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Afferent Connections of the Habenular Complex in the Lizard *Gallotia galloti*

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Key Words

Habenula
Septum
Preoptic area
Lateral hypothalamus
Stria medullaris

Abstract

Afferents to the habenular complex were studied by means of *in vitro* horseradish peroxidase retrograde labeling and anterograde control experiments in the lizard *Gallotia galloti*. The medial habenular nucleus was found to receive abundant afferent fibers from the nucleus of the posterior pallial commissure and the nucleus septalis impar. More restricted input comes from the nucleus eminentiae thalami and the nucleus of the stria medullaris. The lateral habenular nucleus is innervated by various fiber groups originating from the bed nucleus of the anterior commissure, the diagonal band nucleus, the lateral preoptic area, the anterior entopeduncular nucleus, the lateral hypothalamic and mammillary areas, the nucleus of the stria medullaris, the area tegmentalis ventralis and a scattered neuronal subpopulation in the large-celled dorsolateral nucleus of the dorsal thalamus. Habenuropetal fibers generally follow the stria medullaris, but hypothalamic, entopeduncular and dorsal thalamic afferents course through the dorsal peduncle of the lateral forebrain bundle in a transthalamic route. Mesencephalic ventral tegmental afferents ascend through the tractus retroflexus.

Introduction

The connections of the habenular complex have been studied experimentally in mammals [e.g. Herkenham and Nauta, 1977, 1979; McBride, 1981], but they are known only fragmentarily in other vertebrates [*Rana*, Kemali et al., 1980; *Varanus*, Distel and Ebbesson, 1981; Hoogland, 1982]. The habenular circuitry of vertebrates is nevertheless held to be strongly conservative, according to available

data. Afferents from either pallidal or septohypothalamic (limbic) forebrain sources are channelled into the habenular complex through the stria medullaris, ending discretely within its medial or lateral portions (HM, HL). The habenular nuclei project through core and peripheral portions of the fasciculus retroflexus (habenulo-interpeduncular tract) into paramedian forebrain and brainstem limbic formations (septum, hypothalamus, area tegmentalis ventralis, nucleus interpeduncularis, raphe nuclei).

Experimental literature contains scarce data on the sources of afferents to the habenular complex in reptiles [Brauth and Kitt, 1980; Hoogland, 1982]. We have examined this issue by employing *in vitro* horseradish peroxidase (HRP) labeling in young specimens of the lizard *Gallotia galloti*. We previously tested this experimental model by means of *in vitro* labeling of the fasciculus retroflexus, as reported in a companion paper [Díaz and Puelles, 1992]. The present data thus complement earlier observations on the efferent connections of the habenula.

Materials and Methods

Animal care guidelines indicated by Spanish Royal Decree 223/1988 were followed. The experiments were performed on young specimens of *Gallotia galloti* captured in Tenerife. The animals were anesthetized with chloroform or ether and perfused transcardially with Tyrode solution. The brains were quickly removed, dissected free of meninges, and in most cases sectioned midsagittally. Brain halves survived much better *in vitro* than whole brains. In total, 3 whole brains and 35 brain halves were injected with HRP.

Horseradish peroxidase was recrystallized on the tip of fine glass micropipettes, and then applied through the ventricular lining to the habenular complex ($n=10$). Rostral injections involved the lateral habenular nucleus and sectioned fibers passing into the medial habenular nucleus, whereas caudal injections mainly affected the medial habenular nucleus. Control experiments included HRP injections in the anterior commissure and its bed nucleus ($n=3$), lateral preoptic area ($n=3$), nucleus of the stria medullaris ($n=1$), dorsolateral/dorso-medial nuclei of the dorsal thalamus ($n=4$), area tegmentalis ventralis ($n=4$) and interpeduncular nucleus ($n=11$). Additional control cases were provided by our previous experiments labeling the fasciculus retroflexus midway in its dorsoventral course [Díaz and Puelles, 1992].

The injected brains were placed in culture at room temperature in Gibco Minimal Essential Medium (supplemented with penicilline), with constant bubbling of a 95% air and 5% carbon dioxide gas mixture. After 24–35 h, they were fixed with cold 1% glutaraldehyde or 2% glutaraldehyde-1% paraformaldehyde in 0.1 M, pH 7.2 phosphate buffer (4–5 h), and then soaked overnight in cold 30% phosphate-buffered sucrose. Serial frozen or cryostat sections (50–60 μm thick) were cut in the sagittal plane. One whole brain preparation was cut horizontally. The sections were mounted on chrome alum-gelatin-coated slides, briefly air-dried, and reacted with nickel-cobalt-diaminobenzidine, with the addition of one drop of dimethylsulfoxide to each 100 ml incubation medium. All sections were counterstained with cresyl violet. The location of labeled structures was then recorded with a drawing tube or charted on photographs.

Results

The habenular nuclei of the lizard *Gallotia galloti* were found to receive afferents from various forebrain cell groups, as detailed below, as well as from the midbrain area

List of Abbreviations

AT	area triangularis thalami
ATV	area tegmentalis ventralis
BCA	bed nucleus of the commissura anterior
BST	bed nucleus of the stria terminalis
ca	commissura anterior
cpa	commissura pallii anterior
DB	nucleus of the diagonal band
DLI	nucleus dorsolateralis thalami, large-celled part
EPa	nucleus entopeduncularis anterior
ET	nucleus eminentiae thalami
fr	fasciculus retroflexus
GV	nucleus geniculatus ventralis thalami
H	hypothalamus
Hab	habenular complex
Ip	nucleus interpeduncularis
lfbd	lateral forebrain bundle, dorsal peduncle
LHA	lateral hypothalamic area
LMA	lateral mammillary area
LPA	lateral preoptic area
MC	medial cortex
NCPP	nucleus of the commissura pallii posterior
NSM	nucleus of the stria medullaris
R	nucleus rotundus thalami
S	septum
SI	nucleus septalis impar
sm	stria medullaris
toh	tractus olfactohabenularis
VL	nucleus ventrolateralis thalami

tegmentalis ventralis. The experiments consistently labeled the same structures, with small quantitative differences that can be attributed to the varying amount of label deposited. The results are therefore described jointly. The nomenclature of Smeets et al. [1986a] was followed as far as possible.

Injections in the Habenular Complex

A survey of the labeling obtained in a representative case is presented in figures 1–4. The massively labeled stria medullaris can be followed rostrally from the site of injection (asterisk in fig. 1) towards a number of retrogradely labeled cell groups. The nucleus of the posterior pallial commissure (NCPP) appeared intensely stained in a periventricular locus adjacent to the medial cortex (fig. 1, 2). Axons originating here form a compact descending bundle across the caudolateral postcommissural septum, until they sharply bend dorsally into the stria medullaris (fig. 1B, 2B,

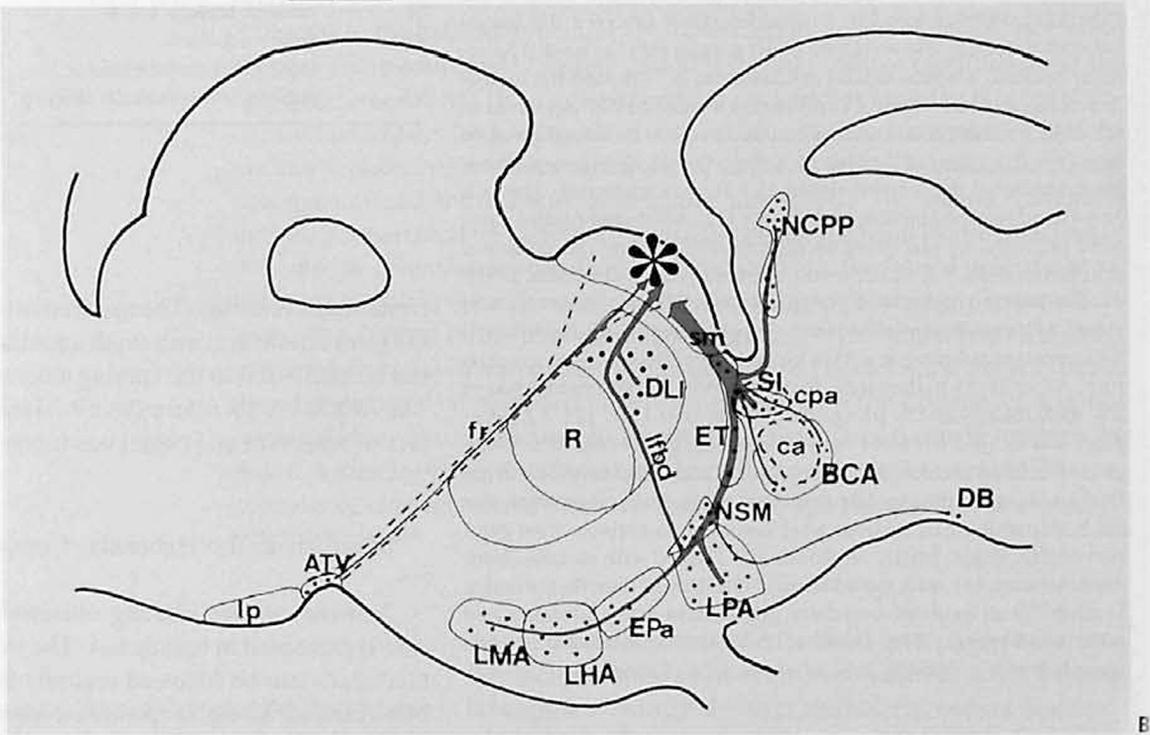
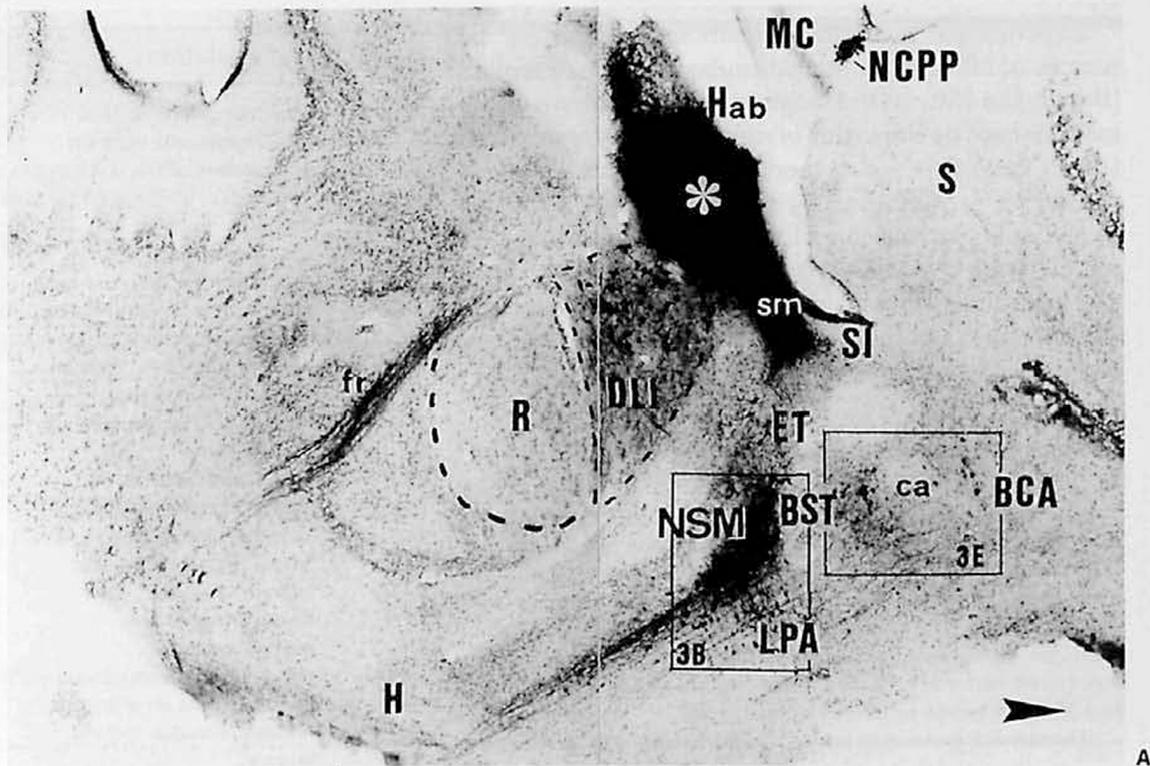


Fig. 1. Habenular afferents and nuclei of origin in the brain of the lizard *Gallotia galloti*. **A)** Photomicrograph ($\times 30$) of medial sagittal section showing site of *in vitro* injection of HRP in the habenula (asterisk) and neurons labeled retrogradely in several prosencephalic grisea (NCPP, SI, ET, BCA, NSM, DLI). Note massive retrograde labeling of stria medullaris (sm) and anterograde labeling of fasciculus retro-

flexus (fr). Areas enclosed in rectangles are shown at higher magnification in figures 3B, E. Arrowhead points rostrally. **B)** Schematic sagittal drawing summarizing the observed pattern of habenular afferents. Black points = retrogradely labeled neurons; red lines = course followed by the various afferents. Asterisk = injection site.

C). As observed in the whole-brain experiment sectioned horizontally, collaterals of these fibers cross the midline via the posterior pallial commissure, entering the contralateral stria medullaris (not shown).

Additional massive projections to the habenula come from the nucleus septalis impar (SI; fig. 1, 3D), the lateral preoptic area, and the nucleus of the stria medullaris (LPA and NSM; fig. 1B, 2A, B, 3A, B, 4A). We have interpreted as nucleus of the stria medullaris a group of medium-sized, labeled neurons that appears just dorsal to the medial forebrain bundle and rostral to nucleus ventromedialis, at the point where the various fiber bundles of the stria medullaris diverge in several directions. These cells can be followed medially nearly to the ventricle. The NSM is sharply delimited rostrally from an unlabeled round neuropile, also extending mediolaterally, which we tentatively interpret as the bed nucleus of the stria terminalis (BST; fig. 1A). Caudally, the NSM is related to area triangularis and a medial extension of nucleus ventrolateralis thalami.

A remarkable dense group of habenulopetal neurons lies ventral to the NSM, practically reaching the ventral surface of the brain at the level of nucleus supraopticus. The labeled cells were located interstitially and orthogonally to the medial forebrain bundle and send their axons into NSM and the stria medullaris. We have interpreted these cells as representing the main portion of the lateral preoptic area (LPA).

More rostrally, sparser labeled neurons were found around the anterior commissure and at the base of the telencephalon. These may be homologous to a vaguely defined bed nucleus of the anterior commissure (BCA; fig. 1, 3A, E) and to the horizontal portion of the nucleus of the diagonal band (DB; fig. 1B), respectively. Their axons pass caudally into the stria medullaris at the level of the eminentia thalami, where a number of retrogradely labeled neurons are also found (ET; fig. 1, 2A, 3A).

Caudal to NSM and LPA, many retrogradely labeled cells were found in the lateral hypothalamic area (LHA; fig. 1B, 2A) and sparse cells in the anterior entopeduncular nucleus (EPa; fig. 1B, 2A, B, 4B) [Smeets et al., 1986a]. The caudalmost hypothalamic cells lie in the lateral mammillary area (LMA; fig. 1B).

A number of scattered cells in the large-celled part of the dorsolateral nucleus of the dorsal thalamus were also labeled (DLI; fig. 1, 3A, C). Fibers coming from the hypothalamus (LMA, LHA, EPa) initially follow the medial forebrain bundle rostrally. However, just before reaching the lateral preoptic area, many of them enter the dorsal peduncle of the lateral forebrain bundle (fig. 1B, 4). After traversing the ventral thalamus, these fibers enter the large-

celled part of the dorsolateral nucleus, collect the axons from labeled neurons within it, and then proceed dorsally towards the habenular complex (fig. 1B, 2A, 4B). A smaller number of hypothalamic fibers follow the lateral forebrain bundle more rostrally and enter the ventral root of the stria medullaris, passing through NSM.

Caudal to the diencephalon, we observed only a small number of retrogradely labeled neurons in the area tegmentalis ventralis; these were superficially located in the midline between the oculomotor nerve roots (ATV; fig. 1).

Since most habenular afferents pass through the lateral habenular nucleus, injections of label restricted to this nucleus invariably produced at least some transport towards all of the forementioned sources of afferents to the habenular complex. By contrast, experiments with injections restricted to the more caudally located medial habenular nucleus ($n=2$) showed no retrograde transport to the bed nucleus of the anterior commissure, nucleus of the diagonal band, anterior entopeduncular nucleus, lateral hypothalamic area, lateral mammillary area, and large-celled part of nucleus dorsolateralis thalami. Retrograde transport to the nucleus of the stria medullaris and the lateral preoptic area was much reduced. Labeling of the nucleus of the posterior pallial commissure, nucleus septalis impar, nucleus eminentiae thalami and area tegmentalis ventralis after deposits restricted to HM was similar to that obtained in the other cases (HM plus HL).

Anterograde transport of label from the habenular nuclei via the fasciculus retroflexus was of course observed in all cases. Since these data coincide with those of our companion report on *in vitro* labeling of this tract [Díaz and Puelles, 1992], they are not further considered here.

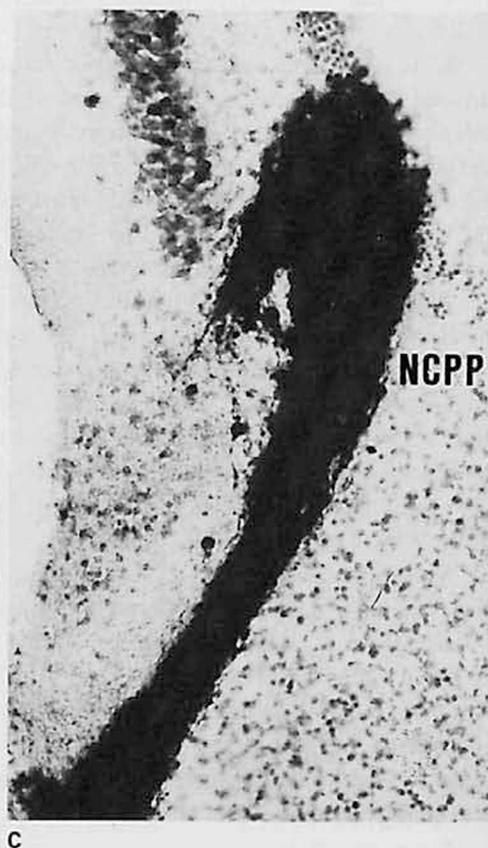
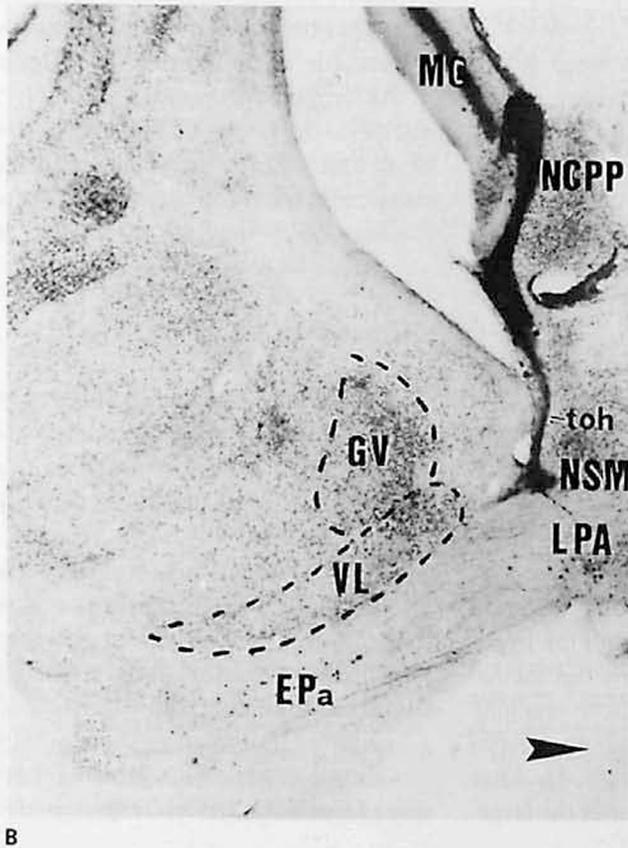
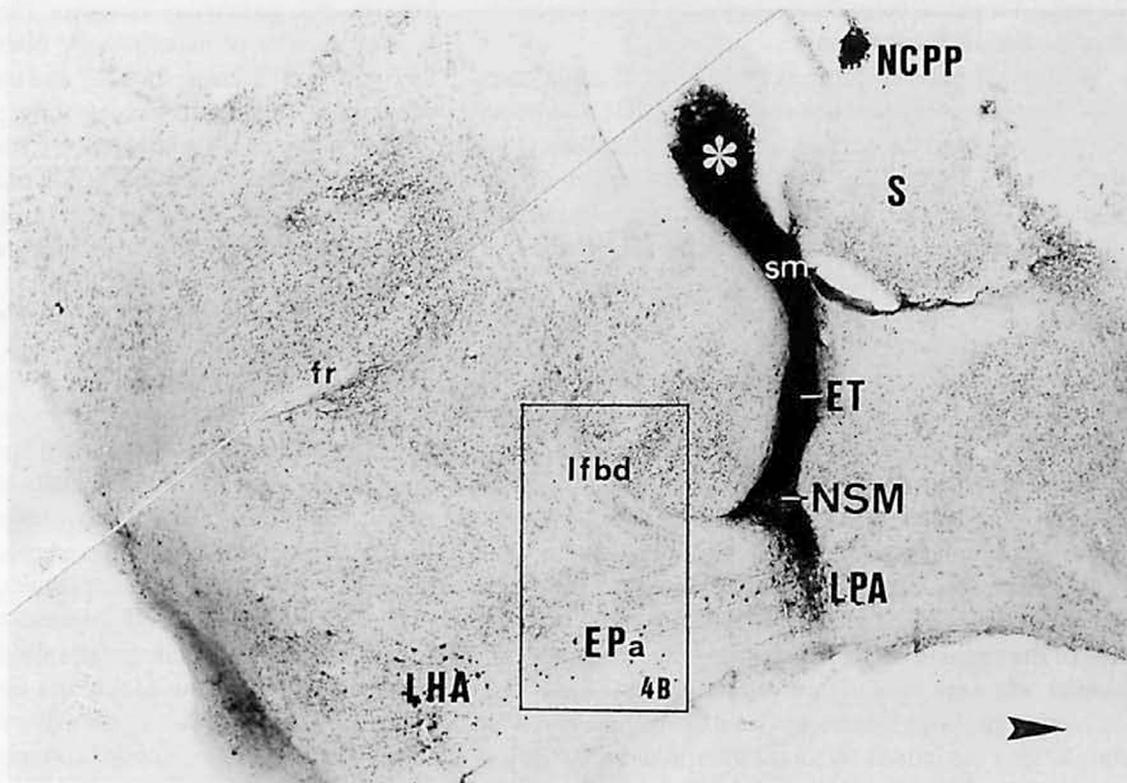
Control Injections

We placed control deposits of HRP at various sites, in order to corroborate the preceding observations by means of anterograde transport and to confirm the apparently differential distribution of afferents to the medial and lateral habenular nuclei.

The HRP-deposits presumably labeling the habenular afferents originating at the bed nucleus of anterior commissure (BCA, $n=3$) produced anterograde labeling of the stria medullaris. Most of these fibers terminate within the lateral habenular nucleus, forming a dense plexus, whereas a few fibers terminate in the medial habenular nucleus.

Control experiments labeling LPA ($n=3$; label also spread medially) showed anterograde transport by way of

A



B

C

the stria medullaris, with sparse terminal labeling of HL, whereas one control injection into NSM produced dense terminal arborizations restricted to HL.

A large injection in the caudobasal telencephalon labeled a bundle of fibers that ascends in the stria medullaris and crosses the midline through the habenular commissure without giving off terminals within the habenular complex. This represents the so-called tractus olfactohabenularis [Crosby, 1917], which was also labeled anterogradely from the massive deposits in the habenula (fig. 2B). Its fibers could be followed inside the telencephalon up to the external amygdaloid nucleus.

We placed four control injections into DLI. Since the tracer was introduced through the ventricular lining, the dorsomedial nucleus and portions of the small-celled part of the dorsolateral nucleus were also labeled. In two experiments (35a, 36a) scarce fibers were seen passing from DLI into HL. In experiment 56a a dense patch of terminals was observed in the caudalmost part of HL. Finally, in experiment 47a, abundant terminals appeared throughout HL. Retrogradely labeled cells were found within DLI, as well as within EPa and HLA, with fibers coursing in the dorsal peduncle of the lateral forebrain bundle.

Control HPR-deposits placed at the area tegmentalis ventralis (n=4) resulted in a few labeled fibers coursing in the fasciculus retroflexus, with terminal arborizations within the HL. Injections into the nucleus interpeduncularis (n=11) revealed no anterograde transport to the habenular complex.

Discussion

Our results in the lizard *Gallotia galloti* corroborate our initial expectation that reptilian habenular afferent circuitry is similar to the circuitry described in mammals [Herkenham and Nauta, 1977; McBride, 1981] and frogs [Kemali et al., 1980]. However, some novel aspects were

observed, as is discussed below. The following sections deal successively with relevant reptilian literature, comparison with mammals and amphibians, and final comments.

Comparison with Other Reptiles

On the whole, the present study corroborates partial data previously reported on prosencephalic afferents to the habenula in reptiles [*Caiman*, Brauth and Kitt, 1980; *Varanus*, Hoogland, 1982]. Some identifications were reformulated (NCPP, NSM, LPA and ATV) and six new afferent grisea were added to the list (BCA, DB, ET, DLI, LHA, LMA). On the other hand, reports of habenular afferents located caudal to the mesencephalon [Brauth and Kitt, 1980; Hoogland, 1982] could not be confirmed.

Septal Area

There are several neuronal populations in the caudal septum that project to the habenula: nucleus septalis impar (SI), nucleus of the posterior pallial commissure (NCPP), bed nucleus of the anterior commissure (BCA) and nucleus of the diagonal band (DB).

Nucleus SI is a bed nucleus of the anterior pallial commissure. This population was named nucleus paraterminalis in *Lacerta* [Frederikse, 1931], or 'bed nucleus of the hippocampal commissure' in *Alligator* [Crosby, 1917; Huber and Crosby, 1926] and *Gekko* [Butler, 1976]. We have followed the terminology of Smeets et al. [1986a]. Hoogland [1982] reported the presence of an unnamed cell group in the caudal septum, dorsal to the anterior commissure, that projects to the habenula in the lizard *Varanus*. This cell mass is clearly the same referred to as SI [Smeets et al., 1986; present results]. Also Brauth and Kitt [1980] observed a comparable projection in *Caiman crocodilus*.

There is scarce experimental information on the nucleus of the posterior pallial commissure (NCPP). This nucleus is illustrated, but not labeled, in the *Gekko* atlas of Smeets et al. [1986; fig. 16, 17]. It appears as a distinct periventricular mass of small cells just below the small-celled medial cortex. In reptiles, a connection of the NCPP with the habenula was inferred from myelin- and fiber-stained material [Herrick, 1910; Cairney, 1926; Kappers et al., 1936] but was not experimentally proved. Brauth and Kitt [1980] reported habenulopetal neurons within the so-called 'ventro-caudal pallial area' in *Caiman crocodilus*, which may correspond to the location of NCPP in that species.

The NCPP projection is bilateral and directed to the medial habenula, with ipsilateral fibers being more numerous. The contralateral fibers cross the midline through the

Fig. 2. Photomicrographs of two sagittal sections (A, B, C) from the same brain but more lateral than fig. 1. B is lateral to A; C is a higher magnification of B. Note the increasing size of NCPP. Mediolaterally, LHA is the most extensive hypothalamic afferent population, while labeled EPa cells appear immersed within the lateral forebrain bundle (lfb). Tractus olfactohabenularis (toh) is seen most laterally, entering the amygdala. Rectangle in A marks detail shown in fig. 4B. Arrowheads in A and B point rostrally. A and B = $\times 30$; C = $\times 200$.

posterior pallial commissure. Lohman and van Woerden-Verkley [1976] reported 'a possible source of the fibers of the posterior pallial commissure ... in the small cells in the mediodorsal cortex ventrally and caudally in the cerebral hemisphere'. They possibly referred in part to NCPP.

Projections to the habenula coming from the bed nucleus of the anterior commissure (BCA) and the nucleus of the diagonal band (DB) had not been described previously in reptiles.

Thalamus

Afferents to the habenula originating from the *ventral thalamus* comprise a dense projection from the nucleus of the stria medullaris (NSM) to the lateral habenular nucleus, with a minor distribution to the medial habenular nucleus, and a sparser projection of nucleus eminentiae thalami (ET) to the whole habenular complex. There are no previous data on ET.

Hoogland [1982] described labeled neurons in the 'area triangularis' after habenular HRP deposits in *Varanus*, which probably correspond to our NSM cells. We think that the area triangularis (AT) is a larger and denser cell population lying immediately caudal to NSM [Smeets et al., 1986a; Medina et al., 1990]. The AT neurons do not lie in the path of the stria medullaris. Both ET and NSM are embedded along the ascending course of the ventral root of the stria medullaris, rostral to the lateral habenular nucleus. In our companion report on fasciculus retroflexus projections in the lizard *Gallotia* [Díaz and Puelles, 1992], we found that some ET neurons send descending axons through this tract. However, the relatively higher number of ET neurons labeled retrogradely following deposits in the habenula indicate that most ET axons do not simply pass by this structure.

The *dorsal thalamus* repeatedly showed a scattered subpopulation of retrogradely labeled neurons in the large-celled part of the dorsolateral nucleus (DLI) following HRP injections into the lateral habenula. The fact that such cells frequently appear in rather ventral portions of the DLI indicates that this labeling is not due to diffusion or dendritic transport. Cairney [1926] described a 'habenulothalamic tract' which contained a component ending within DLI and another component passing through or near nucleus rotundus, entering ventrally into the dorsal peduncle of the lateral forebrain bundle. This is in accord with our data on habenulopetal fibers from DLI, EPa and LHA, which follow this course but in the *opposite* direction.

The DLI in lizards is otherwise known to project to the small-celled part of the medial cortex [Bruce and Butler,

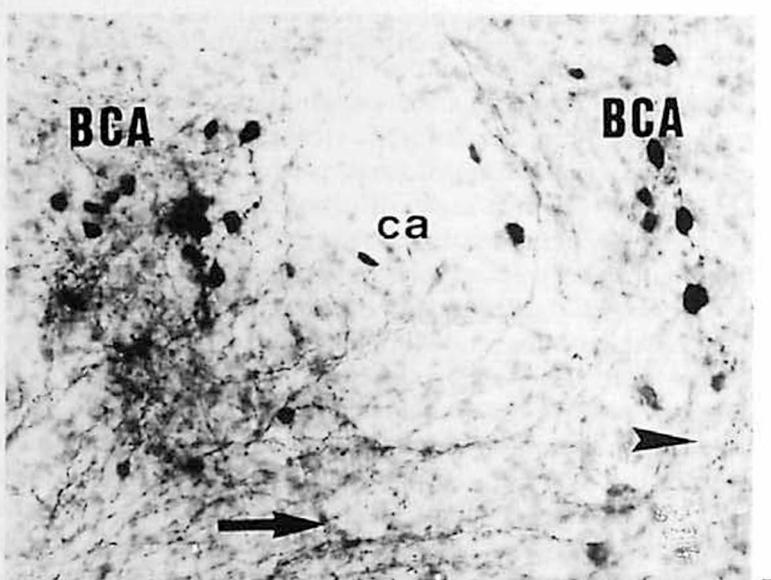
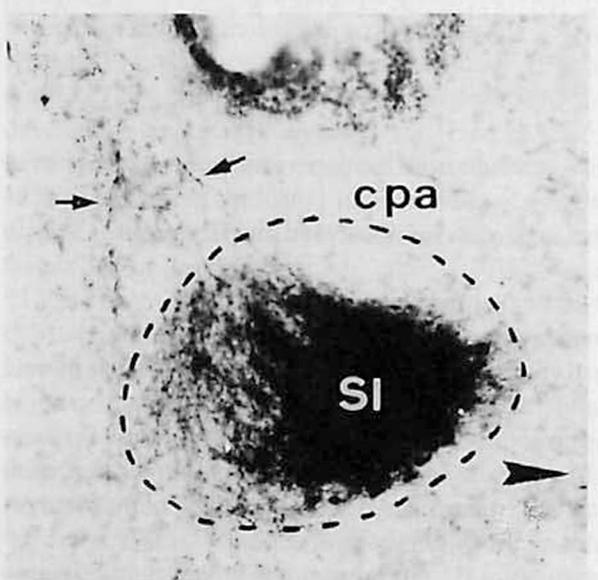
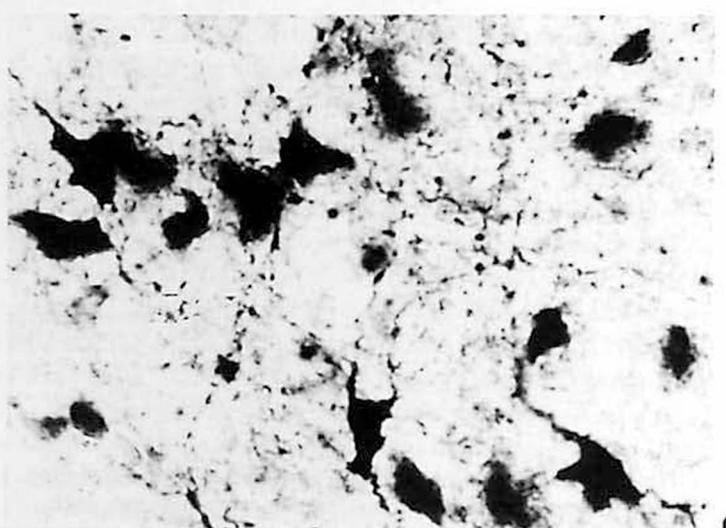
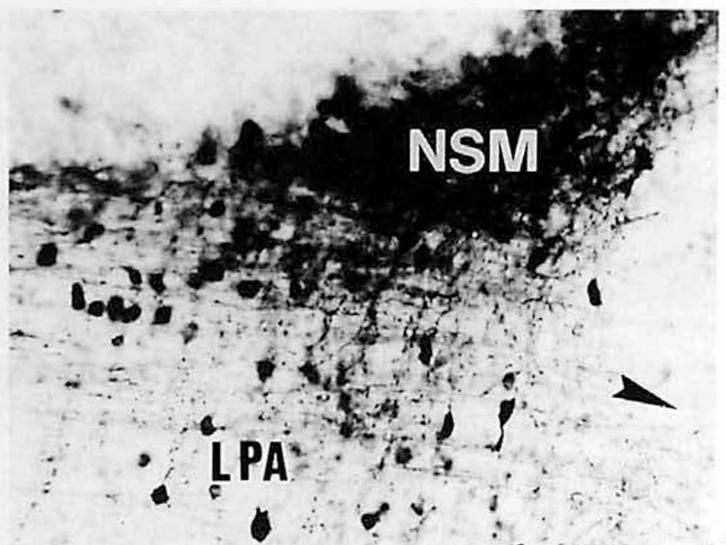
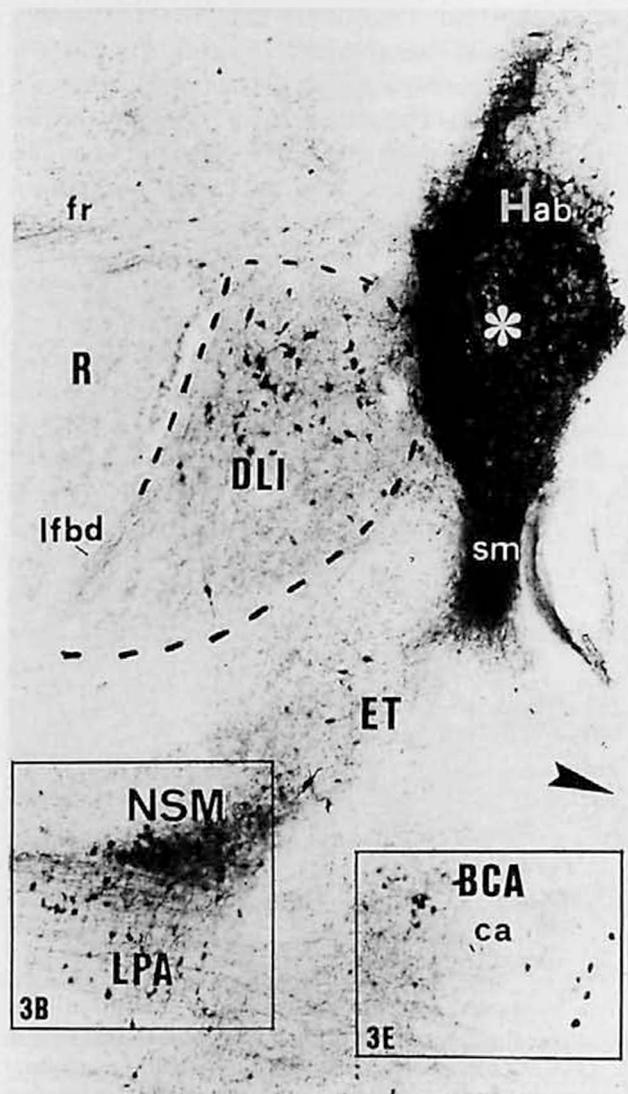
1984; González et al., 1990], and the dorsolateral complex receives projections from the septum, periventricular hypothalamus, anterior entopeduncular nucleus and torus semicircularis pars laminaris [Hoogland, 1982]. These connections are compatible with an interpretation of DLI as participating in limbic circuitry, as is also suggested by its projection to the lateral habenula.

An alternative interpretation of our results is that some DLI axons may course through the stria medullaris on their way towards the medial cortex. This is contradicted by general consensus that the corticopetal fibers course by way of the dorsal peduncle of the lateral forebrain bundle [Lohman and van Woerden-Verkley, 1978; Brauth and Kitt, 1980; Bruce and Butler, 1984; González et al., 1990]. Moreover, we did not observe any fibers following such an anomalous course in our control injections within DL. This negative result is the most informative aspect of these controls, establishing that DLI neurons are, in fact, labeled from the habenula. Otherwise, the anterograde filling of terminals in HL could have resulted from a labeling of LHA and EPa axons coursing through DLI towards HL.

Hypothalamus and Preoptic Area

The HRP injections in the habenula produced retrograde labeling of many neurons placed interstitially in the medial forebrain bundle between the preoptic area and the posterior hypothalamus. We have interpreted these neurons as corresponding to LPA, LHA, and LMA. None of these sources of projections to the HL had been described previously in lizards, although Brauth and Kitt [1980] did report some habenulopetal neurons within the 'dorsal preoptic region' in *Caiman crocodilus*. It should be noted that ascending HL efferent components of the fasciculus retroflexus course through the region occupied by this clon-

Fig. 3. Photomicrographs showing various retrogradely labeled cell groups (SI, DLI, ET, NSM, LPA, BCA) in sagittal view after *in vitro* habenular HRP deposits and transports. **A**) Asterisk = injection site. Note large-sized labeled neurons in DLI. The NSM is contiguous with LPA; both are intimately related to the ventral root of sm and to fibers of the medial forebrain bundle. Labeled fibers of mfb proceed rostrally near the anterior commissure (ca) and terminate in the septum; they represent ascending collaterals of fr (HL efferents). **B**) Higher magnification of NSM and LPA. **C**) Higher magnification of DLI neurons. **D**) The nucleus SI, associated with the anterior pallial commissure, appears labeled both antero- and retrogradely in the most medial sagittal sections. Arrows show SI axons entering the sm. **E**) Detail of neurons labeled in BCA. Note anterograde labeling of surrounding neuropile, probably due to ascending fr collaterals. Arrowheads in **A**, **B**, **D**, **E** point rostrally. **A** = $\times 50$; **B** and **D** = $\times 200$; **C** and **E** = $\times 500$.



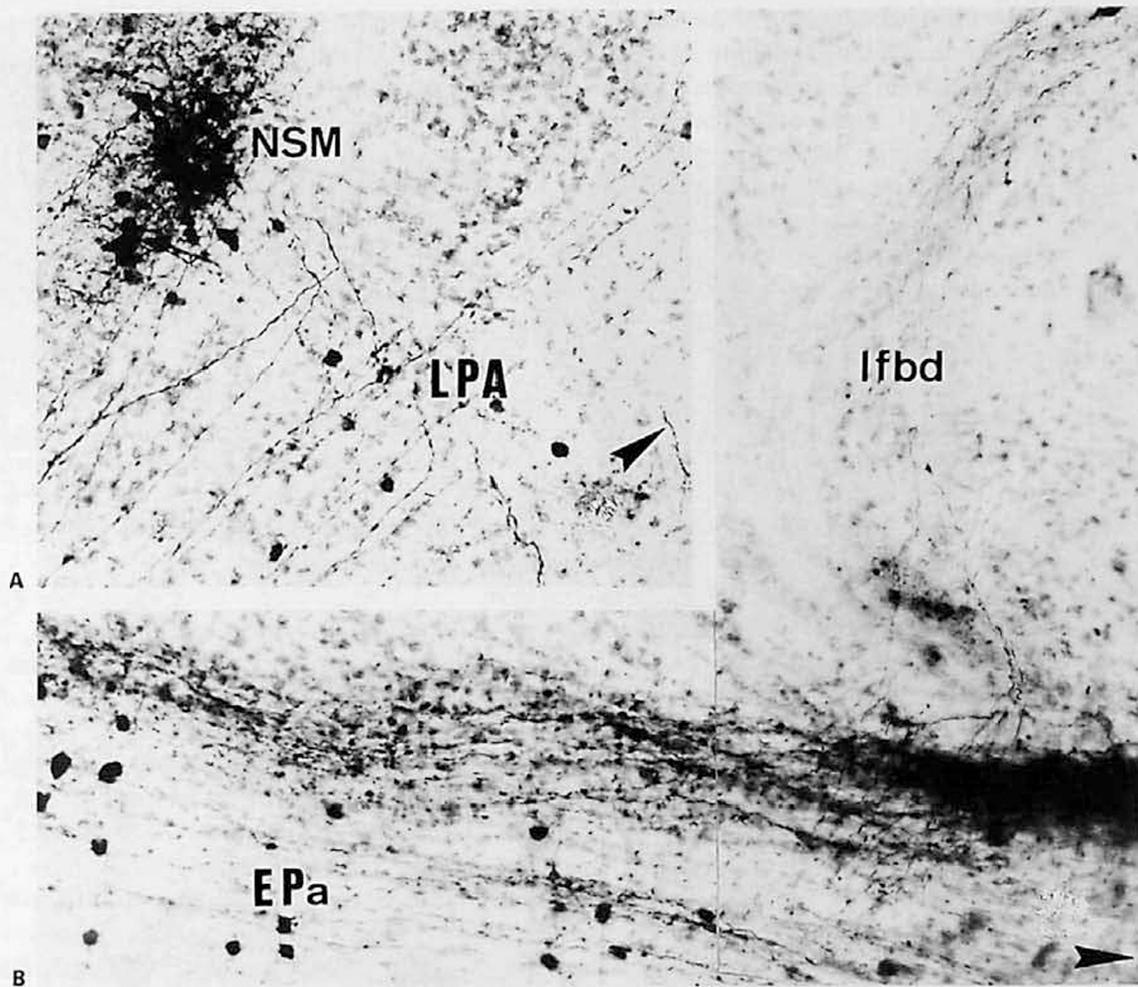


Fig. 4. Photomicrographs of sagittal sections illustrating the course of habenulopedal hypothalamic axons in the mfb in cases where the lateral habenula was labeled with HRP. **A)** Fibers make a sharp ninety-degree turn upon reaching the LPA and cross through NSM to enter into the sm. They possibly contribute terminals to the dense neu-

ropile surrounding labeled NSM neurons. **B)** Fibers originating in LMA, LHA and EPa change course from mfb into the transthalamic route along Ifbd and the boundary region between DLI and nucleus rotundus (R). Arrowheads in **A** and **B** point rostrally. **A** and **B** = $\times 200$.

gated band of habenular afferents, continuing rostral to LPA and into the septal region. This was observed both in the present set of experiments with habenular HRP injections and in the previous series in which the fasciculus retroflexus was injected in the middle of its dorsoventral course [Díaz and Puelles, 1992]. It would be interesting to know whether these axons synapse *en passant* on the habenulopedal neurons, thus closing a feedback circle.

A set of scattered smaller neurons were labeled inside the most lateral portions of the medial forebrain bundle and the adjacent lateral forebrain bundle. Tentatively, we have interpreted these cells as nucleus entopeduncularis anterior (EPa). Relevant topographic references for this

choice were taken from the papers by Smeets et al. [1986a, b] and Russchen and Jonker [1988]. A projection of EPa into the habenula was observed by Hoogland [1982] in *Varanus*.

Ventral Mesencephalon and Rhombencephalon

Small retrogradely labeled neurons located in and alongside the midline between both oculomotor nerve roots represent a habenulopedal component of the area tegmental ventralis (ATV). This population may be compared to cells reported by Hoogland [1982] in the 'interpeduncular nucleus'. Our control deposits of HRP in the latter nucleus ($n = 11$) produced no ascending transport into

the habenula. Other sources of habenular afferents reported by Hoogland [1982] at various places caudal to the mesencephalon (nucleus raphes superior, locus coeruleus, nucleus isthmi pars magnocellularis, nucleus dorsalis motorius nervi vagi, dorsolateral part of the mesencephalic tegmentum) could not be reproduced in our experimental conditions. This might be due either to a limitation in the extent of HRP-transport in our *in vitro* study, or to spread of the label into the overlying cortex in Hoogland's [1982] experiments. Brauth and Kitt [1980] also found some habenulopetal cells in the midline raphe, caudal to the interpeduncular nucleus, in *Caiman*.

The question of the existence of serotonergic afferents to the habenula is somewhat uncertain at the moment. Whereas Braak et al. [1968] described dense serotonin fluorescence in the habenula, but an absence of fluorescent perikarya in the raphe nuclei of *Lacerta viridis* and *Lacerta muralis*, Petkó and Ihionvien [1989] reported an absence of serotonin-immunoreactive fibers in the habenular nuclei of *Lacerta agilis*.

Comparison with Mammals

Afferents from the supracommissural septum to the medial habenula, such as found in the lizard *Gallotia*, are well known in mammals [Nauta, 1956; Powell, 1968; Herkenham and Nauta, 1977; Swanson and Cowan, 1979; Shinoda and Tohyama, 1987; Shinoda et al., 1987]. In particular, the nucleus septalis impar may be compared with the septofimbrial and triangular supracommissural septal populations of mammals. On the other hand, the nucleus of the posterior pallial commissure in reptiles is hardly comparable to any mammalian septal griseum, due to the absence of the corresponding commissure. It might be recalled, however, that an embryological study of the developing NCPP in *Lacerta sicula* [Hetzel, 1975] suggested a ventral thalamic origin of this cell mass. Thus, an embryologically homologous population may nevertheless exist at the boundary zone between ventral thalamus and telencephalon in mammals.

The connection of the bed nucleus of the anterior commissure with the habenula was described by Swanson and Cowan [1979] in rats. In the lizard *Gallotia*, retrograde labeling of scattered BCA neurons was obtained only in massive injections in the habenular complex, suggesting that their number is small. Anterograde control experiments showed predominantly HL is innervated. Perhaps the disputed [see Shinoda and Tohyama, 1987] connections of the bed nucleus of the stria terminalis to the habenula

[Herkenham and Pert, 1980; McLean et al., 1983; van Leeuwen and Caffé, 1983] in mammals are, at least in part, comparable to this BCA connection in the lizard. The cell mass identified as BST in *Gallotia* was never seen to contain labeled cells or fibers.

There is no consensus on the existence of projections from the diagonal band nucleus to the habenula in mammals [Herkenham and Nauta, 1977; Jones and Hartman, 1978; Edwards and Hendrickson, 1981; Parent et al., 1981; Warr et al., 1981]. However, results after kainic acid lesioning [Contestabile and Villani, 1983] or *Phaseolus vulgaris* leucoagglutinin injection into the DB in rats [Tomimoto et al., 1987] seem to confirm this connection. In *Gallotia*, there are only few DB-labeled cells after massive habenular deposits.

The complex formed by the nucleus of the stria medullaris and the eminentia thalami in lizards, located interstitially in the root of the stria medullaris, is anatomically comparable to the nucleus of the stria medullaris in mammals [Scheibel and Scheibel, 1972; Jacobowitz and Palkovits, 1974; Paxinos and Watson, 1986]. Habenular afferents have not been described in this complex.

Projections to the lateral habenular nucleus from the LHA and LPA are also well known in mammals [Swanson, 1976; Herkenham and Nauta, 1977; Parent et al., 1981]. In addition, McBride [1981] reported afferents to HL arising from the posterior hypothalamus, which may be comparable to those from LMA in the lizard *Gallotia*. Veazey et al. [1982] obtained autoradiographic labeling of HL after injecting tritiated aminoacids into the lateral hypothalamus and mammillary region in *Macaca fascicularis*.

Given that the pallidal complex, although obviously present in reptiles [Russchen et al., 1987], is not as well developed as in mammals, it may be premature to draw conclusions on the homology of the anterior entopeduncular habenulopetal population described in reptiles [Hoogland, 1982; present results] and the habenulopetal neurons present in the internal pallidal segment, or entopeduncular nucleus, of mammals [Nauta, 1974; Herkenham and Nauta, 1977; Parent et al., 1981]. Presently, support for this homology is based on (1) relative position with respect to LPA and LHA, (2) location interstitial to the lateral forebrain bundle and (3) projection to the lateral habenula and other dorsal thalamic nuclei [Hoogland, 1982; present results]. Immunocytochemical characterization of EPa in lacertids with respect to substantia P, leucine-enkephaline and GABA [Gottesfeld et al., 1977, 1980, 1981; Gottesfeld and Jacobowitz, 1978; Hayakawa et al., 1981; Shinoda et al., 1984; Shinoda and Tohyama, 1987] may resolve this question definitively.

It is interesting that in *Gallotia* many LHA and EPA fibers course through a transthalamic route towards the habenula, a feature also described in mammals [Iwahori, 1977; McBride, 1981; Shinoda and Tohyama, 1987; Tomimoto et al., 1987]. The habenulopetal neurons present within the dorsal thalamus of *Gallotia*, scattered throughout DLI, have not been reported elsewhere in mammalian or reptilian literature.

Those ATV neurons in *Gallotia* that project to the HL ipsilaterally through the fasciculus retroflexus are possibly homologous to the interfascicular nucleus of the ventral tegmental area in rats. There arises a mesohabenular dopamine pathway which terminates in a restricted caudomedial region of HL [Phillipson and Griffith, 1980; Phillipson and Pycoc, 1982; Skagerberg et al., 1984]. Dopamine-immunoreactive fibers are also present in the habenular region of *Gekko gekko* [Smeets et al., 1986b].

Comparison with Amphibia

Habenular afferents have been studied experimentally in *Rana esculenta* by Kemali et al. [1980]. They found retrograde transport of HRP into the septal area, bed nucleus of the hippocampal commissure, entopeduncular nucleus, nucleus dorsomedialis anterior thalami and periventricular hypothalamus. Since their 'nucleus dorsomedialis anterior' is illustrated lying medial to the entopeduncular nucleus, we estimate that this habenulopetal population is potentially homologous to LPA or NSM cells in *Gallotia*, rather than to DLI.

Final Comments

The foregoing detailed comments lead to the obvious conclusion that the habenular afferent circuitry in a lizard shows considerably overall similarity with the pattern observed in other vertebrates (mammals and anurans). Conservative efferent habenular connections have also been noted [Kemali et al., 1980; Distel and Ebbesson, 1981; Kemali and Guglielmotti, 1982; Kemali and Lazàr, 1985; Díaz and Puelles, 1992]. Similarly, in all species studied, habenular afferents consist mainly of a septal group projecting to the medial habenular nucleus, which discharges through the core of fasciculus retroflexus to the interpeduncular nucleus, and a hypothalamo-preoptic and entopeduncular group projecting to the lateral habenular nucleus. The latter nucleus projects through the mantle portion of the fasciculus retroflexus to dopaminergic mid-

brain tegmental neurons, raphe nuclei, dorsomedial thalamus, hypothalamus, preoptic area, and septum [Distel and Ebbesson, 1981; Díaz and Puelles, 1992]. As we noted above, the possibility exists that the HL efferents may close a feedback circuit, if they terminate *en passant* on its afferent neurons lying along the medial forebrain bundle. A third afferent system, formed by the mesohabenular dopaminergic pathway, apparently also closes a feedback loop with HL. The habenular complex of tetrapods thus emerges as a conservative modulatory station variously interconnected with the limbic system [Skagerberg et al., 1984].

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