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Distribution of Calcitonin Gene-Related Peptide-Like Immunoreactivity in the Brain of the Lizard *Podarcis hispanica*

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ABSTRACT

The present work studies the distribution of calcitonin gene-related peptide-immunoreactive (CGRP-li) neurons and fibers in the brain of a reptile, the lizard *Podarcis hispanica*. CGRP-li perikarya were not present in the telencephalon. In the thalamus, CGRP-li perikarya were restricted to the posteromedial and posterolateral nuclei. In the hypothalamus, CGRP-li cells were found mainly in the supramammillary and mammillary nuclei. In the midbrain and brainstem, CGRP-li cells appeared in the ventral tegmental area, the parabrachial nucleus, and the motor nuclei of the III–VII, IX, X, and XII cranial nerves. Motoneurons of the ventral horn of the spinal cord were also immunoreactive for CGRP. CGRP-li fibers were seen in the telencephalic hemispheres, where a dense plexus of reactive fibers appeared in the septum and in the lateral striatoamygdaloid transition area. From the latter, CGRP-li fibers entered the posterior dorsal ventricular ridge, the cell layer and deep stratum of the ventral lateral cortex, and various amygdaloid nuclei. Parts of the striatum (nucleus accumbens) and pallidum also displayed CGRP-li innervation. In the diencephalon, CGRP-li innervation was observed in parts of the dorsal thalamus and in the periventricular and medial hypothalamus. The pretectum and deep layers of the optic tectum also showed CGRP-li fibers, and numerous CGRP-li fibers were observed in the midbrain central gray, tegmentum, and pons. Some of the sensory fibers of the trigeminal, vagal, and spinal nerves were also CGRP-li. These results show that the distribution of CGRP-li structures in the reptilian brain is similar to that described for other vertebrates and suggest that the thalamotelencephalic CGRPergic projections appear to be conserved among amniote vertebrates. J. Comp. Neurol. 447:99–113, 2002.

Indexing terms: calcitonin gene-related peptide; reptiles; amygdala; thalamoamygdaloid projections; evolution

Calcitonin gene-related peptide (CGRP) is a neuropeptide composed of 37 amino acids, encoded and generated by alternative processing of the mRNA of the calcitonin gene (Amara et al., 1982). This alternative splicing takes place in both the central and the peripheral nervous systems (Rosenfeld et al., 1983). In the central nervous system, the distribution of CGRP-immunoreactive neurons and terminals has been demonstrated in several mammalian species, such as rat, cat, and human (Sakanaka et al., 1985; Seifer et al., 1985; Skofitsch and Jocobowitz, 1985; Inagaki et al., 1986; Harmann et al., 1988; Kruger et al., 1988a,b; Sugimoto et al., 1988), and this work has shown that CGRP is present in specific sensory, motor, and integrative systems, suggesting that it is involved in a variety of functions (see, e.g., Nguyen et al., 1986; Poore and Helmstetter, 1996; for review see van Rossum et al., 1997). The distribution of CGRP has also been studied

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in the brain of some nonmammalian vertebrates (birds: Brauth and Reiner, 1991; Lanuza et al., 2000; amphibians: Petkó and Sánta, 1992; teleost fishes: Batten and Cambre, 1989; Batten et al., 1990; Finger and Kanwal, 1992; elasmobranch fishes: Molist et al., 1995), and these reports suggest that most but not all of the CGRP systems have been well conserved during the evolution of the vertebrate brain.

With the exception of fragmentary data concerning the thalamus of turtles and crocodilians (Brauth and Reiner, 1991; Pritz and Stritzel, 1991) and the spinal cord of turtles (Luthman et al., 1991), there are no data on the distribution of CGRP in the reptilian brain, especially concerning squamate reptiles (lizards and snakes). This type of study is critical to formulating and testing hypotheses concerning the evolution of the vertebrate CGRPergic systems and may shed light on our understanding of the evolution of the vertebrate brain. With regard to this issue, recent data on the expression of homeotic genes during development of the forebrain (Smith-Fernandez et al., 1998; Puelles et al., 2000) have revealed the existence of a new pallial region located ventrally to the lateral (olfactory) pallium but dorsally to the lateral ganglionic eminence (precursor of the dorsal striatum) that has been called "ventral pallium" (Puelles et al., 2000). In mammals, the posterior intralaminar thalamus projects not only to the striatum but also to lateral and ventral pallial derivatives (see discussion in Puelles et al., 2000). These projections have been shown to arise in part from CGRPergic cells (Yasui et al., 1991; suggested by LeDoux and Farb, 1991). Therefore, comparative studies of the distribution of CGRP would be helpful not only for iden-

Abbreviations

ac	anterior commissure
apc	anterior pallial commissure
Acc	nucleus accumbens
ADVR	anterior dorsal ventricular ridge
AHN	anterior hypothalamic nucleus
Amb	nucleus ambiguus
AmbX	vagal part of the nucleus ambiguus
aot	accessory olfactory tract
AT	area triangularis
BST	bed nucleus of the stria terminalis
DB	diagonal band nucleus
DC	dorsal cortex
dh	dorsal horn of the spinal cord
DL	dorsolateral anterior thalamic nucleus
DLA	dorsolateral amygdaloid nucleus
DLH	dorsolateral hypothalamic nucleus
DM	dorsomedial anterior thalamic nucleus
DMC	dorsomedial cortex
Ea	anterior entopeduncular nucleus
flm	fasciculus longitudinalis medialis
fr	fasciculus retroflexus
Fun	nucleus funiculi dorsalis
GC	griseum centrale
GLV	ventral lateral geniculate nucleus
GP	globus pallidus
GT	griseus tectalis
H	habenula
IGL	intergeniculate leaflet
III	nucleus nervi oculomotorii
Ip	interpeduncular nucleus
Isd	nucleus isthmicus diffusus
Ism	nucleus isthmicus magnocellularis
Iss	nucleus isthmicus semilunaris
IV	nucleus nervi trochlearis
JCL	lateral juxtacommissural nucleus
JCM	medial juxtacommissural nucleus
LA	lateral amygdaloid nucleus
Lc	locus coeruleus
LCc	caudal lateral cortex
LCd	dorsal lateral cortex
LCv	ventral lateral cortex
lfb	lateral forebrain bundle
LHA	lateral hypothalamic area
lot	lateral olfactory tract
lttd	lateral descending trigeminal tract
M	medial thalamic nucleus
MA	medial amygdala
MAM	mammillary nucleus
MC	medial cortex
MPO	medial preoptic nucleus
Nac	nucleus of the anterior commissure
Naot	nucleus of the accessory olfactory tract
Nci	nucleus of the commissura infima
Nflm	nucleus of the fasciculus longitudinalis medialis
nIII	nervus oculomotorius
nIV	nervus trochlearis
Nmfb	bed nucleus of the medial forebrain bundle
NOT	nucleus of the basal optic root
Nppc	nucleus of the posterior pallial commissure
NS	nucleus sphericus
Nttd	descending nucleus of the trigeminal nerve
nV	nervus trigeminus
OT	optic tectum
P3	prosomere 3
Pb	parabrachial nucleus
pc	posterior commissure
Pd	nucleus posterodorsalis pretectalis
PDVR	posterior dorsal ventricular ridge
PE	external pretectal nucleus
PG	pretectal geniculate nucleus
PL	posterolateral thalamic nucleus
PM	posteromedial thalamic nucleus
PPC	principal precommissural nucleus
PPO	preoptic periventricular nucleus
Prmc	nucleus profundus mesencephali, pars caudalis
Prmr	nucleus profundus mesencephali, pars rostralis
PVA	anterior periventricular hypothalamus
PVH	paraventricular hypothalamic nucleus
R	red nucleus
Ra	raphe nucleus
RC	retrochiasmatic area
Ris	nucleus reticularis isthmi
Rot	nucleus rotundus
Sa	anterior septal nucleus
SATl	lateral striatoamygdaloid transition area
SATm	medial striatoamygdaloid transition area
sco	subcommissural organ
Sd	dorsal septal nucleus
Sl	lateral septal nucleus
Sm	medial septal nucleus
SN	substantia nigra
Sol	nucleus tractus solitarii
Sp	suprapeduncular nucleus
Ss	nucleus salivatorius superior
St	striatum
SUM	supramammillary nucleus
TSc	torus semircularis, pars centralis
Tu	olfactory tubercle
VAA	ventral anterior amygdala
vh	ventral horn of the spinal cord
VI	nucleus nervi abducens
VII	nucleus nervi facialis
VL	ventrolateral thalamic nucleus
Vm	nucleus motorius nervi trigemini
VMH	ventromedial hypothalamic nucleus
VP	ventral pallidum
VPA	ventral posterior amygdala
VTA	ventral tegmental area
XII	nucleus nervi hypoglossi

tifying the derivatives of the lateral and ventral pallium in the brain of adult mammals but also for recognizing the telencephalic centers that constitute the lateral and ventral pallium in the brain of nonmammals.

The aim of this work is to provide a comprehensive description of the distribution of CGRP-immunoreactive neurons and fibers in the brain of a squamate reptile, the lizard *Podarcis hispanica*. The results suggest that CGRP is present in some pathways (e.g., thalamotelencephalic projections) of the forebrain of all amniote vertebrates that have thus far been studied, whereas the expression of CGRP by other forebrain projection systems is restricted to particular vertebrate groups (e.g., projections from the mammillary hypothalamus).

MATERIALS AND METHODS

For this study, male and female adult specimens of *Podarcis hispanica* (39–57 mm snout-cloaca length) were used. Lizards were captured under license issued by the Valencian Conselleria d'Agricultura i Medi Ambient and maintained in terraria with food and water available ad libitum under natural day/night cycles at 22–30°C. Throughout the experimental work, animals were treated according to the guidelines of the European Community for the treatment of experimental animals.

To study the distribution of CGRP immunoreactivity in the brain of *Podarcis*, animals (n = 6) were anesthetized with an intramuscular injection of 6.5 μl of Ketolar (Park-Davis; ketamine 50 mg/ml, El Prat de Llobregat, Catalunya, Spain) per 1 g body weight. Under deep anesthesia, animals were transcardially perfused with 1–2 ml of saline solution (0.9% NaCl), followed by 15–20 ml of fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Brains were carefully removed from the skull and post-fixed overnight in 4% formaldehyde in phosphate buffer (pH 7.4) at 4°C. The brains were then immersed in buffered 30% sucrose at 4°C until they sank, and 30-μm-thick frontal sections were obtained using a freezing microtome.

The sections were then processed according to the usual immunocytochemical procedure. First, endogenous peroxidase activity was inhibited by incubating the sections for 30 minutes in a 1% H_2O_2 solution. Then, sections were washed three times in Tris (Trizma base; Sigma, St. Louis, MO) buffer, 0.05 M, pH 7.6, with 0.9% NaCl (TBS) and incubated for 24–48 hours at 4°C in a solution containing primary antiserum (rabbit anti-CGRP, Peninsula, San Carlos, CA) diluted (1:8,000) in TBS with 0.3% Triton X-100 (Tx100; Sigma) and 5% normal goat serum (NGS). After rinsing in TBS, sections were transferred to a solution of biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:200 in TBS-Tx100 and NGS 2.5% for 2 hours at room temperature, rinsed in TBS, and incubated with ABC Elite (Vector Laboratories) for 1.5 hours. These sections were washed once in TBS and twice in Tris buffer 0.05 M, pH 7.6 (TB), and finally peroxidase activity was developed using 3,3′-diaminobezidine (DAB; 0.025%; Sigma) diluted in TB and H_2O_2 (0.01%). Sections were immersed in warm 0.25% gelatin (Sigma) diluted in TB, mounted on clean slides, and coverslipped. In one case, two matching series of sections were processed together, and one of them was counterstained with toluidine blue to help locate the labelling.

Control sections were treated identically to the immunolabelled sections except that the primary antiserum was preincubated (overnight at 4°C) with a tenfold excess of blocking peptide (human CGRP; Peninsula) or omitted. Omission of the primary antibody resulted in total absence of labelling. The preincubation with blocking peptide gave rise to some background staining, but no fibers or cell bodies were observed, except for the mesencephalic nucleus of the trigeminal nerve, where faintly labelled perikarya could be observed.

Colchicine pretreatment

In two lizards, visualization of CGRP within neuron cell bodies was enhanced by means of intraventricular injection of colchicine. The animals were anesthetized, and 0.1–0.3 μl of an aqueous solution containing 50 μg/μl of colchicine (Sigma) was injected into the lateral ventricle by means of a Hamilton microsyringe. After 6 days of survival, they were deeply anesthetized and perfused, and their brains were processed for CGRP immunohistochemistry as described above.

Digital photography

Digital images were captured in a Nikon (Eclipse E800) microscope equipped with a digital camera (Nikon DXM1200) using the Nikon ACT-1 software, set at a resolution of 3,840 × 3,072 pixels. The images were later adjusted for size, contrast, and brightness using Adobe Photoshop 5.5, and the panels were prepared with the same software at a resolution of 750 dpi.

RESULTS

The distribution of cell bodies and fibers displaying CGRP-like immunoreactivity (CGRP-li) in the brain of *Podarcis hispanica* is described below as revealed by the application of the described immunohistochemical protocol. For description of the results, we have adapted the atlas by Smeets et al. (1986) for the forebrain and midbrain of the gecko to the brain of *P. hispanica* using the study of the septal nuclei by Font et al. (1995), data from Lanuza et al. (1997, 1998) concerning the cytoarchitecture of the dorsal ventricular ridge (DVR) and amygdala, and the classification of the basal ganglia by Russchen et al. (1987). In dealing with the diencephalon, we have followed the classification of the visual thalamus by Kenigfest et al. (1997) and that by Dávila et al. (2000) for the rest of the thalamus and the pretectum. With regard to the brainstem, we have used the cytoarchitecture proposed by ten Donkelaar et al. (1987) for the brain of *Varanus exanthematicus*, complemented by data on the nuclei of the cranial nerves by Barbas-Henry (1988) and Medina et al. (1993).

CGRP-li cells

Colchicine pretreatment did not alter the distribution of the labelling obtained but resulted in an enhancement of the labelling of some cell bodies. In none of the specimens were CGRP-li cell bodies found in the telencephalon, so we assume that CGRP-li cells are absent in the reptilian telencephalic hemispheres.

In the diencephalon, both the thalamus and the hypothalamus displayed reactive cell bodies (Fig. 1C–F). In the thalamus, faintly CGRP-li cells were found in the ventral aspect of the posteromedial (PM; Figs. 1F, 2B,C) and posterolateral nuclei (PL; Fig. 1G). Colchicine pretreatment was particularly useful for visualizing the morphology of

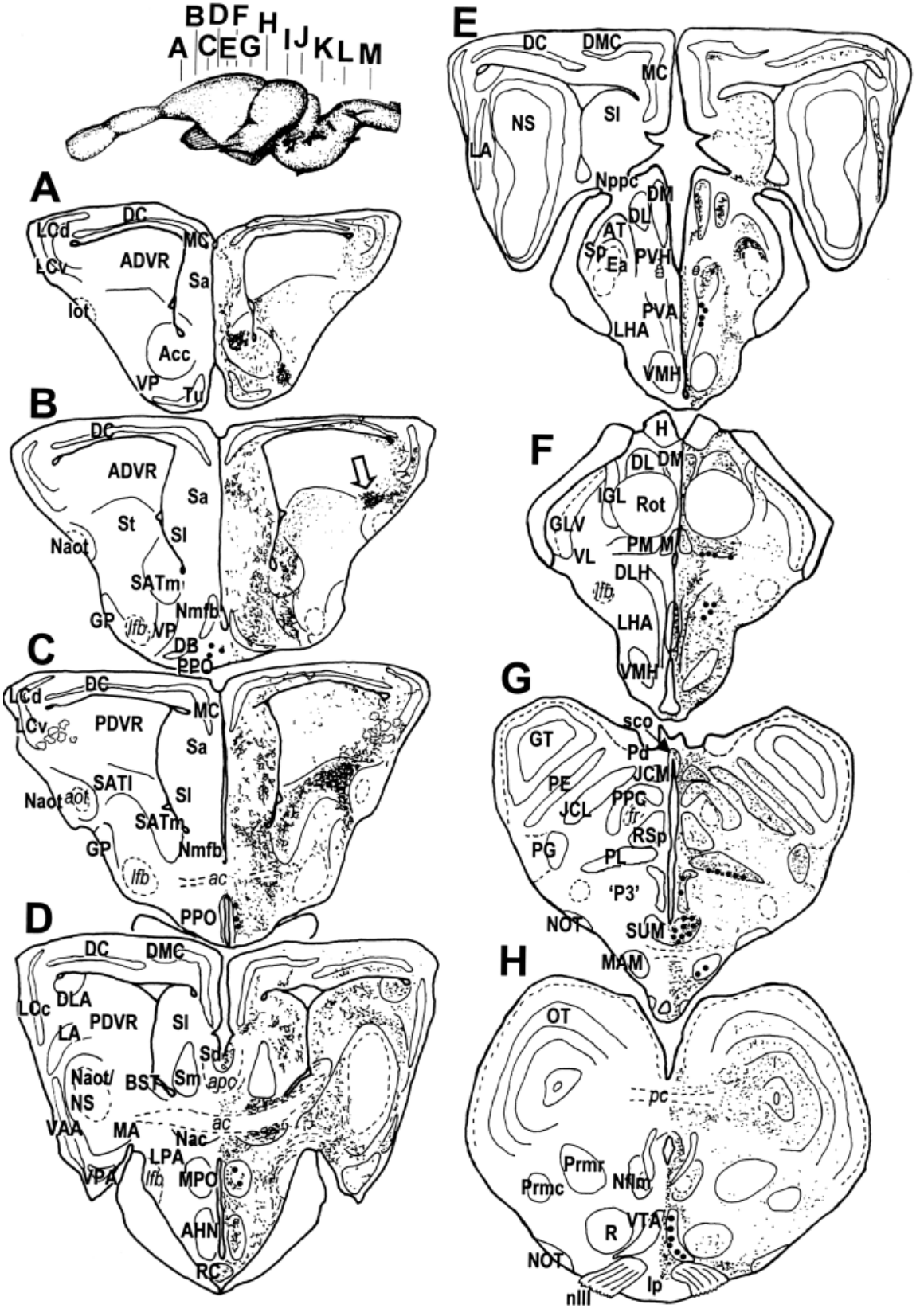
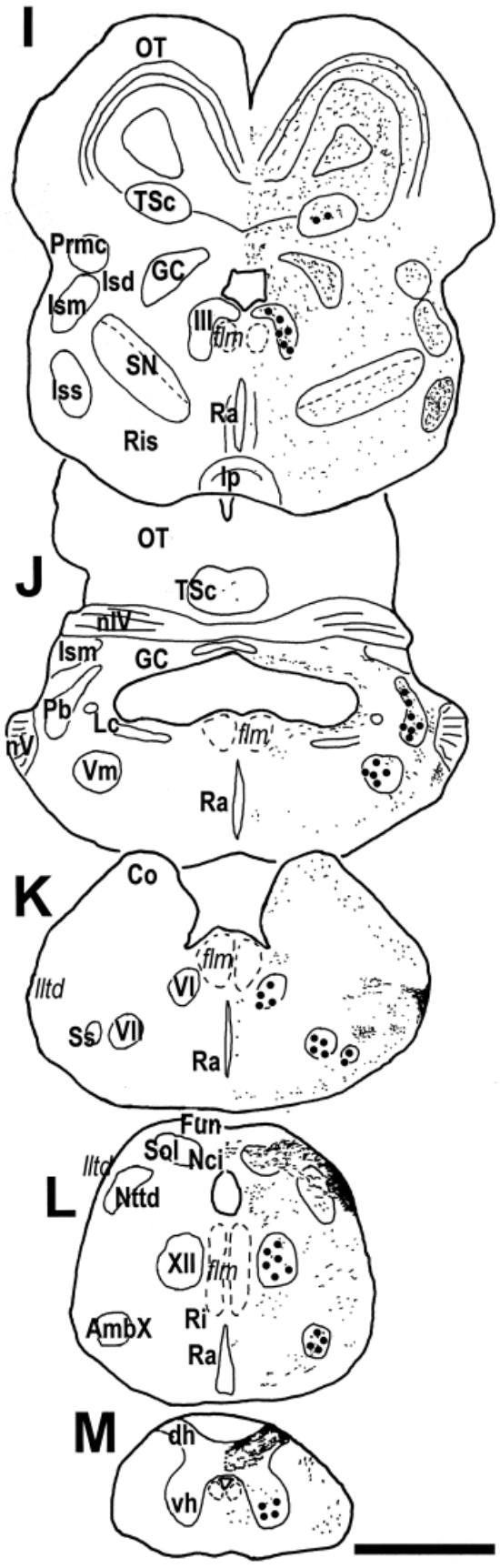


Figure 1 (Continued)



these neurons, which were bipolar cells oriented tangentially to the third ventricle (Fig. 2C). In addition, paraventricular labelled cells were observed ventrally to the caudal levels of the PM and PL in an area that is considered to belong to the basal plate of the third prosomere according to Dávila et al. (2000).

The hypothalamus displayed labelled neurons in four main locations. The most rostral CGRP-li cells were found at preoptic levels (Figs. 1C,D, 2A), mostly in the preoptic periventricular nucleus (PPO) and in the caudal medial preoptic nucleus (MPO). Another group of sparse multipolar cells was visible in the lateral hypothalamic area (LHA) throughout its rostrocaudal axis (Fig. 1F). It should be noted that cell labelling in the MPO and LHA was absent in some animals (this variability was not associated with the colchicine pretreatment). In addition, several faintly CGRP-li cells were located in the anterior periventricular nucleus (PVA; Fig. 1E). In the mammillary hypothalamus, numerous labelled cells appeared in the supramammillary and mammillary nuclei (Figs. 1G, 2D).

The midbrain and brainstem contained numerous CGRP-li cells (Fig. 1G–L). The ventral tegmental area (VTA) showed some intensely labelled cells (Fig. 1H), which apparently showed continuity with the cell group in the supramammillary hypothalamus. In addition, several small and faintly labelled cells were observed in the nucleus centralis of the torus semicircularis (TSc; Fig. 1I). We also observed labelled cell bodies in the parabrachial nucleus (Pb; Figs. 1J, 2F). Labelling was observed in the perikaya of the mesencephalic nucleus of the trigeminal nerve as well, but it is not illustrated, because this nucleus showed labelling also in our preabsorption controls. However, because this labelling has been reported for birds (Lanuza et al., 2000), further research is required to clarify its actual significance.

The remaining CGRP-li cells in the midbrain and brainstem seemed to be motor neurons. Labelling was found in the nuclei of the oculomotor nerves (III, IV, VI; Figs. 1I,K, 2E), including the accessory abducens nucleus. Labelled cells were also visible in the motor nucleus of the trigeminal nerve (Vm; Figs. 1J, 2F) as well as in the motor nuclei of the facial nerve (VII), including the nucleus salivatorius superior (Ss; Fig. 1K). Labelled perikarya were also seen in the nucleus ambiguus (Amb; IX), including its vagal part (AmbX; Fig. 1L), as well as in the nucleus nervi hypoglossi (XII; Fig. 1L). In contrast, no neuronal labelling was observed in the vestibulocochlear neurons. A characteristic feature of motoneurons was the presence of granular labelling restricted to the cell body even in colchicine-treated animals. This kind of labelling was found as well in the cell bodies of the motoneurons in the ventral horn of the spinal cord (Figs. 1M, 2G, 4E).

Fig. 1. **I–M:** Semischematic camera lucida drawing of frontal sections through the brain of *P. hispanica* (A, rostral; M, caudal) showing the distribution of CGRP-li perikarya (solid circles), fibers, and terminals (lines and dots). Arrow in B indicates the patch of CGRP-li fibers at the lateral boundary between the ADVR and the PDVR. The level of each section is indicated on a lateral view of the brain of *Podarcis hispanica*. Scale bar = 500 μm.

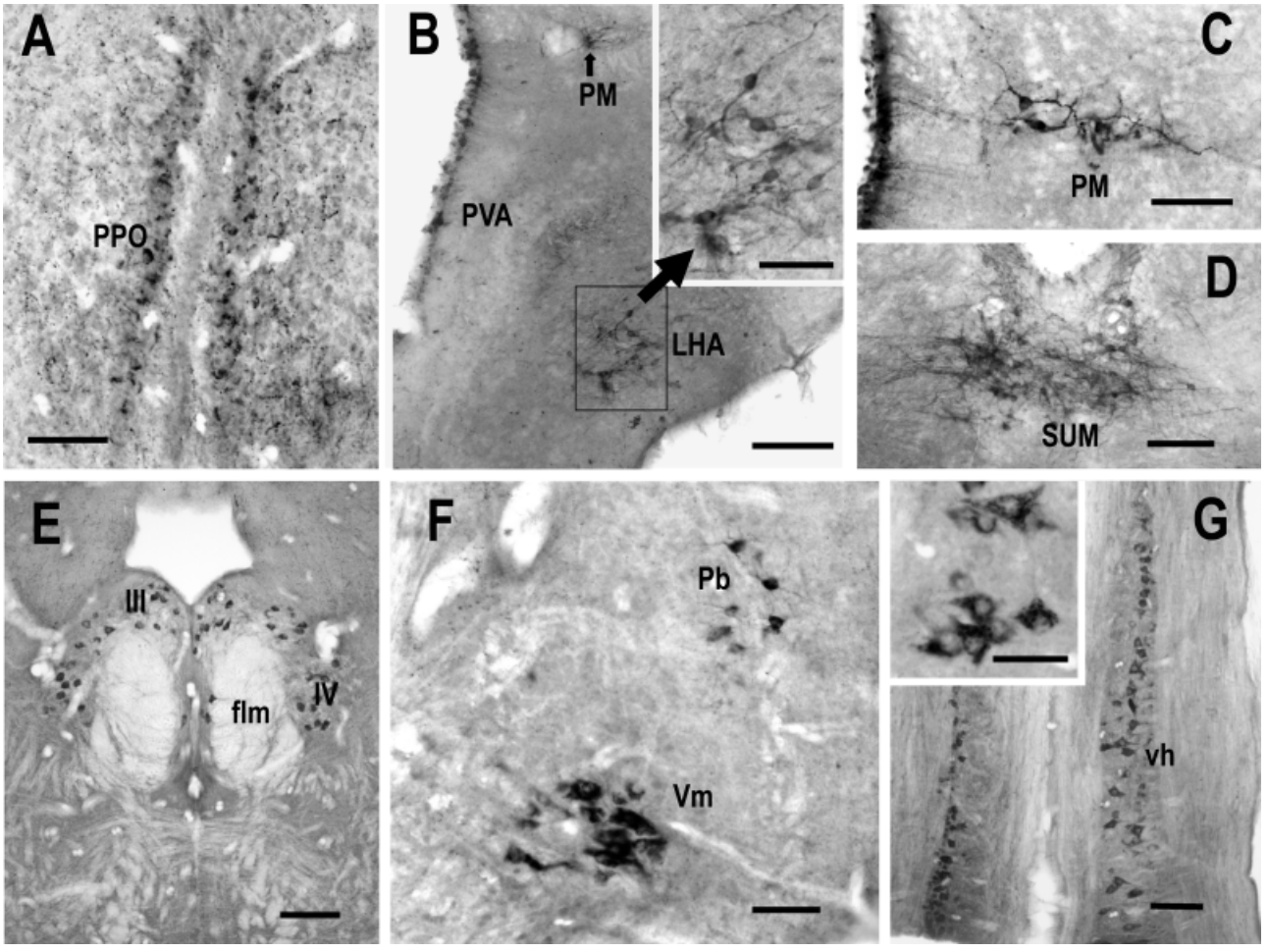


Fig. 2. Distribution of CGRP-li perikarya in the brain of *P. hispanica*. **A:** Low-power photomicrograph of a coronal section through the preoptic hypothalamus, showing CGRP-li perikarya in the preoptic periventricular nucleus (PPO). **B:** Low-power photomicrograph of a coronal section through the intermediate-to-caudal diencephalon of a specimen of *P. hispanica* that was treated with colchicine, showing CGRP-li perikarya in the posteromedial thalamic nucleus (PM; small arrow) and in the lateral hypothalamic area (LHA). The morphology of some labelled cells in the LHA is shown at higher magnification in the **inset**. **C:** High-magnification photomicrograph of CGRP-li cells in PM of a colchicine-treated lizard, showing the bipolar morphology of their dendritic tree. **D:** Photomicrograph showing intensely labelled CGRP-li cells in the supramammillary nucleus. **E:** Photomicrograph illustrating the labelled perikarya in the oculomotor nuclear complex (III). **F:** CGRP-li perikarya in the motor nucleus of the trigeminal nerve (Vm) and in the parabrachial nucleus (Pb). **G:** Photomicrograph of a horizontal section through the spinal cord showing the CGRP-li perikarya located in the ventral horn. A detail of the dense granular labelling typical of motorneurons is shown in the **inset**. Scale bars = 50 μm in A,F, 100 μm in B,D,E,G, 40 μm in C and insets.

CGRP-li fibers

In our material, CGRP-li fibers were present in the forebrain, midbrain, and brainstem (Fig. 1). In every instance, CGRP-li fibers displayed boutons suggesting synaptic contacts.

In the telencephalon, the most rostral CGRP-li fibers were found in the anterior olfactory nucleus, where a few fibers were seen in its medial, ventral, and dorsolateral divisions. Some CGRP-li fibers entered the cortex medially and apparently innervated the inner plexiform and cellular layers of its medial, dorsomedial, and dorsal areas (Fig. 1A,E).

The septum displayed a heterogeneous density of labelled fibers. The lateral (Sl) and anterior septal nuclei (Sa) showed a remarkable population of basket-like figures around unstained somata (Figs. 1A–D, 3D). A number of fibers appeared also in the dorsal septal nucleus (Sd; Fig. 1D) and in the nucleus of the posterior pallial commissure (Nppc; Fig. 1E). Moreover, a moderate density of CGRP-li fibers was visible in the nuclei of the diagonal band (DB) and of the medial forebrain bundle (Nmfb; Fig. 1B,C), which together are thought to constitute the medial septum/diagonal band complex of reptiles (Font et al., 1997).

At the boundary between the anterior (ADVR) and posterior dorsal ventricular ridge (PDVR; see Lanuza et al., 1998, for a cytoarchitectonic description of the limit between these areas), a dense bundle of CGRP-li fibers runs just dorsally to the accessory olfactory tract (arrow in Fig. 1B). From this bundle, fibers seemed to enter the pallial derivatives to innervate portions of the lateral cortex, DVR, and adjacent structures. Noticeably, no CGRP-li

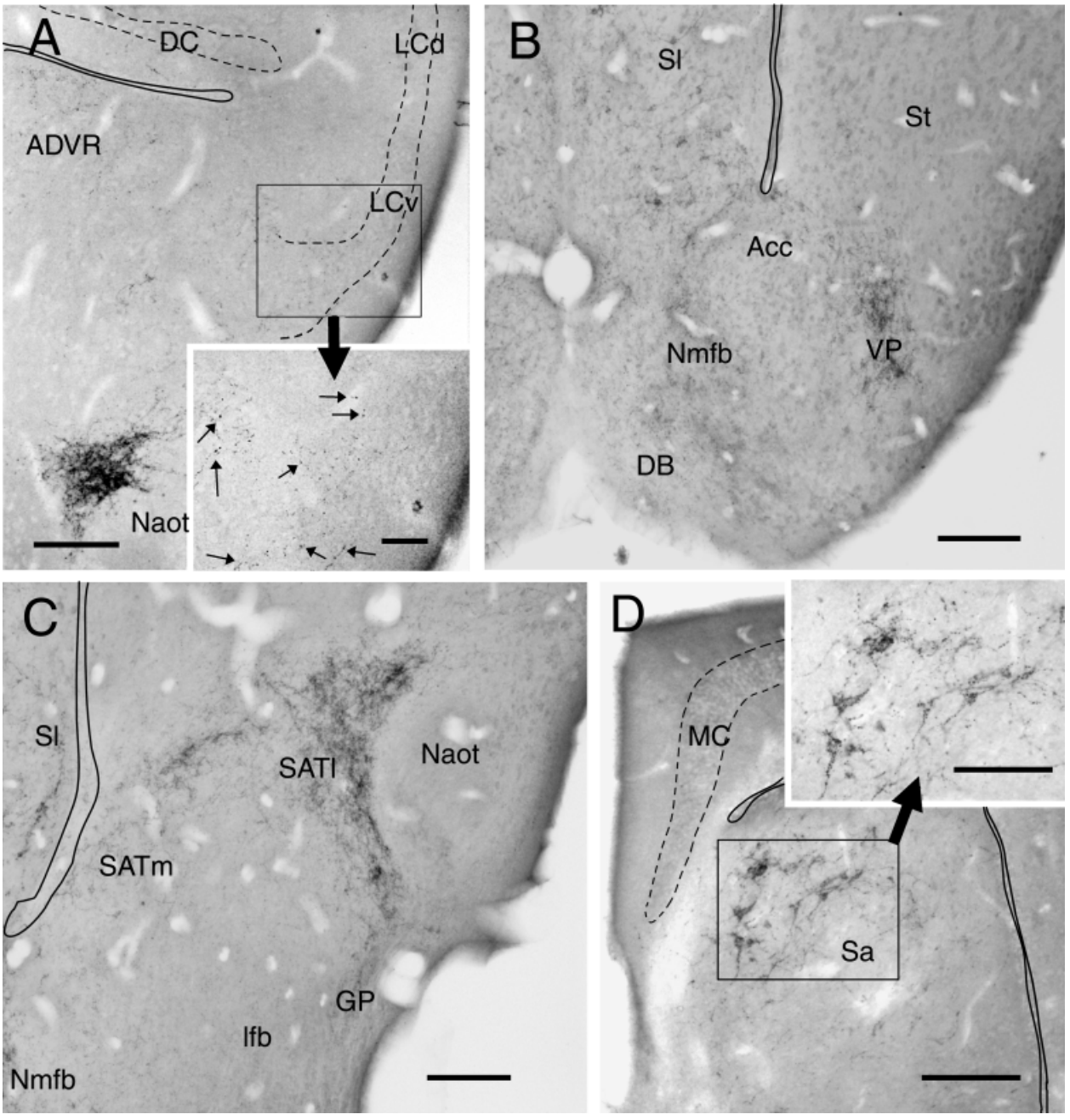


Fig. 3. Photomicrographs illustrating the distribution of CGRP-li fibers in the telencephalon of *P. hispanica*. **A:** Thin CGRP-li fibers and boutons are present in the deep and cell layers of the ventral lateral cortex (arrows in the **inset**). **B:** Immunostaining in the medial basal telencephalon, showing CGRP-li fibers in the caudal pole of the medial nucleus accumbens and in the ventral pallidum. Labelling is also visible in the lateral septum (Sl), where basket-like figures appear, as well as in the diagonal band nucleus (DB) and bed nucleus of the medial forebrain bundle (Nmfb). **C:** Photomicrograph of an immunostained section through the striatoamygdaloid transition area showing the dense CGRP-li innervation of the lateral SAT (SATl) and the relatively lower density of labelling in the medial SAT (SATm). Labelling is also visible in the caudal aspect of the Nmfb as well as in the dorsal aspect of the globus pallidus (GP). The basket-like innervation of the lateral septum (Sl) is also visible. **D:** In the septum, most of the CGRP-li immunoreactive form basket-like figures wrapping immunonegative cell bodies, as shown in detail in the **inset**. Scale bars = 100 μm in A–D, 25 μm in insets.

fibers appeared in the pallial thickening (not shown in Fig. 1; see Kenigfest et al., 1997, for the hodological identification of this structure in *P. hispanica*). Immunoreactive fibers were found in the ventral lateral cortex (LCv), where they gave rise to a moderate innervation of the cell layer and deep stratum (Figs. 1A–C, 3A). In the ADVR, CGRP-li fibers were observed only in its most caudolateral aspect, where innervation was present mainly in the jux-

taventricular layer (Figs. 1B, 3A). With regard to the PDVR, a relatively dense innervation by CGRP-li fibers was observed in its core and around the cell bodies of the most lateral cell clusters (Fig. 1C,D). The plexus of labelled fibers in the core of the PDVR extended into the lateral nucleus of the amygdala (LA). The dorsolateral amygdaloid nucleus (DLA) also showed a moderate-to-low density of thick CGRP-li fibers (Fig. 1D).

The densest plexus of reactive fibers within the amygdala was found just medially to the nucleus of the accessory olfactory tract (Naot) in an area that we have tentatively classified as lateral striatoamygdaloid transition area (SATl; Figs. 1C, 3C). From this dense reactive plexus, fibers entered the medial (MA), ventral anterior (VAA), and ventral posterior (VPA) amygdaloid nuclei (Fig. 1D).

A CGRP-li terminal field rich in basket-like figures was present medially and ventromedially to the SATl in an area that seems to correspond with the striatoamygdaloid transition area as defined originally by Russchen and Jonker (1988) in the gecko. We have named this area the "medial striatoamygdaloid transition area" (SATm; Figs. 1B,C, 3C).

In the basal ganglia, CGRP-li fibers were observed in the nucleus accumbens (Acc; Figs. 1A, 3B), especially in its medial portion. Dense fiber labelling was also observed in the lateral ventral pallidum (Fig. 3B), where some basket-like figures were present. The medial aspect of the dorsal striatum displayed a few CGRP-li fibers, whereas the lateral striatum was virtually unlabelled (Fig. 1A,B). Caudally, we observed some labelled fibers in an area of the lateral basal telencephalon, just lateral to the lateral forebrain bundle, an area that seems to correspond to part of the globus pallidus (GP) as defined by Russchen et al. (1987) in the gecko (Fig. 1C).

In the thalamus, labelled fibers were found in the eminentia thalami and surrounding the subfornical organ. More caudally, CGRP-li fibers appeared in the dorsal tier of the dorsal thalamus (Dávila et al., 2000), where they were heterogeneously distributed (Fig. 1E,F). The dorsomedial anterior thalamic nucleus (DM) showed CGRP-li innervation mainly in its medial aspect at rostral levels (Fig. 1E), whereas labelling was homogeneous at caudal levels (Fig. 1F). The dorsolateral anterior thalamic nucleus (DL) showed a moderate CGRP-li innervation centered in the core of the nucleus (Fig. 1F,G). A moderate CGRP-li innervation was also found in the PM and PL thalamic nuclei (Fig. 1E,F). In contrast, the nucleus rotundus displayed no immunoreactive fibers, whereas other nuclei of the dorsal thalamus, namely, the dorsal lateral geniculate nucleus, the medial thalamic nucleus, and the intergeniculate leaflet showed just a few scattered fibers (Fig. 1E,F).

With few exceptions, the nuclei belonging to the ventral thalamus were virtually devoid of fiber labelling. Thus, no reactive fibers were visible in the ventral lateral geniculate nucleus (GLV) or the nucleus ovalis, and only a few fibers were present in the area triangularis and in the rostral aspect of the ventral part of the ventrolateral thalamic nucleus (VL). However, a moderate density of labelled fibers was observed in the suprapeduncular nucleus (Sp; Fig. 1E).

The hypothalamus displayed CGRP-li fibers throughout its rostrocaudal axis (Fig. 1C–G). As a rule, labelled fibers were more abundant in the periventricular compartment than in the medial and lateral compartments. In the preoptic hypothalamus, CGRP-li fibers were very dense in the preoptic paraventricular nucleus (PPO) and less dense in the medial preoptic nucleus (MPO; Fig. 1C,D). Another relatively dense plexus of reactive fibers occupied the lateral preoptic area (LPO). The anterior hypothalamus showed a dense innervation of the periventricular hypothalamus (Fig. 1E). The anterior and retrochiasmatic (RC) nuclei also showed a moderate density of CGRP-li fibers. Finally, a few labelled fibers were found in the LHA, intermingled with the fibers of the medial forebrain bundle. The most remarkable features of the CGRP immunoreactivity in the tuberal hypothalamus (Fig. 1F) were the dense innervation of the periventricular hypothalamus, including the dorsal hypothalamic area, and the virtual absence of labelling in the ventromedial hypothalamic nucleus (VMH), where only a few reactive puncta were observed. A few fibers surrounded laterally the VMH and provided a faint innervation of the ventral LHA. At caudal diencephalic levels, a profuse innervation of both the supramammillary (SUM) and mammillary (MAM) nuclei (Fig. 1G) was observed. Fibers entered the infundibular recess, where they developed an intricate arborization that was denser next to the juxtaventricular cells.

In the pretectum, a moderate network of CGRP-li fibers was visible just dorsal to the posterior commissure in the posterodorsal nucleus (Pd), and a few fibers extended within the boundaries of the external pretectal nucleus (PE; Fig. 1G). Ventrally to the posterior commissure, a dense field of CGRP-li fibers was found medially in the vicinity of the subcommissural organ, in what probably constitutes the pretectal periventricular gray and in the principal precommissural nucleus (PPC) as well as in the medial (JCM) and lateral juxtacommissural pretectal (JCL) nuclei (Fig. 1G). More laterally, CGRP-li labelling is found in the cell plate of the pretectal geniculate nucleus (PG).

Several centers of the dorsal midbrain displayed CGRP-li fibers (Fig. 1H,I). A moderate density of immunoreactive fibers was observed in the inner layers (2–6) of the midbrain tectum (OT; Fig. 4A), in the juxtaventricular layer of the nucleus laminaris of the torus semicircularis, and with a lower density in its central nucleus (TSc; Fig. 1H,I). More caudally, a high density of reactive fibers was visible in the midbrain central gray (GC; Fig. 1I). In the tegmentum, a high density of CGRP-li fibers was found among the reactive somata of the VTA (Fig. 1H) and a less dense innervation in the reptilian aminergic cell group 8 (RA8) and substantia nigra pars compacta (Fig. 1I). A low density of reactive fibers was visible in the interpeduncular nucleus (especially in its ventral part) and the midbrain raphe nucleus (Fig. 1I).

The isthmic region displayed a nonhomogeneous distribution of CGRP-li fibers. The nucleus isthmi semilunaris showed a dense innervation (Figs. 1I, 4B). The nucleus isthmi diffusus displayed a lower density of reactive fibers, and the remaining isthmic nuclei were apparently devoid of labelling. A dense CGRP-li innervation was found in the parabrachial region, where labelling was moderate in the putative locus coeruleus and dense in the caudal aspect of the central gray (GC; Fig. 1J).

The cerebellum displayed CGRP-li fibers and terminals distributed throughout the granular layer. In the brainstem, a few reactive fibers were visible in the superior raphe nucleus (Fig. 1J). Moreover, a sparse and nonhomogeneous innervation of the reticular formation was ob-

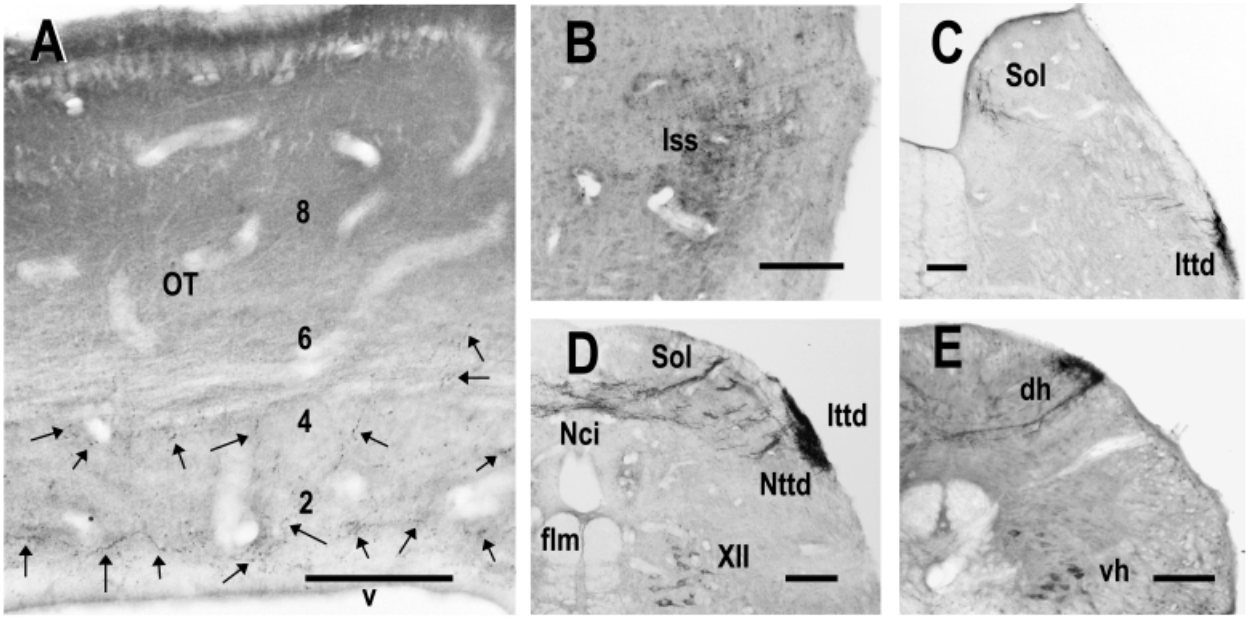


Fig. 4. Photomicrographs illustrating the distribution of CGRP-li fibers in the midbrain, brainstem, and spinal cord of *P. hispanica*. **A:** Detail of the CGRP-li innervation of the midbrain tectum (OT), which is restricted to the deep layers (arrows). **B:** Detail of the CGRP-li innervation of the nucleus isthmicus semilunaris (Iss). **C:** Photomicrograph of a transverse section of the rostral brainstem showing the CGRP-li fiber labelling in the lateral descending trigeminal tract (lttd) and the descending nucleus of the trigeminal nerve (Nttd) as well as in the nucleus of the solitary tract (Sol). **D:** CGRP-li fiber labelling in the descending nucleus of the trigeminal nerve (Nttd) and nucleus of the solitary tract (Sol), including the nucleus of the commisura infima (Nci). Immunoreactive perikarya are also visible in the nucleus of the nervus hipoglossus (XII). **E:** Photomicrograph of a transverse section of the spinal cord showing the CGRP-li fibers in the outer layers of the dorsal horn (dh). CGRP-li motorneurons are visible in the ventral horn (vh). Scale bars = 100 μm.

served, fibers being found mainly in the inferior reticular nucleus (Fig. 1L).

Numerous CGRP-li fibers were located in some of the sensory nuclei of the cranial nerves. In the trigeminal system (Fig. 1J), reactive fibers were scarce, when present, in the principal sensory trigeminal nucleus (not shown). Conversely, a high density of reactive fibers was visible in the lateral descending trigeminal tract (lttd; Fig. 4C), which apparently terminated in the nucleus of the descending trigeminal tract, especially at its most caudal levels (Figs. 1L, 4D). At these levels, CGRP-li fibers also entered the brain from the vagal nerve, which gave rise to a conspicuous terminal field in the nucleus tractus solitarii (Sol) and in the nucleus of the commissura infima (Figs. 1L, 4D).

The spinal cord showed a dense meshwork of CGRP fibers capping dorsally the dorsal horn (Figs. 1M, 4E). From this tract, a few labelled fibers entered the dorsal horn, and some of them reached and even crossed the midline. Although in the cervical spinal cord part of this innervation might arise from the trigeminal system (Barbas-Henry and Lohman, 1986), the presence of this pattern of immunoreactivity at thoracic levels indicates that some of the CGRP-li fibers enter the central nervous system through the spinal nerves.

DISCUSSION

The results obtained confirm the available information about the distribution of CGRP in the thalamus (Brauth and Reiner, 1991; Pritz and Stritzel, 1991) and the spinal cord (Luthman et al., 1991) of reptiles and extends it by showing that CGRP is present in numerous neural systems in the lacertilian brain. Some hypothalamic areas, such as the medial preoptic nucleus and the lateral hypothalamic area, did not display CGRP-li cells in every specimen. When present, immunolabelling of these cells was relatively faint, and this variability might be due to a low concentration of CGRP within the cell bodies, near the minimum detectable by immunocytochemistry, and/or to small variations in this concentration as a result of changes in the turnover or production of the peptide (e.g., changes in synaptic activity). In fact, there is evidence in mammals indicating that the expression of CGRP depends on the levels of sexual steroids (Yang et al., 1998) and can be altered by experimental axotomy (Fukuoka et al., 1997, 1999).

Immunocytochemistry yielded different types of reaction product: granular, usually faint, that consistently appeared in the motor nuclei of the cranial nerves (e.g., Fig. 2E) and homogeneous, variable in intensity, that was consistently observed in the rest of the labelled cell bodies (e.g., labelling in the mammillary bodies; Fig. 2D). These two types of CGRP-immunoreactive labelling, with a very similar distribution, were also observed in the avian brain (Lanuza et al., 2000). The reason for these differences might simply be a lower concentration of CGRP in nuclei where the labelling is faint and granular compared with nuclei where the labelling is dense and homogeneous. An alternative explanation might be the presence of several

species of reptilian CGRP, as is the case in rats and humans (Morris et al., 1984; Amara et al., 1985; Steenbergh et al., 1985), for which the primary antibodies may display different affinities.

Nontelencephalic CGRP-containing systems in reptiles

In lizards, CGRP seems to be found in a subpopulation of primary somatosensory neurons, given that CGRP-li fibers enter the central nervous system through the spinal, vagal, and trigeminal nerves. However, within these nerves, only a portion of the fibers appeared to be CGRP-li; for instance, reactive fibers are very scarce (if present) within the dorsal column and, as a consequence, in the dorsal column nuclei. In the same way, there is but a mild CGRP innervation of the nucleus of the solitary tract, and the principal sensory nucleus of the trigeminal nerve is virtually devoid of labelling. In fact, most of the CGRP-li fibers in the trigeminal system take a descending course through the lttd (Fig. 4D).

A second conclusion that can be drawn from our results is that CGRP is found in motorneurons contributing to both the spinal (Fig. 4E) and the cranial nerves. For instance, CGRP cells are present in the nuclei of the III, IV, and VI nerves as well as in the motor nuclei of the trigeminal and facial nerves; the nucleus ambiguus, including its vagal portion; and the nucleus hypoglossus. Vestibulocochlear motorneurons were not labelled in *P. hispanica*, in contrast to what has been described for mammals (Kawai et al., 1985).

Kawai et al. (1985) suggested that CGRP-li neurons are present in the somatomotor and branchiomotor but not in the visceromotor nuclei. However, this is not the case for birds (Lanuza et al., 2000), and our data indicate that in lizards the vagal part of the nucleus ambiguus (Fig. 1L) as well as the superior salivatory nucleus (Fig. 1K) display CGRP-li. In any case, our findings suggest that CGRP coexists with acetylcholine in some motor and premotor neurons (Medina et al., 1993). This supports the findings by Takami et al. (1985) indicating that CGRP may play a role in regulation of striated muscular activity but suggest a more general action in the modulation of peripheral cholinergic synapses.

CGRP-immunoreactive ascending pathways to the reptilian telencephalon

CGRP-li cells were not seen in the telencephalon of *P. hispanica* even in colchicine-treated animals, so the CGRP innervation of the telencephalon probably has an extratelencephalic origin. As we discuss in this section, likely candidates from which these CGRPergic telencephalic projections originate are the parabrachial nucleus, the thalamic PM and PL nuclei, the caudal hypothalamus, and the VTA.

The parabrachial nucleus has been shown to project to the telencephalon in several reptiles (ten Donkelaar et al., 1987), including areas that display CGRP-li fibers such as the PDVR (Lanuza et al., 1998) and the nucleus accumbens/SAT (Siemen and Künzle, 1994; Pérez-Santana et al., 1997). Several studies in the rat (Shimada et al., 1985a; Schwaber et al., 1988; Yamamo et al., 1988; Yasui et al., 1989) have convincingly demonstrated that CGRP-li neurons in the parabrachial nucleus project to the telencephalon. These results suggest that reptiles, like mammals, possess a CGRPergic component within the parabrachial-telencephalic projection.

In the thalamus, CGRP-li cells were located in the ventral aspect of the PM and PL nuclei (Figs. 1 F,G, 2C). Both nuclei have been shown to project to the telencephalon (Bruce and Butler, 1984; González et al., 1990; Lanuza et al., 1998), including the striatum and DVR. According to Bruce and Butler (1984), the PM and PL together constitute the thalamic somatosensory relay to the DVR. However, only the caudal part of the PM-PL target in the DVR shows CGRP-li fibers (Fig. 1D), suggesting that within the PM-PL CGRP-li cells constitute a subpopulation of cells giving rise to the thalamo-PDVR projection.

Brauth and Reiner (1991) observed that CGRP is present in the thalamotelencephalic auditory pathways in representatives of all amniote vertebrate classes. However, we have observed labelled neurons not in the medial thalamic nucleus of *Podarcis* (homologue to the nucleus reuniens of crocodilians and turtles) but in the ventral aspect of the PM and PL thalamic nuclei. In fact, the thalamic CGRP-li cells observed by Brauth and Reiner (1991) in turtles and crocodilians displayed a similar location, just ventrolateral to nucleus reuniens. On the other hand, in *Podarcis*, CGRP-li fibers were absent in the medial ADVR (which receives the projection from the medial thalamic nucleus) but present in the PDVR and striatum. Therefore, our results indicate that, in lizards, CGRP may be present not in the thalamo-DVR auditory projection but in a multimodal thalamostriatal and thalamoamygdaloid projection.

As discussed by Lanuza et al. (1998), there is evidence indicating that the CGRP-li cell groups (Brauth and Reiner, 1991) in the thalamus of crocodiles (Braford, 1972; Pritz and Stritzel, 1991) and turtles (see Belekhova and Chkheidze, 1992) display a multimodal rather than a pure auditory nature and are, therefore, comparable with the PM-PL of lizards. If this is so, their projection to the DVR would be comparable with the projection from the CGRP-li cell group in the PM-PL to the PDVR of *Podarcis*.

The PM also projects to other telencephalic areas displaying CGRP-li innervation (Lanuza et al., 1998), such as the medial SAT and an area lateral to it and medial to the Naot, identified in this work as lateral SAT (Fig. 3C). The projection to the medial SAT apparently extends rostrally up to the caudal levels of the nucleus accumbens, overlapping with the CGRP-li fiber system in the basal ganglia (Fig. 3B). This fact suggests that the thalamic CGRP-li cell group might contribute to the innervation not only of the PDVR but also of the SAT-Acc continuum. The projection to the lateral SAT also extends rostrally to reach the lateral ADVR and the lateral cortex (Fig. 3A). The origin of this CGRP-li projection is unknown, but, because the nucleus rotundus is clearly immunonegative, the possibility arises that the PM-PL immunoreactive cells give rise to this projection too.

In addition to the dorsal thalamus and parabrachial region, the caudal hypothalamus and VTA may also contribute to the CGRP-li innervation of the telencephalon. Indirect evidence suggests that the CGRP might coexist with dopamine in the VTA projection to the Acc/SAT and septum. On the one hand, reptiles show dopaminergic cell bodies in the VTA and in some species in the mammillary hypothalamus (Smeets, 1994; our unpublished results in *Podarcis*). On the other hand, the Acc/SAT and septum display a dense dopaminergic innervation, which, in the

septum, terminates in the form of perisomatic nests (Font et al., 1995) with a distribution similar to that of the CGRPergic ones.

In all reptilian species studied to date (Bruce and Butler, 1984; Martínez-García and Olucha, 1988), the hypothalamic and tegmental cell groups showing CGRP-li cells project not only to the Acc/SAT and septum but also to the cerebral cortex. This suggests that the caudal hypothalamus and/or VTA might be the source of the sparse CGRP-li innervation of the medial and dorsal cortices. These hypotheses should be confirmed by means of appropriate double-labelling experiments.

CGRP and the comparative neuroanatomy of the forebrain

Our study constitutes the first comprehensive description of the distribution of CGRP in the brain of a reptile, and, consequently, our results have important implications for understanding the evolution of the vertebrate brain. Because stem reptiles gave rise to modern reptiles, birds, and mammals, our work, together with previous studies on the distribution of CGRP in the brain of mammals (Gibson et al., 1984; Kawai et al., 1985; Shimada et al., 1985a,b; Skofitsch and Jacobowitz, 1985; Harmann et al., 1988; Kruger et al., 1988a,b; Sugimoto et al., 1988; Yasui et al., 1989, 1991), birds (Lanuza et al., 2000), and amphibians (Petkó and Sánta, 1992), allows for an analysis of the evolutionary history of CGRPergic systems in tetrapodian vertebrates.

All of the tetrapod species studied thus far (see references above) display a similar distribution of CGRP-li cell bodies that includes the motor neurons of the cranial nerves, the parabrachial area, and a cell group in the ventral aspect of the caudal dorsal thalamus. These data indicate that these CGRPergic systems were probably present in the first amphibians and have been well conserved during the evolution of modern tetrapods.

This analysis strongly suggests that the CGRP-li cell groups of the dorsal thalamus of reptiles, birds, and mammals are homologous and that they were already present in anamniotes, in that a CGRP-li cell group has been reported in the dorsal thalamus of amphibians (Petkó and Sánta, 1992). In the thalamus of mammals, CGRP-li neurons are found in the ventral aspect of the posterior intralaminar complex. This includes the subparafascicular, posterior intralaminar, peripeduncular, posterior limitans, and suprageniculate nuclei (Kawai et al., 1985; Skofitsch and Jacobowitz, 1985; Kruger et al., 1988a,b; Sugimoto et al., 1988; Yasui et al., 1989, 1991). On the other hand, in the avian thalamus, CGRP-li neurons have been found in the shell of the nucleus ovoidalis, nucleus semilunaris paraovoidalis, nucleus dorsolateralis posterior thalami, nucleus dorsointermedius posterior thalami, and nucleus paramedianus internus thalami (Brauth and Reiner, 1991; Bottjer et al., 1997; Lanuza et al., 2000).

Therefore, the PM and PL of lizards would include CGRP-li cell groups homologous to those present in several nuclei of the dorsal thalamus of birds (ovoidalis, semilunaris paraovoidalis, dorsolateralis posterior, dorsointermedius posterior, and paramedianus internus) and to (at least parts of) the posterior intralaminar complex of the thalamus of mammals. This hypothesis is consistent with previous homologies based on hodological (Bruce and Neary, 1995) and neurochemical (Dávila et al., 2000) data. In addition to the presence of CGRP-li neurons and its topographical location within the ventral aspect of the caudal dorsal thalamus, these thalamic nuclei of reptiles, mammals, and birds also share a multimodal nature because of the convergence of a variety of afferents displaying diverse sensory significance.

As discussed above and analyzed in detail by Lanuza et al. (1998), multimodal convergence appears to be common to the CGRP-li cell groups in the thalamus of all reptiles. In mammals, the posterior intralaminar thalamic complex receives auditory projections from the inferior colliculus, somatosensory (probably nociceptive) projections from the spinal cord (LeDoux et al., 1987), visceroceptive afferents from the parabrachial area (Saper and Loewy, 1980; Yasui et al., 1989), and both visual and nonvisual afferents from the deep layers of the superior colliculus (Yamasaki et al., 1986; Linke et al., 1999). In birds, connectional and physiological data indicate that cells in those nuclei displaying CGRP-li perikarya (shell of nucleus ovoidalis, subrotundal-paraovoidal region, and nucleus dorsolateralis posterior; Lanuza et al., 2000) probably convey a mixture of auditory, visual, somatosensory (including visceroceptive), and gustatory information (Cotter, 1976; Gamlin and Cohen, 1986; Funke, 1989; Wild, 1989; Korzeniewska and Güntürkün, 1990; Wild et al., 1990; Durand et al., 1992).

Another feature shared by the CGRP-li cell groups in the thalamus of reptiles, birds, and mammals is a similar set of ascending projections to the subventricular telencephalon, including pallial and subpallial centers (see discussion below). In conclusion, different lines of evidence strongly support the homology of the PM-PL of lizards with the posterior intralaminar complex of the mammalian thalamus and the paraovoidal-subrotundal-dorsolateral posterior thalamus of birds.

In mammals, CGRP-li cells are found in several nuclei from the midline (just medial to the parafascicular nucleus) to the lateral border of the caudal thalamus (peripeduncular nucleus; Kawai et al., 1985; Skofitsch and Jacobowitz, 1985). Studies of the development of the rat dorsal thalamus (Puelles et al., 1992) using calbindin as a neurochemical marker indicate that all the thalamic nuclei displaying CGRP-li cells share a common ontogenetic origin, thus making up a single anatomical unit. In the avian thalamus, CGRP-li cells are also distributed in several nuclei, including medial (periovoidal shell) and lateral (dorsolateral posterior and semilunaris paraovoidalis; Lanuza et al., 2000) ones. Crocodiles, which are close relatives of birds, also display a certain lateral expansion of the CGRP-li cell group. According to Brauth and Reiner (1991), CGRP-li somata are found not only in the nucleus reuniens and diagonalis but also within the boundaries of the nucleus rotundus. Because lizards (this work) and turtles (Brauth and Reiner, 1991) do not show such a strong lateral migration, this lateral expansion of the CGRP-li cell group in the thalamus may have occurred independently in mammals and the evolutionary line leading to modern crocodiles and birds.

Our work has revealed the presence of CGRP-li cells in the mammillary hypothalamus (Fig. 2D) and VTA (Fig. 1H), which may project to the telencephalon, including the septum and nucleus accumbens. The septum and nucleus accumbens of mammals (Skofitsch and Jacobowitz, 1985) and birds (Lanuza et al., 2000) display a dense CGRP-li innervation, with a termination pattern similar to that observed in reptiles (e.g., septal perisomatic baskets; Fig.

3D). Lesion studies in rats (Sakanaka et al., 1985) suggest that septal CGRP-li fibers arise from the anterior hypothalamus. Nevertheless, the VTA of rats displays a few CGRP-li cells (van Rossum et al., 1997), whose ascending fibers probably course in the medial forebrain bundle through the anterior hypothalamus. This fact raises the possibility that in mammals, as probably happens in reptiles, the VTA also contributes to the CGRPergic innervation of the telencephalon.

In any case, CGRP-li cells are present in the mammillary and supramammillary nuclei of lizards (similar results have been observed in the geckonidae *Tarentola mauritanica*; our unpublished results) but have not been reported in other vertebrates. Although at present this seems to be a specific feature of lizards, studies of the distribution of CGRP in the brains of other reptiles and amphibians are required to clarify the comparative significance of these differences.

CGRPergic innervation of the claustroamygdaloid complex of amniote vertebrates

The densest termination area in the reptilian telencephalon is found in the lateral striatoamygdaloid transition area (Fig. 3C). From this dense field of fiber labelling, immunoreactive fibers extend dorsally to enter pallial telencephalic regions such as the PDVR (Fig. 1D) and the ventral and deep lateral cortex in the rostral telencephalon (Fig. 1A,B). This situation is reminiscent of the condition found in both mammals (Yasui et al., 1989, 1991) and birds (Lanuza et al., 2000).

In mammals, the densest plexus of CGRP-li fibers of the telencephalon is present in the amygdalostriatal transition area and lateral (but also medial) portion of the central amygdaloid nucleus (Yasui et al., 1991). Other parts of the amygdala, including the centromedial, basomedial, and lateral amygdala, display a relatively weaker innervation. Finally, a conspicuous CGRPergic innervation is found in the deep perirhinal and insular cortices, including parts of the claustrum. Double-labelling experiments in the rat have demonstrated that the CGRPergic innervation of the amygdala and its transition to the striatum, as well as to the perirhinal and insular cortices, arises from the posterior intralaminar thalamus and parabrachial area (Yasui et al., 1989, 1991). Therefore, these patterns of CGRP innervation, including their thalamic and parabrachial origins, support the theory that the lacertilian SATl is homologous to the mammalian lateral central amygdala and amygdalostriatal transition area. Russchen and Jonker (1988) studied the projections of the basal ganglia of the gecko and concluded that the transition between the medial striatum and the medioventral aspect of the amygdala, which they called *SAT*, constitutes the reptilian counterpart of the mammalian central amygdala. Hodological studies in other lizards (*Podarcis hispanica*; Lanuza et al., 1997) and turtles (Siemen and Künzle, 1994) strongly support this homology. Our data on the distribution of CGRP allow a distinction to be made between a medial and a lateral SAT, the latter displaying a much higher density of CGRP-li fibers. The medial SAT might constitute the reptilian homologue of the medial aspect of the central amygdala.

Our results also indicate that there is a network of CGRP-li fibers connecting the SAT with the nucleus accumbens. This is reminiscent of the condition present in mammals (Kawai et al., 1985; Skofitsch and Jacobowitz, 1985), where a band of CGRP-li fibers in the basal telencephalon, including the dorsal division of the bed nucleus of the stria terminalis, connects the shell of the nucleus accumbens with the dense terminal field of the central amygdala. This pattern of reactivity matches the so-called extended amygdala (see Alheid et al., 1995). Therefore, in the basal telencephalon of reptiles, the continuum of structures made up by the nucleus accumbens rostrally, the SAT caudally, and the intermediate area joining both nuclei might be considered as the reptilian extended amygdala, as suggested by both its location and its pattern of CGRP immunoreactivity. More studies are needed to check this hypothesis, although our preliminary results on the connections of this area (Martínez-García et al., 1993; Novejarque et al., 2000) and similar studies in other reptiles (Russchen and Jonker, 1988; González et al., 1990; Siemen and Künzle, 1994) lend support to this view.

From the dense terminal field in the lateral SAT, CGRP-li fibers run dorsally to enter the DVR and a few adjoining structures, namely, the lateral and dorsolateral amygdaloid nuclei and the ventral and deep lateral cortex. In mammals, pallial CGRP-li innervation is restricted to parts of the basolateral amygdaloid complex (which includes its basal, accessory basal, and lateral nuclei) and to the deep insular and perirhinal cortices, including the claustrum (Yasui et al., 1989, 1991). Therefore, our results concerning CGRP immunoreactivity in the telencephalon of *Podarcis* are consistent with the idea that the caudal aspect of the DVR (including the PDVR and the lateral and dorsolateral amygdaloid nuclei) and the deep LCv might include the reptilian counterparts of the mammalian basolateral amygdaloid complex and claustrum, respectively. This view again fits previous hypotheses based on connectional studies in different reptiles (Lanuza et al., 1998). For instance, the PDVR and the lateral and dorsolateral amygdaloid nuclei, like the mammalian basolateral amygdaloid complex (Yasui et al., 1991; see Price, 1995), constitute the target for a multimodal thalamic projection arising from the medial (dorsomedial anterior thalamus) and caudoventral dorsal thalamus (PM-PL), which, according to our results, includes a CGRP-li component. In addition, as with parts of the mammalian basolateral amygdaloid complex (Price et al., 1991; Petrovich et al., 1996), parts of the PDVR and LA of lizards project to the ventromedial hypothalamus through the stria terminalis (Bruce and Neary, 1995; Lanuza et al., 1997).

The PDVR and LA of lizards receive a convergent input from the three sensory areas of the anterior DVR (Andreu et al., 1996; Lanuza et al., 1998). Similarly, the amygdala of mammals receives a multimodal input from the temporal (associative) neocortex. Data on developmental gene expression in the mammalian and reptilian cerebral hemispheres (Smith-Fernandez et al., 1998) indicate that the reptilian DVR and the mammalian neocortex are not homologous structures. However, because both the reptilian ADVR and the mammalian associative cortex provide a highly processed multimodal input to the amygdala, these two structures seem to be functionally analogous.

Other parts of the reptilian amygdala, such as its olfactory (ventral anterior amygdala) and vomeronasal (medial amygdala and nucleus sphericus) divisions, as in their mammalian counterparts (namely, the cortical anterior and posterior amygdalae and the medial amygdaloid nu-

cleus; Price et al., 1987), also display a relatively weak CGRP-li innervation (Yasui et al., 1991).

Our results may also be helpful for making comparisons between the reptilian and the avian DVR. As described by Lanuza et al. (2000), CGRP-li fibers are very scarce (if present) in the primary sensory areas (ectostriatum, nucleus basalis, and field L2) of the chicken and quail DVR. Conversely, a dense innervation is found in the intermediate neostriatum from which fibers extend into parts of the ventral hyperstriatum and of the caudal neostriatum. These areas are thought to constitute the associative DVR of birds (Rehkamper and Zilles, 1991; Metzger et al., 1998). This is very similar to the situation in reptiles, in which CGRP-li fibers are generally absent in the primary sensory regions of the ADVR (Fig. 1A,B) but densely innervate the ventral lateral cortex, PDVR, and adjoining areas. The latter apparently constitute the associative DVR of reptiles (Belekhova and Chkheidze, 1991, 1992; Chkheidze and Belekhova, 1992; Andreu et al., 1996; Lanuza et al., 1998).

Recent data on the expression of homeotic genes during development of the mammalian, avian, and reptilian telencephalon (Smith-Fernandez et al., 1998) indicate that most of the reptilian DVR is a ventral pallial derivative (as defined by Puelles et al., 2000), but the avian DVR has a dual ontogenetic origin. Whereas the ventral hyperstriatum develops as part of the lateral pallium, the neostriatum is a ventral pallial structure (Puelles et al., 2000). Thus, the caudal neostriatum of birds seems to be equivalent to the reptilian PDVR, with which it shares not only its caudal position within the ventral pallium (Smith-Fernandez et al., 1998; Puelles et al., 2000) but also its associative nature (see above) and its moderate CGRP-li innervation. The dense CGRP-li terminal field in the intermediate neostriatum of birds seems to be the equivalent to the patch of CGRP-li fibers found in lizards on the boundary between the ADVR and the PDVR (Fig. 1B). Finally, the avian ventral hyperstriatum is a lateral pallial structure, which also receives a moderate CGRP-li innervation. These features seem to be accomplished by the deep stratum of the ventral lateral cortex rostrally and the dorsolateral amygdaloid nucleus caudally. Although there is evidence indicating that the avian ventral hyperstriatum is an associative center (Rehkamper and Zilles, 1991; Metzger et al., 1998), data on the sensory nature of the reptilian DLA and ventral lateral cortex are lacking, and further studies on the histochemistry, connections, and development of these structures are needed to check their possible homologies.

In conclusion, CGRP-li thalamotelencephalic systems seem to play similar roles in the pallium of reptiles, birds, and mammals, namely, to modulate a multimodal thalamic input to the associative "cortical" areas. These cortical areas would include the reptilian and avian DVR (plus portions of the ventral lateral cortex of reptiles) and the basolateral amygdala and parts of the claustral complex of mammals. The DVR of both birds and reptiles displays primary sensory areas, which show scarce (if any) CGRP innervation, so that its counterpart in the claustroamygdaloid complex of mammals is at present unclear. Detailed studies on the development, histochemistry, and connections of the claustroamygdaloid complex of mammals are needed to assess the comparative significance of the sensory DVR of sauropsids.

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