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Thalamo-Telencephalic Pathways in the Fire-Bellied Toad *Bombina orientalis*

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ABSTRACT

It was suggested that among extant vertebrates, anuran amphibians display a brain organization closest to the ancestral tetrapod condition, and recent research suggests that anuran brains share important similarities with the brains of amniotes. The thalamus is the major source of sensory input to the telencephalon in both amphibians and amniote vertebrates, and this sensory input is critical for higher brain functions. The present study investigated the thalamo-telencephalic pathways in the fire-bellied toad *Bombina orientalis*, a basal anuran, by using a combination of retrograde tract tracing and intracellular injections with the tracer biocytin. Intracellular labeling revealed that the majority of neurons in the anterior and central thalamic nuclei project to multiple brain targets involved in behavioral modulation either through axon collaterals or en passant varicosities. Single anterior thalamic neurons target multiple regions in the forebrain and midbrain. Of note, these neurons display abundant projections to the medial amygdala and a variety of pallial areas, predominantly the anterior medial pallium. In *Bombina*, telencephalic projections of central thalamic neurons are restricted to the dorsal striato-pallidum. The bed nucleus of the pallial commissure/thalamic eminence similarly targets multiple brain regions including the ventral medial pallium, but this is accomplished through a higher variety of distinct neuron types. We propose that the amphibian diencephalon exerts widespread influence in brain regions involved in behavioral modulation and that a single dorsal thalamic neuron is in a position to integrate different sensory channels and distribute the resulting information to multiple brain regions. J. Comp. Neurol. 508:806–823, 2008.

Indexing terms: amphibians; thalamus; thalamic eminence; pallium; dorsal striato-pallidum; intracellular labeling

Brain centers in the telencephalon have been implicated in many aspects of higher brain functions, which depend on sensory input for initiation and fine-tuning of behavior. Therefore, the sensory pathways to the telencephalon have been studied across animals in the hope of elucidating the neural basis of behavioral differences among vertebrates. One major brain center known to transmit sensory information to the telencephalon is the thalamus. Previous investigations in amphibians demonstrated the existence of two thalamic pathways ascending in parallel to the telencephalon: a pathway from the central and lateral dorsal thalamus predominantly to the striatum and possibly to the central and lateral amygdala (sensu Moreno and González, 2004, 2005) coursing through the lateral forebrain bundle, and a pathway from the anterior dorsal thalamus to the medial and central amygdala (sensu Roth et al., 2004), septum and pallium coursing through the medial forebrain bundle (Kicliter and Northcutt, 1975; Kicliter, 1979; Wilczynski and Northcutt, 1983; Neary, 1984, 1990; Wicht and Himstedt, 1986; Hall and Feng, 1987; Roth and Grunwald, 2000; Roth et al., 2003; Endepols et al., 2004). Both pathways likely transmit no unimodal, but rather multimodal, sensory information to the telencephalon (Roth et al., 2003; Laberge and Roth, 2007). The most anterior part of the diencephalon, which includes the thalamic eminence, also appears to project to

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the telencephalon in amphibians (Northcutt and Ronan, 1992; Krug et al., 1993; Endepols et al., 2005).

The organization of the amphibian forebrain is a topic of contention. Consequently, the presentation of data requires the choice of one anatomical framework over existing alternatives. Here, the organizational framework of the anuran telencephalon recently used in our studies of the fire-bellied toad was chosen (Roth et al., 2004; Laberge and Roth, 2007; Roth et al., 2007). Accordingly, the subpallial part of the lateral telencephalic walls caudal to the accessory olfactory bulbs (extended vomeronasal amygdala excluded; see below) represents the dorsal striato-pallidum as defined by Endepols and colleagues (2004). It assumes that dorsal striatum and dorsal pallidum form a continuous structure unified by the presence of dendrites in the striatal neuropil and bordered caudally by the amygdaloid complex. The amphibian amygdaloid complex is here defined as consisting of three functional components: 1) autonomic-limbic associative (medial); 2) vomeronasal (lateral); and 3) main olfactory (cortical) (Laberge et al., 2006).

The medial amygdala is thought to extend rostrally in the subpallium forming the bed nucleus of the stria terminalis (BNST), whereas the lateral amygdala extends rostrally above the dorsal striato-pallidum forming an extended vomeronasal amygdala. The amphibian pallium is divided into rostral, medial, dorsal, lateral, and ventral subdivisions, as proposed in Roth and colleagues (2007), except that the ventral division of the ventral pallium is now considered mainly of subpallial origin after Moreno and González (2007).

We consider all telencephalic regions in amphibians part of the limbic system, with the exception of the dorsal striato-pallidum. The amphibian limbic system additionally comprises the olfactory pathways, preoptic area/hypothalamus, anterior thalamus, and midbrain structures such as the dopaminergic neurons projecting to the nucleus accumbens found in the tuberculum posterius and ventral tegmentum (Marín et al., 1995). Pallial regions displaying multimodal sensory responses are interpreted as limbic because there is no evidence for unimodal regions receiving thalamic input in amphibians (Supin and Gusel'nikov, 1965; Vesselkin et al., 1971; Mudry and Capranica, 1987; Hall and Feng, 1987; Feng et al., 1990; Roth and Grunwald, 2000; Roth et al., 2003; Westhoff et al., 2004; Laberge and Roth, 2007), thereby preventing the opportunity for higher order associations between unimodal sensory regions in the pallium, as is seen in the mammalian association cortices and association thalamic nuclei processing isocortical input.

Butler (1994) proposed that two major divisions of the dorsal thalamus, i.e., the lemnothalamus receiving mostly unimodal lemniscal input and the collothalamus receiving input from the midbrain roof, underwent a dual elaboration in amniote (i.e., mammals and sauropsids) ancestors, followed by additional elaboration of the lemnothalamus in the mammalian lineage. The amphibian thalamus was regarded as relatively small and simply organized, with the anterior nucleus homologous "as a field" to amniote lemnothalamic nuclei and the central and lateral nuclei homologous to amniote collothalamic nuclei. Although the latter appears to be justified, because at least the central dorsal thalamic nucleus receives substantial input from the tectum and projects to the lateral telencephalon via the lateral forebrain bundle, the former is weakened by the fact mentioned above that the anterior dorsal thalamus apparently transmits no unimodal input. Therefore, the concept of a lemnothalamic pathway in anurans is not tenable from a functional viewpoint and the role of the thalamic sensory projections to the pallium in amphibians remains to be determined.

Abbreviations

I	olfactory nerve
II	optic nerve
III	oculomotor nerve
V	trigeminal nerve
VIII	octaval nerve
IX-X-XI	glossopharyngeal-vagal-accessory nerve bundle
2Sp	second spinal nerve
AC	anterior commissure
aEN	anterior entopeduncular nucleus
AMY	amygdala
AT	anterior thalamic nucleus
BNPC	bed nucleus of the pallial commissure
c	caudal
CB	cerebellum
COA	cortical amygdala
CT	central thalamic nucleus
DP	dorsal pallium
DT	dorsal thalamus
DHYP	dorsal hypothalamus
EN	entopeduncular nucleus
gcl	granule cell layer of the olfactory bulb
H	habenula
HYP	hypothalamus
LA	lateral amygdala
lfb	lateral forebrain bundle
LP	lateral pallium
LS	lateral septum
LT	lateral thalamic nucleus
m	medial
MA	medial amygdala
mcl	mitral cell layer of the olfactory bulb
mfb	medial forebrain bundle
MO	medulla oblongata
MOB	main olfactory bulb
MP	medial pallium
MS	medial septum
NA	nucleus accumbens
NPC	nucleus of the posterior commisssure
OT	optic tectum
PAL	pallidum
PC	pallial commissure
PIT	pituitary gland
POA	preoptic area
PT	pretectum
OT	optic tectum
rP	rostral pallium
SCN	suprachiasmatic nucleus
SPTA	striato-pallial transition area
STR	striatum
STR/DPAL	dorsal striato-pallidum
TE	thalamic eminence
TEG	tegmentum
TOR	torus semicircularis
TP	tuberculum posterius
v	ventral
VHYP	ventral hypothalamus
VL	ventrolateral thalamic nucleus
VT	ventral thalamus

Accordingly, a better description of the amphibian thalamus and its projection pattern could help establish the likely situation prior to the phylogenetical divergence of amniotes and thus highlight the neural substrate available for both major amniote radiations before evolutionary modification of their forebrains. Further, additional investigations of amphibians and vertebrates basal to them are needed in order to clarify whether amphibian brains are effectively close to the ancestral tetrapod situation or whether modifications through phylogenetical secondary simplification make comparisons with amniotes unreliable (Roth et al., 1994). Even though thalamo-telencephalic pathways have been studied in many vertebrate groups, the anatomical methods used generally lacked single cell resolution. Because single neurons are known to display axon collaterals or en passant connections in many brain regions, previous reports using tract tracing of connections might have missed important aspects of the organization of brain connectivity.

Here, we studied the thalamo-telencephalic pathways in *B. orientalis* by means of retrograde and intracellular biocytin labeling in an attempt to highlight ancestral characters of the sensory pathways to the telencephalon in tetrapod vertebrates as well as some principles of organization of the sensory pathways in a relatively simple vertebrate brain. Accordingly, the present study details the anatomy of the bed nucleus of the pallial commissure (BNPC), thalamic eminence (TE), and anterior thalamic neurons in order to confirm their dendritic morphology and axonal projection sites. Additionally, the results are synthesized with reanalyzed data of Roth and colleagues (2003) to update the state of knowledge about the thalamo-telencephalic pathways in *B. orientalis*.

MATERIALS AND METHODS

Forty adult fire-bellied toads *B. orientalis* were used in the present study. For synthesis purposes, additional thalamic neurons included in the study by Roth and colleagues (2003) were examined in greater detail regarding their projection pattern and type of axonal arborization and are included in Figure 8. The animals were bought from a local supplier (Tropenhaus, Hamburg, Germany) or taken from a breeding colony at our institute. The experimental procedures were approved by the veterinary office of the Ministry of Health of the state of Bremen, Germany.

All experiments were carried out in vitro in isolated brain preparations. After deep anesthesia by exposure to a solution of 0.5% tricaine methanesulfonate, the animals were quickly decapitated, the lower jaw was removed, and the skull was opened from the roof of the mouth to enable brain dissection. The dissection was performed in Ringer's solution consisting of Na^+ 129 mM, K^+ 4 mM, Ca^{2+} 2.4 mM, Mg^{2+} 1.4 mM, Cl^- 115 mM, HCO_3^- 25 mM, glucose 10 mM, perfused with 95% O_2/5% CO_2 until a pH of 7.3 was measured. Intracellular labeling was made by using glass micropipettes filled with 2% biocytin (Sigma-Aldrich, St. Louis, MO) dissolved in 0.3 M KCl. Briefly, the brain was split longitudinally and the medial surface was approached. One intracellular injection was made on each side. The brain was continuously perfused with oxygenated Ringer's solution (6 ml/min) at a temperature of 14–18°C.

The impedance of the electrodes was 80–150 MΩ. The electrodes were advanced in steps of 1 or 2 μm, and a 200-ms hyperpolarizing current of 0.2 nA was applied every second. When a nerve cell membrane was penetrated, the potential dropped to −20 to −65 mV. For injection of biocytin, a pulsed current of 1 nA was applied for 4 minutes. For tract tracing, biocytin crystals were applied directly to the lightly lesioned surface of the brain outside of the Ringer's bath. Examples of retrograde labeling after biocytin applications in the toad telencephalon were taken from a larger study comprising 73 tract tracing experiments to be published elsewhere (S. Mühlenbrock-Lenter, unpublished data). Biocytin applications in the medial pallium (n = 15), dorsal pallium (n = 23), olfactory amygdala region (n = 5), and dorsal striato-pallidum (n = 2) were considered. After biocytin injection or crystal application, the brains were stored in oxygenated Ringer's solution for 3 hours at room temperature and then at 4°C overnight. On the next day, the brains were fixed in 2% paraformaldehyde and 2% glutaraldehyde, and then 50-μm-thick transverse sections were cut on a Vibratome.

Biocytin was visualized by means of an avidin-biotin-horseradish peroxidase complex (Vector Standard Kit, Vector, Burlingame, CA) by using diaminobenzidine (DAB; Sigma) as chromogen with heavy metal intensification (Adams, 1981). Sections were lightly counterstained with cresyl violet, dehydrated in ethanol, cleared in xylene, and coverslipped. In one experiment, retrograde tract tracing was performed by simultaneous applications of biocytin in the dorsal striato-pallidum and the fluorescent tracer tetramethylrhodamine (TMR; Molecular Probes, Eugene, OR) in the medial septum (n = 3). Retrograde labeling was then visualized by using fluorescence microscopy. For that purpose, biocytin was revealed by overnight incubation in a complex of streptavidin-Oregon green 488 (Molecular Probes, Eugene, OR).

Reconstructions of labeled neurons were made by hand with the aid of a camera lucida. Photomicrographs using the light microscope were scanned with a digital camera (AxioCam HR, Zeiss, Jena, Germany) at a resolution of 3,900 × 3,090 pixels, whereas fluorescent photomicrographs were scanned with a different camera (DFC 350 FX, Leica Microsystems, Wetzlar, Germany) at a resolution of 1,392 × 1,040 pixels. Brightness and contrast were optimized by using Photoshop 6.0 (Adobe Systems, San Jose, CA).

RESULTS

Retrograde tract tracing

Figure 1 displays retrograde labeling in the diencephalon in four representative tract-tracing experiments by using biocytin. Application of biocytin crystals to the medial pallium (Fig. 1A) resulted in retrogradely filled neurons in the anterior thalamic nucleus and, additionally, in the BNPC and thalamic eminence (BNPC/TE) when the ventral part of the medial pallium was included in the application site. A few backfilled neurons were also observed in the dorsal suprachiasmatic nucleus, whereas no backfilled neurons occurred in the central or lateral dorsal thalamic nucleus. Application of biocytin restricted to the dorsal pallium (Fig. 1B) exclusively labeled neurons in the anterior dorsal thalamic nucleus at diencephalic levels. Application of biocytin to the white matter of the olfactory amygdala (vomeronasal and main olfactory parts

included; Fig. 1C) produced abundant retrograde labeling in the dorsolateral magnocellular preoptic area. This labeling was due to dendritic uptake of biocytin because neurons in the dorsolateral magnocellular preoptic area send many dendrites into the accessory olfactory neuropil (Roth et al., 2004). Only a few ventral thalamic neurons were additionally backfilled in the diencephalon, but no labeled neurons occurred in the dorsal thalamus. Application of biocytin to the dorsal striato-pallidum (Fig. 1D) massively labeled neurons in the lateral and central thalamic nuclei. In addition, a few neurons were seen in the ventral thalamus, preoptic area, and suprachiasmatic nucleus, but not in the anterior dorsal thalamic nucleus.

In another retrograde labeling experiment (Fig. 2), different tracers (biocytin, TMR) were applied to the medial septum and dorsal striato-pallidum simultaneously and were visualized by fluorescence microscopy. The pattern of backfilled neurons following biocytin application to the dorsal striato-pallidum was as described above, i.e., such neurons were found in the lateral and central, but not in the anterior dorsal thalamic nucleus. Neurons backfilled following TMR application to the medial septum were observed in the anterior thalamic nucleus, ventral preoptic area, and ventral suprachiasmatic nucleus. Importantly, no double-labeled cells were observed.

Intracellular labeling

Injection of biocytin into single cells situated in the following regions displayed projections to the telencephalon: 1) BNPC/TE; 2) anterior; 3) central; and 4) ventral thalamic nuclei. Neurons in the lateral thalamic nucleus were not injected. Examples of reconstructions and photomicrographs of labeled neurons for each region are shown in Figures 3–7. Axonal projection sites were determined by light microscopic observation of axonal terminals, collaterals, or en passant fiber varicosities in the whole brain, excluding the spinal cord. The present section refers to neuron types (TH1, 2, 3, etc.) according to Roth and colleagues (2003) for guidance. Such neuron types fulfill a major hodological criterion, but the neurons included exhibit inherent variability in additional axonal targets. Importantly, discrete subtypes could not be unequivocally identified for each neuron within a cluster. Therefore, the present description does not aim at specifying discrete subtypes within a major neuron type.

Anterior thalamic nucleus. Thirteen neurons or clusters of neurons (two to four neurons) were injected in the anterior thalamic nucleus for a total of 33 neurons. Tables 1 and 2 list the distribution of axonal projection sites from these neurons, and Figures 3 and 7A exemplify a typical cluster of anterior thalamic neurons. The principal axonal targets are the rostral, medial, and dorsal pallium, septum, medial amygdala, preoptic area/suprachiasmatic hypothalamus, tuberculum posterius/dorsal hypothalamus region (Fig. 7B), thalamus, and pretectum/optic tectum. Projection sites reached in only one case were the BNPC/TE, entopeduncular nucleus, ventral tegmentum, parabrachial nucleus, and medulla oblongata. Axons of anterior thalamic neurons also pass through the dendrites of nucleus accumbens neurons situated in the rostral ventromedial telencephalon (G. Roth, unpublished observation). The average number of axonal projection sites per neuron/neuron cluster for anterior thalamic neurons was 6.6 ± 2.3 (mean ± SD; 5 sites/single neuron and 6.8 sites/cluster). Axon collaterals in the septum, medial amygdala, and preoptic area/suprachiasmatic nucleus were frequent. Local axonal branches sometimes displayed evident varicosities within the thalamus. Descending axonal branches that targeted the pretectum and optic tectum ran dorsally. In one case, these axons were observed to descend in a dorsomedial position all the way to the caudal optic tectum, curve behind the latter, and ascend back in a ventrolateral position. Anterior thalamic neurons targeted the white matter of diverse pallial regions and combination of regions, as shown in Table 2.

Neurons from the Roth and colleagues (2003) study situated in the anterior dorsal thalamus of *Bombina* belonging to type TH3 were re-examined with special emphasis on the presence of additional weakly labeled neurons, the number of axons running inside the forebrain bundle, and additional targets of axon collaterals outside of the telencephalon. The number of labeled axons was 29, all running via the medial forebrain bundle to medial telencephalic targets. This number corresponded to 24 heavily and 5 weakly labeled neurons (the latter not reported in Roth et al., 2003) situated in the anterior dorsal thalamic nucleus. It was confirmed that axons of 13 neurons terminated in the ventral or dorsal portion of the medial pallium and 9 neurons in the dorsal pallium after sending collaterals to the medial pallium and in two of these cases invading the lateral pallium. In two cases, axons terminated in the rostral pallium, as defined above. Five axons ascending via the medial forebrain bundle terminated in the lateral septum, not invading the medial pallium. Because the number of these axons corresponds to the number of weakly labeled neurons inside the anterior dorsal thalamus, we cannot exclude that these neurons were insufficiently labeled and would have terminated in the medial or dorsal pallium after complete labeling. Of the 29 neurons, 24 extensively arborized the axon in the vicinity of the tuberculum posterius, and 4 neurons sent collaterals to the hypothalamus. The neurons all displayed varicosities or axon collaterals on their way through the suprachiasmatic/preoptic region, in the medial amygdala and septum. No axons or axon collaterals to other midbrain or medullar targets were observed.

Thus, there is a close correspondence between the newly injected neurons and the re-examined group TH3 of Roth and colleagues (2003). Additional projection sites of TH3 neurons injected in the present study are the pretectum, optic tectum, and medulla oblongata. Varicose fibers could also be observed in the ipsilateral thalamus, adding to the previous observation that axons collaterals ended in or in the vicinity of the contralateral neuropil of Bellonci.

Figure 3F shows that axons of most anterior thalamic neurons followed a particular course in which at first they descend caudally in the dorsal diencephalon before taking a sharp ventral turn toward the dorsal hypothalamus/tuberculum posterius region, where they display collaterals and fiber varicosities. This is followed by another sharp rostral turn ascending in a ventral position within the medial forebrain bundle up to the rostral part of the telencephalon, where they take up a dorsal course toward the dorsal septum and pallium. Some of the most rostrally situated anterior thalamic neurons had axon branches that followed both the path just described as well as a direct rostrodorsal path to the pallium similar to the course of telencephalic projections of BNPC/TE neurons (see below). Interestingly, the neuron clusters targeting the posterior parts of the pallium did so through axonal branches taking the rostrodorsal route.

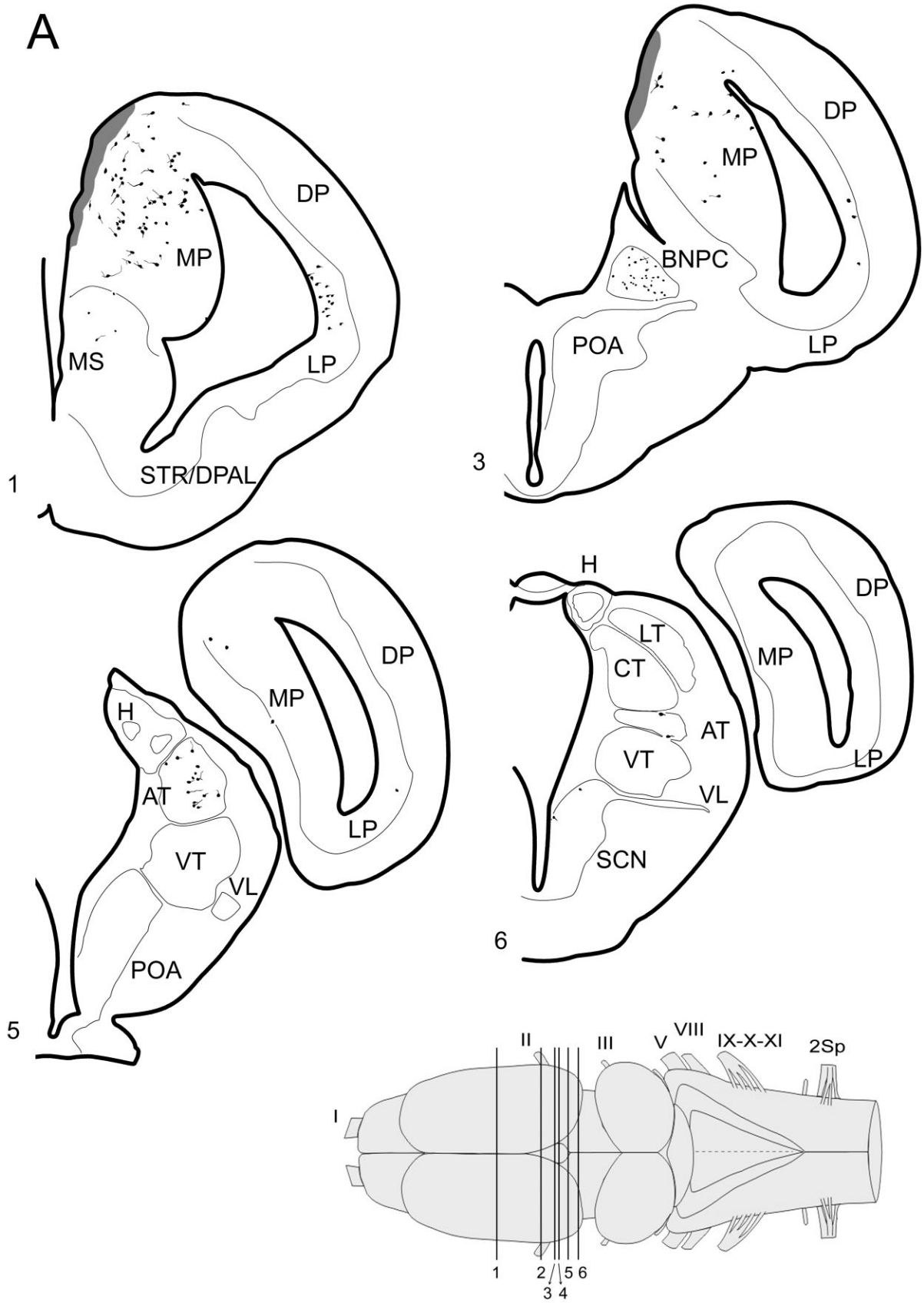

Fig. 1. Retrograde labeling of diencephalic neurons after application of crystalline biocytin in four telencephalic sites. The sites of biocytin application in the medial pallium (A), dorsal pallium (B), olfactory amygdala (C), and dorsal striato-pallidum (D) are illustrated in gray, whereas the somata of retrogradely filled neurons and proximal dendritic portions are in black. Applications in the medial and dorsal pallium labeled somata in the rostral and caudal parts of the anterior thalamic nucleus. Note that this nucleus is found between the central nucleus and the ventral thalamus caudally. Applications that included the ventral part of the medial pallium additionally labeled small somata in the BNPC/TE. Application in the olfactory amygdala neuropil labeled somata in the dorsolateral preoptic area through dendritic uptake. Numbers to the left of each section indicate the level of transverse section, which is illustrated schematically on the drawing of the dorsal view of the toad brain. In D, level 5* showing the anterior part of the lateral thalamic nucleus is found slightly caudal to level 5 in A–C. For abbreviations, see list. Scale bar = 500 μm in C (applies to A–D).

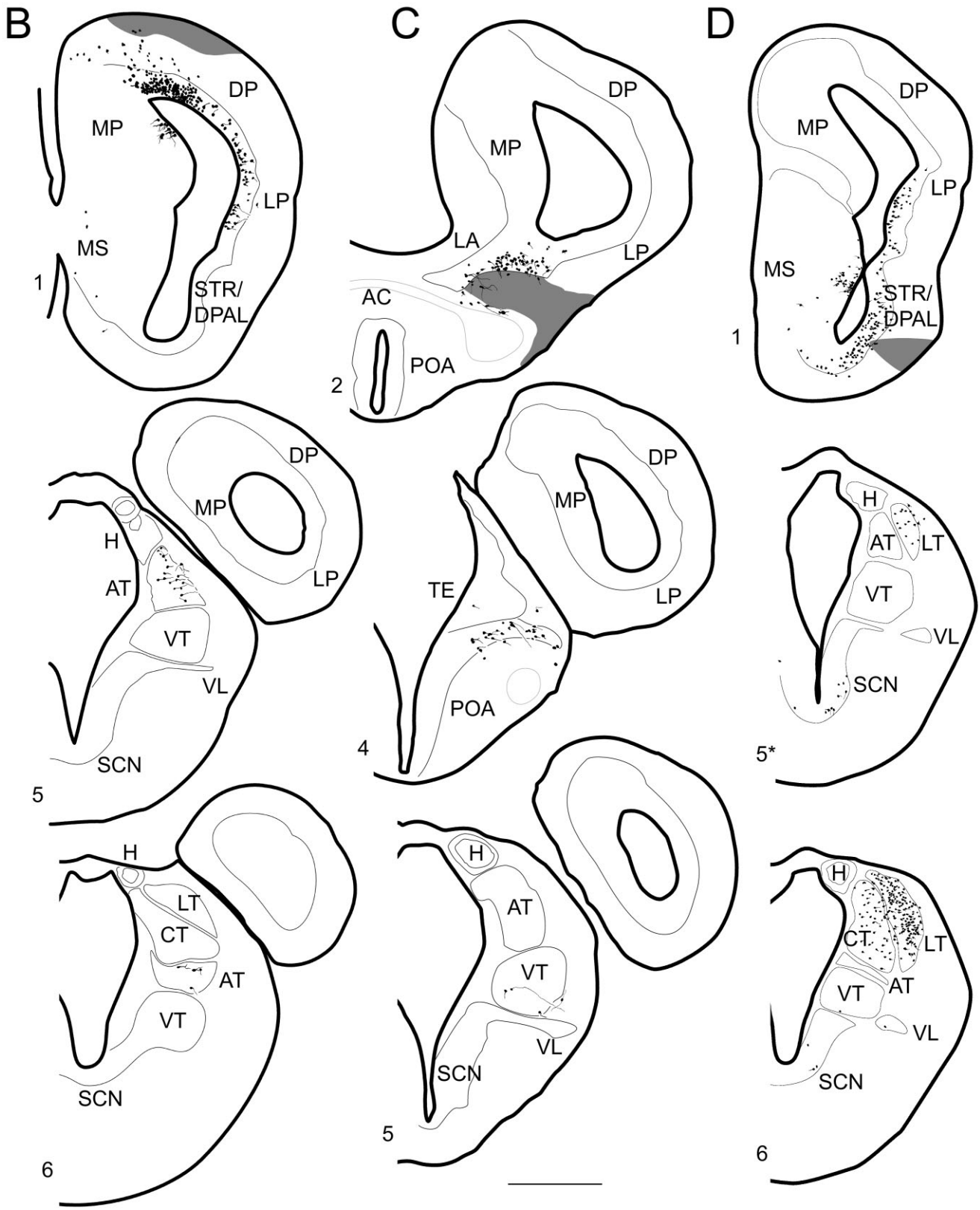

Figure 1. (Continued)

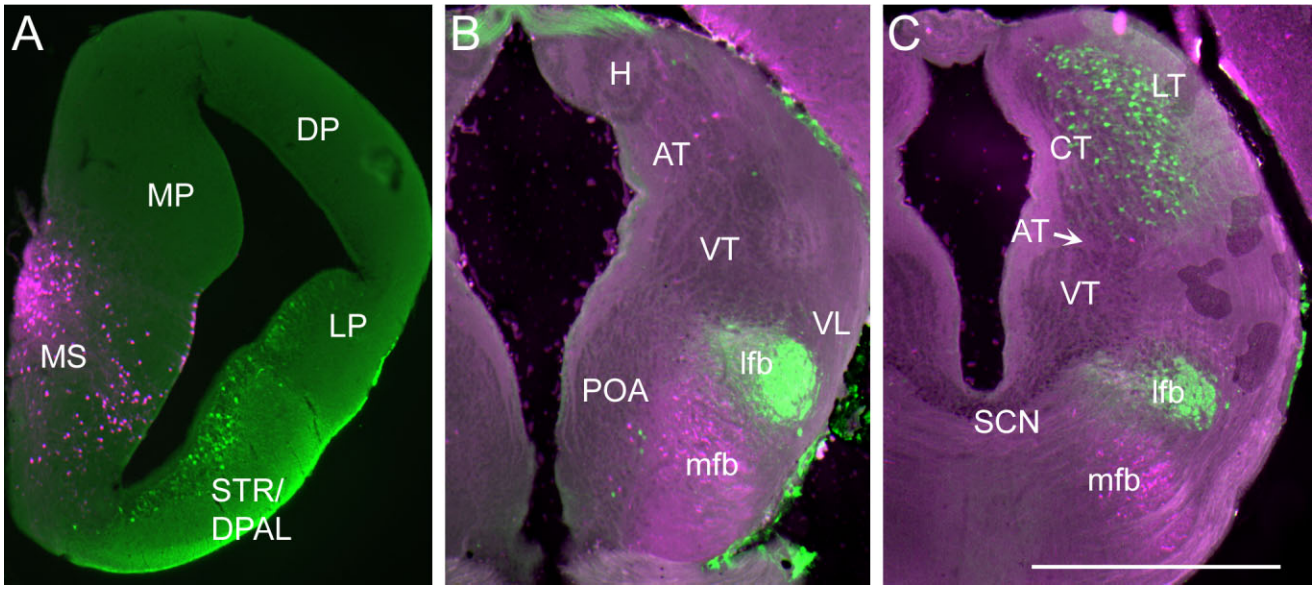

Fig. 2. Retrograde labeling of diencephalic neurons after simultaneous application of two different tracers in the medial and lateral telencephalon. **A:** Sites of application of tetramethylrhodamine (magenta) in the medial septum and biocytin (green) in the dorsal striatopallidum. **B:** Combined magenta/green fluorescence image showing backfilled neurons at the level of the anterior thalamic nucleus. **C:** Combined magenta/green fluorescence image showing backfilled neurons at the level of the central thalamic nucleus. For abbreviations, see list. Scale bar = 500 μm in C (applies to A–C).

Dendrites of anterior thalamic neurons generally displayed a low or medium amount of spines (e.g., Fig. 7C). Their arborization pattern varied greatly. Some dendritic trees invaded the lateral and medial white matter as well as the gray matter of multiple divisions of the dorsal thalamus between the BNPC rostrally and the most caudal part of the posterior dorsal thalamus. The white matter lateral to the rostral ventral thalamus was also sometimes covered. Other neurons displayed dendrites that were more restricted, particularly the neurons found in the most rostral part of the anterior thalamic nucleus, which for the most part had dendrites confined to the rostral diencephalon, although a few dendritic branches sometimes reached the posterior dorsal thalamus. Only a few neurons in the caudal part of the anterior thalamic nucleus sent a substantial number of dendrites into the retino-recipient neuropils. The reader is referred to Roth and colleagues (2003) for a complete description of the retino-recipient regions in the thalamus of *B. orientalis*. Briefly, three regions within the ventral thalamic white matter receive retinal afferents: 1) the neuropil of Bellonci situated laterally between the dorsal and ventral thalamus; 2) the corpus geniculatum thalamicum situated below the neuropil of Bellonci; and 3) the posterior thalamic neuropil.

Bed nucleus of the pallial commissure and thalamic eminence. Intracellular injections of biocytin in the BNPC and thalamic eminence turned out to be very difficult because of the limited extent of these brain regions and the small size of neurons. Thirteen neurons/neuron clusters (1–8 neurons) were injected in the thalamic eminence for a total of 31 neurons. Two single neurons and two clusters consisting of two neurons were injected in the BNPC. We recently presented evidence that the BNPC and thalamic eminence form a continuum in the anterior diencephalon in a position rostral to the ventral and anterior thalamic nuclei (see Fig. 9 in Laberge and Roth, 2007). Figures 4 and 7D show a neuron in the thalamic eminence that projects to the medial pallium. Table 1 lists the distribution of axonal projection sites for both thalamic eminence and BNPC neurons combined. The principal axonal targets were in limbic regions, as observed with anterior thalamic neurons, but additional projections to the habenula were observed as well as local connections of the thalamic eminence to BNPC. BNPC neurons were the only ones observed to invade the region of the pallial commissure with axonal networks. One BNPC neuron targeted the dorsal habenular neuropil, whereas three clusters of thalamic eminence neurons targeted the ventral habenular neuropil. Projections to the habenula were sometimes very strong. One cluster comprised two BNPC interneurons. Neurons of the BNPC and thalamic eminence displayed similar axonal projection patterns, with the exceptions noted above for the BNPC and the fact that only thalamic eminence neurons displayed projections to the preoptic area, midbrain, and medulla oblongata.

These distinctions should be taken with caution because we could only produce a small sample of BNPC neurons. The BNPC/TE neurons projecting to the medial pallium targeted mostly the periventricular part within the gray matter of the ventral medial pallium, where very fine varicose fibers were seen (Fig. 7E). Axons of BNPC/TE neurons that ascended to the telencephalon followed a direct rostrodorsal course toward the medial part of the caudal telencephalon. In most cases, these axons targeted the septum or ventral medial pallium in the caudal half of the telencephalon, but in one case a BNPC neuron had an axonal projection covering the whole rostrocaudal extent of the ventral medial pallium between the soma and the level of the caudal border of the accessory olfactory bulb, which was used to delimit the beginning of the rostral pallium (rP) proper. Projection sites reached in only one

TABLE 1. Frequency Distribution of Axonal Projection Sites of Anterior Thalamic and Thalamic Eminence Neurons[1]

Area	No. of sites
AT (n = 13)	
rP	7
MP	12
DP	5
Septum	9
mAMY	11
AC	2
POA	4
SCN	4
DT	2
VT	2
HYP	8
TP	8
PT	2
OT	2
BNPC/TE (n = 17)	
MP	3
Septum	3
PC	2
BNPC	5
POA	2
Habenula	4[2]
DT	6
SCN	8
HYP	6
TP	2
vTEG	3

[1]Only sites where at least two clusters displayed projections are shown. For abbreviations, see list.
[2]Three in ventral and one in dorsal neuropil.

TABLE 2. Distribution of Pallial Projection Sites for Anterior Thalamic Neurons (n = 12)[1]

Projection site	No. of clusters	Rostrocaudal distribution[2]
vMP[3]	1	Anterior only
MP	1	Anterior only
vMP, rP	4	Rostral-anterior
MP, rP	1	Rostral-anterior
MP, DP, rP	1	Rostral-anterior
vMP, DP, rP	1	Rostral-anterior
MP, DP	3	Anterior-posterior (2), Anterior only (1)

[1]For abbreviations, see list.
[2]The rostrocaudal territories within the pallium were defined as in Roth and colleagues (2007). Briefly, the rostral pallium extends between the most rostral pole of the pallium and the level of the accessory olfactory bulb. The anterior zone extends between the rostral pallium and the level of the commissures, whereas the posterior zone includes most of the telencephalic hemispheres caudal to the level of the commissures. It is important to note that most of the neurons targeting the anterior zone of the medial or dorsal pallium had axons restricted to the most rostral part of these regions.
[3]Ventral medial pallium refers to axons that terminate in the most ventral part of the white matter of the medial pallium including the cell-free zone between the medial pallium and dorsal septum.

case were the optic tectum, parabrachial nucleus, and medulla oblongata. The average number of axonal projection sites per neuron/neuron cluster was 3.1 ± 1.7 (3 sites/single neuron and 3.3 sites/cluster) in the thalamic eminence and 1.8 ± 1.7 (2.5 sites/single neuron and 1 site/cluster) in the BNPC.

Dendritic trees of BNPC/TE neurons were sparse, only sometimes reaching outside the BNPC/TE into the amygdala, rostral dorsal thalamus, rostral ventral thalamus (particularly caudal thalamic eminence neurons), or dorsal preoptic area/suprachiasmatic nucleus. The dendrites were mostly spineless (e.g., Fig. 7F), with the exception of some large neurons surrounding the BNPC, which displayed medium-spiny dendrites. Both gray and white matter surrounding the BNPC/TE were covered.

Central and ventral thalamic nuclei. Central and ventral thalamic neurons have already been described in *B. orientalis* (Roth et al., 2003). In the present study, four additional neurons/neuron clusters were injected in the central thalamic nucleus and one in the ventral thalamic nucleus. Two central thalamic clusters corresponded to type TH1 projecting to the dorsal striato-pallidum and one to type TH 2.2, the latter with projections to the caudal dorsal pallidum (central amygdala of Moreno and González, 2005), medial amygdala/ventral pallidum region, and posterior entopeduncular nucleus (see Roth et al., 2003, for classification). One cluster of five central thalamic neurons projecting to the dorsal striato-pallidum, tuberculum posterius, posterior entopeduncular nucleus, and nucleus isthmi likely included neurons of types TH1 and TH4. The neuron injected in the ventral thalamus could be classified as type TH6, because it projected only to the ventral hypothalamus. An example of a cluster of central thalamic neurons and the extent of its axonal projections to the dorsal striato-pallidum and tuberculum posterius is illustrated in Figure 5. Abundant fiber collateralization and varicosities were observed along the whole rostrocaudal extent of the dorsal striato-pallidum.

The previous description of contacts with the central and lateral amygdala (Roth et al., 2003) is revised for contacts with the caudal dorsal pallidum because axonal contacts are visible and augment in the rostral direction beginning just rostral to level C in Figure 5, which corresponds to the arborization of dendritic fields of neurons in the caudal dorsal pallidum, not the amygdala (see Fig. 2 in Laberge et al., 2006). The tuberculum posterius is targeted by descending axonal branches. The average number of axonal projection sites of central thalamic neurons calculated including neurons from Roth and collaborators (2003) was 2.7 ± 1.1 (2.7 sites/single neuron and 2.7 sites/cluster) with the dorsal striato-pallidum as the sole telencephalic projection site. Additional descending projection sites were the thalamus, entopeduncular nucleus, tuberculum posterius, hypothalamus, tegmentum, pretectum, optic tectum, and nucleus isthmi.

Over the course of our intracellular labeling studies in the diencephalon of *B. orientalis*, only two injected neuron clusters in the ventral thalamus displayed ascending projections to the telencephalon, which is in line with the small number of backfilled neurons seen in that region in Figure 1. An example of such a cluster of ventral thalamic neurons projecting to the superficial white matter of the caudal pallidum/anterior entopeduncular nucleus region is shown in Figure 6. Dendrites of central thalamic neurons covered the gray and white matter of most of the dorsal thalamus in the posterior region, with only few branches reaching into the retino-recipient neuropils, whereas dendrites of ventral thalamic neurons massively invaded the laterally and ventrolaterally situated retino-recipient neuropils as described previously (Roth et al., 2003).

Summary of intracellular labeling data

Figure 8 summarizes the data on ascending projections to the telencephalon obtained by intracellular biocytin injections in the fire-bellied toad for both the present study and 50 clusters of neurons from the Roth et al. (2003) study. It shows the location of the injected somata according to their type of ascending projections to the telencephalon. It is noteworthy that the rostralmost transition between the anterior and central nuclei is abrupt.

DISCUSSION

The data presented here highlight five diencephalic divisions that display ascending projections to the telen-

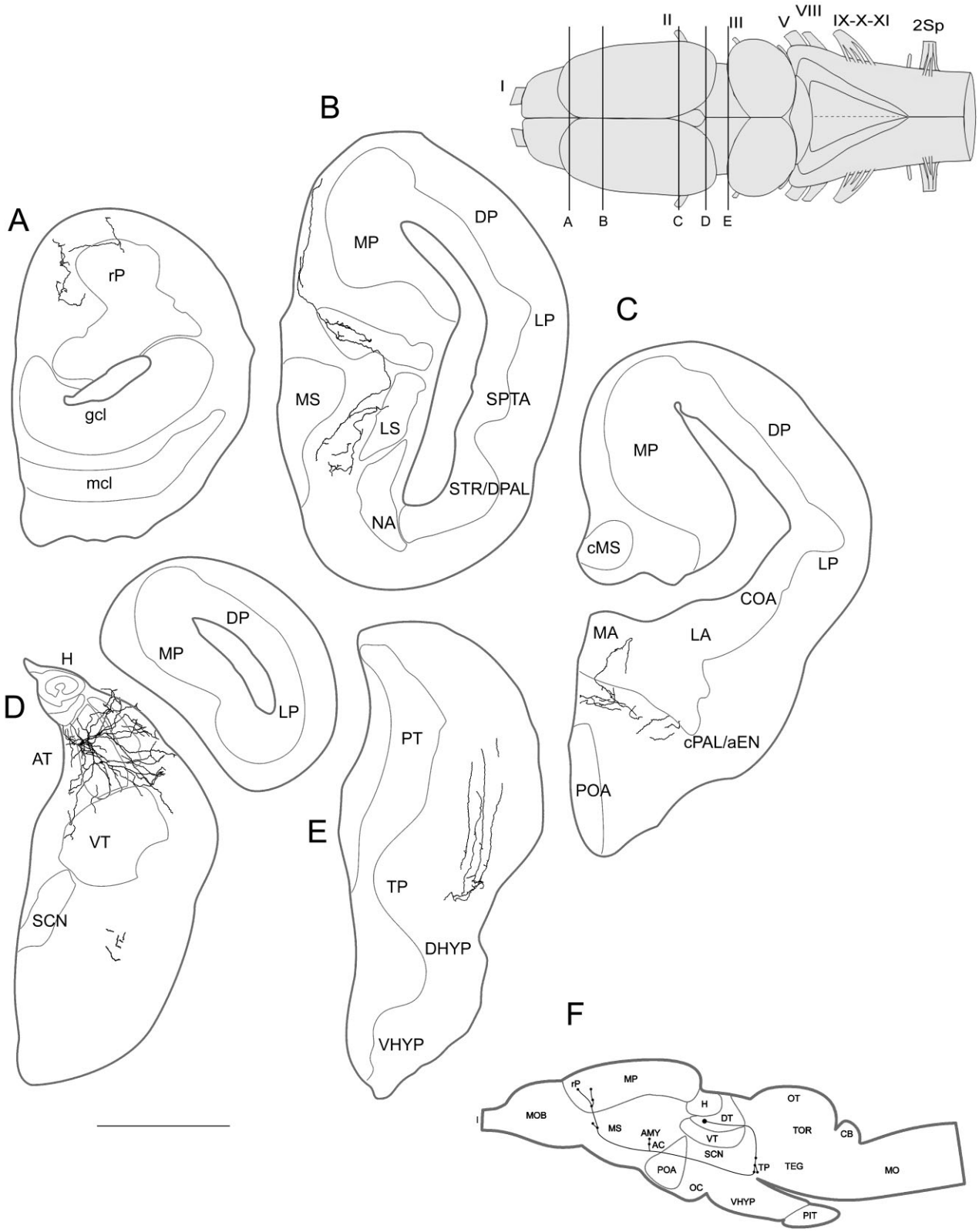

Fig. 3. Camera lucida reconstruction of representative anterior thalamic neurons. The cluster resulting from one intracellular biocytin injection is composed of four neurons. Axon collaterals or varicosities were observed in the pallium, septum, medial amygdala, and tuberculum posterius. **A–E:** Levels of transverse section on which the neuron cluster is displayed are indicated by letters on the schematic dorsal view of the toad brain. Dendrites extending outside the outline of the section were situated caudal to the somata, where the brain was wider. **F:** A schematic medial sagittal view of the brain illustrates the particular axonal pathway of the majority of anterior thalamic neurons; axons first descend caudally in the dorsal diencephalon before taking a sharp ventral turn toward the tuberculum posterius followed by another sharp turn rostrally. Then they ascend in a ventral position in the medial forebrain bundle up to the rostral part of the telencephalon, where they take up a dorsal course toward the pallium. For abbreviations, see list. Scale bar = 500 μm in D (applies to A–E).

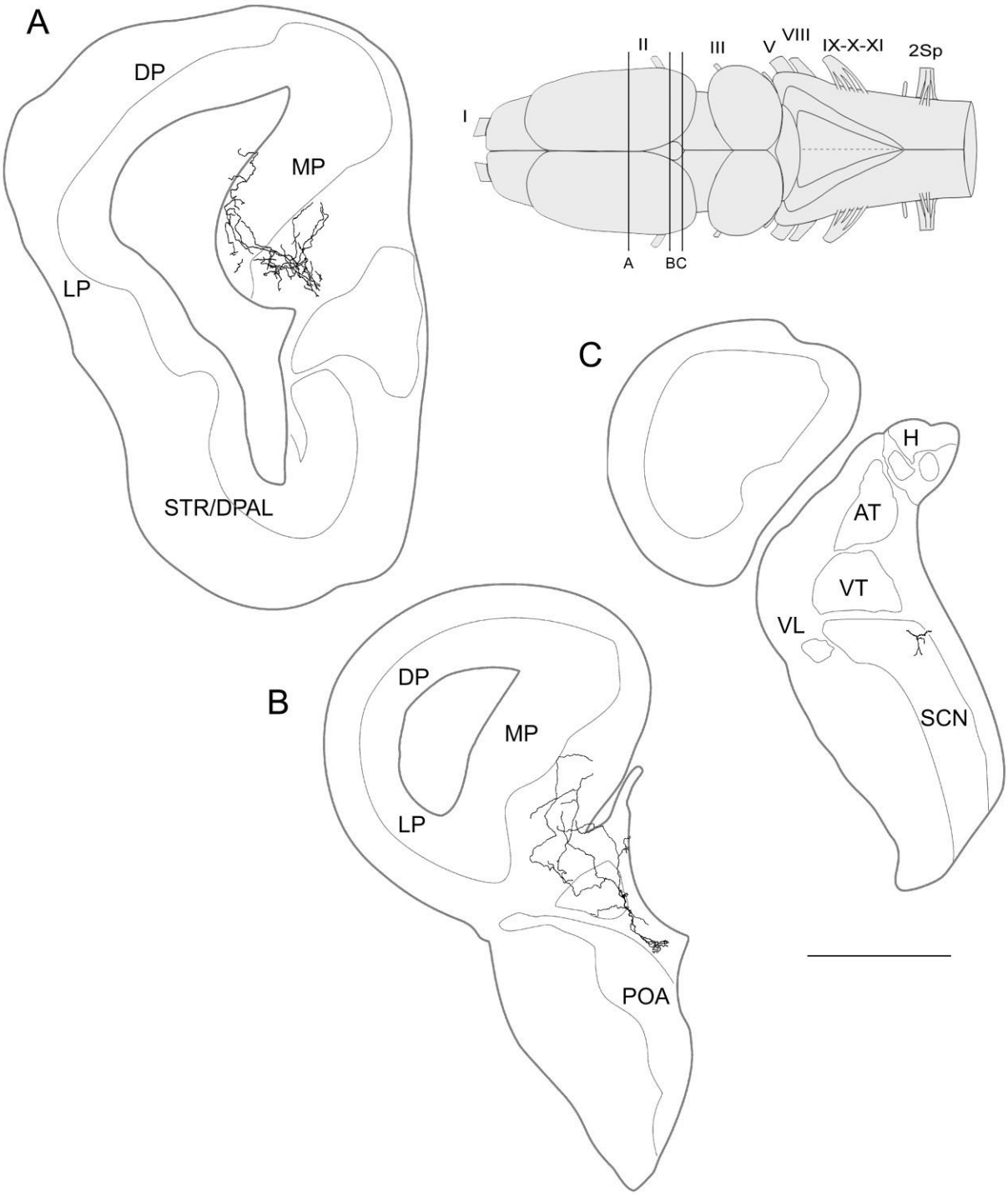

Fig. 4. Camera lucida reconstruction of a thalamic eminence neuron projecting to the medial pallium. This neuron also displayed axonal processes in the pallial commissure region and the dorsal suprachiasmatic nucleus. **A–C:** Levels of transverse section on which the neuron is displayed are indicated by letters on the schematic dorsal view of the toad brain. Axon branches extending inside the ventricle were situated anterior to the section outlined, where the medial pallium extended more laterally. For abbreviations, see list. Scale bar = 500 μm in C (applies to A–C).

cephalon in *B. orientalis*. Ascending projections from the anterior dorsal thalamic nucleus target predominantly the medial subpallium and rostral, medial and dorsal pallium, whereas those from the central thalamic nucleus target exclusively the lateral subpallium. Further, intracellular labeling showed that dorsal thalamic neurons tar-

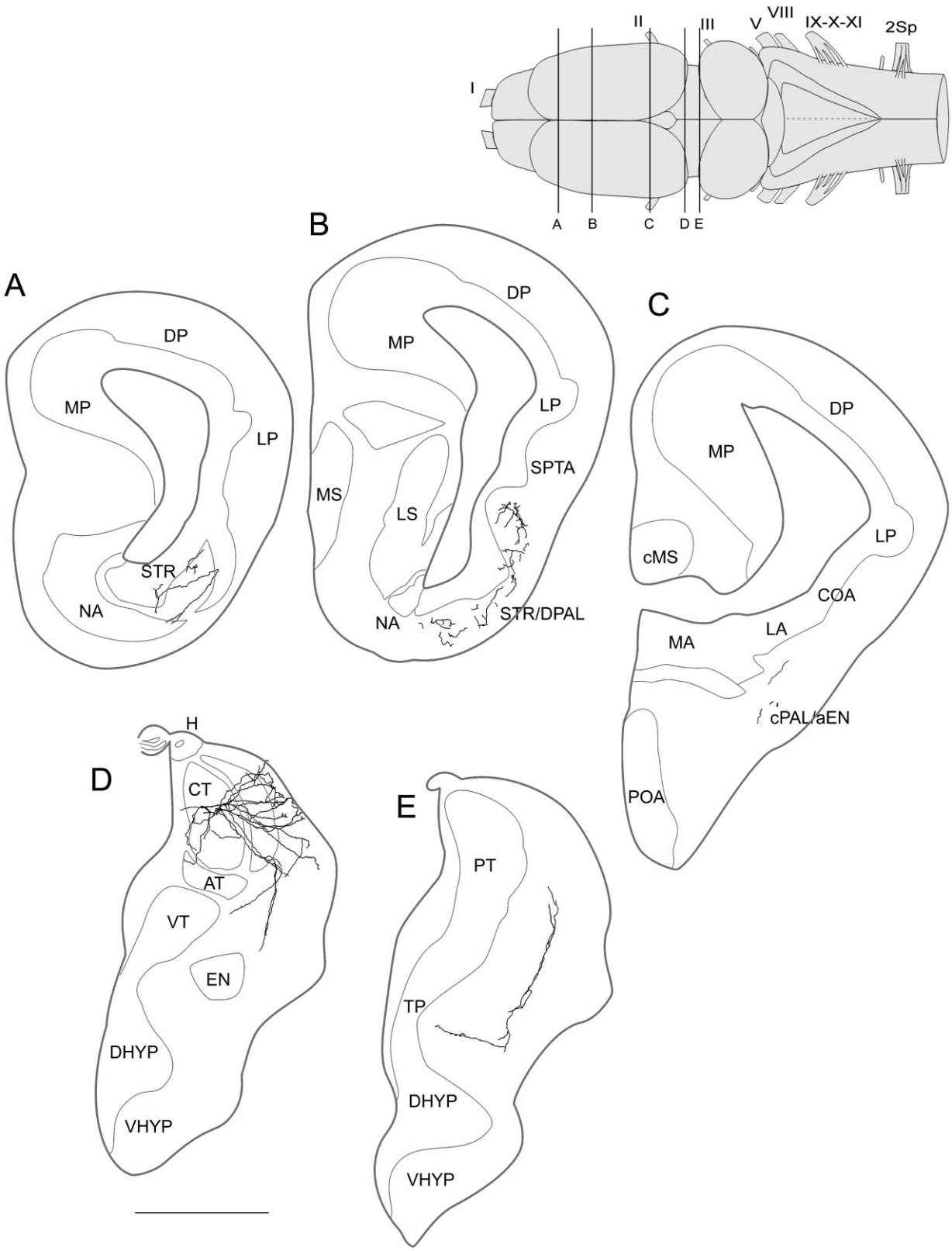

Fig. 5. Camera lucida reconstruction of central thalamic neurons. The cluster resulting from one intracellular biocytin injection is composed of two neurons. Axon terminals or varicosities were observed in the whole rostrocaudal extent of the dorsal striato-pallidum and tuberculum posterius. **A–E:** Levels of transverse section on which the neuron cluster is displayed are indicated by letters on the schematic dorsal view of the toad brain. For abbreviations, see list. Scale bar is 500 μm in D (applies to A–E).

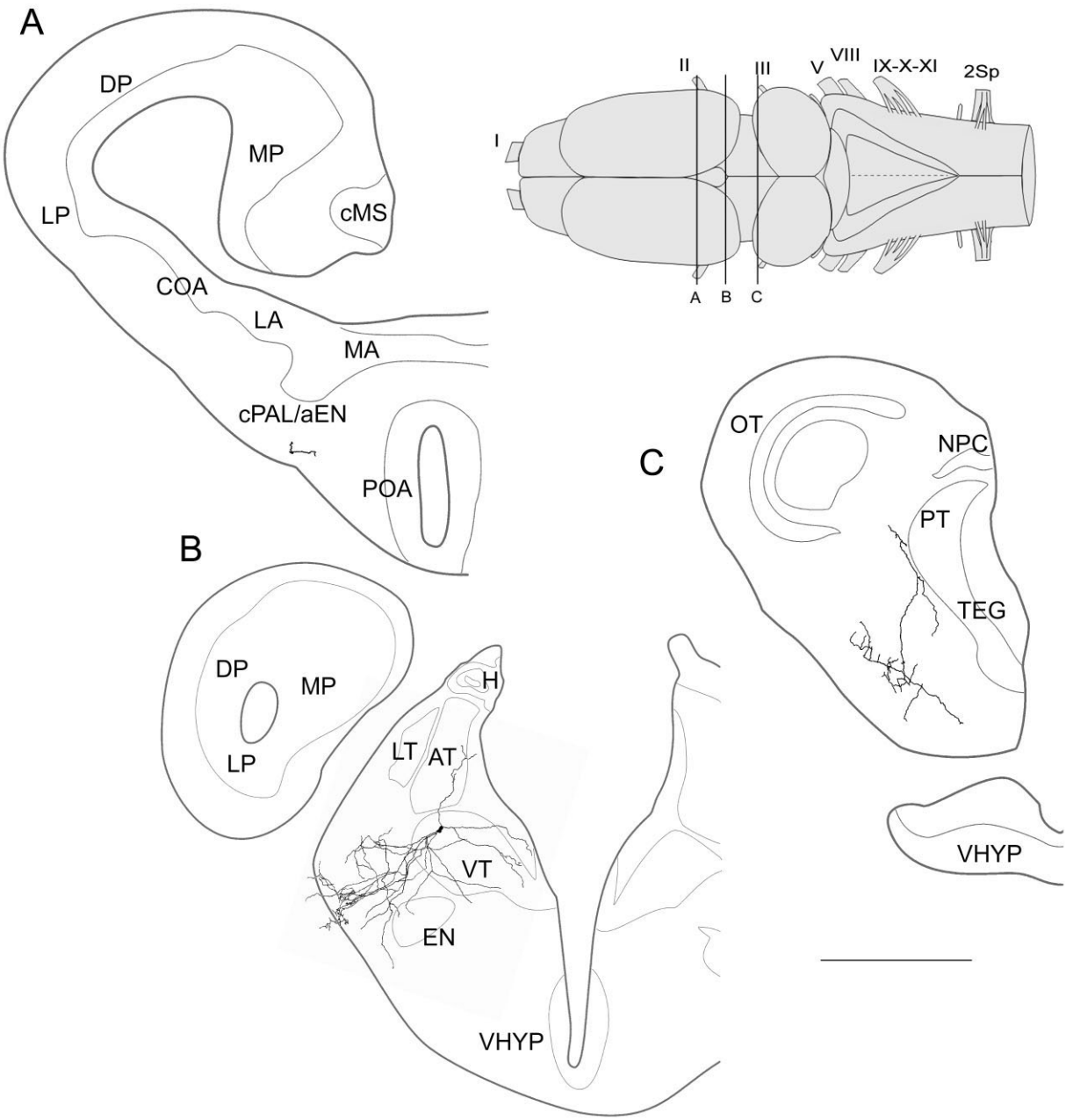

Fig. 6. Camera lucida reconstruction of ventral thalamic neurons projecting to the telencephalon. The cluster resulting from one intracellular biocytin injection is composed of two neurons. It was included, but not pictured, in a previous study (Roth et al., 2003). Axon terminals were observed in the superficial part of the caudal pallidum/anterior entopeduncular nucleus region, caudal ventral thalamus, and tegmentum. **A–C:** Levels of transverse section on which the neuron cluster is displayed are indicated by letters on the schematic dorsal view of the toad brain. Dendrites extending outside the section outline were situated caudal to the somata, where the brain was wider. For abbreviations, see list. Scale bar = 500 μm in C (applies to A–C).

get a great number of widespread brain regions through axon collaterals, en passant contacts, and terminals. The bed nucleus of the pallial commissure/thalamic eminence contains some neurons that project to the ventral portion of the medial pallium and septum. The ventral thalamic nucleus displays a few neurons projecting to the superficial part of the caudal telencephalic subpallium. The lateral dorsal thalamic nucleus projects to the lateral subpallium, but the site of axonal termination of these neurons is not precisely known because they were not studied systematically by using intracellular labeling. Definitely, this nucleus does not project to the pallium. Neurons in the ventral part of the preoptic area/suprachiasmatic nucleus (part of the secondary prosencephalon) also project to the telencephalon in anuran amphibians, although the site of axonal termination of these

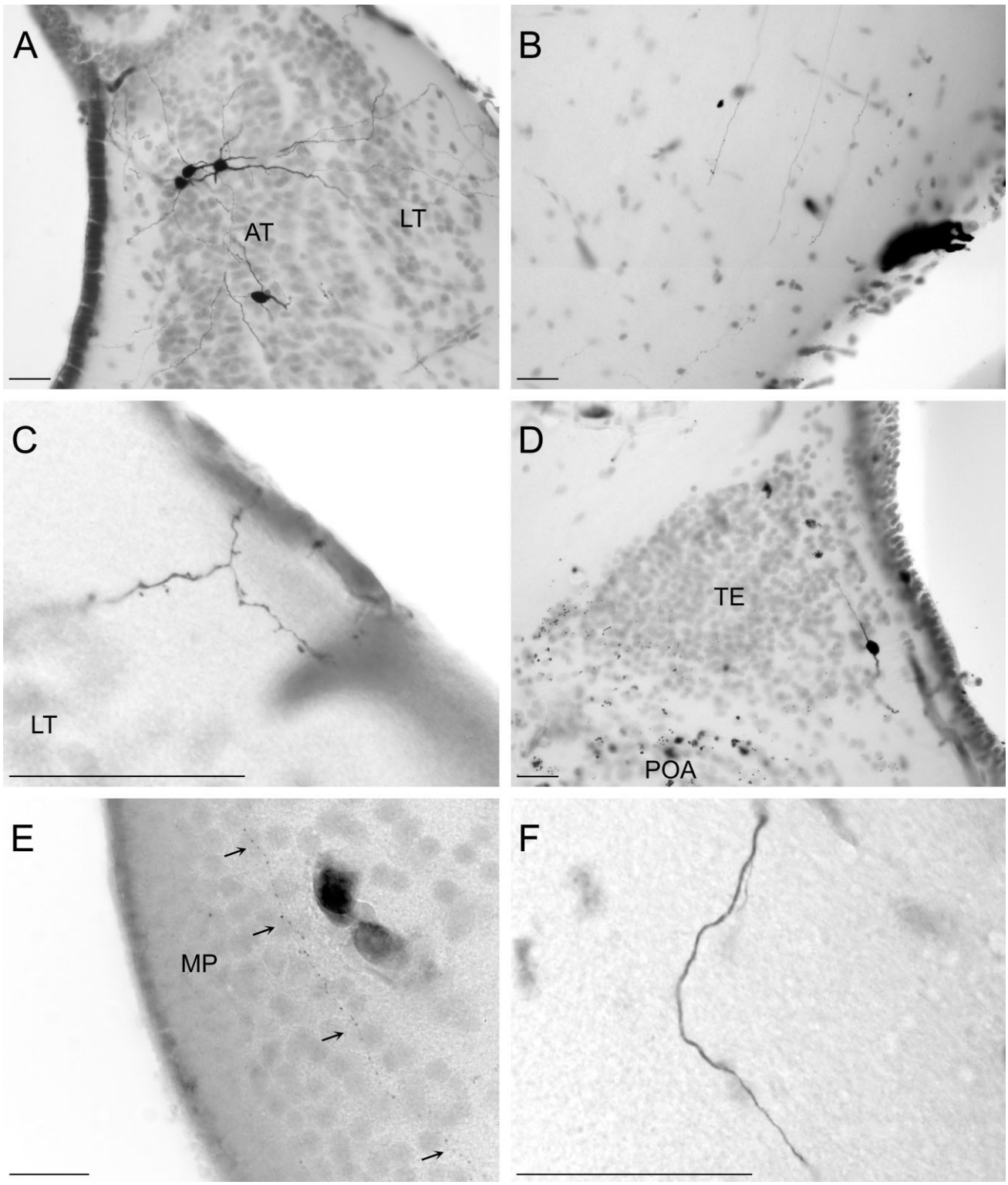

Fig. 7. Photomicrographs of diencephalic neurons. **A:** Somata of the four anterior thalamic neurons illustrated in Figure 3D. **B:** Axons and collaterals of the same neurons as in A in the white matter of the tuberculum posterius/dorsal hypothalamus region. Two micrographs were adjoined in order to show the fibers above and below in focus. **C:** Medium-spiny dendritic branches of the same neuron as in A in the white matter of the anterior dorsal thalamus. **D:** Soma of the neuron in the thalamic eminence illustrated in Figure 4B. **E:** Varicose fiber in the medial pallium of the same neuron as in D. **F:** Smooth dendrite belonging to the same neuron as in D in the white matter ventral to the bed nucleus of the pallial commissure. For abbreviations, see list. Scale bar = 50 μm in A–F.

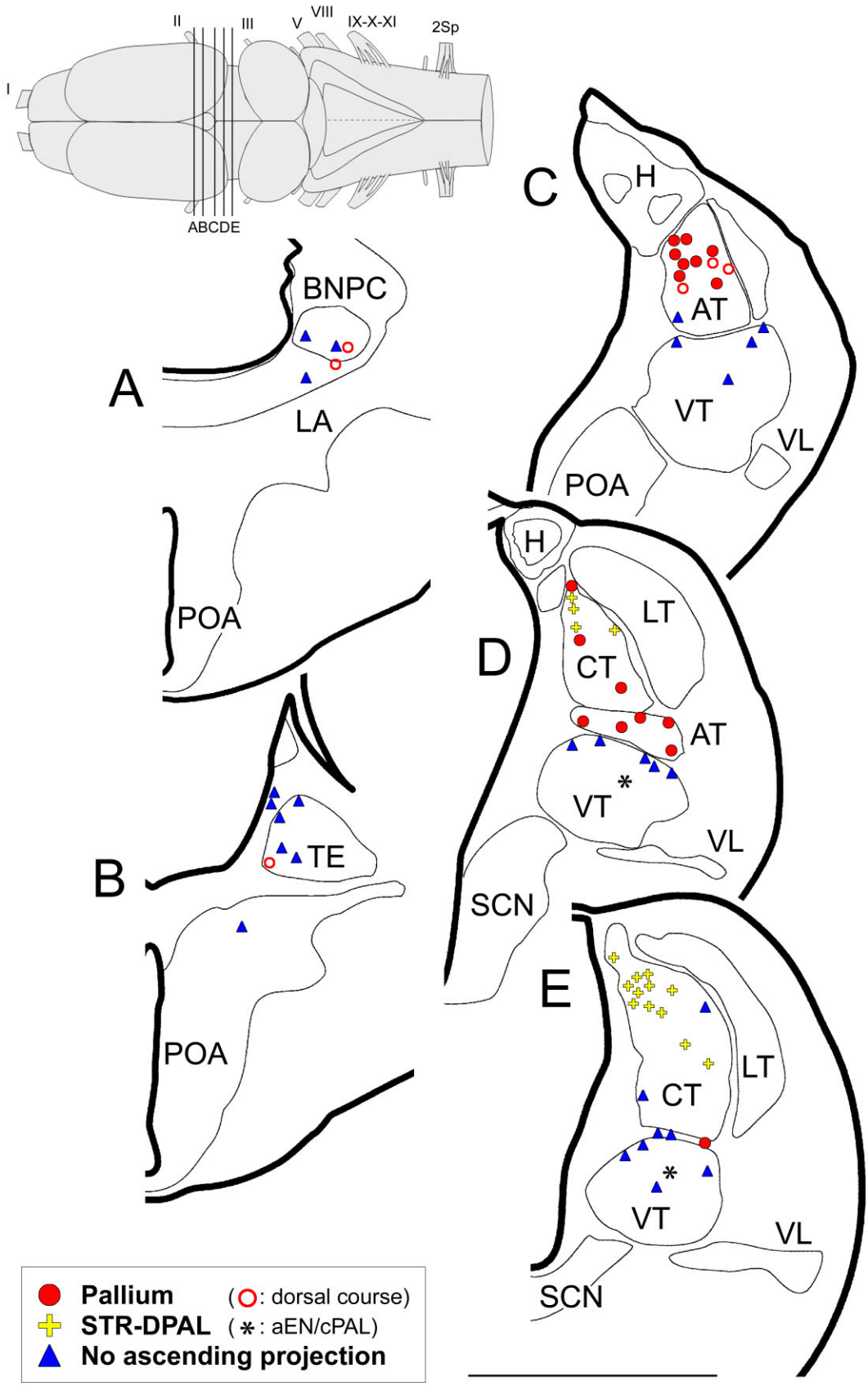

Fig. 8. Location of somata of diencephalic neurons according to their ascending projections to the telencephalon. All neurons described in the present study as well as 50 clusters of neurons described in Roth et al. (2003) were included. Somata of neurons projecting to the pallium are represented by red dots. Filled red dots stand for ascending pallial projections that course through the medial forebrain bundle as in Figure 3F, whereas open red dots stand for projections that course dorsally at the level of the pallial commissure without a caudal turn as in Figure 4. Somata of neurons projecting to the dorsal striato-pallidum are represented by yellow crosses. Stars represent the two clusters of neurons in the ventral thalamus that project to the superficial caudal pallidum/anterior entopeduncular nucleus. Blue triangles represent soma location of neurons that have no ascending projections to the telencephalon. For the sake of clarity, labelings found on the left side were projected on the right side of the brain. Levels of transverse section are indicated by letters on the schematic dorsal view of the toad brain. For abbreviations, see list. Scale bar = 500 μm in E (applies to A–E).

neurons is unknown (Wilczynski and Northcutt, 1983; Marín et al., 1997a; present study). Importantly, the use of simultaneous applications of two different tracers showed that the ascending thalamic pathways to the lateral and medial telencephalon originate from distinct neuron populations.

Methodological considerations

It is often difficult to ascertain axonal contacts by using anterograde tract tracing, especially when dealing with short collaterals or fiber varicosities. Definitive proof of synaptic contacts by axonal varicosities is not demonstrated here, but previous studies have shown that varicosities indeed represent synaptic sites (Anderson and Martin, 2001; Sabo et al., 2006). Here, the combination of retrograde tract tracing and intracellular labeling greatly facilitated the determination of axonal projection sites. For example, in addition to pallial projections, projections of the anterior thalamic nucleus to the lateral subpallium were previously reported in anurans (Neary, 1990). By using anterograde tract tracing in *B. orientalis*, biocytin applications in this part of the diencephalon often displayed projections to both the pallium and lateral subpallium, but applications restricted to the rostral portion did not show axons in the lateral subpallium (Laberge and Roth, 2007). In the present study, intracellular labeling showed that anterior thalamic and central thalamic neurons display segregated telencephalic projections despite their close proximity within the diencephalon. A disadvantage of the intracellular labeling method is that neurons are not always perfectly filled, and in such cases cannot be included in the analysis. Unfortunately, the latter means that the completion of a data set using intracellular labeling requires many specimens. Additionally, single intracellular injections of biocytin often produce multiple labeling of neurons at the site of injection. This phenomenon might be due to leakage of biocytin at the injection site or transcellular biocytin transport via gap junctions (Dermietzel and Spray, 1993) or via chemical synapses (Luo and Dessem, 1996).

It was previously assumed that all neurons within a cluster possessed the same axonal projection pattern (Roth et al., 2003). The present report is mostly concerned with a detailed description of thalamic axonal projections. The injections that resulted in the labeling of a single neuron, although in limited number, were used to generalize our conclusions to the level of the single neuron. The re-examination of the 2003 data revealed that all neurons injected in the anterior dorsal thalamic nucleus (including the weakly labeled ones) sent axons via the medial forebrain bundle to medial telencephalic targets, and all darkly labeled neurons terminated in the pallium fulfilling the criterion for type TH3 neurons sensu Roth et al. (2003). Apart from the TH3 criterion mentioned above, we could not ascertain whether each neuron within a cluster exhibited the same pattern of additional axonal projections.

Comments on the anatomical framework of the present study

The organizational scheme of the amphibian telencephalon used in the present study is not universally accepted. Other investigators proposed that the most caudal part of the dorsal pallidum, as defined here, represents the central (autonomic) amygdala (Bruce and Neary, 1995a; Moreno and González, 2005). We gave evidence of a distinct group of neurons found more medially that fulfills the criteria for an autonomic amygdala (Roth et al., 2004; Mühlenbrock-Lenter et al., 2005) and suggested that a central amygdala in the caudal pallidum as proposed by other authors, if amygdala at all, might represent a lateral component of the autonomic amygdala (Laberge et al., 2006). Moreno and González (2003, 2004, 2005) have used an alternative nomenclature based on their proposed homologies with the mammalian amygdala. According to these authors, our medial, lateral, and cortical amygdala correspond to the BNST, medial amygdala, and lateral amygdala, respectively. Another point of dispute for investigators of the amphibian amygdala regards the presence or absence of a structure homologous to the basolateral amygdaloid complex of amniotes. The ventral part of the lateral pallium as defined by Bruce and Neary (1995a) or the lateral amygdala of Moreno and González (2004) present many similarities with ventropallial derivatives of the basolateral complex and are suggested to be in a position to associate visceral and sensory information from all modalities.

However, we argued that visceral and thalamic inputs to this lateral part of the amphibian amygdala were too weak for such a function (Laberge et al., 2006), which is supported by our observation that responses to sensory modalities other than olfaction appear to be restricted to the medial part of the anuran amygdala (Laberge and Roth, 2007). This divergence of opinion might result from differences in preferred concepts of homology. Here, we tend to favor a concept of homology that essentially includes equivalent function of brain regions across groups.

Few investigators have analyzed rostrocaudal differences among pallial regions in amphibians. Two studies investigating that problem in anurans reached the conclusion that the most rostral part of the pallium forms a region on its own (Veenman et al., 1989; Roth et al., 2007). Veenman and collaborators (1989) defined the rostral pallium as the rostral one-third of the entire pallium, whereas we restricted it to the region between the rostral pole of the pallium and the level of the caudal border of the accessory olfactory bulb (Roth et al., 2007), which is the usage for the present report.

Connectivity of the anterior diencephalon in *B. orientalis*

The anterior dorsal thalamus in *B. orientalis* displays different types of neurons projecting to the pallium, with axons running either through the medial forebrain bundle after a caudal detour through the tuberculum posterius/hypothalamic region (majority of neurons), or directly through a rostrodorsal route. Because neurons with axonal branches following the rostrodorsal route were found in the most rostral anterior thalamus, and only these axonal branches targeted the posterior parts of the pallium, a topographical relationship of pallial projections may exist within the anterior thalamic nucleus. The dorsal thalamus in amphibians is known to transmit sensory input to the telencephalon (Karamian et al., 1966; Vesselkin et al., 1971; Laberge and Roth, 2007). In anurans, there are reports of afferents to the anterior dorsal thalamus from the medial pallium (Northcutt and Ronan, 1992; Roth et al., 2007), preoptic area, suprachiasmatic nucleus, ventral hypothalamus, posterior entopeduncular nucleus, posterior thalamus, optic tectum (G. Roth and U.

Dicke, unpublished observations), torus semicircularis, and dorsal column nuclei (Northcutt, 1995). Therefore, the anterior dorsal thalamic nucleus is in a good position either to integrate multiple sensory modalities as well as visceral influences or to transmit already integrated multimodal information.

Thalamic input to the lateral part of the amygdala could not be observed in *B. orientalis* in the present and a previous study (Laberge and Roth, 2007). However, scattered dorsal thalamic input to the lateral amygdala was detected in *Hyla* (Fig. 5D in Endepols et al., 2004), *Rana*, and *Xenopus* (*Rana* LPv and LPi in Neary, 1990; Moreno and González, 2004) prompting the latter authors' proposal of a homology with the multimodal area of the amniote basolateral complex. Species differences could be present, but in any case it is doubtful that such weak connections are of functional significance (cf. Neary, 1990). Moreno and González (2005) also proposed a central amygdala occupying what is considered here the most caudal portion of the dorsal pallidum. Neurons in this region display dendrites that course rostrally, invading the dorsal striatal neuropil, which is the target of central thalamic axons. Amygdala neurons in *B. orientalis* send their dendrites caudal to the dorsal striatal neuropil (Roth et al., 2004), not in a position to receive central thalamic input. However, dendrites of the medial amygdala (autonomic-limbic associative), as defined here, are in a good position to receive input from the anterior thalamic nucleus.

BNPC/TE projections to the telencephalon do not appear to transmit sensory information (Laberge and Roth, 2007), and no thalamic input is seen there in *B. orientalis*. Because BNPC/TE receives input from the medial and dorsal pallium (Roth et al., 2007), it could be involved in a loop between the pallium and limbic regions, as its main outputs are to the medial telencephalon, preoptic area/hypothalamus, dorsal thalamus, limbic midbrain, and habenula. Axonal projection zones of BNPC/TE neurons within the medial pallium differ from the projections of the anterior dorsal thalamus providing distinct means for these regions to modulate pallial activity. Elucidation of the role of BNPC/TE is complicated by its absence in adult amniotes (Wullimann and Mueller, 2004).

Comparisons with other vertebrates

In turtles, three divisions of the anterior thalamus are recognized, viz., the dorsomedial anterior, dorsolateral anterior, and lateral geniculate nuclei, each of which targets different pallial territories following a medial to lateral pattern in the sense that the dorsomedial anterior nucleus projects to the medial cortex, the dorsolateral anterior nucleus to the dorsomedial cortex, and the lateral geniculate nucleus to the dorsal cortex (Ulinski, 1990; Zhu et al., 2005). Turtles also possess a collothalamic nucleus rotundus, which projects to the striatum as well as to the dorsal ventricular ridge (Hall and Ebner, 1970; Ulinski, 1990; Cordery and Molnár, 1999). The situation found in turtles generally corresponds with the mammalian pattern of anterior/midline dorsal thalamic nuclei targeting limbic cortices, a lateral geniculate nucleus targeting the dorsal pallium, and collothalamic nuclei targeting the striatum and pallium (Price, 1995; Hoover and Vertes, 2007). However, in turtles, intralaminar nuclei targeting the striatum have not been identified (Butler, 1994), and the lateral geniculate target is found in a rostral position, whereas in mammals it is found caudally. In lizards, the lateral geniculate nucleus and the similarly placed nucleus intercalatus appear to project to the pallial thickening instead of the dorsal cortex, and the remaining thalamo-cortical input (medial, dorsal, and lateral) originates from the dorsolateral anterior nucleus (Bruce and Butler, 1984; Martinez-Garcia and Lorente, 1990; Ulinski, 1990; ten Donkelaar, 1998; Guirado and Dávila, 2002).

Overall, when compared with amphibians, amniote vertebrates display an increased number of thalamic nuclei as well as additional thalamo-telencephalic pathways. Conservation of thalamo-pallial/cortical pathways is seen, but anterior thalamic projections through the lateral forebrain bundle and collothalamic collaterals to the pallium are greatly enhanced in amniotes (Butler, 1994; Northcutt, 1995). We previously proposed that thalamic input to the pallial amygdala also underwent a major expansion in amniotes (Laberge et al., 2006). Are the medial subpallial and hypothalamic/tubercular projections of the anterior thalamus, which are seen in *Bombina*, characteristics shared by all tetrapods? Anterior thalamic projections to the medial subpallium have been described in a turtle (Siemen and Künzle, 1994; Zhu et al., 2005), and projections to the hypothalamus might also exist (Belekhova, 1991; Bruce and Neary, 1995b). In mammals, midline thalamic nuclei target the nucleus accumbens, septum, and prefrontal cortex, and evidence exists that single neurons target multiple sites (Otake and Nakamura, 1998; Van der Werf et al., 2002; Vertes et al., 2006). Some hypothalamic projections were also observed, but projections to the substantia nigra/ventral tegmental area (supposed tuberculum posterius homologues) were not seen.

Wicht and Northcutt (1998) suggested that pallial afferents from both the dorsal thalamus and posterior tuberculum were common features of ancestral craniates but that tubercular afferents were lost in lampreys. In ray-finned fishes, dorsal thalamic projections to the telencephalon are mostly to the subpallium, and ascending sensory pathways to the pallium originate principally from the preglomerular nuclei (Holmes and Northcutt, 2003; Folgueira et al., 2004; Yamamoto and Ito, 2005; Huesa et al., 2006; Northcutt, 2006). Most investigators considered the preglomerular nuclei to be derived from the posterior tuberculum (Butler, 1994; Northcutt, 1995), suggesting that a reduction of pallial sensory afferents from the posterior tuberculum has occurred in tetrapod ancestors. However, it has recently been proposed that the preglomerular nuclei of teleosts could represent migrated parts of the dorsal thalamus homologous to the dorsal thalamic regions projecting to the telencephalon in other vertebrates (Yamamoto and Ito, 2005; Ishikawa et al., 2007). Accordingly, the anuran thalamo-telencephalic pathways can be considered either as distinct or homologous when compared with teleosts, depending on the different views of the origin of the teleost preglomerular complex.

A new report, however, favors multiple developmental origins of the fish preglomerular complex (Northcutt, in press). Further work is clearly needed on this topic. Preglomerular nuclei in teleosts appear to encode unimodal sensory information to some extent (Prechtl et al., 1998; Yamamoto and Ito, 2005), which is incompatible with previous studies in amphibians suggesting that dorsal thalamic sensory input reaching the telencephalon represents integrated multimodal information (Vesselkin et al., 1971; Roth et al., 2003; Westhoff et al., 2004; Laberge and

Roth, 2007). Thus, an alternative hypothesis would be that unimodal thalamic sensory pathways are a common feature of teleosts and amniotes and that such pathways have been lost in amphibians, possibly in the context of pedomorphosis. If the latter were the case, then we would have to expect that the formation of these pathways is a late ontogenetic event, because such events are most severely affected by pedomorphic loss (cf. Roth et al., 1994).

Functional significance

The likely multimodal sensory nature of the amphibian dorsal thalamus is reminiscent of the mammalian limbic thalamus and could prove to be a useful model of the latter, the function of which is poorly understood at this time. In light of the data, the amphibian dorsal thalamus can be regarded as a center that integrates sensory information and distributes it widely throughout the brain in regions preferentially involved in behavioral modulation. Projections of the anterior thalamus to the pretectum/tectum could be involved either in the modulation of sensory perception or in attention. The connectivity of the BNPC/TE suggests that it is in a good position for coordination of the limbic system in general, in line with pallial information, and is possibly involved in modulation of limbic activity along with the anterior thalamus. Its projections to the limbic midbrain and habenula could indicate involvement in negative reward signals, as suggested by recent findings in mammals (Matsumoto and Hikosaka, 2007; Gruber et al., 2007). The collateral projections of dorsal thalamic neurons to the tuberculum posterius/ventral tegmentum also appear to be in a good position for modulation of the amphibian limbic midbrain and its dopaminergic neurons (Marín et al., 1995, 1997b), but it is unclear whether tubercular or hypothalamic neurons are targeted. It will be important to determine the role of the anterior thalamus and BNPC/TE in appetitive/aversive learning in amphibians, and the possible functional differences between pallial projection fields of anterior thalamic neurons also deserve interest.

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