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Septal Complex of the Telencephalon of Lizards: III. Efferent Connections and General Discussion

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ABSTRACT

The projections of the septum of the lizard *Podarcis hispanica* (Lacertidae) were studied by combining retrograde and anterograde neuroanatomical tracing. The results confirm the classification of septal nuclei into three main divisions. The nuclei composing the central septal division (anterior, lateral, medial, dorsolateral, and ventrolateral nuclei) displayed differential projections to the basal telencephalon, preoptic and anterior hypothalamus, lateral hypothalamic area, dorsal hypothalamus, mammillary complex, dorsomedial anterior thalamus, ventral tegmental area, interpeduncular nucleus, raphe nucleus, torus semicircularis pars laminaris, reptilian A8 nucleus/substantia nigra and central gray. For instance, only the medial septal nucleus projected substantially to the thalamus whereas the anterior septum was the only nucleus projecting to the caudal midbrain including the central gray. The anterior and lateral septal nuclei also differ in the way in which their projection to the preoptic hypothalamus terminated. The midline septal division is composed of the dorsal septal nucleus, nucleus septalis impar and nucleus of the posterior pallial commissure. The latter two nuclei projected to the lateral habenula and, at least the nucleus of the posterior pallial commissure, to the mammillary complex. The dorsal septal nucleus projected to the preoptic and periventricular hypothalamus and the anterior thalamus, but its central part seemed to project to the caudal midbrain (up to the midbrain central gray). Finally, the ventromedial septal division (ventromedial septal nucleus) showed a massive projection to the anterior and the lateral tuberomammillary hypothalamus.

Data on the connections of the septum of *P. hispanica* and *Gecko gekko* are discussed from a comparative point of view and used for better understanding of the functional anatomy of the tetrapodian septum. J. Comp. Neurol. 401:525–548, 1998.

Indexing terms: limbic system; reptiles; comparative neuroanatomy; territorial behavior.

The septal complex is one of the main components of the vertebrate telencephalon and is a key component of the so-called limbic telencephalon (Swanson, 1983). There are three main reasons to include this medial telencephalic structure in the limbic system. First, it is a multimodal noncortical center which is directly involved in neither the control of motor activity nor the processing of sensory information. Second, it plays a substantial role in the control of complex forms of behavior that include a strong emotional component, and it is an important center for the regulation of several important physiological processes (DeFrance, 1976). Third, these functions are performed by means of its interconnections with other forebrain limbic centers such as the hypothalamus and the hippocampal cortex (Swanson and Cowan, 1979).

Both lesions and electrical stimulation have been used in mammals to study the role of the septum in physiology and behavior. The results of these experiments suggest

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multiple roles of the septum that include aggressive-defensive behaviors (Grossman, 1976; Lisciotto et al., 1990), regulation of hydrosaline homeostasis (Bridge, 1976; Gordon and Johnson, 1981), food intake (King and Nance, 1986), heat homeostasis (Cooper, 1987; Lee et al., 1989), relief of fear (Thomas, 1988), and learning/withholding of appetitive behaviors (McCleary, 1961, 1966). These results suggest a functional heterogeneity of the septum, but, up to now, no clear subdivisions of the septum have been proposed by using functional criteria. This is mainly due to the lack of detailed studies on the septal connections in many vertebrates and, consequently, to the lack of a solid anatomical background in most of the functional studies of the vertebrate telencephalic septum.

The understanding of the mechanisms underlying these functions requires a detailed knowledge of the anatomical pathways that subserve the septal influence on behavior and physiology. This requires a detailed analysis of the septal projections in appropriate experimental species. In this context, there are advantages in studying the septum of squamate reptiles (lizards and snakes). First, in this reptilian group the septum reaches as much as 10% of the whole telencephalic volume (Platel, 1980). Second, the telencephalon of reptiles displays a relatively simple organization (as compared to that of mammals and birds), thus making results easier to study and interpret. Finally, the key position of reptiles in the phylogenetic tree of vertebrates makes the study of the septum of extant reptiles very useful for understanding the evolution of the vertebrate forebrain and, more specifically, of the limbic telencephalon. This is very important taking into account that the limbic forebrain is classically viewed as an old structure of the vertebrate telencephalon (Northcutt, 1981).

Therefore, in the present account we have studied the efferent projections of the different septal nuclei in the old-world lizard *Podarcis hispanica* (Lacertidae). To do so, we have used the same strategy as in the previous article in this series: First, anterograde tracers have been applied to the different nuclei of the septum to determine the termination areas of each septal projection by studying the fiber labeling present in the whole brain. Then, tracers have been injected into areas showing labeling in the previous experiments, and the retrograde transport into the septum has been studied to identify the cells of origin of the main septal projections.

Anterograde tracer injections in several septal nuclei have also been performed in another lacertilian species belonging to a different family, namely *Gecko gekko* (Geckonidae). The results obtained in both species are similar and give a solid picture of the efferent connections of the septum of lizards. These data are discussed from comparative and functional viewpoints.

MATERIALS AND METHODS

The experimental design used in the present work consists of two kinds of tract-tracing experiments in *P. hispanica*. In a first step, iontophoretic injections of either *Phaseolus vulgaris*-leucoagglutinin (PHA-L; Vector, Burlingame, CA), biotinylated dextranamine (BDA, 10,000

Abbreviations

ac	anterior commissure
Acc	nucleus accumbens
AH	anterior hypothalamus
AT	area triangularis
Bst	bed nucleus of the stria terminalis
CG	central gray
DB	diagonal band nucleus
DC	dorsal cortex
DH	dorsal hypothalamus
DHA	dorsal hypothalamic area
DLA	dorsolateral anterior thalamic nucleus
DLAl	dorsolateral anterior thalamic nucleus, large-celled part
DLAs	dorsolateral anterior thalamic nucleus, small-celled part
DLH	dorsolateral hypothalamic nucleus
DM	dorsomedial cortex
DMA	dorsomedial anterior thalamic nucleus
DVR	dorsal ventricular ridge
ET	eminentia thalami
H	habenula
Hl	lateral habenula
Hm	medial habenula
IIId	nucleus nervi oculomotori, pars dorsalis
Inf	infundibulum
IP	interpeduncular nucleus
IPd	interpeduncular nucleus, pars dorsalis
LC	lateral cortex
lfb	lateral forebrain bundle
LHA	lateral hypothalamic area
Lp	lentiform thalamic nucleus, pars plicata
LPA	lateral preoptic area
LTM	lateral tuberomammillary area
MAM	mammillary nucleus
MC	medial cortex
MP	medial preoptic nucleus
MPA	medial preoptic area
Nac	nucleus of the anterior commissure
Nmfb	bed nucleus of the medial forebrain bundle
Nppc	nucleus of the posterior pallial commissure
NS	nucleus sphericus
op	optic tract
OV	nucleus ovalis
PA	preoptic area
PHA-L	*Phaseolus vulgaris*-leucoagglutinin
PO	paraventricular organ
PP	periventricular preoptic nucleus
PT	posterior thalamus
PV	periventricular hypothalamic nucleus
R	raphe nucleus
RA8	reptilian A8 nucleus
RC	retrochiasmatic nucleus
Rot	nucleus rotundus
Sa	anterior septal nucleus
SC	suprachiasmatic nucleus
ScO	subcommissural organ
Sd	dorsal septal nucleus
Sdc	central part of the dorsal septal nucleus
Sdd	dorsal part of the dorsal septal nucleus
Sdl	dorsolateral septal nucleus
Si	nucleus septalis impar
sh	septo-hypothalamic tract
Sl	lateral septal nucleus
Sm	medial septal nucleus
sm	stria medullaris
Sn	substantia nigra
SO	supraoptic nucleus
St	striatum
sth	septo-thalamic tract
SUM	supramammillary nucleus
Svl	ventrolateral septal nucleus
Svm	ventromedial septal nucleus
TM	tectum mesencephali
TS	torus semicircularis
TSc	torus semicircularis pars centralis
TSl	torus semicircularis pars laminaris
Vm	nucleus motorius nervi trigemini
VMH	ventromedial hypothalamic nucleus
VTA	ventral tegmental area

TABLE 1. Anterograde Labeling After Tracer Injections in the Septum of *P. hispanica*[1]

Case	Injection site	Bas Tel	Nac	LPA	MPA	MP	PP	SO	Rost Thal	HI	DMA	DLA	ScO	Lp
P9219	Sa, Sl, Svm, Sdl	+++	++	++	+++	++	++	+++	++	++	++	+	++	+
P9226	Sa, Sm, Sd													
P9249	Sa, Sl, Sm, Sdl													
P9259	Sa, Sl, Sm, Sd													
H92100	Sa, Sl, Sdl, Nac													
P9243	Sa	+++	+++	++	0	0	0	0	+	0	0	0	0	+
R9434	Sa, Sdl	+++	+++	++	0	0	0	0	+	0	0	0	+	++
H8812	Sa, Sdl	+++	+++	++	0	0	0	0	++	~	~	~	+	++
B9509	Sl (r)	+++	+++	+	+++	+	0	0	++	+	+	0	++	+
B9519	Svm, Sl, (Sa[2])	+++	+++	+	+++	+	+	~	++	++	++	+	++	+
H9271	Si, Sd	+++	+++	++	++	++	+++	++	~	+++	~	~	++	++
B9507	Sdl, Sl (d), DM	++	++	+	+	0	0	0	++	0	0	0	0	0

Case	Injection site	AH	DLH/ DHA	LHA	PV	PO	Mam	VTA	IPd	Sn/ RA8	R	TSI	TM	CG
P9219	Sa, Sl, Svm, Sdl	++	++	++	++	++	++	++	+	+	+	+	~	+
P9226	Sa, Sm, Sd													
P9249	Sa, Sl, Sm, Sdl													
P9259	Sa, Sl, Sm, Sd													
H92100	Sa, Sl, Sdl, Nac													
P9243	Sa	+(1)	+	+	0	~	++	+++	+	0	+	+	0	+
R9434	Sa, Sdl	+(1)	++	++	0	~	++	+++	+	+	+	++	0	++
H8812	Sa, Sdl	+(1)	++	++	0	~	++	++	+	+	+	++	0	++
B9509	Sl (r)	+	++	++	++	+	++	~	0	0	0	~	~	0
B9519	Svm, Sl, (Sa[2])	+++	++	++	++	++	++	++	+	+	+	+	+	+
B9271	Si, Sd	++	++	++	+++	+++	++	++	+	++	+	++	+	++
B9507	Sdl, Sl (d), DM	+	+	++	+	~	++(1)	++	++	+	+	0	0	+

[1]0, no labeling; ~, very scarce labeling (1 or 2 fibers); +, scarce fiber labeling; ++, moderate fiber labeling; +++, dense fiber labeling; Bas. Tel, basal telencephalon; d, dorsal; l, lateral; Mam, mammillary hypothalamus; r, rostral; Rost. Thal, rostral thalamus. For other abbreviations, see list.
[2]Intense and abundant retrograde labeling.

TABLE 2. Retrograde Labeling in the Septum of *P. hispanica* After Extraseptal Tracer Injections[1]

Case	Injection site	Sa	Sl	Sm	Sdd	Sdc	Sdl	Svm	Svl	Si	Nppc
P9242	Nmfb, DB	++	++	+	0	0	++	0	0	0	++
H9288	Nac	+	++	+	0	0	+	0	0	0	0
B9518	Rostral Thalamus (AT, OV, ET)	+	++(r)	+	+	0	+	0	0	++	+++
H9298	DMA, H	0	+(r)	++	+	0	0	0	0	+++	+++
H9303	H	0	0	0	0	0	0	0	0	+++	+++
B9521	Preoptic area	++	++	0	+	+	+	++	+	0	+
H9289	Preoptic area, AH	+	++	0	0	0	0	+++	+	0	0
B9520	AH	++	++	++	++	+	0	+++	+	++	+
B9421	PV (d), DHA, AH	++	++(d)	+	0	+	+	0	0	++	+++
B9457	PV, PO	+(c)	+(c)	0	+	(+)	+(c)	0	0	0	0
B9416	PV (v), VMH	+	0	0	+	+	0	0	0	++	++
H9204	LHA, MAM	++(r)	+(r)	0	0	0	0	0	+(r)	0	0
B9404	LHA (d)	++(r)	~(r)	0	0	~	0	0	0	0	0
H9322	SUM, MAM	++	++	+	+	+	+	0	+	0	+
H9201	MAM (m, c)	++	++	+	+	+	+	0	+	0	+
H9205	MAM (l)	+	+	0	+	+	+	++	++	0	++
H8742/H8810	Rostral midbrain tegmentum MAM (c)	++	++	+	++	+++	+++	0	+	0	+
H9267	VTA	+(r)	0	0	0	0	0	0	0	0	0
H9050	Sn, RA8, reticular formation, CG	+	0	0	0	++	+	0	0	0	0

[1]0, no labeling; ~, very scarce labeling (1 or 2 cells); +, scarce cell labeling; ++, moderate retrograde labeling; +++, dense cell labeling; c, caudal; d, dorsal; l, lateral; r, rostral. For other abbreviations, see list.

MW; Molecular Probes, Eugene, OR), horseradish peroxidase (HRP; Sigma type VI, St. Louis, MO), or rhodamine-labeled dextran amine (RDA, 10,000 MW, Molecular Probes) were placed into different septal nuclei to study the resulting anterograde transport. In the second group of experiments, HRP, PHA-L, or BDA were injected in different forebrain and midbrain centers, and the retrograde labeling found in the septum was studied. This allowed us to check whether the labeling found in the first set of experiments was really due to transport from the septum and to identify the cells of origin of the main septal efferents. As explained in the opening section, iontophoretic injections of BDA were placed in the main septal nuclei of the gecko to study the anterograde transport to the site of termination of their efferent projections.

Thirty-two adult specimens of *P. hispanica* (45–60 mm snout–vent) and six adult specimens of *G. gekko,* both sexes, were used for this study. In *P. hispanica,* tracers were applied to the septum (n = 12), basal telencephalon (n = 2), hypothalamus (n = 11), thalamus and epithalamus (n = 3), and midbrain tegmentum (n = 4). The location of the injection site and semiquantitative report of the anterograde and retrograde labeling found after these injections are shown in Tables 1 (septal injections) and 2 (extraseptal injections). In *G. gekko* BDA injections were centered in the anterior (n = 3) and lateral (n = 3) septal nuclei (Table 3).

The animals considered here were processed between 1986 and 1995. They were treated throughout according to the guidelines of the European Community and Spanish Ministry of Agriculture on the use, handling, and care of experimental animals.

For tracer injections and the histochemical procedure we have followed the protocol described in the previous work in this series (Font et al., 1997). Briefly, tracers were injected iontophoretically from a 10% (HRP and BDA), a

TABLE 3. Anterograde Labeling After Tracer Injections in the Septum of *Gekko gecko*[1]

Case	Injection site	Bas. Tel.	Nac	LPA	MPA	Rost. Thal./ DMA	ScO	Lp	AH	DLH/ DHA	LHA	PV/ PO	Mam	VTA	Sn/ RA8/ IPd	TSI/ CG
R15	Sa (r)	0	++	++	0	0	0	++	+	++	+	0	++	++	++	++
R21, R25	Sa (c)	+	++	++	0	0	0	0	+	+	+	0	++	++	+	+
R26, R27	Sl	++	+++	0	++	++	+	+	++	++	++	+	++	+	0	0
R28	Sl (r), (Sa, v)	++	+++	0	++	+	0	0	+++	++	++	0	++	++	+	+

[1]0, no labeling; +, scarce fiber labeling; ++, moderate fiber labeling; +++, dense fiber labeling; Bas. Tel, basal telencephalon; c, caudal; Mam, mammillary hypothalamus; r, rostral; Rost. Thal, rostral thalamus; v, ventral. For other abbreviations, see list.

5% (RDA and some BDA injections), or a 2.5% (PHA-L) solution in appropriate buffers (0.05 M TRIS buffer pH 8.6 for HRP; 10 mM phosphate buffer pH 7.4 for BDA, RDA, and PHA-L). Positive pulses (2–5 μA; 7 seconds ON/7 seconds OFF) generated by a current source (Direlec, Madrid, Spain) were given for 5–20 minutes, to deliver tracers from micropipettes of 12 to 50 μm inner-diameter tip.

After appropriate survival times (7 days for HRP and 12 days for the remaining tracers), animals were transcardially perfused with 4% paraformaldehyde in 0.1 M neutral phosphate buffer, usually supplemented with up to 0.5% glutaraldehyde. Tracers were detected in free-floating, frontal sections (40-μm-thick) obtained with a freezing microtome. For the histochemical detection of HRP, 3,3′-diaminobenzidine (DAB) was used as a chromogen (in 50 mM TRIS buffer, pH 8.0), usually darkened with nickel salts (up to 0.4% of nickel ammonium sulphate). For detection of PHA-L the ABC indirect immunoperoxidase technique was employed, followed by development of the peroxidase label with DAB. The BDA was detected by means of incubation in the ABC complex (Vector) followed by histochemical detection of the HRP as indicated above. For RDA, sections were directly mounted, air-dried, and coverslipped with mowiol (Osborn and Weber, 1982). Then, labeling was visualized by means of an epifluorescence microscope with an appropriate filter set (Leica N2.1).

RESULTS

To describe the results we will first report the anterograde labeling observed outside the septum after septal injections of tracers. The second section describes the retrograde labeling within the different septal nuclei after extraseptal injections. Throughout the results, the reference number of each experiment is preceded by a letter indicating the kind of tracer employed (P, PHA-L; H, HRP; B, BDA; R, RDA).

For the description of our results we will use the terminology of Font et al. (1995, 1997) for the septal nuclei, of Smeets et al. (1986) for the other forebrain centers, and of ten Donkelaar et al. (1987) for the midbrain structures.

Anterograde labeling after tracer injections into the septal complex of *P. hispanica*

Large injections. Large injections of PHA-L and HRP (P9219, P9226, P9249, P9259, and H92100) affected several septal nuclei: anterior (Sa), lateral (Sl), medial (Sm), dorsolateral (Sdl), and dorsal (Sd) septal nuclei (although not all of them affected exactly the same nuclei; see Table 1). The general pattern of labeling was similar in all these cases. Figure 1 shows the distribution of fiber labeling in lizard P9249, in which a PHA-L injection was applied to the septum after cortical ablation. This case is used as a representative example of these injections. Labeled fibers were found in the basal telencephalon, hypothalamus, dorsomedial thalamus, epithalamus, and in several midbrain areas.

In the telencephalon, intense anterograde labeling was seen in the nucleus of diagonal band (DB) and in the nucleus of medial forebrain bundle (Nmfb). This labeling showed a caudal continuation into the nucleus of the anterior commissure (Nac), and a few labeled fibers could be seen to enter the bed nucleus of stria terminalis (Bst). Caudal to Nac, labeled fibers split into two main tracts that reached the preoptic hypothalamus (septo-hypothalamic pathway) and the eminentia thalami (septo-thalamic tract), respectively (Fig. 2A), although a few labeled fibers were also observed in the stria medullaris.

Labeling in the septo-hypothalamic tract was composed of several fiber bundles that crossed the entire hypothalamus, throughout its rostrocaudal extent, giving rise to termination fields in several hypothalamic nuclei and areas. In the preoptic hypothalamus labeled fibers were present in the lateral (LPA) and medial (MPA) preoptic areas, and medial (MP, not shown) and periventricular (PP) preoptic nuclei. A remarkable network of labeled varicose fibers was also observed around the supraoptic nucleus (SO). The anterior hypothalamus showed terminal labeling in all its extension, but fiber labeling was especially dense in the periventricular area. All this labeling was bilateral with ipsilateral prevalence.

In the tuberal hypothalamus, labeled beaded fibers appeared bilaterally in the dorsal hypothalamic area (DHA) and in the dorsolateral hypothalamic nucleus (DLH), as well as in the lateral hypothalamic area (LHA) and in the periventricular hypothalamic nucleus (PV), where labeling was restricted to the juxtaventricular neuropile. A small number of labeled fibers left the PV and entered the ventromedial hypothalamic nucleus (VMH; not shown). Other labeled fibers could be traced into the mammillary hypothalamus, where they apparently innervated the ipsilateral supramammillary nucleus (SUM) and, bilaterally, the mammillary nuclei (MAM). Labeling is especially dense in the lateral neuropile of the infundibular recess (Fig. 2B), in an area that we call lateral tuberomammillary area (LTM: Lanuza et al., 1997). Some varicose fibers could be followed further caudally within the midbrain at the level of the ventral tegmental area (VTA) and interpeduncular nucleus (IP). On the other hand, a few axons from SUM, VTA, and IP took an ascending course, thus reaching the posterior thalamus and pretectum (lentiform thalamic nucleus, pars plicata; Lp) and the laminar part of the torus semicircularis (TSl).

However, the bulk of the labeled fibers that reached the thalamus and epithalamus used the septo-thalamic tract. Most of these fibers seemed to enter the eminentia thalami just behind the Nac, ran caudally into the dorsomedial

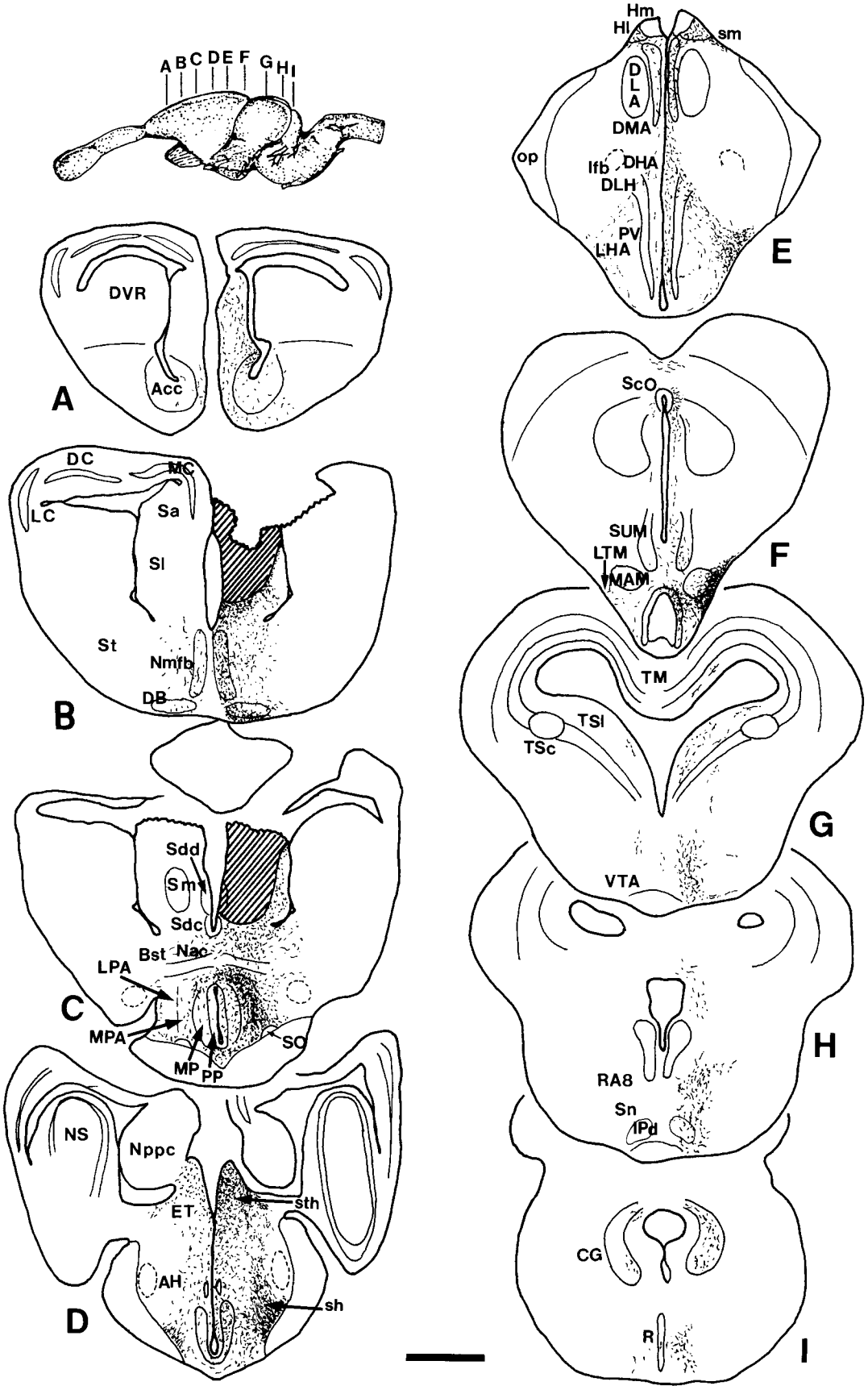

Fig. 1. **A–I:** Semi-schematic camera lucida drawings of frontal sections through the brain of *P. hispanica* showing the anterograde labeling that resulted from a *Phaseolus vulgaris*-leucoagglutinin (PHA-L) ionophoretic injection (hatched area) in the anterior (Sa), lateral (Sl), medial (Sm), dorsal (Sd), and dorsolateral (Sdl) septal nuclei (case P9249). For other abbreviations, see list. Scale bar = 500 µm.

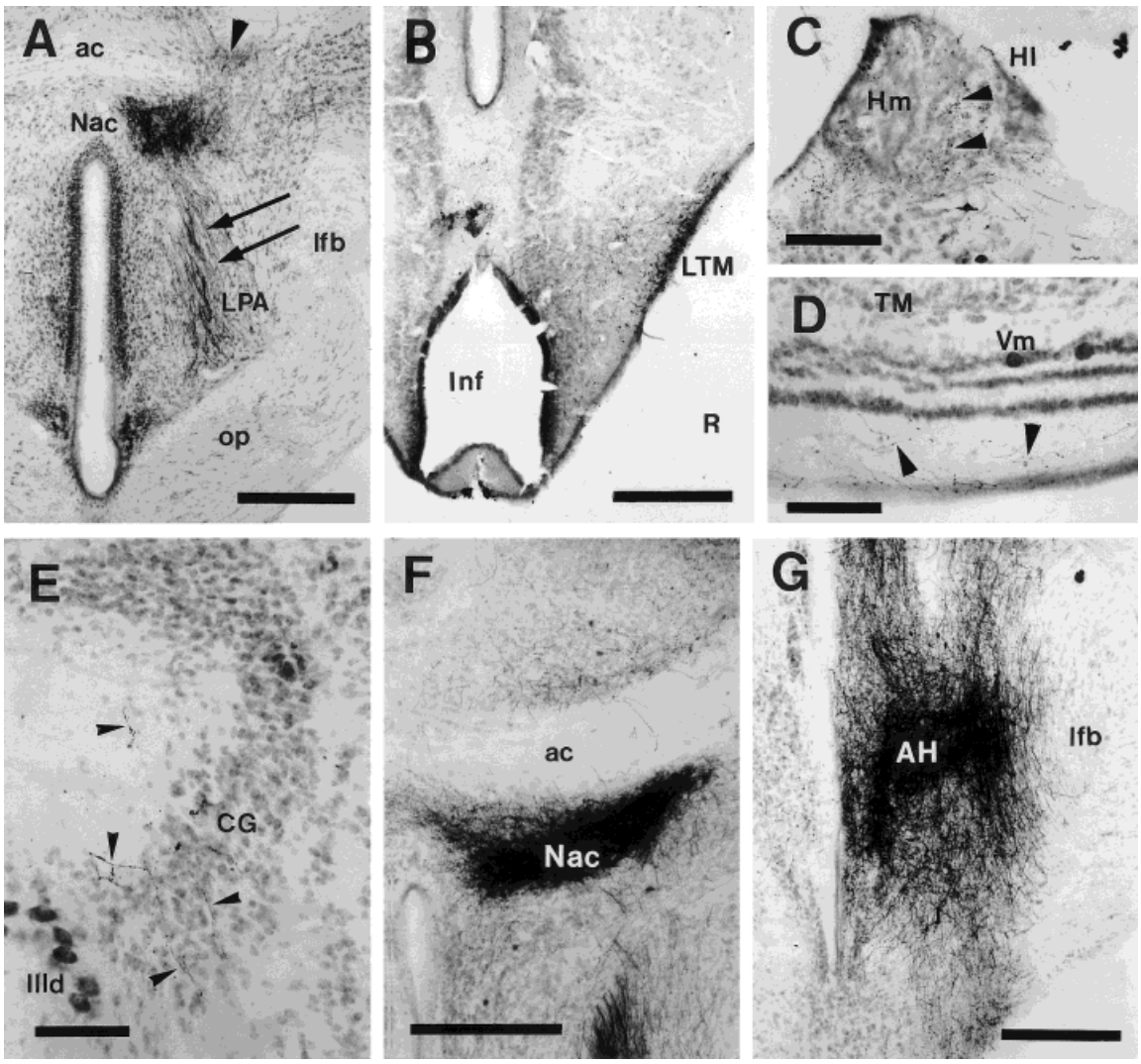

Fig. 2. Photomicrographs illustrating the anterograde labeling resulting from tracer injections in the septal nuclei of *P. hispanica*. **A:** After septal injections of tracers, fibers are present in two tracts that split just caudal to the nucleus of anterior commissure (Nac): the septo-thalamic (arrowhead) and septo-hypothalamic (double-arrow) tracts (case B9519). **B:** Labeling is visible in the lateral tuberomammillary area (LTM) after a PHA-L large injection in the septum (case P9249). **C:** Terminal-like labeling is present in the habenula, mainly in the lateral habenula (Hl; arrowheads) after a horseradish peroxidase (HRP) injection which involved the nucleus septalis impar (Si) and Sd (case H9271). **D:** Large injection in the septum of *P. hispanica* (case P9249) gives rise to labeling of beaded fibers in layer 2 of the ipsilateral midbrain tectum (TM; arrowheads). **E:** Fiber labeling is visible in the midbrain central gray (CG; arrowheads) wherever the injection site involved the anterior septal nucleus. **F:** Labeling in the Nac (ventral to the anterior commissure) is specially prominent after injections restricted to the rostral Sl (case B9509). **G:** A dense network of labeled fibers is visible in the anterior hypothalamus (AH), just medial to the lateral forebrain bundle, after those injections which involved the ventromedial septal nucleus (Svm; case B9519). For other abbreviations, see list. Scale bars = 200 μm in A,B,F,G and 100 μm in C,D,E.

anterior thalamic nucleus (DMA), and a few of them entered the dorsolateral anterior nucleus (DLA). In the epithalamus dense fiber labeling was found in the lateral habenula (Hl; Fig. 2C), although a few labeled fibers were also present in the medial habenula (Hm). Although we were unable to follow the labeled fibers from the injection site in the septum to the habenula, we assume that, according to Díaz and Puelles (1992), this projection courses through the stria medullaris where, as described above, some labeled fibers were present. From the epithalamus and thalamus a low number of labeled fibers reached the pretectum, dorsal to the posterior commissure (Fig. 1F).

At the level of the caudal DMA, fibers from the septo-thalamic tract joined the ascending bundle that left the septo-hypothalamic tract at the meso-diencephalic boundary as described above. From the posterior thalamus they ran caudally to give rise to a dense terminal field around the subcommissural organ (ScO) and, even more caudally, to a scarce terminal field in the deepest layers (2–5) of the tectum mesencephali (TM; Fig. 2D) and in the other midbrain centers such as the torus semicircularis (juxtaventricular layer) and central gray (CG; Fig. 2E).

Small injections. In one case (R9434) an RDA injection was confined to the Sa and Sdl at slightly precommissural levels (Fig. 3). This injection gave rise to labeled fibers

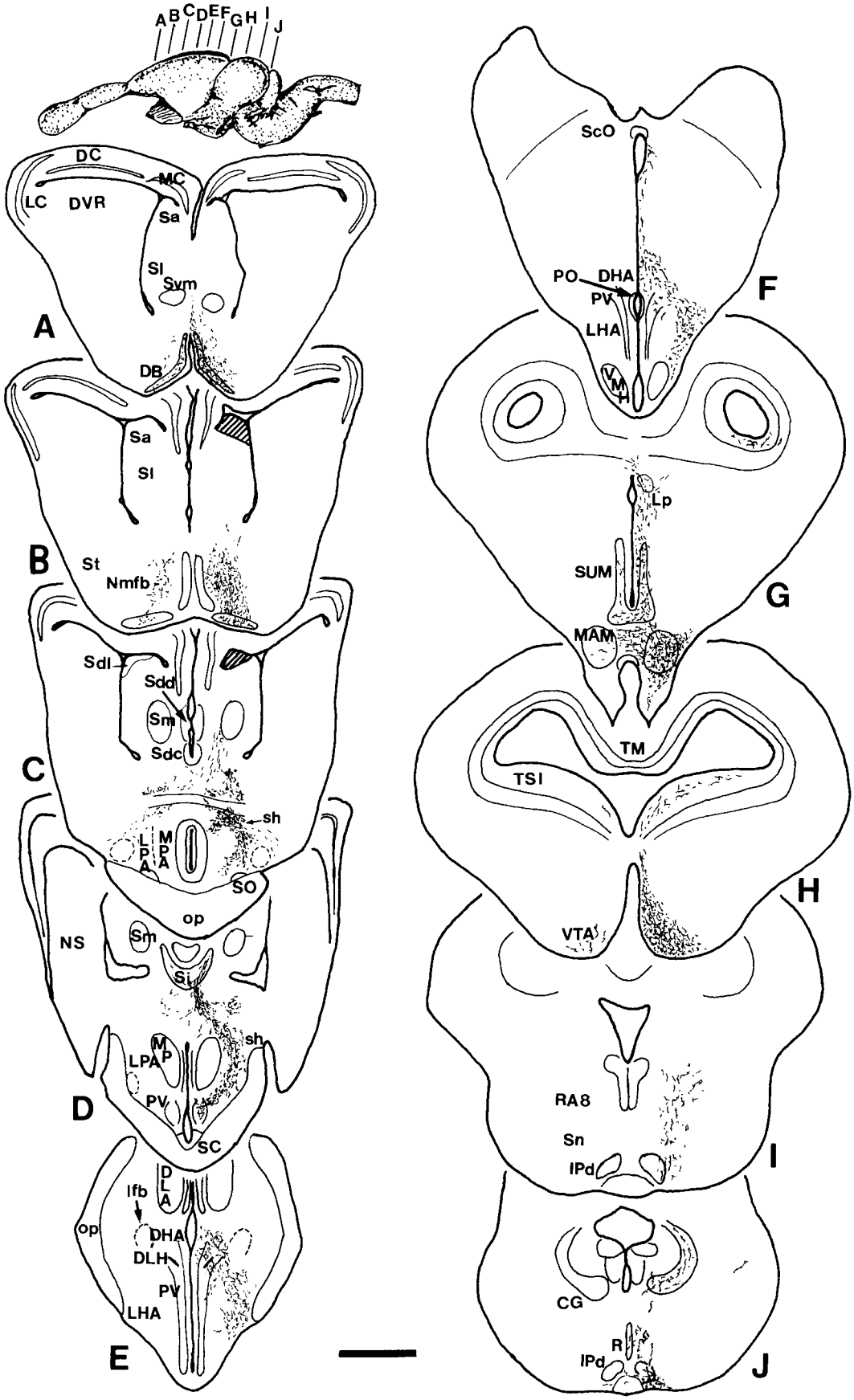

Fig. 3. **A–J:** Anterograde labeling in the brain of a specimen of *P. hispanica* which received an iontophoretic injection of rhodamine-labeled dextranamine (hatched area) into the anterior septal nucleus and the rostral edge of the dorsolateral septal nucleus (case R9434). For abbreviations, see list. Scale bar = 500 µm.

within the basal telencephalon like the large injections described above. However, ventral to the anterior commissure, fibers in the septo-hypothalamic tract bent laterally to innervate the lateral hypothalamus at preoptic (LPA), anterior and tuberal (LHA) levels, whereas in the periventricular and medial hypothalamus labeling was restricted to the DHA and DLH. At mammillary levels, labeled fibers were very dense in the SUM and MAM. In contrast to the big injections described above, the LTM did not show a remarkable amount of labeling. Another important differential feature of this injection was the virtual absence of labeled fibers in the septo-thalamic pathway, and therefore in the thalamus, epithalamus, and tectum. Other midbrain centers showed a pattern of labeling similar to the big injections but labeled fibers were relatively denser in the Lp, TSl, CG, interpenduncular nucleus, pars dorsalis (IPd), and, especially, in the VTA.

Injection P9243 was restricted to the Sa and the medial cortex at commissural levels, but the Sdl was not involved in the injection site. Because the medial cortex does not project out of the telencephalon (Olucha et al., 1988; Hoogland and Vermeulen VanderZee, 1993) it is clear that the extratelencephalic labeled fibers found in this case arise from cells in the Sa. In fact, the whole pattern of anterograde labeling was virtually identical with the one in case R9434, but fiber labeling was relatively scarce in the Lp, TSl, CG, and IPd. Therefore, it can be assumed that the Sdl is the main origin of the septal projections to these nuclei.

A restricted injection into the rostral part of the lateral septal nucleus (B9509) resulted in a pattern of fiber labeling slightly different from that following large injections (Fig. 4). In the telencephalon labeling was scarce, whereas the highest concentration of labeled fibers was found in the ventral part of the nucleus of the anterior commissure (Fig. 2F). In the preoptic hypothalamus labeled fibers were abundant in medial preoptic area, whereas only a few labeled fibers could be seen in the lateral preoptic area and medial preoptic nucleus. In fact, the periventricular preoptic nucleus was devoid of any labeling. In the anterior and tuberal hypothalamus the pattern of labeling was virtually identical with that of large injections, except for the presence of a few fibers surrounding the VMH. In the mammillary hypothalamus, labeling is almost absent in the mammillary nucleus, whereas the density of labeled fibers was high in the LTM. In contrast to large injections, labeling in the midbrain was very scarce since only a few labeled fibers could be seen in the VTA, torus, and tectum. However, the presence of a dense terminal field next to the subcommissural organ should be stressed.

Another BDA injection (B9519) was centered in the ventromedial septal nucleus, although a part of the rostral Sl was also affected by the injection. It should be noticed that the anterior septal nucleus showed an important retrograde labeling mainly at rostral levels (Fig. 5A) that could be responsible for part of the anterograde labeling found. Moreover, the presence of retrogradely labeled cells in the cortex and hypothalamus makes interpretation of fiber labeling somewhat difficult. As expected, labeling in this case was largely similar to that found after large injections, but three main differences are worth noticing (Fig. 5), which should be attributed either to the injection into the Svm, to the presence of retrogradely labeled cells in the cortex, or to sparing of the Sd by the injection.

First, a very dense bilateral (with ipsilateral predominance) field of labeled fibers was present in the anterior hypothalamus, just medial to the ventral peduncle of the lateral forebrain bundle (Fig. 2G), where retrogradely labeled cells were also present. This labeling strongly suggests a specific projection from the Svm to the anterior hypothalamus (as confirmed by the intense retrograde labeling in the Svm after hypothalamic injections). Second, a few labeled fibers were found in the ipsilateral striatum, which were probably due to labeling of axonal collaterals of retrogradely labeled neurons in the dorsal cortex (Hoogland and Vermeulen-VanderZee, 1989). And third, labeling was very scarce, if present, in the SO, which showed dense fiber labeling after large injections which also included the Sm and Sd. Taking into account that the SO was not labeled after restricted injections into either the Sa or Sl, and not in this case either, a projection can be inferred from the Sm or Sd to the SO.

In one case (H9271) HRP was injected into the nucleus septalis imparis (Si) and the central part of dorsal septal nucleus (Sdc). Because the injection site also involved the rostral pole of the DMA, labeling in the septo-thalamic pathway could not be studied in this case. Some of the efferents of the Si/Sd can be inferred, however, using a control injection in the DMA (case H9298): Areas receiving a projection from the Si/Sd would show fiber labeling in case H9271 but not in case H9298.

Comparing the results of these two cases, it can be assumed that anterograde transport from the Si and Sdc resulted in fiber labeling in the preoptic, anterior, tuberal, and mammillary hypothalamus. Although labeling was observed throughout the medio-lateral axis of the hypothalamus, case H9271 differed with respect to other septal injections in the presence of a dense terminal field in the periventricular neuropile.

In the midbrain labeling was present in several nuclei that were devoid of it after the injection restricted to the DMA, namely the laminar part of the torus semicircularis, the substantia nigra and reptilian aminergic group 8 (Sn/RA8), and the central gray, as well as the inner layers of the optic tectum.

Retrograde labeling in the septal complex of *P. hispanica* after extraseptal injections

Injections in the basal telencephalon. Following an HRP injection into the ventral part of the Nac (H9288, Fig. 6A), retrogradely labeled cells were mainly observed in the precommissural aspect of the Sl, although a few cells were also present in the anterior septal nucleus (Fig. 7A) and in the rostral Sdl. The Sm also showed some labeled cells, but as the micropipette track crossed this nucleus, it is difficult to ascertain whether or not this labeling arose from the injection site.

Another injection was centered in the Nmfb, but the micropipette track also affected the rostral Sa and Sl. This injection showed labeled cells in the Sa and Sl (far from the injection site) as well as in the Sm, Sdl, and Nppc. Labeling in these septal nuclei cannot be attributed to retrograde transport from the pipette track in the rostral Sa and Sl, because retrograde labeling was not observed in either of these nuclei after big injections in the Sl that gave rise to retrograde labeling in other forebrain areas (see Font et al., 1997). As interesting as the presence of this retrograde labeling is the absence of labeled neurons in the Si, Sd—in

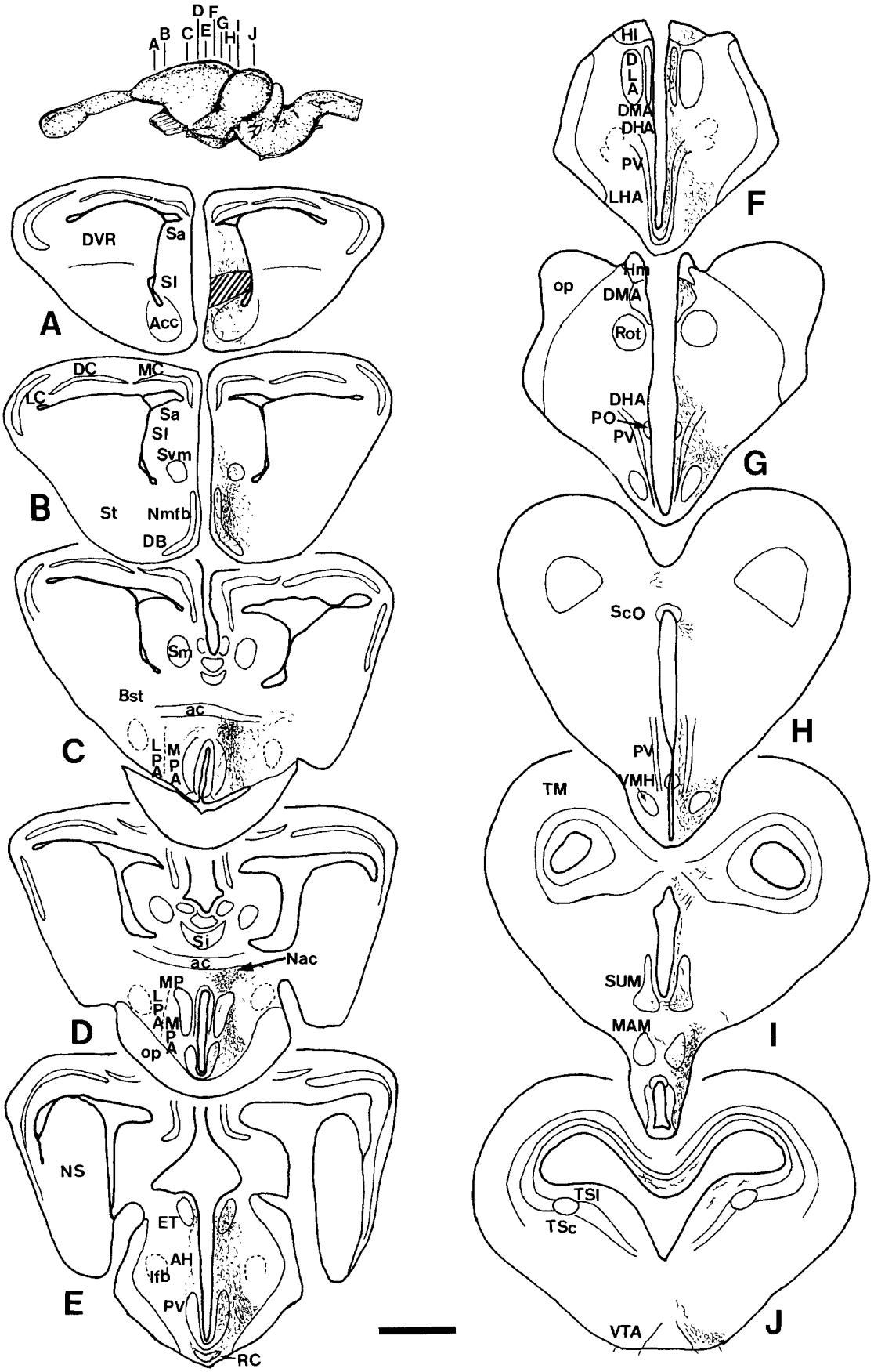

Fig. 4. **A–J:** Anterograde labeling resulting from a biotinylated dextranamine (BDA) injection (hatched area) restricted in the rostral part of the lateral septal nucleus (Sl) of *P. hispanica* (case B9509). For abbreviations, see list. Scale bar = 500 µm.

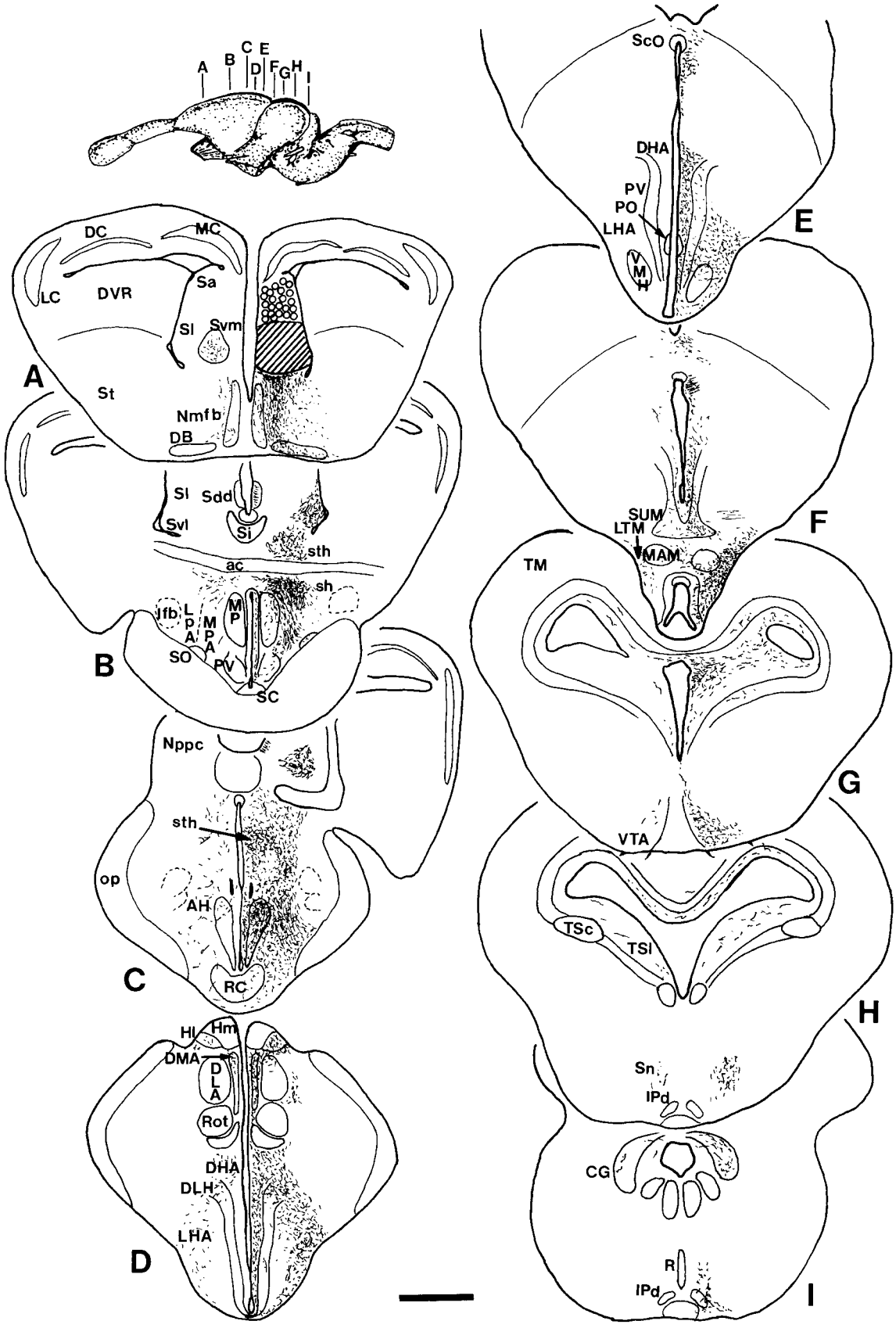

Fig. 5. **A–I:** Anterogradely BDA-labeled fibers after an injection (hatched area) into the ventromedial septal nucleus and the rostral part of Sl of *P. hispanica* (case B9519). Open circles indicate retrograde labeling that was observed in the septum. For abbreviations, see list. Scale bar = 500 µm.

its central (Sdc) and dorsal (Sdd) parts—and Svm, which could indicate that these nuclei do not project to the Nmfb.

Injections in the thalamus and epithalamus. In case B9518, a BDA injection was placed in the most rostral part of the thalamus (area triangularis, nucleus ovalis, and eminentia thalami; Fig. 6B), retrograde labeling was found within the Sl, although a few labeled neurons were also present in the Sa, Sm, Sdd, Sdl, and Si. However, the densest population of labeled cells was found within the Nppc (Fig. 7B).

An HRP injection was placed in the dorsomedial anterior thalamus and habenular nuclei (H9298, Fig. 6C). The largest number of HRP-positive cells was found in the Si (Fig. 7D), where apparently every cell of the nucleus was labeled, and in the nucleus of posterior pallial commissure (Nppc). The medial septal nucleus also displayed a moderate density of retrogradely labeled somata (Fig. 7D), whereas in the Sl a few scattered labeled neurons were found at precommissural levels.

In contrast, after an HRP injection restricted to the lateral habenula (H9303), septal retrograde labeling was restricted to the Si and Nppc.

Injections in the hypothalamus. A large BDA injection in the preoptic area (B9521; Fig. 6D) gave rise to a high number of retrograde labeled cells in all of the septal nuclei except for the Si and Sm.

Two injections were located in the anterior hypothalamus. In one case, HRP was injected in the medial anterior hypothalamus, just medial to the lfb, but apparently diffused into the anterior periventricular hypothalamus (H9289, Fig. 6E). Labeled cells were observed mainly in the ventromedial septal nucleus (Svm; Fig. 7C) and the rostral part of the lateral septum. In addition, the Sa contained a few scattered labeled cells. In the other case, the injection site was larger and comprised the whole mediolateral extent of the anterior hypothalamus (B9520; Fig. 6F); in addition to the labeling described for the previous case, labeled cells were also present in the Sm, Svl, Si, Sd, and (a few scattered cells) in the Nppc. In the Sa the number of labeled cells was higher than in case H9289. It is worth noting that in this case, apparently all of the cells in the Svm were retrogradely labeled.

In another case, BDA was injected in the dorsal periventricular hypothalamus at anterior-to-tuberal levels (B9421, Fig. 6G). Many BDA-positive cells were identified in both Sa and dorsal aspect of the Sl, mainly at rostral levels (Fig. 7E). Labeled cells were observed as well in the central part of the Sd and Sdl, and a few labeled cells could be seen in the Sm. In the Si and Nppc there were a large number of labeled cells.

Several injections were placed in the tuberal hypothalamus. One of them (B9457; Fig. 6H), which received a small BDA injection in the periventricular nucleus close to the paraventricular organ, showed scattered labeled cells in the Sa, Sl, Sdl, and Sd, at commissural levels. Another BDA injection was centered in the ventromedial hypothalamic nucleus and the ventral aspect of the periventricular hypothalamic nucleus (B9416, Fig. 6I). In this case, a few labeled cells observed in the precommissural Sa displayed a Golgi-like labeling (Fig. 7F) so that their whole spiny dendritic tree was visible. A few retrograde labeled neurons were also found in the Sd and a higher number in the Si and Nppc.

In one case in which HRP was injected into the lateral hypothalamic area and lateral mammillary bodies (H9204, Fig. 6J) labeled cells were seen throughout the precommissural Sa, and a few cells were also present in the Sl and Svl. Another BDA injection was centered in the dorsal part of the lateral hypothalamic area (B9404; Fig. 6K). As a result, retrograde labeling in the septum was virtually restricted to the precommisural Sa.

Peroxidase injections were given in the mammillary complex of three animals (H9322, H9201, and H9205). In each instance the largest number of labeled cells was found in the Sa and Sl. However, the remaining septal nuclei also displayed an important number of labeled cells excluding the Svm and Si (Fig. 6L). In case H9205, in which the injection involved the lateral MAM and the area just lateral to it (lateral tuberomammillary area), some differences were evident: The retrograde labeling in the Svl and Nppc was more abundant, whereas the Sm was free of labeling. Moreover, there was a high number of retrograde neurons in the Svm.

Midbrain injections. Large injections in the rostral midbrain tegmentum, also encompassing the caudal mammillary bodies (H8742 and H8810), showed, as expected, a pattern of retrograde labeling similar to the one reported above for injections in the mammillary hypothalamus (Fig. 6M). It should be noticed, however, that retrogradely labeled cells were especially abundant in the Sdl and Sdc.

In one case (H9267, Fig. 6N), a small HRP injection was given in the ventral tegmental area and the interpeduncular nucleus. Labeled cells could only be observed in the precommissural Sa. On the other hand, a large HRP injection (H9050) in the caudal midbrain that affected the Sn, RA8, reticular formation, and central gray rendered retrograde labeling in the Sa, Sdc, and Sdl (Fig. 6O).

Anterograde labeling after tracer injections into the central division of septal complex of *G. gecko*

In the gecko, BDA injections were centered in the anterior (R15, R21, and R25) and lateral (R26, R27, and R28) septal nuclei. When injections were placed in the rostral part of Sa (Fig. 8; case R15), labeled fibers were observed in the Nac, lateral preoptic area, anterior hypothalamus, tuberal hypothalamus (DLH, DHA, LHA), mammillary complex (mainly in the supramammillary nucleus), and VTA. Labeled fibers run further caudally, as happens after similar injections in *P. hispanica*, to the medial aspect of the midbrain (TSl, IP, substantia nigra [Sn]/RA8, CG, and raphe nucleus [R]). Injections located in the caudal Sa (R21) displayed few differences: Labeled fibers were present in the basal telencephalon (DB and Nmfb), and the number of labeled fibers in the caudal mesencephalon was lower.

Injections restricted to the Sl (Fig. 9; case R27) displayed anterograde labeling in the basal telencephalon, eminentia thalami, dorsomedial anterior thalamic nucleus, medial preoptic area, anterior hypothalamus, tuberal hypothalamus, mammillary complex (mainly in the lateral tuberomammillary area), and also a few labeled fibers in the VTA; labeling was not observed at more caudal levels. Injections that involved the ventral aspect of the rostral Sl (R28, where in *P. hispanica* the Svm is located) showed a dense meshwork of labeled fibers with plenty of varicosities in the anterior hypothalamus.

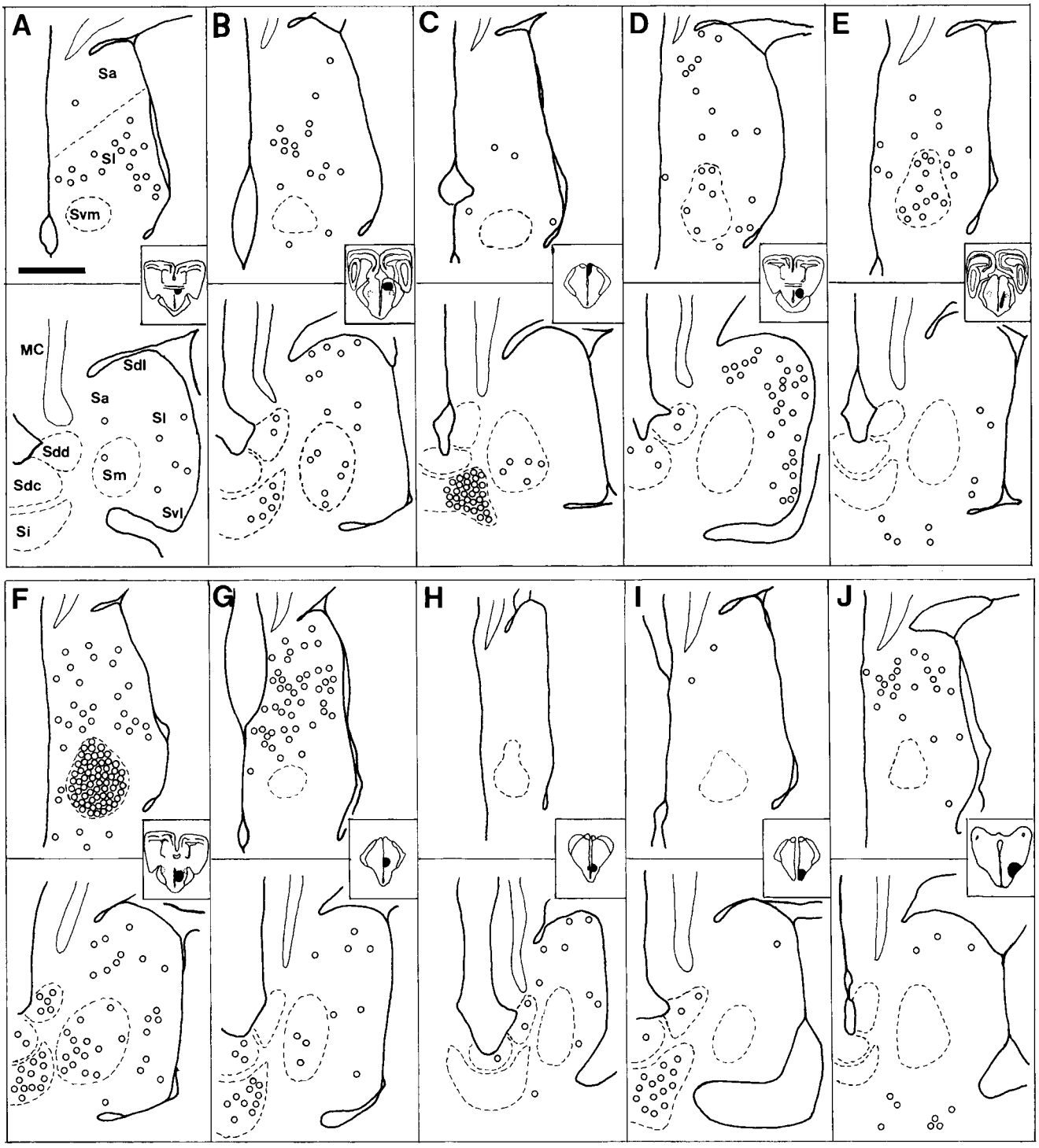

Fig. 6. Semi-schematic camera lucida drawings of two frontal sections through the septal complex of *P. hispanica* (top: precommissural level; bottom: commissural level) showing retrograde labeling (open circles) after different extraseptal injections. In each case, the injection site is indicated in small drawings of frontal sections through the diencephalon or midbrain (see **insets**). **A:** Injection of horseradish peroxidase (HRP) in the ventral part of the nucleus of anterior commisure (Nac; case H9288). **B:** BDA injection in the rostral thalamus (area triangularis, nucleus ovalis, and eminentia thalami; case B9518). **C:** Injection of HRP in the dorsomedial anterior thalamic nucleus and habenula (case H9298). **D:** A large BDA injection in the preoptic area (case B9521). **E:** HRP injection in the anterior hypothalamus (case H9289). **F:** BDA injection which comprised the whole mediolateral extent of anterior hypothalamus (case B9520). **G:** BDA injection into the dorsal periventricular hypothalamus at anterior-to-tuberal levels (case B9421). **H:** Small BDA injection in the periventricular hypothalamic nucleus, close to the paraventricular organ (case B9457). **I:** BDA injection centered in the ventromedial hypothalamic nucleus and the most ventral part of periventricular hypothalamic nucleus (case B9416). **J:** HRP injection in the lateral hypothalamic area and lateral mammillary body (case H9204). **K:** BDA injection centered in the dorsal part of the lateral hypothalamic area (case B9404). **L:** HRP injection into the mammillary complex (case H9201). **M:** Large HRP injection in the rostral midbrain tegmentum (case H8810). **N:** HRP injection in the ventral tegmental area and interpeduncular nucleus (case H9267). **O:** Large HRP injection in the caudal midbrain that affected the substantia nigra (Sn), reptilian aminergic group (RA8), reticular formation, and CG (case H9050). For abbreviations, see list. Scale bar = 200 µm.

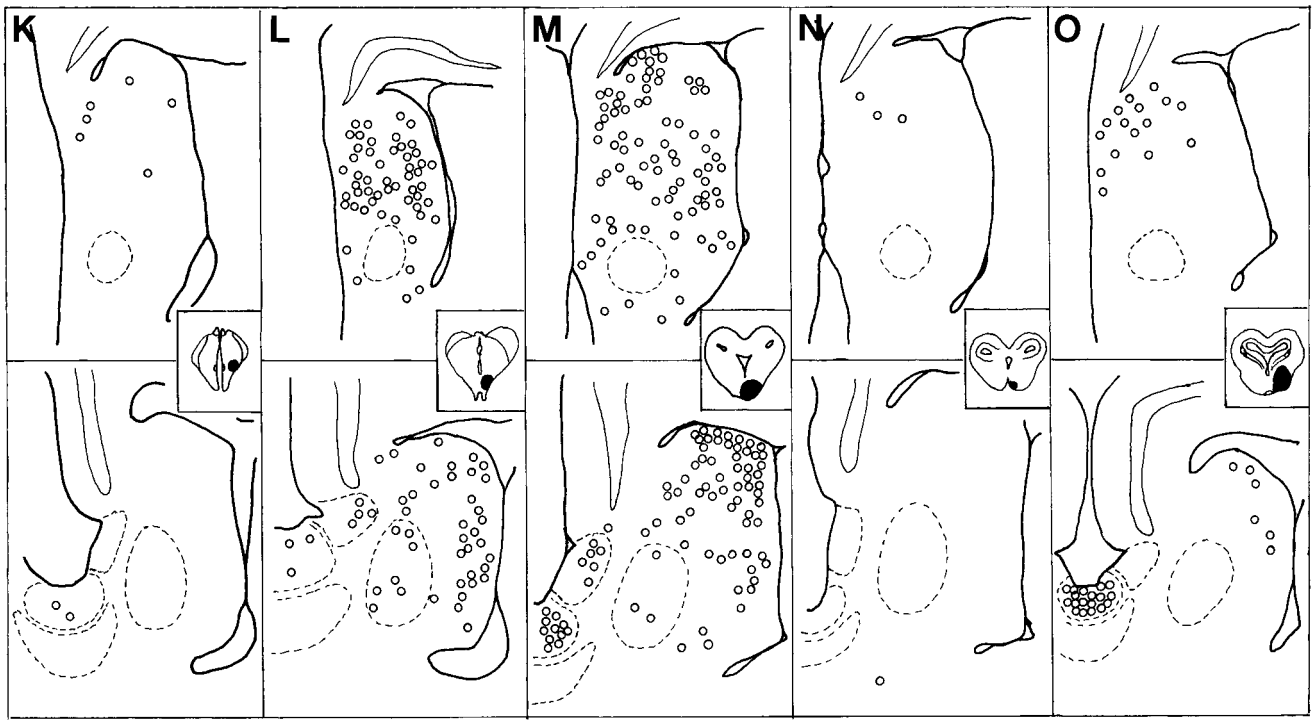

Figure 6 (Continued)

DISCUSSION

The application of intra-axonic neuroanatomical tracers to the study of the efferents of the septum of lizards is a complex task owing to several technical problems (Font et al., 1997). We have solved some of these problems by tracing most of the projections twice: first using anterograde transport and then by means of retrograde transport. This strategy is useful to overcome problems such as uptake of tracers by passing fibers and those derived from the large size of the injection relative to the small brain of lizards, which makes it very difficult to restrict the injection sites to single septal nuclei.

Using this strategy we have been able to trace, with certainty, the main efferents of the different septal nuclei of *P. hispanica* including their origin in the septum and their termination in the forebrain and midbrain. The injections of BDA in the septum of the gecko, which has a larger brain, were restricted to a few septal nuclei (Sa and Sl) and were easier to interpret. From these results it can be concluded that, in spite of the differences in the organization of the septal complex in the two distant species of lizards (*Podarcis* and *Gekko*), their connections are very similar (with minor differences) and reflect a general pattern of efferents of the telencephalic septum of lizards (and maybe of other reptiles).

Once the connections of the reptilian septum have been defined, they are discussed from a comparative point of view. On the basis of the available anatomical data, we discuss the roles that the septum might play, as a key center of part of the limbic forebrain, in the control of behavior. In this respect, the anatomical heterogeneity of the septum is correlated with the diversity of its putative functions.

Projections of the septal nuclei of lizards

Previous works specifically devoted to the study of the septal projections in reptiles (Hoogland et al., 1978; Sligar and Voneida, 1981; Belekhova and Nemova, 1988; Nemova, 1988) considered the septum as a unitary structure and, therefore, neglected the anatomical heterogeneity of this telencephalic area. In view of its chemoarchitectonical heterogeneity (Font et al., 1995) and its afferent connections (Font et al., 1997), the septum of *Podarcis hispanica* has been divided into three main domains: the central septal division (Sa, Sl, Sm, Sdl, Svl, and Nppc), the midline septal division (Si and the Sd, which is composed of a central and dorsal parts—Sdc and Sdd, respectively), and the ventromedial septal division (Svm). Our data on the efferent connections of the septum in this species, and the efferents of the main central nuclei (Sa and Sl) of the septum of the gecko, give further support to this classification of the septal nuclei and can be helpful in speculating about the roles of the septum in behavior and physiology.

Efferent projections of the central septal division. Considering together our results of retrograde and anterograde tracing experiments in *Podarcis* and *Gekko,* the main projections of the central septal division of lizards can be drawn. The efferent connections of the central septal division terminate (as defined by means of anterograde transport) in the basal telencephalon (but see below) including the Nac, in the preoptic, anterior, tuberal, and mammillary hypothalamus, and in the midline thalamus. At mesencephalic levels, some of the injections into the central septal division label the midbrain ventral tegmental area, interpeduncular nucleus, and raphe nucleus, as well as more dorsal structures such as the TSl and the CG.

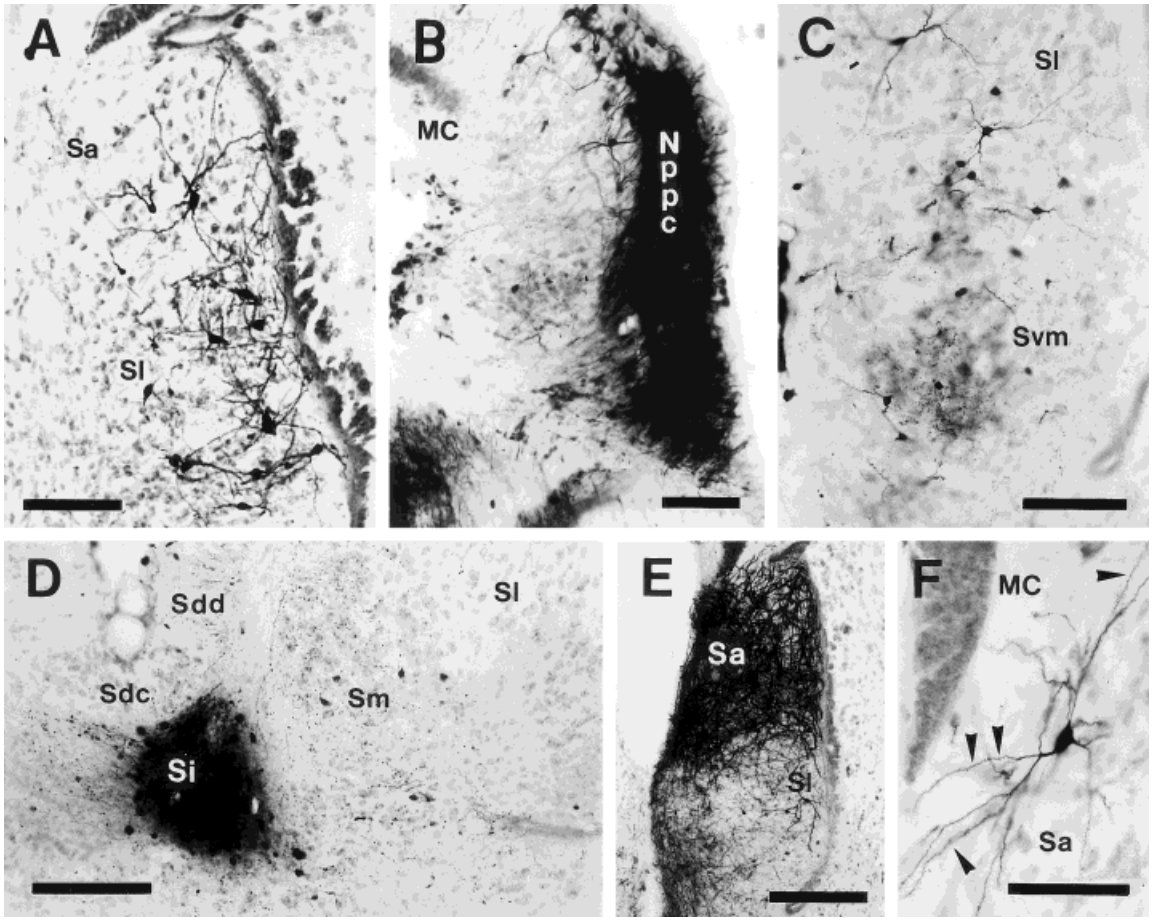

Fig. 7. Photomicrographs of frontal sections through the septal complex showing the retrograde labeling in the septum after different extraseptal injections. **A:** Labeled cells in the Sl and a few labeled cells in the Sa following an injection in the ventral part of Nac. **B:** High number of retrogradely labeled cells in the nucleus of posterior pallial commissure (Nppc) after a BDA injection in the rostral thalamus. **C:** Labeled cells in the Svm after an HRP injection in the anterior hypothalamus. **D:** Large number of HRP-positive cells in the Si following an injection in the dorsomedial thalamic nucleus and habenula. The Sm and the dorsal part of the Sd also display a few retrogradely labeled somata. **E:** BDA-positive cells in both Sa and dorsal aspect of Sl at a rostral level after an injection in the dorsal periventricular hypothalamus. **F:** An intensely labeled cell in the precommissural Sa after a BDA injection in the ventromedial hypothalamic nucleus and the most ventral part of the periventricular nucleus. Arrowheads indicate dendritic spines. For other abbreviations, see list. Scale bars = 100 µm in A,B,C,D, 200 µm in E, and 50 µm in F.

Our results confirm and expand the view pointed out by Hoogland et al. (1994) and supported by Bruce and Neary (1995a,b), according to which the two main nuclei of the central septal division, namely the Sa and Sl, display a differential pattern of efferent projections. According to the anterograde tracing experiments in both *Podarcis* and *Gekko,* the Sa mainly projects to the LPA (Figs. 3, 8, 10), whereas the Sl projects mainly to the MPA (Figs. 4, 9, 10). Another important difference in the descending projections of the Sa and Sl is only clear in the gecko, in which the basal telencephalon apparently does not receive a substantial projection from the rostral (enlarged) aspect of the Sa (see Fig. 8), whereas injections in the caudal Sa (R21, R25; see Table 3) do show labeling of varicose fibers in the basal telencephalon. These differences are not present in *Podarcis,* in which labeling in the basal telencephalon is present after every injection in the central septal central division. The possibility arises, therefore, that the Sa of *Gekko,* which is especially well developed, displays some compartmentalization (as revealed by its projections to the basal telencephalon) not observed in the Sa of *Podarcis.*

Another noticeable difference between the Sa and Sl is found in both *Podarcis* and *Gekko.* The Sa (and probably the Sdl) projects weakly to the anterior hypothalamus but massively to the tubero-mammillary region and to several midbrain nuclei (VTA, IP, R, TSl, and CG). This view is supported by the results of the application of retrograde tracers to the hypothalamus of the gecko (see Fig. 6 in Bruce and Neary, 1995a). On the other hand, the Sl appears to project massively to the rostral hypothalamus (preoptic and anterior levels), as demonstrated by using retrograde tracing in the gecko (see Fig. 4 in Bruce and Neary, 1995b), only weakly to the tubero-mammillary hypothalamus, and not at all to the midbrain (see Bruce and Neary, 1995a, and Fig. 6 in Bruce and Neary, 1995b).

Finally, both retrograde and anterograde transport (in *Podarcis* and *Gekko*) indicate that the Sl projects to the anterior thalamus (mainly to the DMA), whereas the Sa does not. However, the main source of projections to the

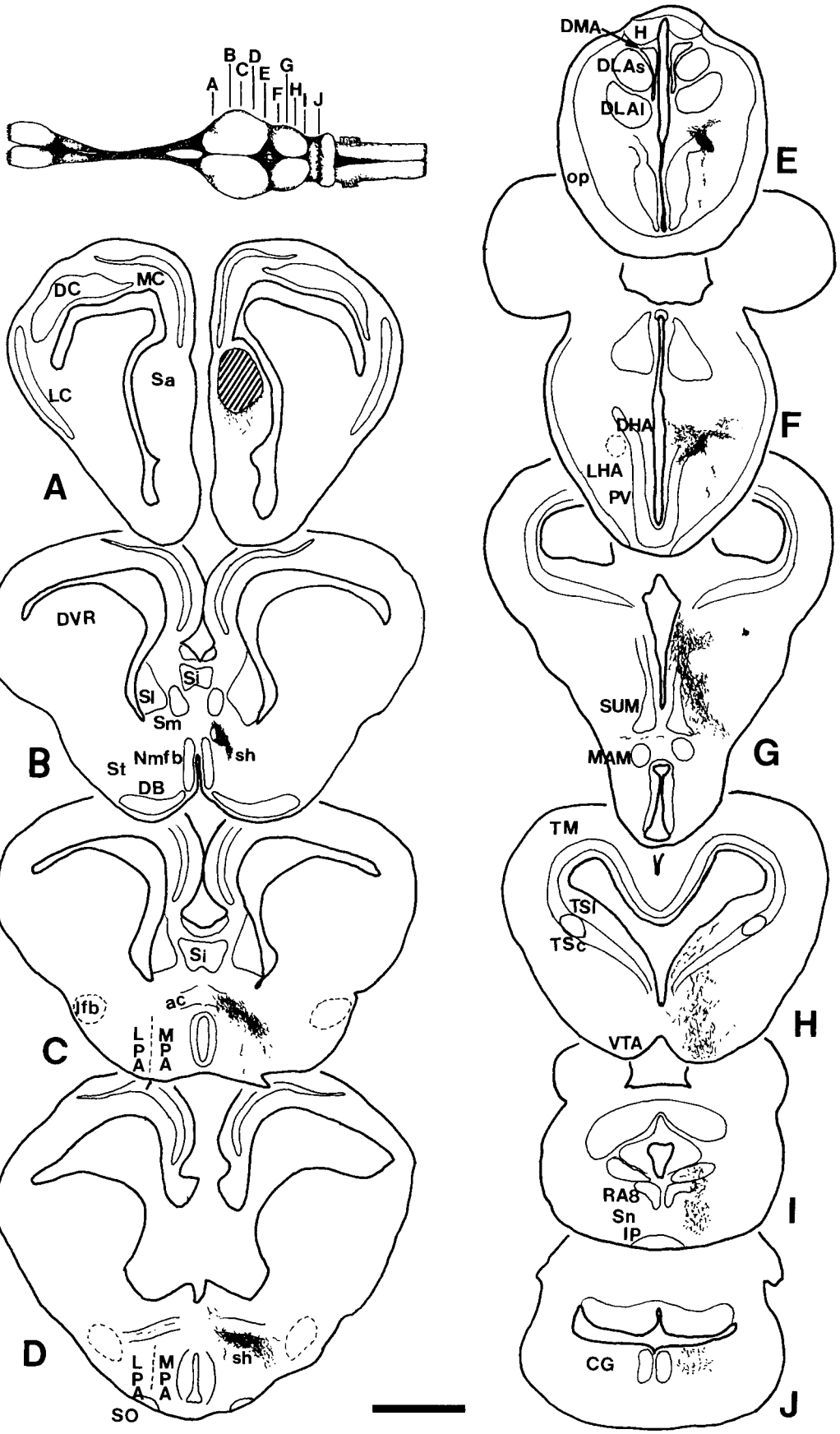

Fig. 8. **A–J:** Semi-schematic camera lucida drawings of frontal sections through the brain of *G. gecko* showing the anterograde labeling that resulted from a BDA iontophoretic injection (hatched area) in the anterior septal nucleus (case R15). For abbreviations, see list. Scale bar = 1 mm.

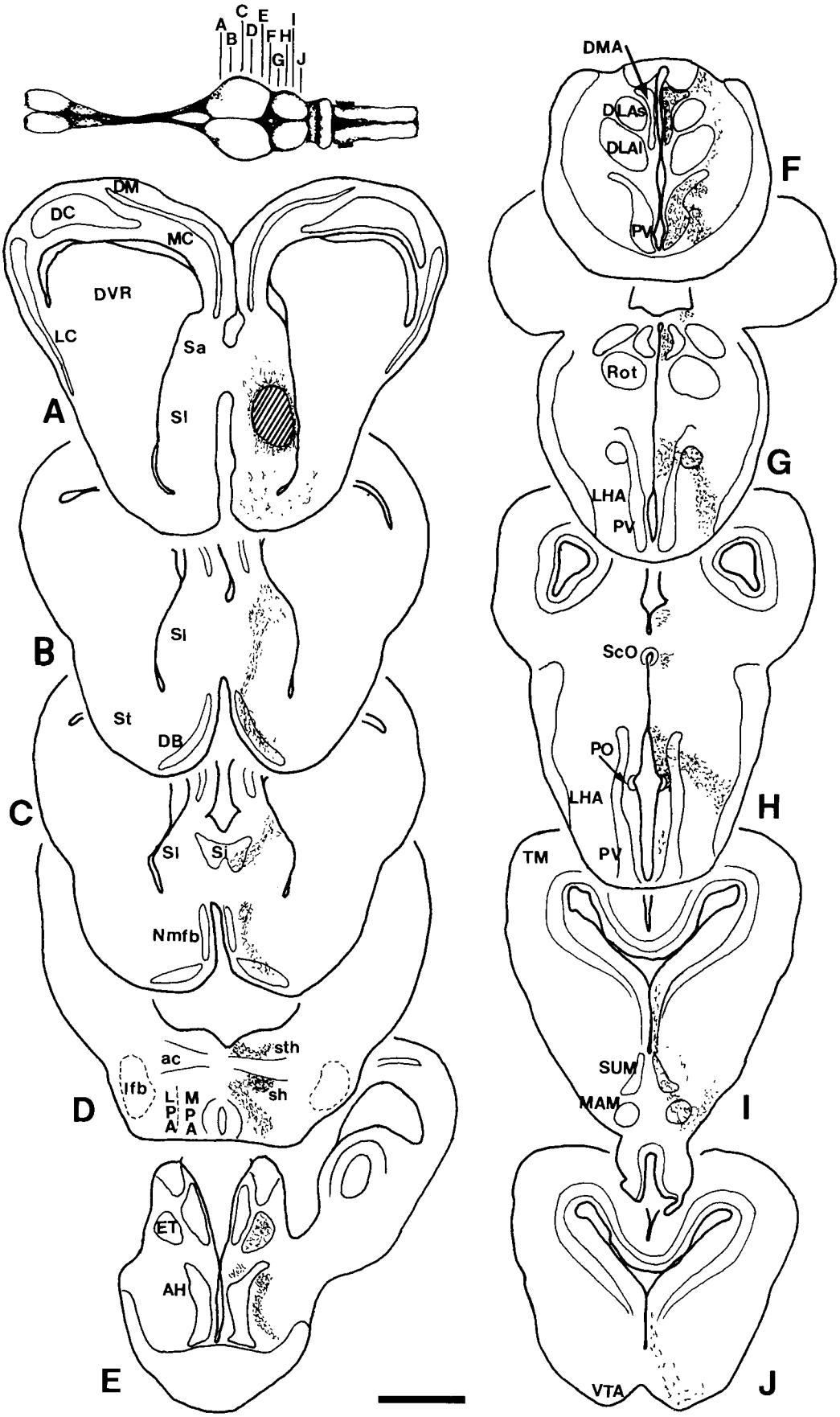

Fig. 9. **A–J:** Anterograde labeling in the brain of a lizard *G. gecko* after BDA injection (hatched area) in the Sl (case R27). For abbreviations, see list. Scale bar = 1 mm.

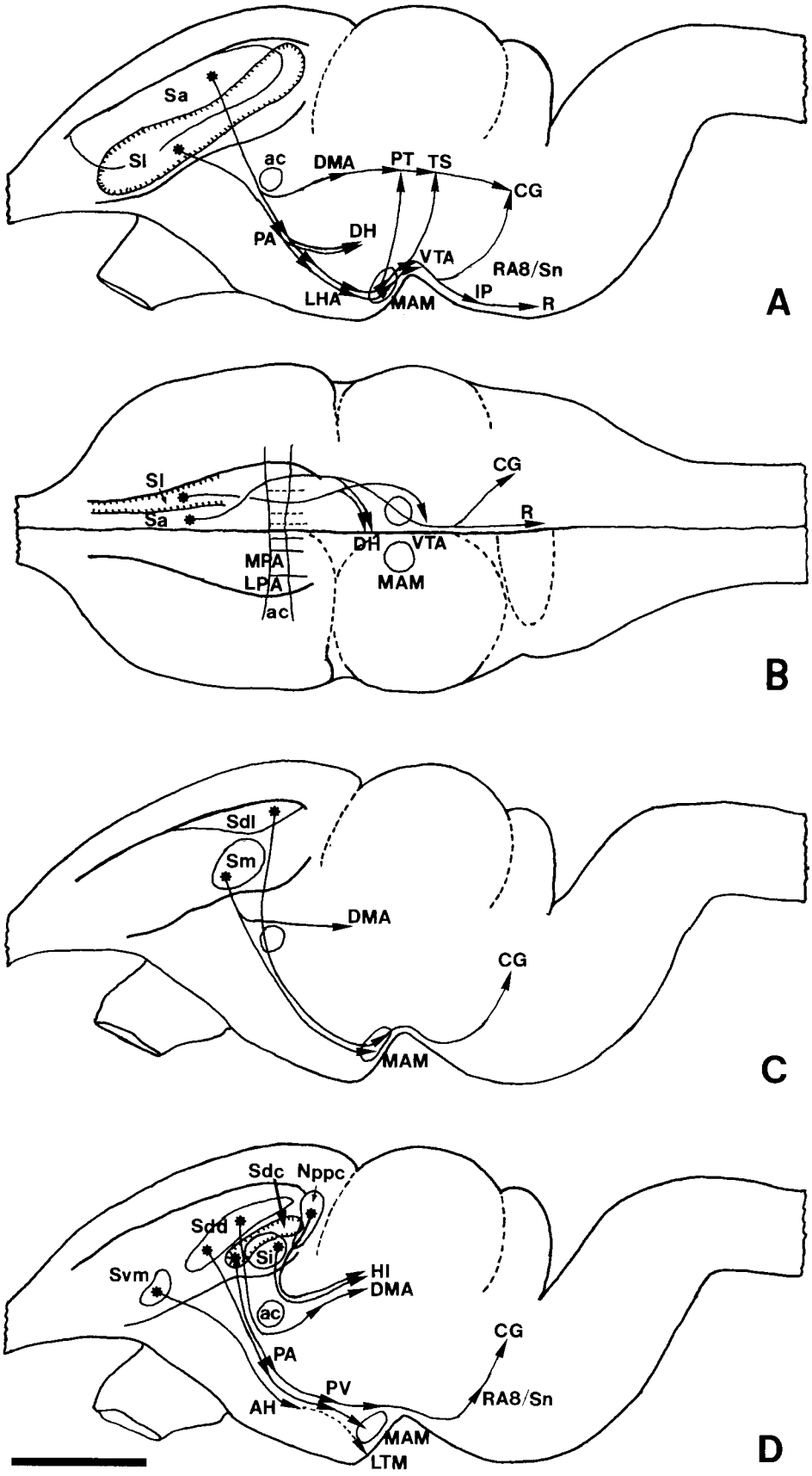

Fig. 10. Summarizing scheme of the efferent projections from the septum of *P. hispanica* according to our results of anterograde and retrograde tracing. Projections from the Sa and Sl are represented in two different drawings using a sagittal (**A**) and horizontal (**B**) view to show clearly the differential topography displayed by the projections of both nuclei. The projections from the remaining nuclei of the central septal division are represented in a single sagittal view (**C**). The projections of midline and ventromedial septal divisions are represented in a single drawing (sagittal view; **D**). For abbreviations, see list. Scale bar = 1 mm.

anterior thalamus is the Sm, which projects only scarcely to the hypothalamus but massively to the rostral (eminentia thalami) and dorsomedial anterior thalamus (DMA) and to part of the DLA. This septothalamic projection has also been described in *Varanus* by means of retrograde transport of HRP (Hoogland, 1982).

Efferent projections of the midline septal division. Throughout the present work we were unable to make a restricted injection to the midline septal division. However, from the results of retrograde labeling after extraseptal injections and from injections in the septum that involved the Si and/or Sd plus other septal nuclei, some of the efferent projections of the midline septal division can be inferred.

In our injections involving the Si (and Sm) of *Podarcis* (H9271), anterograde labeling is present in the habenula and anterior thalamus. On the other hand, injections restricted to the epithalamus (P9303) display dense retrograde labeling in the Si and Nppc. These findings confirm the results of retrograde labeling by Hoogland (1982) and Díaz and Puelles (1992), who already described a septohabenular projection in other lizards arising from these nuclei. According to Díaz and Puelles (1992), this projection courses via the stria medullaris and terminates in the medial habenula; in contrast, our results of anterograde labeling indicate that the above-mentioned septal nuclei project also to the lateral habenula. However, in some of our injections (e.g., B-9519, see Fig. 5) labeling in the lateral habenula might be due to transport from retrogradely labeled cells in the nucleus of the anterior commissure (see Fig. 4 in Font et al., 1997) as suggested by the results of Díaz and Puelles (1992). Further investigation is required to clarify this topic. The fact that the Nppc projects to the habenula, together with its topographical situation among the fibers of the posterior pallial commissure, suggests that this nucleus should be included as a part of the midline septal division, rather than the central division.

An intriguing result of our work is the presence of retrograde labeling in the Si and Nppc after several injections involving medial structures of the diencephalon (B9518, B9521, B9520, B9421, B9416; see Fig. 6). We interpret this labeling as due to uptake of the tracers at the level of the habenula or the septothalamic tract and/or stria medullaris (in which some labeled fibers are visible), because in all these cases the micropipette entered the diencephalon across the habenula or immediately rostral to it.

Although we have not made restricted injections into the Sd, extraseptal injections give an idea of its principal projections. On the one hand, every injection involving the periventricular hypothalamus at either anterior (B9520) or tuberal levels (B9416 and B9457) as well as big injections into the mammillary hypothalamus (H8810, BH9201, H9205, and H9322) give rise to labeled cells in both subnuclei of the Sd (Sdd and Sdc). However, more caudal injections indicate differential projections of both subnuclei. Retrograde labeling is found in the Sdc but not in the Sdd, as a result of caudal injections in the lateral midbrain, probably involving most of the CG and Sn/RA8. Therefore, the whole Sd seems to project to the periventricular hypothalamus and mammillary bodies, whereas the Sdc apparently projects specifically to the lateral midbrain.

As a conclusion, the midline septal division shows a characteristic pattern of efferents, which clearly differs from that of the central division. Moreover, the different nuclei of the midline septum display specific projections to the epithalamus, hypothalamus, and midbrain (Fig. 10D).

Efferent projections of the ventromedial septal division. This division is composed of a single nucleus, the Svm, which receives its main input from the anterior hypothalamus (Font et al., 1997). The results of both anterograde (B9519) and retrograde tracing (H9289 and B9510) indicate that this projection is reciprocated by a massive descending pathway from the Svm to the anterior hypothalamus. Again, the presence of labeled neurons in the Svm after tracer injections in the preoptic hypothalamus (B9521) might be interpreted as being due to uptake by fibers-of-passage.

The Svm appears to contribute to the projection to the LTM, because dense anterograde labeling is observed in the LTM after injections in the Svm (B9519), and many labeled neurons are present in the Svm when tracer injections involved the LTM (H9205).

In *Gekko,* the injections that affected the rostro-ventral part of lateral septal nucleus (R28), where the Svm is found in *Podarcis*, displayed dense anterograde labeling in the anterior hypothalamus. This fact and histochemical data (Font et al., 1995) suggest that the presence of an Svm is a characteristic feature of the septum of all lizards. This nucleus is characterized by a projection to the anterior hypothalamus (this work), a strong reactivity to acetylcholinesterase, and a dense innervation by fibers immunoreactive for serotonin (Font et al., 1995) and neuropeptide Y (Salom et al., 1994), even in those species (e.g., the gecko) in which an Svm is not easy to identify in Nissl-stained sections.

Comparative remarks

The septal complex of lizards has a chemoarchitectonical organization (Font et al., 1995) and a set of afferent connections (Font et al., 1997) similar to those of the dorsal septum of birds (Krayniak and Siegel, 1978a; Székely and Krebs, 1996) and the lateral and posterior divisions of the mammalian septum (Swanson and Cowan, 1979; Staiger and Nürnberger, 1991). Concerning the efferent connections, our results and fragmentary data in other reptiles (*Tupinambis:* Hoogland et al., 1978; Sligar and Voneida, 1981; *Varanus,* Hoogland, 1982; *Ophisaurus:* Belekhova and Nemova, 1988; *Gekko:* Hoogland et al., 1994; Bruce and Neary, 1995a,b; *Testudo:* Nemova, 1988; *Gallotia:* Díaz and Puelles, 1992) suggest that, as expected, tetrapods also share a common pattern of septal efferents.

Although detailed studies on the projections of the septum of birds are not available, big ^{3}H-leucine injections (Krayniak and Siegel, 1978b) and big deposits of lipophilic tracers (Balthazart et al., 1994) within the boundaries of the "dorsal" septum reveal a pattern of projections similar to the one described in lizards (this work). The main projections of the avian septum course through two different pathways comparable to the septo-thalamic and septo-hypothalamic tracts of lizards (compare Fig. 10 of this work and Fig. 7A in Krayniak and Siegel, 1978b): The first one innervates the midline thalamus, whereas the septohypothalamic tract innervates the whole hypothalamus and the ventral tegmentum. As in lizards, the septohypothalamic tract contributes to the innervation of the posterior thalamus and dorsal midbrain (CG and adjacent reticular

formation). The lack of more precise neuroanatomical studies using small injections of modern tracers in different parts of the avian septum makes a more detailed comparison difficult.

Mammals constitute the only group of tetrapods for which detailed studies of the septal connections have been performed (see Jakab and Leranth, 1995; Risold and Swanson, 1997). In spite of the differences in the organization of the septum between reptiles and mammals, many similarities arise when one compares the septal afferents and efferents in both groups. These similarities suggest a scheme of homologies between both structures. As we discussed in a previous report (Font et al., 1997) the lacertilian septum as considered in our work is only comparable to the lateral and posterior septum of the mammalian telencephalon, whereas the medial septum of mammals has a counterpart in part of the basal telencephalon of reptiles including the DB and Nmfb.

The lateral septum of mammals coincides with the central division of the reptilian septum in its topographically organized afferents from the hippocampal cortex (Siegel et al., 1974; Font et al., 1997; Risold and Swanson, 1997). Moreover, the different parts of the lateral septum of mammals project massively to the hypothalamus (Meibach and Siegel, 1977; Swanson and Cowan, 1979; Krayniak et al., 1980). According to Staiger and Nürnberger (1991), in the golden hamster the lateral septal projection to the hypothalamus is topographically organized: The dorsal subnucleus of the lateral septum projects to the most lateral hypothalamic regions, whereas the projection from the ventral lateral septum terminates in a more medial position within the hypothalamus. This situation clearly recalls the pattern of hypothalamic projections from the different nuclei of the central division of the reptilian septum. The Sa shows a pattern of projections to the hypothalamus similar to that in the dorsal subnucleus of the mammalian lateral septum, that is to say, mainly to the lateral aspect of the hypothalamus; on the other hand, the Sl, like the ventral subnucleus of the mammalian lateral septum, projects mainly to medial centers within the hypothalamus. In spite of the use of a different compartmentalization of the lateral septum, the results of Risold and Swanson (1997) indicate a similar situation in the rat.

Another hodological feature shared by the mammalian lateral septum and the central division of the reptilian septum is the projection to the midbrain. According to Risold and Swanson (1997) a projection terminating in the VTA, periaqueductal gray, interfascicular nucleus, and neighboring areas arises in the rat from the dorsal division of the caudal lateral septum and the septohippocampal nucleus. Therefore, these divisions of the rat lateral septum stand out as the best candidates for homologues to the reptilian Sa (and Sdl), which are the main sources of projections to the midbrain (see Results). Because the reptilian Sa receives a projection from the reptilian homologue of the mossy fibers (a zinc-enriched projection from the medial cortex: see Font et al., 1995, 1997) it has been considered as a nucleus specific to the septum of reptiles with no counterpart in the mammalian brain (Bruce and Neary, 1995b). However, our data on the projections of the reptilian septum suggest that the Sa is comparable to the dorsal subnucleus of the mammalian lateral septum, and that the presence of a direct input from the reptilian counterpart of the mammalian "fascia dentata" is due to a relative expansion of this zinc-enriched projection in the reptilian brain.

Finally, the other main target of the lateral septum efferents is the midline thalamus (paraventricular and parataenial nuclei: Swanson and Cowan, 1979; Staiger and Nürnberger, 1991). In mammals this projection arises mainly from the intermediate subdivision of the lateral septum (see levels 9.8 and 9.4 in Fig. 1 of Staiger and Nürnberger, 1991; see Figs. 11 and 14 of Risold and Swanson, 1997). In the reptilian brain, a similar projection mainly arises from the Sm, the third main nucleus of the central septal division.

So it appears that the lateral septum of mammals and the central division of the reptilian septum show a comparable general pattern of both afferents and efferents and a similar scheme of compartmentalization (dorsal subnucleus/Sa; intermediate subnucleus/Sm; ventral subnucleus/Sl). However, some differences are found between reptiles and mammals when the projections of each specific subdivision of the lateral septum are compared.

In mammals, the posterior septal division (septofimbrial and triangular nuclei) projects through the stria medullaris mainly to the medial habenular nucleus (Swanson and Cowan, 1979; Kawaja et al., 1990). Our results indicate that this projection also exists in *Podarcis,* and it arises from two nuclei of the midline septal division, the Si and Nppc. Therefore, this area could be compared to the posterior septum of mammals because it is composed of nuclei associated with the main fiber tracts crossing the septum (hippocampal or pallial commissures and the fornix), and it displays a prominent projection to the epithalamus. However, an important difference exists between reptiles and mammals: whereas in mammals the septal projection terminates in the medial habenula (but see Risold and Swanson, 1997), in reptiles it seems to reach mainly the lateral habenula. Further investigation is needed to clarify whether this fact reflects differences in the organization of the septum, the habenula, or both structures, especially because Díaz and Puelles (1992) claim that the septo-habenular projection terminates in the medial habenula in a close-related lizard (*Gallotia*).

The other nucleus of the midline septal division of reptiles, the Sd, has been compared to the septofimbrial nucleus of mammals (Halpern, 1980; see Font et al., 1995). Although both nuclei share some histochemical features (serotonergic innervation and presence of enkephalinergic cells and fibers: see Font et al., 1995) and a non-topographical hippocampal (cortical) input, their efferent projections appear partially different. The mammalian septofimbrial nucleus projects to the tuberomammillary nucleus (Ericson et al., 1991); in *Podarcis* injections of tracers in the mammillary hypothalamus give rise to retrograde labeling of cells in the Sd, thus supporting the homology of the Sd and septofimbrial nucleus. However, the septofimbrial nucleus of mammals displays an important projection to the habenula (Swanson and Cowan, 1979), whereas the reptilian Sd does not (no retrograde labeling is found in the Sd after an HRP injection restricted to the habenula, as observed in case H9303; Hoogland, 1982; Díaz and Puelles, 1992). Furthermore, anterograde and retrograde transport experiments in *Podarcis* indicate that the Sd, especially Sdc, projects caudally to the ventral midbrain.

This seems, therefore, to confirm that a general resemblance does exist between the midline division of the lacertilian septum and the posterior division of the mam-

malian one, although both structures display a somewhat different pattern of compartmentalization with respect to the organization of their projections.

Using topographical criteria, the mammalian equivalent to the ventromedial division of the lacertilian septum should be looked for in the ventralmost aspect of the lateral septum (LSv). The most remarkable features of the LSv are its massive (and specific) projection to the anterior hypothalamus (Swanson and Cowan, 1979; Swanson et al., 1987; Risold and Swanson, 1997) and an afferent projection from part of the amygdala (Krettek and Price, 1978; Risold and Swanson, 1997) including the bed nucleus of the stria terminalis (Staiger and Nürnberger, 1989). This last nucleus is rich in neuropeptide Y (Allen et al., 1984). In *Podarcis* the Svm seems involved in a similar circuit (septum-anterior hypothalamus-amygdala: Font et al., 1997) and is densely innervated by fibers which are immunoreactive for neuropeptide Y (Salom et al., 1994). The origin of this NPY innervation remains unknown. In spite of these similarities, further investigation is needed before any solid conclusion is drawn on the presence of a counterpart of the Svm in the mammalian septum.

It is interesting that the amygdaloid and septal projections to the hypothalamus do not overlap in reptiles (Lanuza et al., 1997; this work) or in mammals (Swanson and Cowan, 1979; Canteras et al., 1992). An exception to this rule is found, however, in the ventromedial septum. In fact, the Svm of reptiles (this work) and the ventral aspect of the lateral septum of mammals (Staiger and Nürnberger, 1991) project to the LTM, a hypothalamic area that is also reached by projections arising from the medial amygdaloid nucleus, the amygdalo-hippocampal transition area, and bed nucleus of the stria terminalis in mammals (Ericson et al., 1991) and from part of the amygdala in lizards (Lanuza et al., 1997). Therefore, the ventromedial septum stands out as a septal area that, together with the amygdala, controls the activity of the tuberomammillary hypothalamus. The relationship of the ventromedial septum with the amygdala is stressed by the presence in reptiles (Font et al., 1997) and mammals (Canteras et al., 1992; Staiger and Nürnberger, 1989) of direct projections from the amygdala (bed nucleus of the stria terminalis and medial amygdala) to the ventromedial septal division and the LSv, respectively.

General discussion: Functional aspects of the vertebrate septal complex

Our studies on the cytoarchitecture, chemoarchitecture, and connections of the reptilian septum (Font et al., 1995, 1997; this work) indicate that, in spite of some minor differences, a common scheme of organization and connections is recognized for the septum of reptiles and mammals (as well as birds and maybe amphibians). Therefore, it seems that the septum, as a part of the limbic forebrain, has been well conserved during the evolutionary history of tetrapods. This fact indicates that the septo-hippocampal limbic forebrain plays a basic role within the vertebrate brain.

However, lesion experiments and electrical or chemical stimulation of the septum, which have been extensively used in mammals (see De France, 1976; Gordon and Johnson, 1981; King and Nance, 1986; Lisciotto et al., 1990) to investigate the roles of this structure on physiology and behavior, have not clarified the functions of the septum. From these experiments, many different roles concerning very different physiological functions and behaviors have been proposed for the septum of vertebrates, but, to date, there is no hypothesis that puts all these functions together in an appropriate neuroanatomical background.

According to our work, the main reason for these difficulties might be found in the complexity of the septum and in the fact that most of the experimental works that aimed to analyze the functions of the septum did not consider the cytoarchitectonic complexity of the telencephalic septum, but usually viewed it as a single, uniform structure. On account of our anatomical data (and those of others), some of the functional studies can be reinterpreted to suggest a hypothesis on the functional anatomy of the septal complex of amniote vertebrates.

In this respect, functional studies on the mammalian septum, which were mainly focused on the central division, conclude that the lateral septum is involved in the expression of apetitive behaviors (such as food or water intake), aggressive/defensive behavior, or even in neuroendocrine aspects of reproductive physiology (see references above). Although functional investigations of the septum in non-mammals are scarce, a few studies using electrical stimulation of the brain (Distel, 1978) or septal lesions (Berk and Heath, 1975; Tarr, 1977; Krohmer and Crews, 1987) in reptiles are compatible with the view that the reptilian septum is involved in similar functions as the mammalian one.

Presumably, these functions are mediated by the projections of the central (lateral) septum to different areas of the hypothalamus and midbrain. In fact, some of the targets for the descending projections of the septum are known to be involved in aggression (e.g., the ventral tegmental area: Adams, 1986; Kruk, 1991), in defensive behavior or fleeing (periaqueductal gray; Shaikh and Siegel, 1989), food intake (lateral hypothalamus, Rolls, 1986), water intake (e.g., lateral preoptic area: Blass and Epstein, 1971; Blank and Wayner, 1975), or reproductive physiology and sexual behavior (lateral tuberal hypothalamus: Li et al., 1989, 1990; medial preoptic area: Malsbury, 1971; Hansen et al., 1982). Because, according to our data and to those of others (Swanson and Cowan, 1979; Staiger and Nürnberger, 1991; Risold and Swanson, 1997), discrete zones of the central septum project differentially to these areas in the hypothalamus and midbrain, the septum might be thought of as a "distributor" of behaviors: The probability that these behaviors are expressed would depend on the regional or spatial pattern of septal activity at each moment.

The pattern of septal activity in every instant obviously depends on the septal inputs. As described before (Font et al., 1997) the central septal division (lateral septum in mammals) is dominated by a massive (putatively excitatory) projection from the hippocampal cortex which shows a clear topography, according to which the stimulation of a small portion of the hippocampus would result in the increased activity of septal cells in a stripe of the septum covering its whole rostro-caudal axis (Font et al., 1997). Different lines of evidence, including anatomical, physiological, and/or behavioral data, in both mammals (Staiger and Nürnberger, 1989; Muller, 1996; Nishijo et al., 1997) and non-mammals (Krayniak and Siegel, 1978a; Krebs et al., 1989; Székely and Krebs, 1996), suggest that the

hippocampal projection provides the central septal division with information on the location of the animal in its environment.

Therefore, the position of the central septal division within the circuitry of the limbic forebrain (see Fig. 11) allows it to play a crucial role in deciding which behavior should be expressed as a function of the location of the animal. Therefore, it might well be involved in territorial aspects of behavior. This would explain the high diversity of behaviors elicited by electrical stimulation of the central septal division, as well as the inconsistent effects that lesioning the lateral septum has on the expression of natural behaviors in the laboratory (Grossman, 1976), where variables such as territoriality are difficult to control.

Although admittedly speculative, this hypothesis fits the few results available on the functions of the septum and of its main afferents. On the one hand, the results of lesion of the lateral septum in birds indicate that the lateral septal area is responsible for the differences in courtship and aggression between territorial and colonial species (Goodson et al., 1997). On the other hand, in a recent work Kollak-Walker et al. (1997) report that defeat after an agonistic encounter explosively increases the expression of c-fos mRNA in the lateral septum of male hamsters.

Considering our results together (Font et al., 1995, 1997; this work) the ventromedial and midline divisions of the lacertilian septum appear as clearly different to the central division concerning cytoarchitecture, chemoarchitecture, and connections. Therefore, it is logical to assume that their functions also differ.

Like the central division, the midline septal division (putative homologue of the mammalian posterior septum) also receives a direct input from the hippocampal cortex, but it is not organized in a topographical manner, is not rich in heavy metals (see Font et al., 1997) and, therefore, is probably not glutamatergic. Both divisions of the septum also differ considerably with regard to their efferents: Whereas the central septum projects to the hypothalamus, anterior thalamus, and midbrain, the midline septum projects mainly to the epithalamus (habenula), as well as to some caudal nuclei in the midbrain (central gray, aminergic groups RA8, and substantia nigra; Fig. 12, left panel).

In fact, according to Grossman (1976) lesions of the anterior and posterior septum affect behavior differentially, although the roles of the midline/posterior septum on behavior and physiology are far from clear. For instance, although many functional studies suggest a role for the mammalian posterior septum on water intake (Lubar, 1969; Blass and Hanson, 1970), it is important to keep in mind that the posterior septum is placed next to the subfornical organ (which is just caudal to the triangular nucleus: Akert et al., 1961), whose role as a central integrator of water balance is well known (Oldfield and McKinley, 1995). Therefore, it is possible that some of the functions attributed to the posterior septum are carried out by the subfornical organ, especially taking into account that in reptiles the subfornical organ, as defined by means of CRF-immunoreactivity, seems to extend rostrally within the Sdd (Mancera et al., 1991). Therefore the role attributed to the midline/posterior septum in water balance as a consequence of lesion experiments might be due to its close proximity to the subfornical organ. Further research is needed to check this possibility.

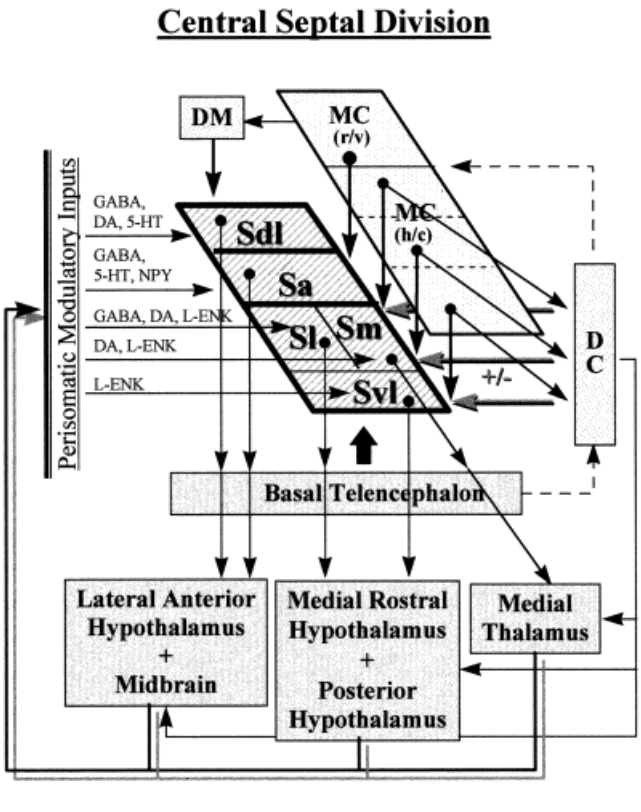

Fig. 11. Summarizing diagram of the circuitry of the central division of the septum in lizards as inferred from our results and Olucha et al. (1988). DA, dopamine; GABA, γ-aminobutyric acid; 5-HT, serotonin; L-ENK, Leu-enkephalin; NPY, neuropeptide Y; h/c, horizontal and caudal medial cortex; r/v, rostral and ventral medial cortex; for other abbreviations, see list. See text for details.

Concerning the ventromedial septal division, represented in lizards by the Svm, a similar cell aggregate is not evident in the mammalian septum. However, as discussed above, hodological data indicate some similarities between the reptilian Svm and the ventralmost aspect of the rostral lateral septum of mammals (LSv). The connections of the ventromedial septum (Fig. 12, right panel) clearly indicate that it must be functionally different from the central and midline septal divisions. For instance, it displays specific reciprocal connections with a NPY-rich cell group in the anterior hypothalamus, and it also seems to project to the lateral tuberomammillary hypothalamus (Fig. 12, right panel). Moreover, the Svm seems to receive a projection from an area of the amygdala (Bst and ventral anterior amygdala: Font et al., 1997) that also projects to the anterior hypothalamus and LTM (see Lanuza et al., 1997). This fact suggests that the ventromedial septum and the amygdala take part in the control of some as yet unknown functions through their projections to the hypothalamus. Among these functions, one can suggest those carried out by NPY, such as the control of sexual behavior and feeding (mammals: Allen et al., 1984; reptiles: Salom et al., 1994) or the control of neurosecretion (Danger et al., 1990; Li et al., 1990). Obviously, research is needed to put our anatomical data in the appropriate functional context.

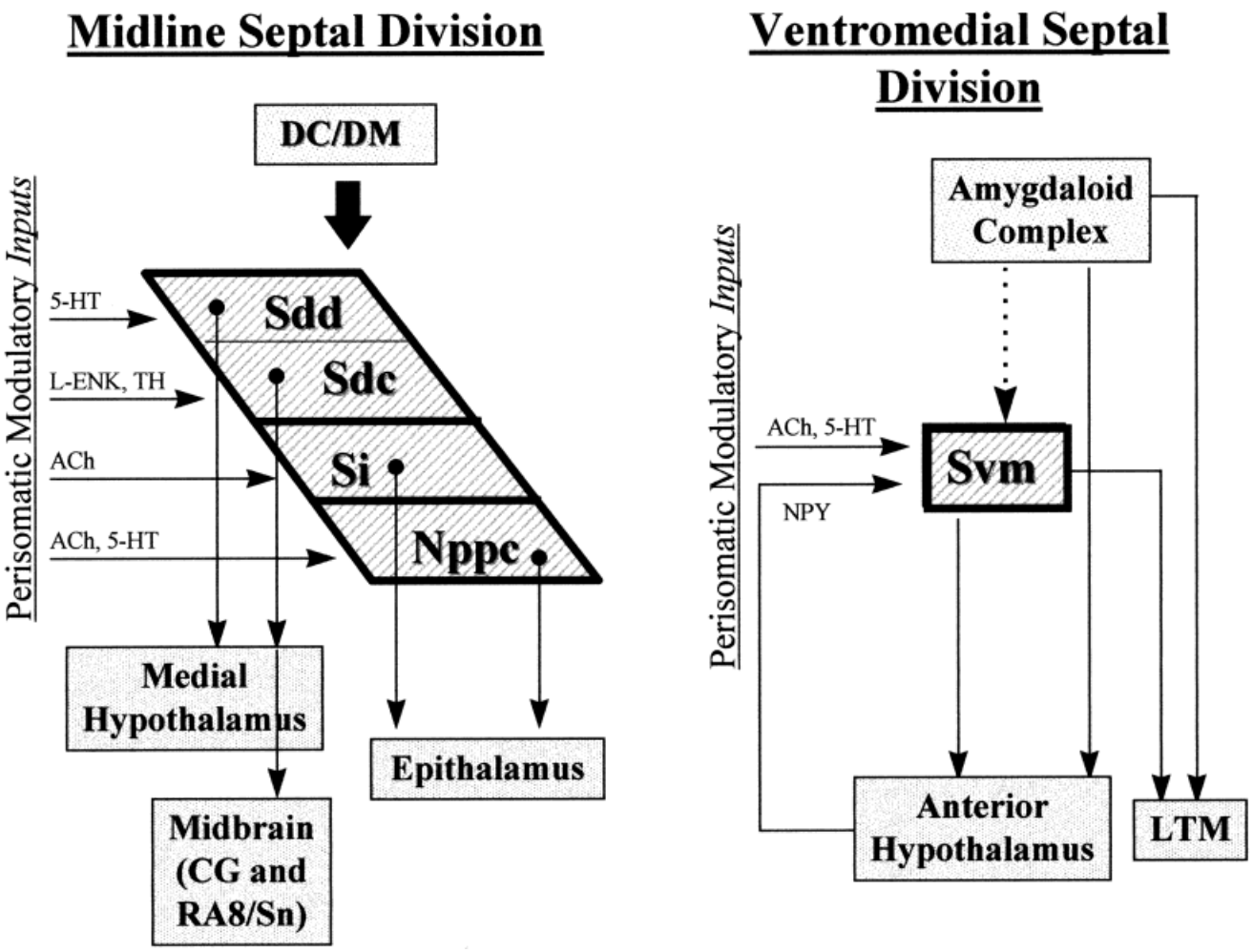

Fig. 12. Diagram of the main connections of the midline (left panel) and ventromedial (right panel) septal divisions of lizards, according to our data. Ach, acetylcholine; 5-HT, serotonin; L-ENK, Leu-enkephalin; NPY, neuropeptide Y; TH, tyrosine hydroxylase; for other abbreviations, see list. See text for details.

CONCLUSIONS

Our work has allowed further compartmentalization of the septum in tetrapodian vertebrates. As a consequence, the septum cannot be considered as a unitary structure anymore, but as comprising at least three anatomical divisions that display different connections and, therefore, are involved in different functions. Indirect evidence suggests a role of the central septal division on territorial aspects of motivation. Future research should carefully consider the anatomical complexity of the vertebrate septum and the social organization (namely the colonial or territorial organization) of the experimental species, in the design of any investigation of the role of the central septum on behavior and the functions accomplished by the remaining two divisions in the septum of tetrapods.

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