. Evol., *26:* 71–90.

Fitzpatrick, D., M. Conley, G. Luppino, M. Matelli, and I.T. Diamond (1988) Cholinergic projections from the midbrain reticular formation and the parabigeminal nucleus to the lateral geniculate nucleus in the tree shrew. J. Comp. Neurol., *272:* 43–67.

Foster, R.E., and W.C. Hall (1975) The connections and laminar organization of the optic tectum in a reptile, *Iguana iguana.* J. Comp. Neurol., *163:* 397–426.

Franzoni, M.F., J: Thibault, A. Fasolo, M.G. Martinoli, F. Scaranari, and A. Calas (1986) Organization of tyrosine-hydroxylase immunopositive neurons in the brain of the crested newt, *Triturus cristatus carnifex.* J. Comp. Neurol., *251:* 121–134.

Gamlin, D.R., and D.H. Cohen (1988) The retinal projections to the pretectum in the pigeon *(Columba livia).* J. Comp. Neurol., *269:* 1–17.

Gläsener, G., W. Himstedt, R. Weiler, and C. Matute (1988) Putative neurotransmitters in the retinae of three urodele species *(Triturus alpestris, Salamandra salamandra, Pleurodeles waltli).* Cell Tissue Res., *252:* 317–328.

González, A., and F.T. Russchen (1988) Connections of the basal ganglia in the lizard *Gekko gecko. In* The Forebrain of Reptiles: Current Concepts of Structure and Function (ed. by W.K. Schwerdtfeger and W.J.A.J. Smeets), Karger, Basel, pp. 50–59.

González, A., and W.J.A.J. Smeets (1991) A comparative analysis of dopamine- and tyrosine hydroxylase-immunoreactivities in the brain of two amphibians, the anuran *Rana ridibunda* and the urodele *Pleurodeles waltii.* J. Comp. Neurol., *303:* 457–477.

Graybiel, A.M., N. Brecha, and H.J. Karten (1984) Cluster-and-sheet pattern of enkephalin-like immunoreactivity in the superior colliculus of the cat. Neuroscience, *12:* 191–214.

Gruberg, E.R., E. Kicliter, E.A. Newman, L. Kass, and P.H. Hartline (1979) Connections of the tectum of the rattlesnake *Crotalus viridis:* An HRP study. J. Comp. Neurol., *188:* 31–42.

Hallanger, A.E., A.I. Levey, H.J. Lee, D.D. Rye, and B.H. Wainer (1987) The origins of cholinergic and other subcortical afferents to the thalamus in the rat. J. Comp. Neurol., *262:* 105–124.

Hayden, S.A., J.W. Mills, and R.M. Masland (1980) Acetylcholine synthesis by displaced amacrine cells. Science, *210:* 435–437.

Hoogland, P.V., and E. Vermeulen-Van der Zee (1990) Distribution of choline acetyltransferase immunoreactivity in the telencephalon of the lizard *Gekko gecko*. Brain Behav. Evol., *36:* 378–390.

Houser, C.R., G.D. Crawford, R.P. Barber, P.M. Salvaterra, and J.E. Vaughn (1983) Organization and morphological characteristics of cholinergic neurons: an immunocytochemical study with a monoclonal antibody to choline acetyltransferase. Brain Res., *266:* 97–119.

Illing, R.-B. (1990) Choline acetyltransferase-like immunoreactivity in the superior colliculus of the cat and its relation to the pattern of acetylcholinesterase staining. J. Comp. Neurol., *296:* 32–46.

James, W.M., and W.L. Klein (1985) Alpha-Bungarotoxin receptors on neurons isolated from turtle retina: molecular heterogeneity of biopolar cell. J. Neurosci., *5:* 352–361.

Kah, O., and P. Chambolle (1983) Serotonin in the brain of the goldfish *Carassius auratus*. An immunocytochemical study. Cell Tissue Res., *234:* 319–333.

Karten, H.J., and N. Brecha (1980) Localization of substance P immunoreactivity in amacrine cells of the retina. Nature, *282:* 87–88.

Karten, H.J., and N. Brecha (1983) Localization of neuroactive substance in the vertebrate retina: Evidence for lamination in the inner plexiform layer. Vision Res., *23:* 1197–1205.

Kása, P. (1986) The cholinergic systems in brain and spinal cord. Progr. Neurobiol., *26:* 211–272.

Keyser, K., L.R.G. Britto, and H.J. Karten (1987) Catecholaminergic ganglion cells in the retina of the pigeon. Soc. Neurosci. Abstr., *13:* 1058.

Khachaturian, H., M.E. Lewis, and S.J. Watson (1983) Enkephalin systems in diencephalon and brainstem of the rat. J. Comp. Neurol., *220:* 310–320.

Kimura, H., P.L. McGeer, J.H. Peng, and E.G. McGeer (1981) The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat. J. Comp. Neurol., *200:* 151–201.

Kuljis, R.O., and H.J. Karten (1982) Laminar organization of peptide-like immunoreactivity in the anuran optic tectum. J. Comp. Neurol., *212:* 188–201.

Kuljis, R.O., and H.J. Karten (1983) Modification in the laminar organization of peptide-like immunoreactivity in the anuran optic tectum following deafferentiation. J. Comp. Neurol., *217:* 239–251.

Kuljis, R.O., and H.J. Karten (1986) Substance P-containing ganglion cells become progressively less detectable during retinotectal development in the frog *Rana pipiens*. Proc. Natl. Acad. Sci. USA, *83:* 5736–5740.

Lázár, G. (1978) Application of cobalt-filling technique to show retinal projections in the frog. Neuroscience, *3:* 725–736.

Lázár, G., P. Toth, G.Y. Csank, and E. Kicliter (1983) Morphology and location of tectal projection neurons in frog: a study with HRP and cobalt-filling. J. Comp. Neurol., *215:* 108–120.

Levey, A.I., A.E. Hallanger, and B.H. Wainer (1987) Choline acetyltransferase immunoreactivity in the rat thalamus. J. Comp. Neurol., *257:* 317–332.

Maley, B.E., M.L. Frick, A.I. Levey, B.H. Wainer, and R.P. Elde (1988) Immunohistochemistry of choline acetyltransferase in the guinea pig brain. Neurosci. Lett., *84:* 137–142.

Medina, L. (1991) Estudio ontogenético e inmunohistoquímico de los centros visuales primarios en el lagarto *Gallotia galloti*. Ph. D. Dissertation. Universidad de La Laguna, Canary Islands, Spain.

Medina, L., and W.J.A.J. Smeets (1991) Comparative aspects of the basal ganglia-tectal pathways in reptiles. J. Comp. Neurol., *308:* 614–629.

Meek, J., and H.W.J. Joosten (1989) Distribution of serotonin in the brain of the mormyrid teleost *Gnathonemus petersii*. J. Comp. Neurol., *281:* 206–224.

Meek, J., H.W.J. Joosten, and H.W.M. Steinbusch (1989) Distribution of dopamine immunoreactivity in the brain of the mormyrid teleost *Gnathonemus petersii*. J. Comp. Neurol., *281:* 362–383.

Merchenthaler, I., G. Lázár, and J.L. Maderdrut (1989) Distribution of proenkephalin-derived peptides in the brain of *Rana esculenta*. J. Comp. Neurol., *281:* 23–39.

Mesulam, M.M., E.J. Mufson, A.I. Levey, and B.H. Wainer (1984) Atlas of cholinergic neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry. Neuroscience, *12:* 669–686.

Moffett, J.R., L.C. Williamson, M. Palkovits, and M.A.A. Namboodiri (1990) N-Acetylaspartylglutamate: A transmitter candidate for the retinohypothalamic tract. Proc. Natl. Acad. Sci. USA, *87:* 8065–8069.

Moffett, J.R., L.C. Williamson, J.H. Neale, M. Palkovits, and M.A.A. Namboodori (1991) Effect of optic nerve transection on N-acetylaspartylglutamate immunoreactivity in the primary and acessory optic projection system in the rat. Brain Res., *538:* 86–94.

Moore, R.Y., A.E. Halaria, and B.E. Jones (1978) Serotonin neurons of the midbrain: ascending projections. J. Comp. Neurol., *180:* 417–438.

Morrison, J.H., and S.L. Foote (1986) Noradrenergic and serotoninergic innervation of cortical, thalamic, and tectal visual structures in Old and New World monkeys. J. Comp. Neurol., *243:* 117–138.

Mufson, E.J., P.H. Desan, M.M. Mesulam, B.H. Wainer, and A.I. Levey (1984) Choline acetyltransferase-like immunoreactivity in the forebrain of the red-eared pond turtle *(Pseudemys scripta elegans)*. Brain Res., *323:* 103–108.

Naik, R.D., M. Sar, and W.E. Stumpf (1981) Immunohistochemical localization of enkephalin in the central nervous system and pituitary of a lizard, *Anolis carolinensis*. J. Comp. Neurol., *198:* 583–601.

Nakagawa, S., Y. Hasegawa, T. Kubozono, and K. Takumi (1988) Substance P-like immunoreactive retinal terminals found in two retinorecipient areas of the Japanese monkey. Neurosci. Lett., *93:* 32–37.

Northcutt, R.G., and A.B. Butler (1974) Evolution of reptilian visual systems: Retinal projections in a nocturnal lizard, *Gekko gecko* (Linnaeus). J. Comp. Neurol., *157:* 453–466.

Osborne, N.N., T. Nesselhut, D.A. Nicholas, S. Patel, and A.C. Cuello (1982) Serotonin-containing neurons in vertebrate retinas. J. Neurochem., *39:* 1519–1528.

Papadopoulos, G.C., and J.G. Parnavelas (1990) Distribution and synaptic organization of dopaminergic axons in the lateral geniculate nucleus of the rat. J. Comp. Neurol., *294:* 356–361.

Parent, A., L. Descarries, and A. Beaudet (1981) Organization of ascending serotoninergic systems in the adult rat. A radioautographic study after intraventricular administration of (^{3}H)5-hydroxytryptamine. Neuroscience, *6:* 115–138.

Parent, A., and R.G. Northcutt (1982) The monoamine-containing neurons in the brain of the garfish, *Lepisosteus osseus*. Brain Res. Bull., *9:* 189–204.

Parent, A., D. Poitras, and L. Dubé (1984) Comparative anatomy of central monoaminergic systems. *In* Classical Transmitters in the CNS (ed. by A. Björklund and T. Hökfelt), Handbook of Chemical Neuroanatomy, Vol. 2, Part I. Elsevier, Amsterdam, pp. 409–439.

Parent, A., D. Paré, Y. Smith, and M. Steriade (1988) Basal forebrain cholinergic and noncholinergic projections to the thalamus and brainstem in cats and monkeys. J. Comp. Neurol., *277:* 281–301.

Reiner, A. (1987) The distribution of proenkephalin-derived peptides in the central nervous system of turtles. J. Comp. Neurol., *259:* 65–91.

Reiner, A., and H.J. Karten (1982) Laminar distribution of the cells of origin of the descending tectofugal pathways in the pigeon *(Columba livia)*. J. Comp. Neurol., *204:* 165–187.

Reiner, A., H.J. Karten, and N.C. Brecha (1982a) Enkephalin-mediated basal ganglia influences over the optic tectum: immunohistochemistry of the tectum and the lateral spiriform nucleus in pigeon. J. Comp. Neurol., *208:* 37–53.

Reiner, A., N.C. Brecha, and H.J. Karten (1982b) Basal ganglia pathways to the tectum: the afferent and efferent connections of the lateral spiriform nucleus of the pigeon. J. Comp. Neurol., *208:* 16-36.

Reiner, A., J.E. Krause, K.T. Keyser, W.D. Eldred, and J.F. McKelvy (1984) The distribution of substance P in turtle nervous system: A radioimmunoassay and immunohistochemical study. J. Comp. Neurol., *226:* 50–75.

Reperant, J., J.P. Rio, D. Miceli, and M. Lemire (1978) A radioautographic study of retinal projections in type I and type II lizards. Brain Res., *142:* 401–411.

Reperant, J., D. Miceli, J.P. Rio, and C. Weidner (1987) The primary optic system in a microphtalmic snake *(Calabaria reinhardti)*. Brain Res., *408:* 233–238.

Ricciuti, A.J., and E.R. Gruberg (1985) Nucleus isthmi provides most tectal choline acetyltransferase in the frog *Rana pipiens*. Brain Res., *341:* 399–402.

Ritchie, T.C., C.A. Livingston, M.G. Hughes, D.J. McAdoo, and R.B. Leonard (1983) The distribution of serotonin in the CNS of an elasmobranch fish: immunohistochemical and biochemical studies in the atlantic stingray, *Dasyatis sabina*. J. Comp. Neurol., *221:* 429–443.

Roberts, B.L., G.E. Mederith, and S. Maslam (1989) Immunocytochemical analysis of the dopamine system in the brain and spinal cord of the european eel, *Anguilla anguilla*. Anat. Embryol., *180:* 401–412.

Roth, G., C. Naujoks-Manteuffel, and W. Grunwald (1990) Cytoarchitecture of the tectum mesencephali in salamanders: a Golgi and HRP study. J. Comp. Neurol., *291:* 27–42.

Russchen, F.T., and A.J. Jonker (1988) Efferent connections of the striatum and the nucleus accumbens in the lizard *Gekko gecko*. J. Comp. Neurol., *276:* 61–80.

Scalia, F. (1976) The optic pathway of the frog. Nuclear organization and connections. *In* Frog Neurobiology (ed. by R. Llinas and W. Precht). Springer, New York, pp. 386–406.

Schmidt, A., G. Roth, and M. Ernst (1989) Distribution of substance P-like, leucine-enkephalin-like, and bombesine-linke immunoreactivity and acetylcholinesterase activity in the visual system of salamanders. J. Comp. Neurol., *288:* 123–135.

Schroeder, D.M. (1981) Retinal afferents and efferents of an infrared sensitive snake, *Crotalus viridis*. J. Morphol., *170:* 29–42.

Schütte, M., and R. Weiler (1988) Mesencephalic innervation of the turtle retina by a single serotonin-containing neuron. Neurosci. Lett., *91:* 289–294.

Smeets, W.J.A.J. (1988) Distribution of dopamine immunoreactivity in the forebrain and midbrain of the snake *Python regius:* a study with antibodies against dopamine. J. Comp. Neurol., *271:* 115–129.

Smeets, W.J.A.J. (1991) Comparative aspects of the distribution of substance P and dopamine immunoreactivity in the substantia nigra of amniotes. Brain Behav. Evol., *37:* 179–188.

Smeets, W.J.A.J., P.V. Hoogland, and P. Voorn (1986a) The distribution of dopamine immunoreactivity in the forebrain and midbrain of the lizard *Gekko gecko:* an immunohistochemical study with antibodies against dopamine. J. Comp. Neurol., *253:* 46–60.

Smeets, W.J.A.J., P.V. Hoogland, and A.H.M. Lohman (1986b) A forebrain atlas of the lizard *Gekko gecko*. J. Comp. Neurol., *254:* 1–19.

Smeets, W.J.A.J., A.J. Jonker, and P.V. Hoogland (1987) Distribution of dopamine in the forebrain and midbrain of the red-eared turtle, *Pseudemys scripta elegans*, reinvestigated using antibodies against dopamine. Brain Behav. Evol., *30:* 121–142.

Smeets, W.J.A.J., and H.W.M. Steinbusch (1988) Distribution of serotonin immunoreactivity in the forebrain and midbrain of the lizard *Gekko gecko*. J. Comp. Neurol., *271:* 419–434.

Smeets, W.J.A.J., and H.W.M. Steinbusch (1989) Distribution of noradrenaline immunoreactivity in the forebrain and midbrain of the lizard *Gekko gecko*. J. Comp. Neurol., *285:* 453–466.

Smeets, W.J.A.J., and H.W.M. Steinbusch (1990) New insights into the reptilian catecholaminergic systems as revealed by antibodies against the neurotransmitters and their synthetic enzymes. J. Chem. Neuroanat., *3:* 25–43.

Sorenson, E.M., D. Parkinson, J.L. Dahl, and V.A. Chiappinelli (1989) Immunohistochemical localization of choline acetyltransferase in the chicken mesencephalon. J. Comp. Neurol., *281:* 641–657.

Steinbusch, H.W.M. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat. Cell bodies and terminals. Neuroscience, *6:* 557–618.

Takada, M., Z.K. Li, and T. Hattori (1988a) Collateral projection from the substantia nigra to the striatum and superior colliculus in the rat. Neuroscience, *25:* 563–568.

Takada, M., Z.K. Li, and T. Hattori (1988b) dopaminergic nigrotectal projection in the rat. Brain Res. *457:* 165–168.

ten Donkelaar, H.J., G.C. Bangma, H.A. Barbas-Henry, R. de Boer-van Huizen, and J.G. Wolters (1987) The brainstem in a lizard, *Varanus exanthematicus*. Adv. Anat. Embryol. Cell Biol., *107:* 1–168.

Tumosa, N., F. Eckenstein, and W.K. Stell (1984) Immunocytochemical localization of putative cholinergic neurons in the goldfish retina. Neurosci. Lett., *48:* 255–259.

Tumosa, N., and W.K. Stell (1986) Choline acetyltransferase immunoreactivity suggests that ganglion cells in the goldfish retina are not cholinergic. J. Comp. Neurol., *244:* 267–275.

Tumosa, N., W.K. Stell, C.D. Johnson, and M.L. Epstein (1986) Putative cholinergic interneurons in the optic tectum of goldfish. Brain Res., *370:* 365–369.

Ueda, S., Y. Nojyo, and Y. Sano (1984) Immunohistochemical demonstration of the serotonin neuron system in the central nervous system of the bullfrog *Rana catesbeiana*. Anat. Embryol., *169:* 219–229.

Ueda, S., and Y. Sano (1986) Distributional patterns of serotonin-immunoreactive nerve fibers in the lateral geniculate nucleus of the rat, cat and monkey *(Macaca fuscata)*. Cell Tissue Res., *243:* 249–253.

Ueda, S., Y. Takeuchi, and Y. Sano (1993) Immunohistochemical demonstration of serotonin neurons in the central nervous system of the turtle *(Clemmys japonica)*. Anat. Embryol., *168:* 1–19.

Vecino, E., and P. Ekstöm (1990) Distribution of met-enkephalin, leu-enkephalin, substance P, neuropeptide Y, FMRFamide, and serotonin immunoreactivities in the optic tectum of the atlantic salmon (*Salmo salar* L.). J. Comp. Neurol., *299:* 229–241.

Wallace, M.T., A.J. Ricciuti, and E.R. Gruberg (1990) Nucleus isthmi: its contribution to tectal acetylcholinesterase and choline acetyltransferase in the frog *Rana pipiens*. Neuroscience, *35:* 627–636.

Weiler, R., and J. Ammermüller (1986) Immunocytochemical localization of serotonin in intracellulary analyzed and dye-injected ganglion cells of the turtle retina. Neurosci. Lett., *72:* 147–152.

Weiler, R., and M. Schütte (1985) Morphological and pharmacological analysis of putative serotoninergic bipolar and amacrine cells in the retina of a turtle, *Pseudemys scripta elegans*. Cell Tissue Res., *241:* 373–382.

Welker, E., P.V. Hoogland, and A.H.M. Lohman (1983) Tectal connections in *Python reticulatus*. J. Comp. Neurol., *220:* 347–354.

Wolters, J.G., H.J. ten Donkelaar, and A.A.J. Verhofstad (1984) Distribution of catecholamines in the brain stem and spinal cord of the lizard *Varanus exanthematicus:* an immunohistochemical study based on the use of antibodies to tyrosine hydroxylase. Neuroscience, *13:* 469–493.

Wolter, J.G., H.J. ten Donkelaar, H.W.M. Steinbusch, and A.A.J. Verhofstad (1985) Distribution of serotonin in the brain stem and spinal cord of the lizard *Varanus exanthematicus:* an immunohistochemical study. Neuroscience, *14:* 169–193.

Wolters, J.G., H.J. ten Donkelaar, and A.A.J. Verhostad (1986) Distribution of some peptides (substance P, leu-enkephalin, met-enkephalin) in the brain stem and spinal cord of a lizard, *Varanus exanthematicus*. Neuroscience, *18:* 917–946.

Wulle, I., and H.-J. Wagner (1990) GABA and tyrosine hydroxylase immunohistochemistry reveal different patterns of colocalization in retinal neurons of various vertebrates. J. Comp. Neurol., *296:* 173–178.

Zottoli, S.J., K.J. Rhodes, and E.J. Mufson (1987) Comparison of acetylcholinesterase and choline acetyltransferase staining patterns in the optic tectum of the goldfish *Carassius auratus*. Brain Behav. Evol., *30:* 143–159.

Zottoli, S.J., K.J. Rhodes, J.G. Corrodi, and E.J. Mufson (1988) Cholinergic projections from the nucleus isthmi and nucleus reticularis mesencephali to the optic tectum in the goldfish *(Carassius auratus)*. J. Comp. Neurol., *273:* 385–398.