THE JOURNAL OF COMPARATIVE NEUROLOGY 482:176–200 (2005)

Connectivity and Cytoarchitecture of the Ventral Telencephalon in the Salamander *Plethodon shermani*

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ABSTRACT

The cytoarchitecture and axonal connection pattern of centers in the ventral telencephalon of the salamander *Plethodon shermani* were studied using biocytin for anterograde and retrograde labeling of cell groups, as well as by intracellular injections. Application of biocytin to the main and accessory olfactory bulbs identified the olfactory pallial regions and the vomeronasal portion of the amygdala, respectively. According to our results, the amygdala of *Plethodon* is divided into (1) a rostral part projecting to visceral and limbic centers and receiving afferents from the dorsal thalamus, and (2) a caudal part receiving accessory olfactory input. The striatopallial transition area (SPTA) lies rostrodorsally to the caudal (vomeronasal) amygdala and is similar in connections and possibly in function. The rostral striatum has few descending projections to the medulla, whereas the intermediate striatum sends strong projections to the tegmentum and medulla. The caudal striatum has strong ascending projections to the striatum and descending projections to the ventral hypothalamus. The dendritic trees of neurons labeled below the striatum and in the SPTA spread laterally from the soma, whereas dendrites of striatal neurons converge into the laterally situated striatal neuropil. In the caudal amygdala, three distinct types of neurons are found differing in dendritic arborization. It is concluded that, hodologically, the rostral part of the urodele amygdala corresponds to the central and basolateral amygdala and the caudal part to the cortical/medial amygdala of mammals. The urodele striatum is divided into a rostral striatum proper, an intermediate dorsal pallidum, and a caudal part, with distinct connections described here for the first time in a vertebrate. J. Comp. Neurol. 482:176–200, 2005.
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Indexing terms: amphibians; neuroanatomy; amygdala; biocytin labeling

The telencephalon of salamanders is characterized by a low degree of cellular migration, except in the medial pallium and an almost complete absence of nuclei with distinct boundaries. Despite this situation, the urodele ventral telencephalon is believed to possess a septum in the ventromedial telencephalon below the medial pallium, a striatum in the lateral wall of the telencephalon below the lateral pallium, and an amygdala in the ventral caudal telencephalon (Northcutt and Kicliter, 1980; Northcutt, 1981). Recent work proposed a dorsal and ventral pallidum to be likewise present in amphibians and gave a new interpretation of the amygdalar complex (Medina and Reiner, 1995; Marín et al., 1997a,c, 1998; Moreno and González, 2003). A recent study helped clarify further the organization of the striatopallidal complex in anurans: the caudal part of what was considered the striatum in a wider sense by Marín and collaborators is now proposed to be part of the dorsal pallidum (Endepols et al., 2004).

The anatomical description of the amygdala is complicated by the presence of pallial and subpallial cell groups with different functions (see Swanson and Petrovich, 1998; McDonald, 2003). Using a comparative approach,

Grant sponsor: Deutsche Forschungsgemeinschaft; Grant number: SFB 517; Grant sponsor: National Science Foundation; Grant number: IBN 0110666; Grant sponsor: Hanse Institute for Advanced Study.

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Received 10 December 2003; Revised 8 July 2004; Accepted 1 October 2004

DOI 10.1002/cne.20430

Published online in Wiley InterScience (www.interscience.wiley.com).

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Bruce and Neary (1995) argued that the common ancestors of reptiles and mammals had a well-developed limbic system, in which the basic subdivisions and connections of the amygdala were already present, i.e., a portion with long descending projections to the hypothalamus and to autonomic–visceral centers in the medulla, a portion that receives main olfactory input and one receiving accessory olfactory (vomeronasal) input, both projecting to the hypothalamus, as well as a nonolfactory portion that likewise projects to the hypothalamus and receives input from the dorsal thalamus. It follows that the connections of the amygdala with the brainstem, hypothalamus, olfactory systems and dorsal thalamus would serve evolutionarily conserved functions. Thus, the study of the anatomy and connectivity of the amygdala and related centers in the ventral telencephalon in urodeles could help elucidate the basic structural and functional ancestral condition of the amygdala and the striatopallidal complex.

However, the knowledge of the urodele ventral telencephalon derives mostly from interpretations of anatomical observations of normal brain morphology (Fish, 1895; Herrick, 1910 [and references therein], 1921, 1948; Northcutt and Kicliter, 1980) and histochemistry (Dubé and Parent, 1982; Taban and Cathieni, 1983; Fasolo et al., 1990; González and Smeets, 1991, 1992; Naujoks-Manteuffel et al., 1994; Dicke et al., 1997; Lowry et al., 1997; Marín et al., 1997b, 1998; Beltramo et al., 1998). The few experimental studies conducted in salamanders give only a partial picture of the ventral telencephalon (Kokoros and Northcutt, 1977; Finkenstädt et al., 1983; Wicht and Himstedt, 1986, 1988; Naujoks-Manteuffel and Manteuffel, 1988; Dubé et al., 1990; Fasolo et al., 1990; Sassoè-Pognetto et al., 1995; Marín et al., 1997a,c; Dicke et al., 1998; Roth and Grunwald, 2000).

The present study attempts to describe the anatomy and connectivity of the ventral telencephalon and its neighboring lateral regions in the terrestrial salamander *Plethodon shermani* by anterograde and retrograde tracing experiments using the tracer biocytin, with application methods that avoid the problem of uptake by fibers of passage at the application site, as well as by intracellular injection of biocytin to reveal morphology of neurons and details of the axonal projection pattern. The present work likewise is performed to establish the neuroanatomical framework necessary for future investigations of responses to courtship pheromones in plethodontid salamanders (Rollmann et al., 1999).

MATERIALS AND METHODS

The experiments were carried out in 84 specimens of the salamander *Plethodon shermani*, previously known as *Plethodon jordani* (Highton and Peabody, 2000). The animals were collected at the Highlands Biological Station, Highlands, North Carolina (collecting permit Dr. Lynne Houck). All experiments 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. Animals were anesthetized by exposure to carbon dioxide gas for 10 minutes in a closed plastic box, put in a Petri dish, and perfused transcardially with 20 ml of cold oxygenated Ringer's solution (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, pH 7.4). A stream of carbon dioxide was maintained over the skin of the animal throughout that procedure. 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 isolated brain was kept in Ringer's solution as mentioned above.

The identification of olfactory and vomeronasal input is important for defining essential parts of the amygdaloid complex. Labeling of the central projections of the main and accessory olfactory bulbs of salamanders with the neurotracer horseradish peroxidase was performed previously in our laboratory (Schmidt and Roth, 1990). These investigations were repeated in the present study for three reasons: (1) biocytin is a more sensitive neurotracer than horseradish peroxidase, (2) the previous work used *P. jordani* before this species complex was divided into multiple species without the possibility of knowing if the animals now used are from the same species, (3) it is now possible to achieve tracer application completely restricted to the main or accessory olfactory bulb, unlike in our previous study. Tracing of axonal projections of popu-

Abbreviations

A	amygdala
AC	anterior commissure
AOB	accessory olfactory bulb
AOHT	anterior olfactohabenular tract
AOT	accessory olfactory tract
below VCP	region below the ventral cellular prominence
c	caudal
caudal pole	caudal pole of the telencephalon
CB	cerebellum
DH	dorsal hypothalamus
DP	dorsal pallium
DPAL	dorsal pallidum
DT	dorsal thalamus
DTEG	dorsal tegmentum
H	habenula
LCHT	lateral corticohabenular tract
LOT	lateral olfactory tract
LP	lateral pallium
MO	medulla oblongata
MOB	main olfactory bulb
MOT	medial olfactory tract
MP	medial pallium
NA	nucleus accumbens
OT	optic tectum
P	pallium
POA	preoptic area
POC	postoptic commissure
PT	pretectum
r	rostral
S	septum
SC	spinal cord
SPTA	striatopallial transition area
STR	striatum
TEL	telencephalon
TP	tuberculum posterius
VCP	ventral cellular prominence
VH	ventral hypothalamus
VPAL	ventral pallidum
VT	ventral thalamus
VTEG	ventral tegmentum

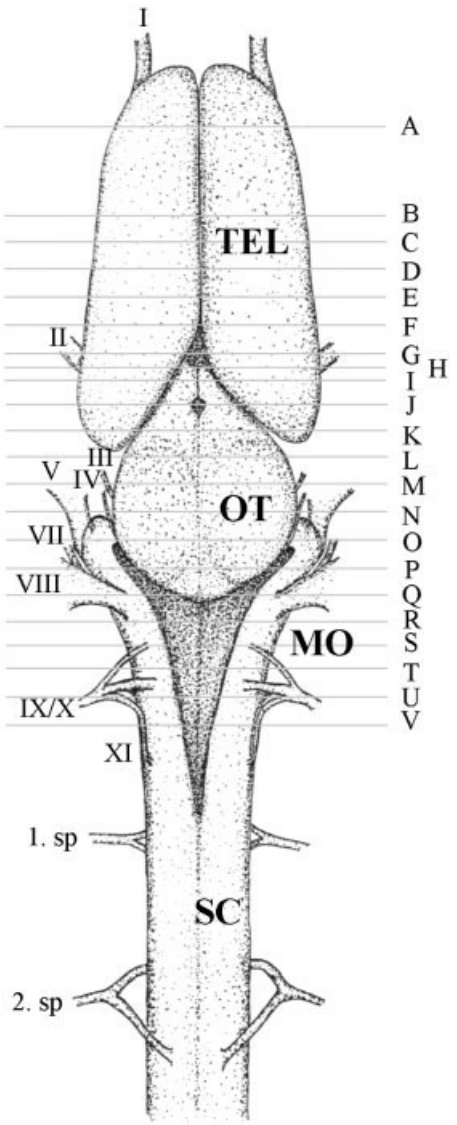

Fig. 1. Gross morphology of the salamander brain. Dorsal view. Rostrocaudal levels of transverse section used in the present study are indicated by letters. The distance between sections is 250 μm, except between A and B (750 μm) and G and H and I (125 μm): Cranial (roman numerals) and first two spinal (sp) nerves are also indicated. For abbreviations, see list.

TABLE 1. Tract-Tracing: Summary of Biocytin Application Sites[1]

Sample size	Application method	Experiment	Application site
3	C (2), M (1)	A	MOB
7	C (3), M (4)	A	AOB
5	C	A	VCP/below VCP
3	C	A	STR + MP
3	C	A	VCP + ventral STR
2	C	A	cA + caudal STR
2	C	A	SPTA + LP + caudal pole
1	C	A	LP
1	C	A	Caudal STR
1	C	A	SPTA + dorsal STR
1	C	A	SPTA + dorsal STR + LP
1	C	A	SPTA + LP + DP
1	C	A	SPTA + dorsal STR + LP + cA
1	C	A	SPTA + LP + caudal to AOB
2	C	R	MP
2	C	R	POA
2	C	R	VH
2	C	R	MO (caudal to CB)
1	C	R	SC

[1]C, biocytin crystal application; M, biocytin microinjection; A, anterograde tract-tracing; R, retrograde tract-tracing. For other abbreviations, see list.

lations of neurons was achieved by application of biocytin crystals (Sigma-Aldrich, St. Louis, MO) to the brain surface. In some cases, microinjections of 2% biocytin dissolved in 0.3 M potassium chloride were made in the olfactory bulbs with a nanoliter injector (World Precision Instruments, Sarasota, FL). For application of crystalline biocytin, the brains were exposed to the air and dried with the help of paper tissue, before a small lesion was made with a glass micropipette on the site of application. Biocytin application to the olfactory bulbs was made from the external surface, whereas application to telencephalic regions was made from the medial surface. The medial pallium was cut out in cases of application of biocytin to the dorsal-most telencephalon. This method has the advantage of avoiding the uptake of tracer by passing fiber tracts, as happens with applications from the external surface. However, the brains have to be split into halves, and projections to the contralateral side by means of the commissures cannot be studied. Retrograde filling of neurons projecting to the medial pallium, preoptic area, and ventral hypothalamus was made by applying biocytin to the external surface of these brain regions after multiple lesions with a glass micropipette. To reveal the location of neurons projecting to the brainstem and spinal cord, the brain was cut immediately caudal to the cerebellum and obex, respectively, and biocytin was generously applied to the cut surface. Ten minutes were allowed for biocytin uptake, while the brains were exposed to the air. Afterward, the brains were stored in Ringer's solution for 4 hours at room temperature and at 4°C overnight. Brains were then fixed in a solution of 2% paraformaldehyde–2% glutaraldehyde, embedded in 4.4% gelatin, and 50-μm-thick transverse sections were cut on a Vibratome. Biocytin was visualized by means of an avidin–biotin–peroxidase complex (Vectastain standard kit, Vector Laboratories, Burlingame, CA) using diaminobenzidine (Sigma) as chromogen with heavy-metal intensification (Adams, 1981). Sections were lightly counterstained with 0.1% cresyl violet, dehydrated in ascending ethanol concentrations, cleared in xylene, and cover-slipped with Eukitt (Kindler O. & Co., Freiburg, Germany).

For intracellular labeling, glass micropipettes were filled with 2% biocytin dissolved in 0.3 M potassium chloride. The impedance of the electrodes ranged between 80 and 160 MΩ. The brain was split longitudinally, and brain halves were fixed to the floor of the recording chamber. The brain was penetrated from the medial surface. The brain was continuously perfused with oxygenated Ringer's solution (6 ml/minute) at a temperature of 14–18°C. A hyperpolarizing current of 0.2 nA was applied for 200 msec every second, while the electrode was moved dorsoventrally in small steps with the help of a hydraulic three-axis micromanipulator (model ONO-131, Narishige, Tokyo, Japan). Cell membranes were penetrated by application of a slight overcompensating current (tickling). A drop of −20 to −60 mV of the membrane potential had to remain stable before a biocytin injection was made

by iontophoresis (1 nA pulsed current for 4 minutes). Usually, only one injection was made in each half of the brain. After injection, the brains were stored in Ringer's solution at room temperature for 4 hours and at 4°C overnight. Brains were processed as described above for tract-tracing. Labeled neurons were reconstructed by hand with the help of a camera lucida (Carl Zeiss, Inc., Germany), scanned, and graphically processed in Photoshop 6.0 (Adobe Systems, Inc., San Jose, CA). The photomicrographs presented were scanned with a digital camera (AxioCam HR, Carl Zeiss, Inc.).

RESULTS

The vagueness of the established structural boundaries of the urodele brain prompted us to use mostly general terms in the description of our results. The nomenclature of the telencephalon is based on Northcutt and Kicliter (1980), with the following exceptions: the pars ventralis of the lateral pallium (lateral prominence) is being referred to as the striatopallial transition area (SPTA) sensu Marín et al. (1997a,c), the pars dorsalis of the lateral pallium is the lateral pallium proper, the pars medialis of the amygdala is divided into the ventral cellular prominence (VCP) and the region below it (below VCP), and the pars lateralis of the amygdala is called the caudal amygdala. The nomenclature of the remainder of the brain is as in Herrick (1948). Figure 1 represents a schematic dorsal view of the salamander brain with the rostrocaudal levels of sections used in the figures and throughout the text illustrated.

Olfactory projections to the forebrain

Table 1 lists the number of animals used and methods of biocytin application in experiments on the projection pattern of the main and accessory olfactory bulb. As shown in Figure 2, the efferent axons of the main olfactory bulb extend within the white matter of the rostral pallium (Fig. 2B; nucleus olfactorius anterior of Herrick [Herrick, 1948]), where they carry numerous fiber varicosities. At the level of the rostral accessory olfactory bulb, the projections of the main olfactory bulb split into a dorsal and a ventral bundle, which represent the rostral portion of the lateral and medial olfactory tracts, respectively (Fig. 2C). The lateral olfactory tract takes a progressively more lateral position, as it runs caudally in the telencephalon and eventually occupies a position between the lateral and dorsal pallium at the level of the rostral SPTA (Fig. 2E). Starting at the level of the lamina terminalis, a portion of the fibers of the lateral olfactory tract takes a ventral path through the white matter, extends beyond the caudal amygdala, and then courses dorsally into the habenular commissure (Fig. 2G–I). Fibers not part of a tract, carrying many varicosities suggesting synaptic terminals, are seen in the dorsal pallium beginning at the level of the rostral striatum and in the caudal pole of the dorsal, lateral and ventral telencephalic surfaces (Fig. 2E–K). There are fewer fibers carrying varicosities in the lateral pallium below the lateral olfactory tract. The fibers seen in the habenula bear few varicosities and are clearly part of a tract (Fig. 2I).

The contralateral projections of the main olfactory bulb course immediately ventrally after passing through the habenula and then turn rostrally as a small darkly stained bundle called the anterior olfactohabenular tract by Herrick (1921). This ventral ascending tract courses upward to the rostral pallium at the level of the main olfactory bulb, where fibers run dorsally within the medial white matter as seen on the ipsilateral side, albeit not in the same direction (Fig. 2B). A small amount of varicosities is visible in these fibers in the rostral pallium. Fibers of the anterior olfactohabenular tract are the source of a tract directed caudally in the position of the lateral olfactory tract on the ipsilateral side. In one animal, this tract could be followed caudalward up to the level of the caudal amygdala, where it became faint (Fig. 2H,I). A contralateral olfactory tract in the same position was named the lateral corticohabenular tract by Herrick (1948). However, the latter olfactory tract was thought to be an exclusively ascending projection.

The medial olfactory tract takes a sharp turn from the medial to the ventrolateral surface of the telencephalon at the level of the caudal accessory olfactory bulb. Below the accessory olfactory bulb, as well as in the nucleus accumbens and to a lesser extent in the lateral septum, some fibers with numerous varicosities are observed below the lateral surface (Fig. 2C,D). The medial olfactory tract eventually gives off a few fibers all along the lateral wall of the telencephalon from the most ventral part to the SPTA dorsally (Fig. 2E–H, fibers not charted). Some of these fibers are axons of retrogradely filled neurons found in the VCP. A small number of fibers of the medial olfactory tract merge ventrally with the fibers of the lateral olfactory tract projecting into the habenular commissure. For sake of clarity, only the most important projections are charted in Figure 2.

Figure 3 shows the projection of the accessory olfactory bulb. The accessory olfactory tract divides into a large dorsolateral component extending along the SPTA and a small ventral component starting at the rostral striatum and extending lateral to the striatal neuropil (Fig. 3D–G). Both components of the accessory olfactory tract join immediately caudal to the striatum and form a dense terminal neuropil in the white matter overlying the caudal amygdala in the caudal telencephalon extending up to the level of the posterior habenula (Fig. 3H,I). Varicosities are abundant in the terminal neuropil of the accessory olfactory tract, but are also present to a lesser extent along the entire accessory olfactory tract.

Connectivity of the ventral telencephalon: tract-tracing

Table 1 lists the sites and number of applications of crystalline biocytin in anterograde and retrograde tracing experiments. Table 2 summarizes the axonal projection sites from eight selected biocytin applications.

Anterograde tracing. Little retrograde labeling was observed, when crystalline biocytin was applied from the medial surface of the brain directly onto the layer of somata, when compared with applications from the external surface that damage passing fiber tracts (not shown). Figures 4, 5, and 6 show the results of biocytin application restricted to the region below the VCP, the anterior part of VCP and the posterior part of VCP, respectively. These three regions share descending projections to the preoptic area, the caudal pole of the telencephalon (caudal pole), the dorsal thalamus/habenular region, the dorsal hypothalamus, and the ventral tegmentum. They appear to send projections to the septum/nucleus accumbens region, although the caudal portion of the region below the VCP

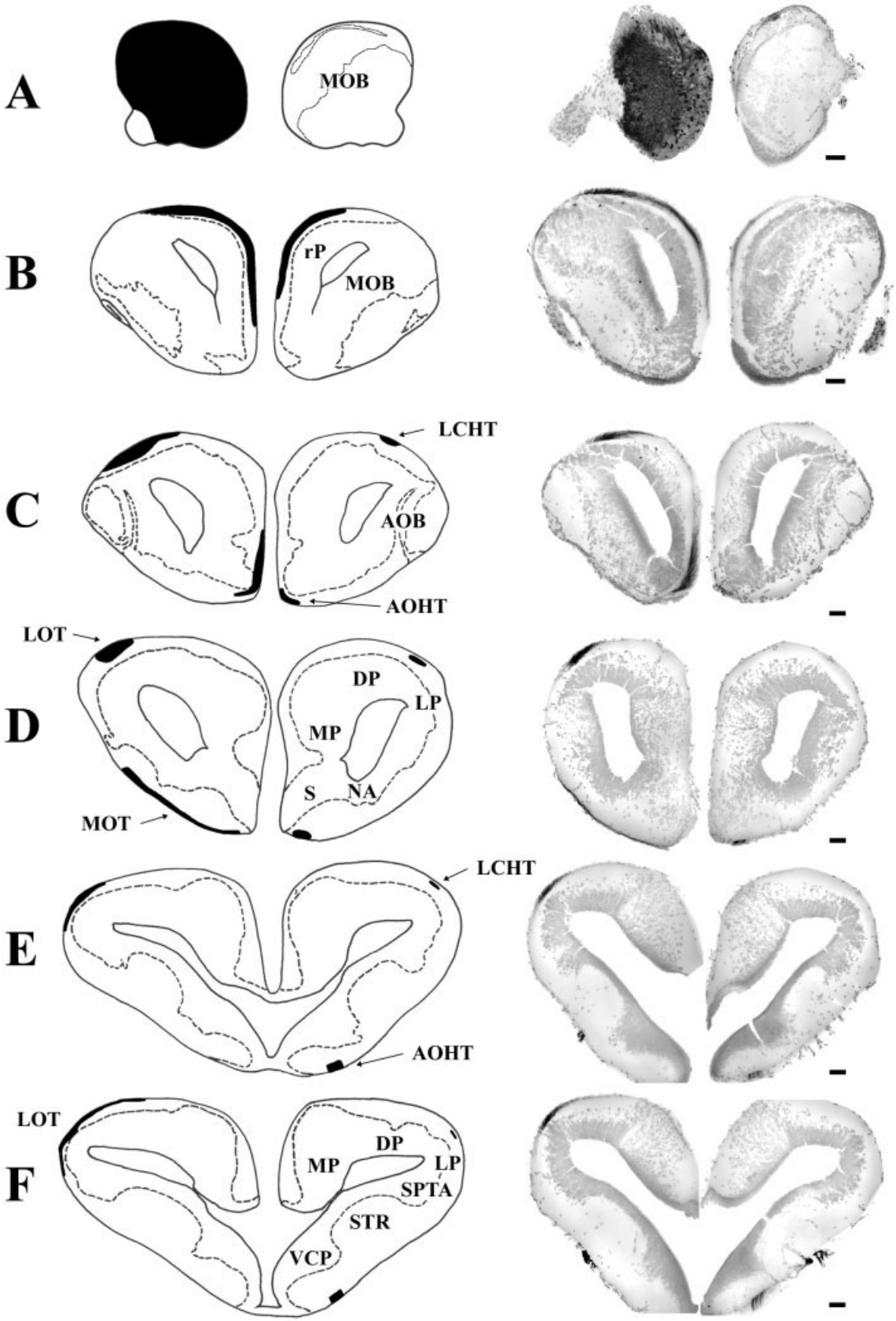

Fig. 2. Efferents of the main olfactory bulb (MOB) in *Plethodon shermani*. **A–K:** Schematic illustrations (left column) and photomicrographs (right column) showing the bilateral forebrain projection of the MOB after application of crystalline biocytin restricted to the left MOB. Rostrocaudal levels A–K corresponds to levels A–K in Figure 1. The photomicrograph on the right in K represents a higher magnification of axonal terminals of the MOB in the lateral part of the left caudal pole of the telencephalon. For other abbreviations, see list. Scale bars = 100 μm in A–K.

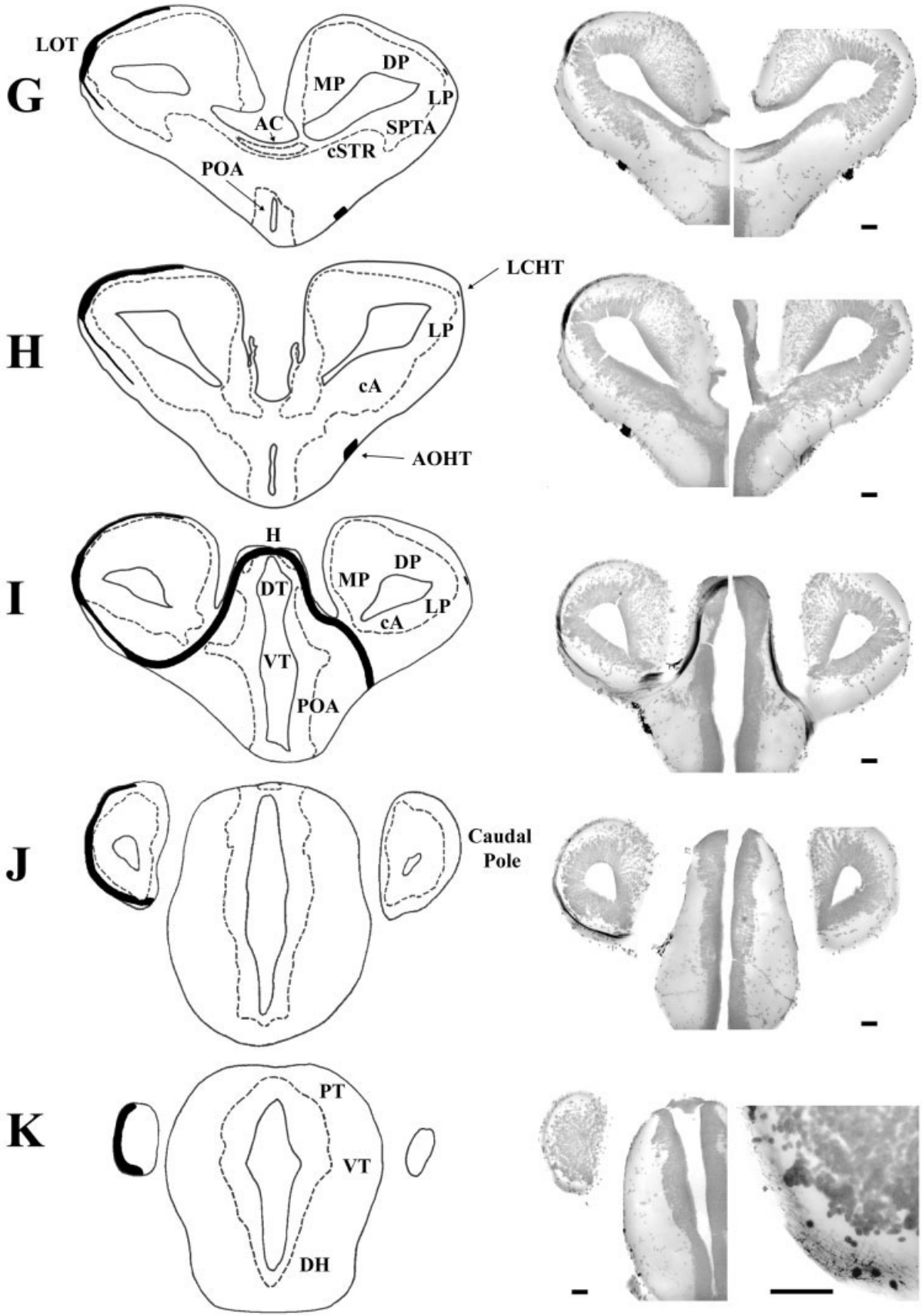

Figure 2 (Continued)

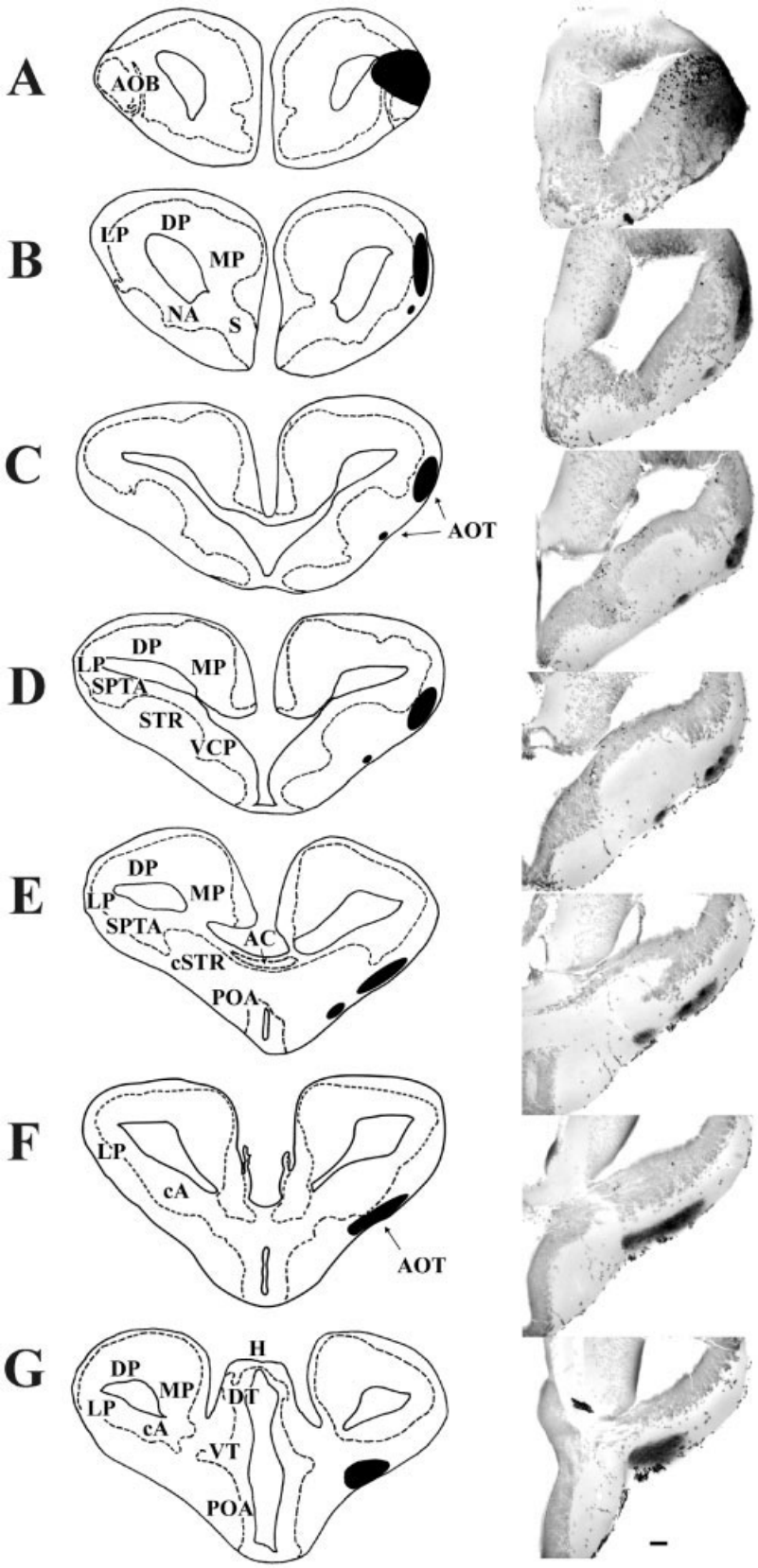

Fig. 3. Efferent pathway of the accessory olfactory bulb (AOB) in *Plethodon shermani*. **A–G:** Schematic illustrations (left column) and photomicrographs (right column) showing the ipsilateral forebrain projection of the AOB after application of crystalline biocytin restricted to the right AOB. A–G correspond to rostrocaudal levels C–I in Figure 1. For abbreviations, see list. Scale bar = 100 μm in G (applies to A–G).

TABLE 2. Crystal Applications: Projection Sites of Different Regions of the Telencephalon in *P. shermani*[1]

Axonal projection sites	Biocytin application sites							
	Below VCP	Anterior VCP	Posterior VCP	Ventral STR + MP	Caudal STR	cA + caudal STR	SPTA + LP + dorsal STR	LP
MOB	+	++	++	−	−	−	++	−
rP	−	++	++	−	−	−	−	−
AOB	−	−	++	−	−	−	++	++
rMP	−	−[2]	++	n.a.	−	−	−	−
Caudal to AOB	−	−	−	−	−	++	−	−
S/NA	+	?	++	++	−	+	++	++
STR	−	−	?	n.a.	+++	++	++	++
SPTA	−	−	−	−	−	?	n.a.	−
VCP/below VCP	n.a.	n.a.	n.a.	++	−	+	++	−
DP	+	++	−	−	−	−	n.a.	−
LP	−	−	++	−	++	++	n.a.	++
AC	−	−	−	−	++	++	++	−
POA	+++	++	++	++	−	++	++	−
Caudal pole	++	++	++	−	−	++	++	−
DT/H	+++	++	++	++	−	−	++	−
VT	+++	−	++	−	−	−	−	−
PT	++	−	−	++	−	−	−	−
OT	−	++	−	+	+	−	+	−
POC	−	−	+	−	−	−	++	−
TP	?	−	−	++	+	+	++	−
DH	+++	++	++	++	−	+	−	−
VH	++	+++	+	++	++	++	++	++
VTEG	++	++	++	++	+	−	++	+
CB	−	−	−	++	−	−	−	−
MO	−	+	++	+++	−	−	++	−

[1]Projection intensity: none (−), weak (+), moderate (++), strong (+++), ? (difficult to assess), n.a. (not applicable). For abbreviations, see list.
[2]This region has a moderate projection to the medial part of the caudal pole of the telencephalon.

constitutes only weak input. The anterior and posterior parts of the VCP send ascending projections to the main olfactory bulb and the rostral pallium. Only the region below the VCP and the posterior VCP project to the ventral thalamus. The former projects to the lateral part of the ventral hypothalamus, whereas the anterior VCP projects to the white matter of the entire ventral hypothalamus. The region below the VCP is also characterized by a projection to the pretectum, the anterior VCP by projections to the dorsal pallium and the optic tectum, the posterior VCP by projections to the accessory olfactory bulb, rostral medial pallium, lateral pallium, and medulla.

Some projections originating in the caudal amygdala can be deduced by comparing two biocytin applications, one encompassing the caudal amygdala plus part of the caudal striatum and the other one restricted to the caudal striatum (Table 2). Only when the caudal amygdala is involved, projections to the region caudal to accessory olfactory bulb, preoptic area, and caudal pole are seen in addition to weak projections to the septum–nucleus accumbens region, VCP/below VCP, and dorsal hypothalamus. Our best biocytin applications to the rostral or intermediate striatum always spilled over to portions of the ventral medial pallium close to the application site. They always revealed long descending projections to the tuberculum posterius, ventral tegmentum, and medulla (Table 2). The origin of these long descending projections is attributed to the striatum, because in salamander descending projections of the medial pallium do not extend beyond the hypothalamus (Westhoff and Roth, 2002). By using the same criterion, a projection to the dorsal thalamus, pretectum, and the vicinity of the cerebellum can be attributed to the striatum. On the other hand, biocytin application restricted to the caudal striatum revealed descending projections only to the medial ventral hypothalamic neuropil, in addition to a strong ascending projection to the middle layer of the striatal neuropil, fibers entering the anterior commissure and a moderate projection to the lateral pallium (Fig. 7). A common feature of biocytin applications encompassing the SPTA is the presence of projections to the main olfactory bulb and postoptic commissure (POC in Table 2). Biocytin application restricted to the caudal lateral pallium yielded projections to the accessory olfactory bulb, septum–nucleus accumbens region, striatum, rostral lateral pallium, and medial ventral hypothalamus (Table 2).

Retrograde tracing. Figure 8 shows the location of the somata of neurons with projections to the medulla, preoptic area, ventral hypothalamus, or medial pallium, as revealed by retrograde tracing. After biocytin application to the rostral medulla (Fig. 8-1), somata are found in the nucleus accumbens and more dorsally in the region rostral to the striatal neuropil, although to a lesser extent. Only a small number of neurons are backfilled in the very rostral part of the striatum (Fig. 9A) compared with the great density of neurons in the intermediate striatum (Fig. 9B). The border between the striatum, VCP, and SPTA can be seen in Figure 9B. Neurons projecting to the medulla are also found in the posterior VCP (Fig. 9B). The onset of the caudal striatum is easily detectable, because neurons projecting to the medulla are absent in this region (Fig. 9C). The intermediate part of the striatum, with abundant long descending projections, occupies most of the rostrocaudal extent of the striatum, except for small portions at its rostral and caudal ends. Caudal to the telencephalon, neurons projecting to the medulla were labeled in the thalamus, tuberculum posterius, tegmentum, tectum, and cerebellum. Despite the good quality of retrograde tracing, labeled neurons were not found rostral to the region of the dorsal hypothalamus/ventral thalamus in the animal used to study the neurons projecting to the spinal cord (not shown).

After biocytin application to the ventral hypothalamus (Fig. 8-2), retrogradely filled neurons are found in the nucleus accumbens and the anterior VCP (Fig. 9D), representing two distinct cellular groups. Other retrogradely filled neurons can be seen in the caudal and intermediate stria-

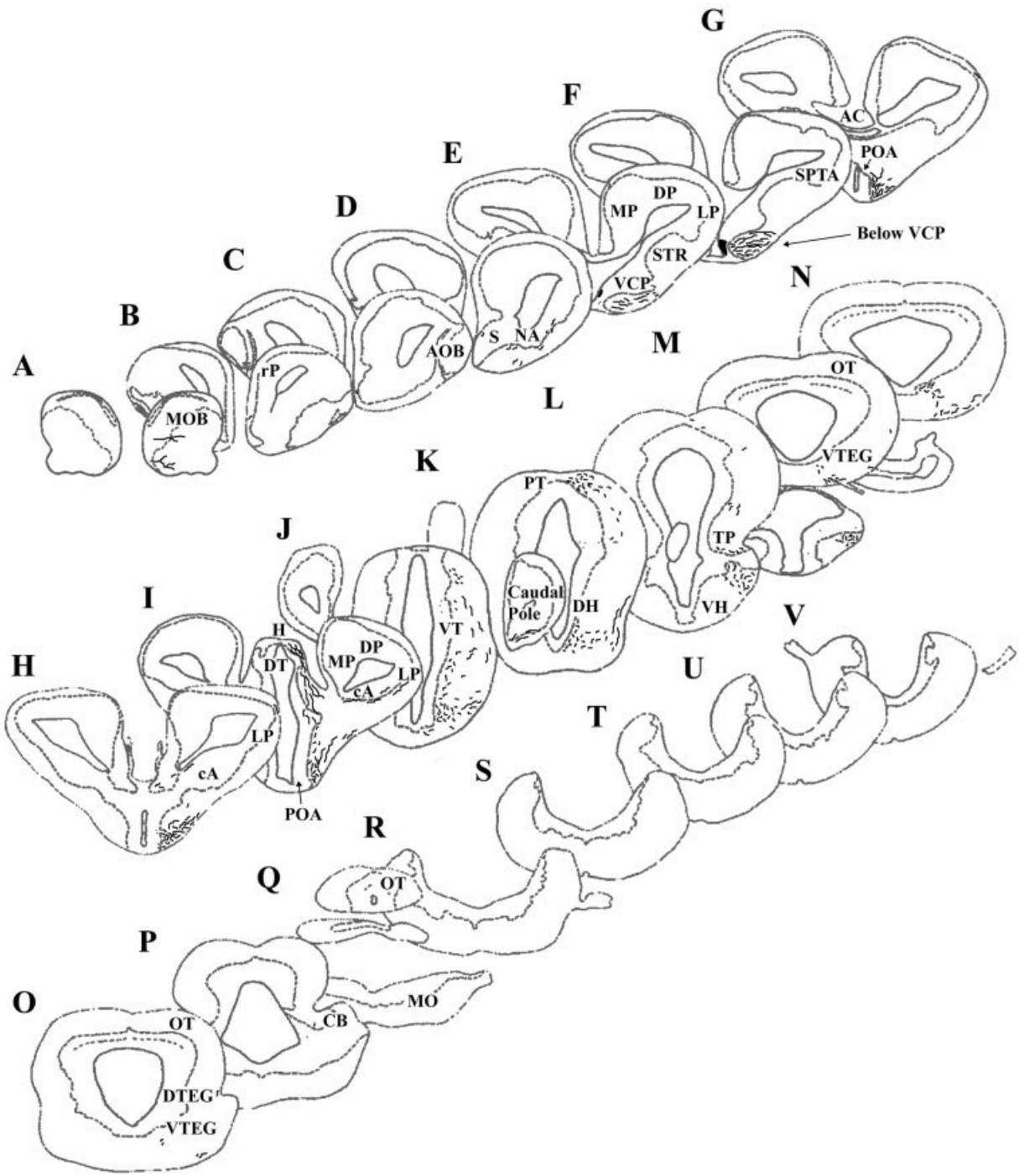

Fig. 4. **A–V:** Axonal projections of the region below the ventral cellular prominence (below VCP). Schematic illustration of the dendrites and axons labeled by application of crystalline biocytin to below VCP. The blackened region in the cellular layer indicates the site of application of biocytin; dots represent retrogradely filled neurons. Rostrocaudal levels A–V correspond to levels A–V in Figure 1. For abbreviations, see list.

tum, the SPTA, in the caudal amygdala, especially in the transition region with the preoptic area, and in the caudal lateral pallium, where they are abundant at rostrocaudal level I. Caudal to the telencephalon, neurons projecting to the ventral hypothalamus are abundant in the preoptic area and dorsal hypothalamus and present in smaller numbers in the pretectum, ventral thalamus, tuberculum posterius, optic tectum, and median raphe nucleus.

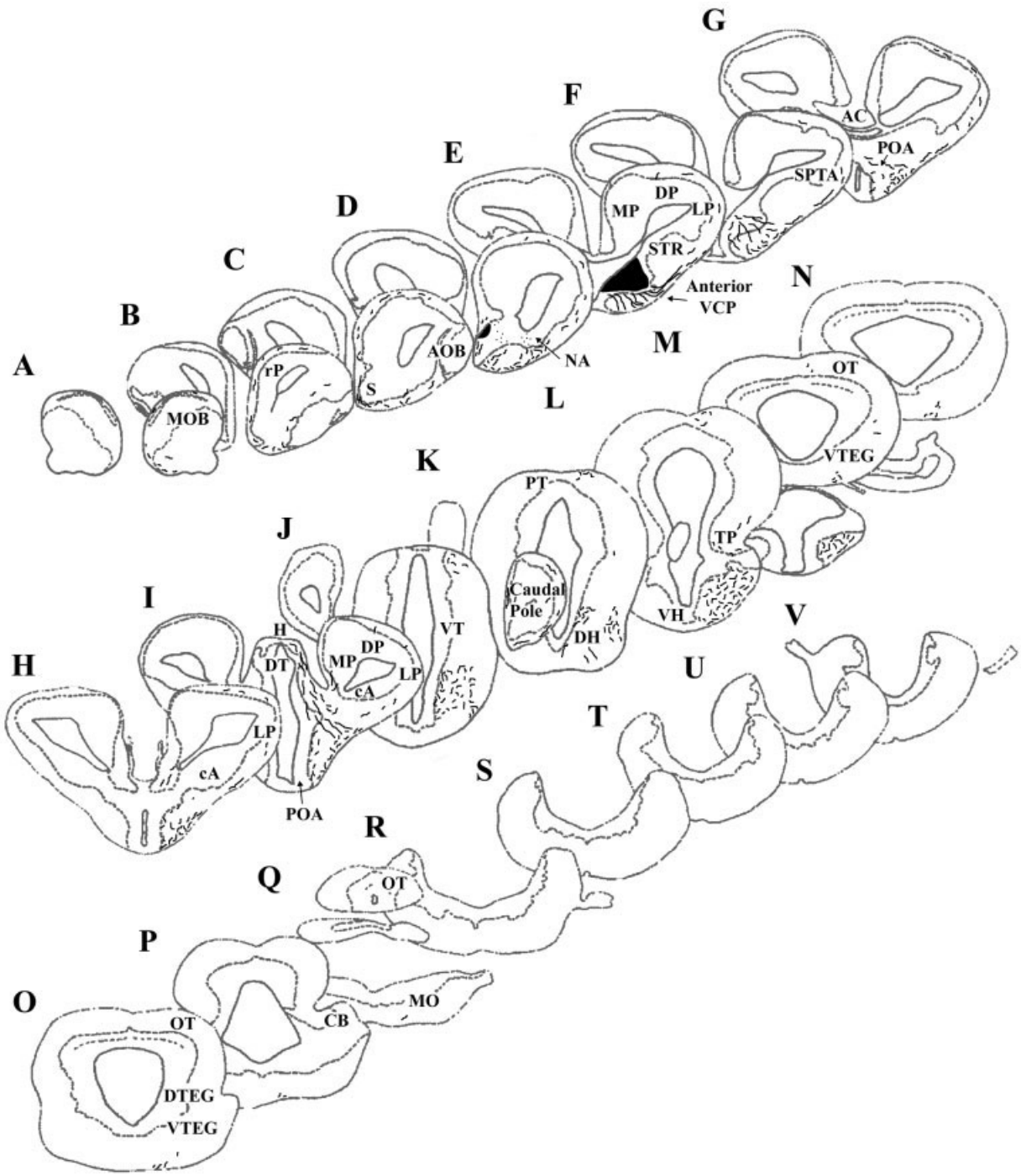

Fig. 5. **A–V:** Axonal projections of the anterior part of the ventral cellular prominence. Schematic illustration of the dendrites and axons labeled by application of crystalline biocytin to the anterior part of the ventral cellular prominence. Rostrocaudal levels A–V correspond to levels A–V in Figure 1. For abbreviations, see list.

Neurons retrogradely filled after biocytin application to the preoptic area are found in the septum, nucleus accumbens, along the VCP/below VCP (Fig. 9E), SPTA, caudal amygdala, and caudal lateral pallium (Fig 8-3). Caudal to the telencephalon, neurons projecting to the preoptic area are also seen in the dorsal hypothalamus, dorsal thalamus, ventral hypothalamus, and what appears to be the parabrachial nucleus of salamanders.

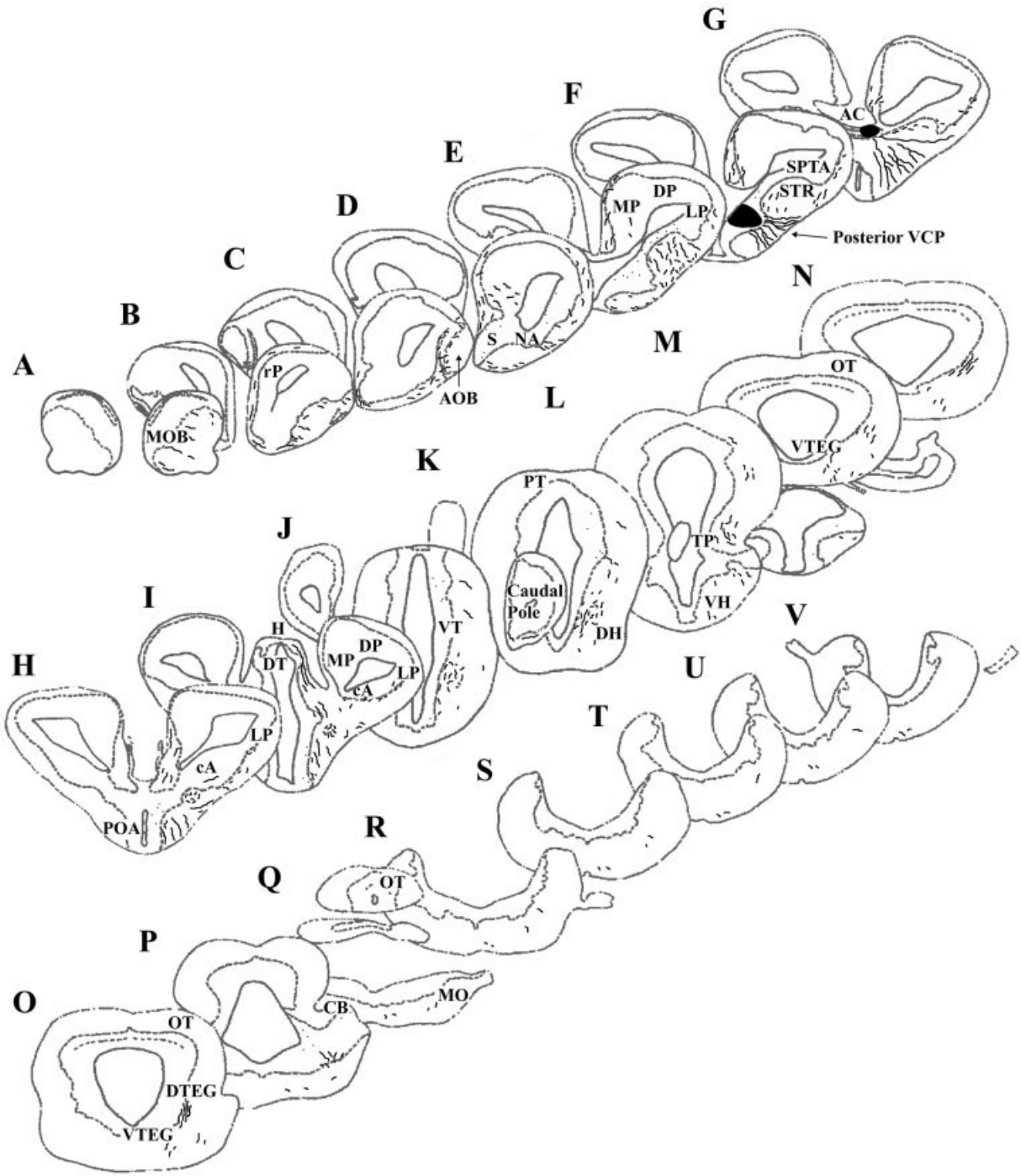

Fig. 6. **A–V:** Axonal projections of the posterior part of the ventral cellular prominence. Schematic illustration of the dendrites and axons labeled by application of crystalline biocytin to the posterior part of the ventral cellular prominence. Rostrocaudal levels A–V correspond to levels A–V in Figure 1. For abbreviations, see list.

Neurons filled retrogradely after biocytin application to the medial pallium are found in the dorsal pallium, posterior VCP, caudal pole, ventral tegmentum, and in smaller numbers in the ventral thalamus, dorsal thalamus, and median raphe (Fig. 8-4). The group of amygdalar neurons projecting to the medial pallium occupies a medial position in the posterior VCP (Fig. 9F). This group of neurons extends caudally and forms a thin band located between the anterior and hippocampal commissures. Unilateral application of biocytin to the

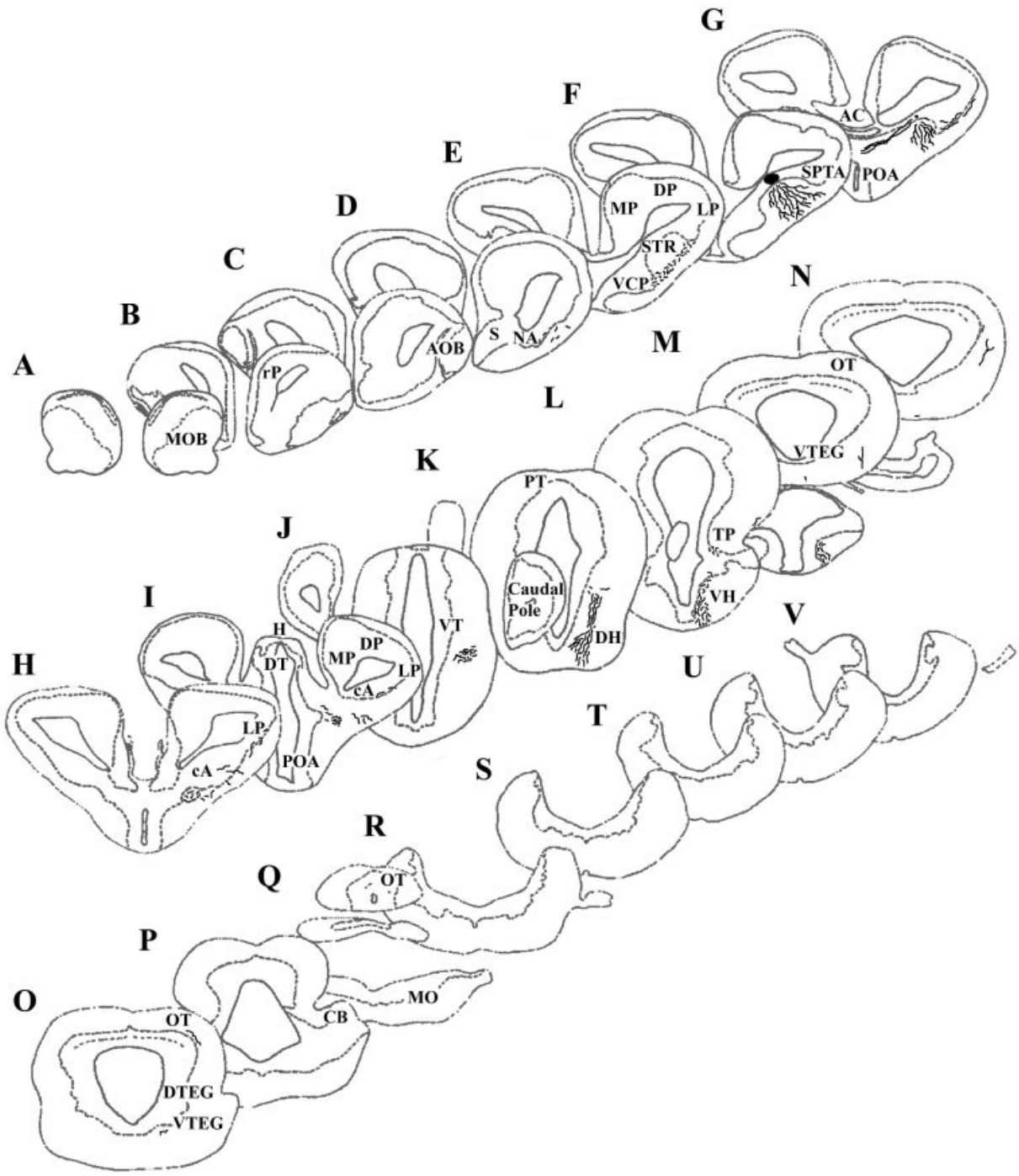

Fig. 7. **A–V:** Axonal projections of the caudal striatum. Schematic illustration of the dendrites and axons labeled by application of crystalline biocytin to the caudal striatum. The site of biocytin application, indicated by the blackened region in the cellular layer, is found mostly between levels F and G. Rostrocaudal levels A–V are as in Figure 1. For abbreviations, see list.

medial pallium in a split brain yielded a much smaller number of neurons in the posterior VCP than application to the medial pallium in an intact brain suggesting that most of these neurons have projections to the contralateral medial pallium.

Connectivity of the ventral telencephalon: intracellular labeling

In the ventral telencephalon, except the rostral septum and nucleus accumbens region, which was not investi-

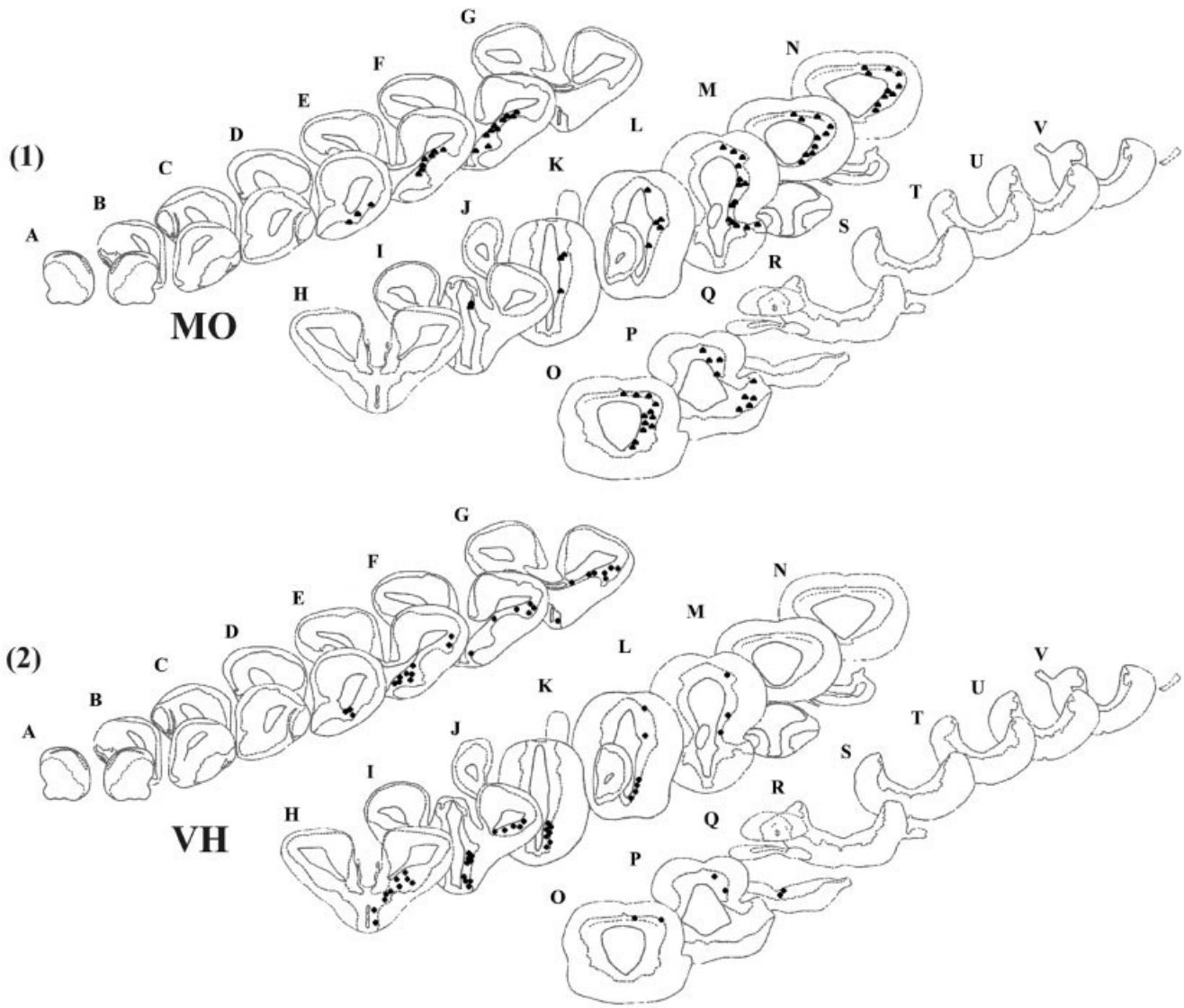

Fig. 8. Summary of the retrograde labeling experiments. General location of somata retrogradely filled by application of biocytin to four different brain regions: (**1**) rostral medulla applications (triangles), (**2**) ventral hypothalamus applications (crosses), (**3**) preoptic area applications (circles), and (**4**) medial pallium applications (squares). Labeled somata are projected onto the right side of the brain. Rostrocaudal levels A–V are as in Figure 1. For abbreviations, see list.

gated here, a total of 174 neurons were labeled either singly (n = 17) or in clusters, with 23 clusters consisting of 2, 13 clusters of 3, 8 clusters of 4, 2 clusters of 5, and 1 cluster of 6, 7, 8, or 9 neurons each. Clusters typically consisted of closely neighboring somata, which were most probably labeled by the uptake of leaking biocytin or by electrical coupling. In cases where simultaneously labeled neuron somata were separated by some distance, it is possible that biocytin was either transferred by means of electrical or chemical synapses (Dermietzel and Spray, 1993; Luo and Dessem, 1996) or by uptake by means of adjacent dendrites. Labeled neurons typically send axon collaterals to multiple brain regions and have axons that often display varicosities indicating synaptic contacts along their course. Therefore, the assessment of an axonal projection site was decided by the presence of axonal terminals or of synaptic varicosities in a brain region.

Cytoarchitecture. Neurons labeled in the VCP/below VCP region have dendritic trees directed toward the lateral surface of the telencephalon (Fig. 10A,B). Somata are somewhat larger in the VCP compared to the region below. Neurons in the VCP have dendrites that carry a moderate amount of spines. Some VCP neurons extend dendritic branches dorsally into the ventral striatal neuropil and ventrally into the lateral region below the VCP. Neurons with somata below the VCP have smooth dendrites that are restricted to the white matter below the VCP. Neurons labeled in the striatum have dendritic trees that are generally confined to the striatal neuropil (Fig. 10C–E). Consequently, neurons in the ventral, dorsal, and

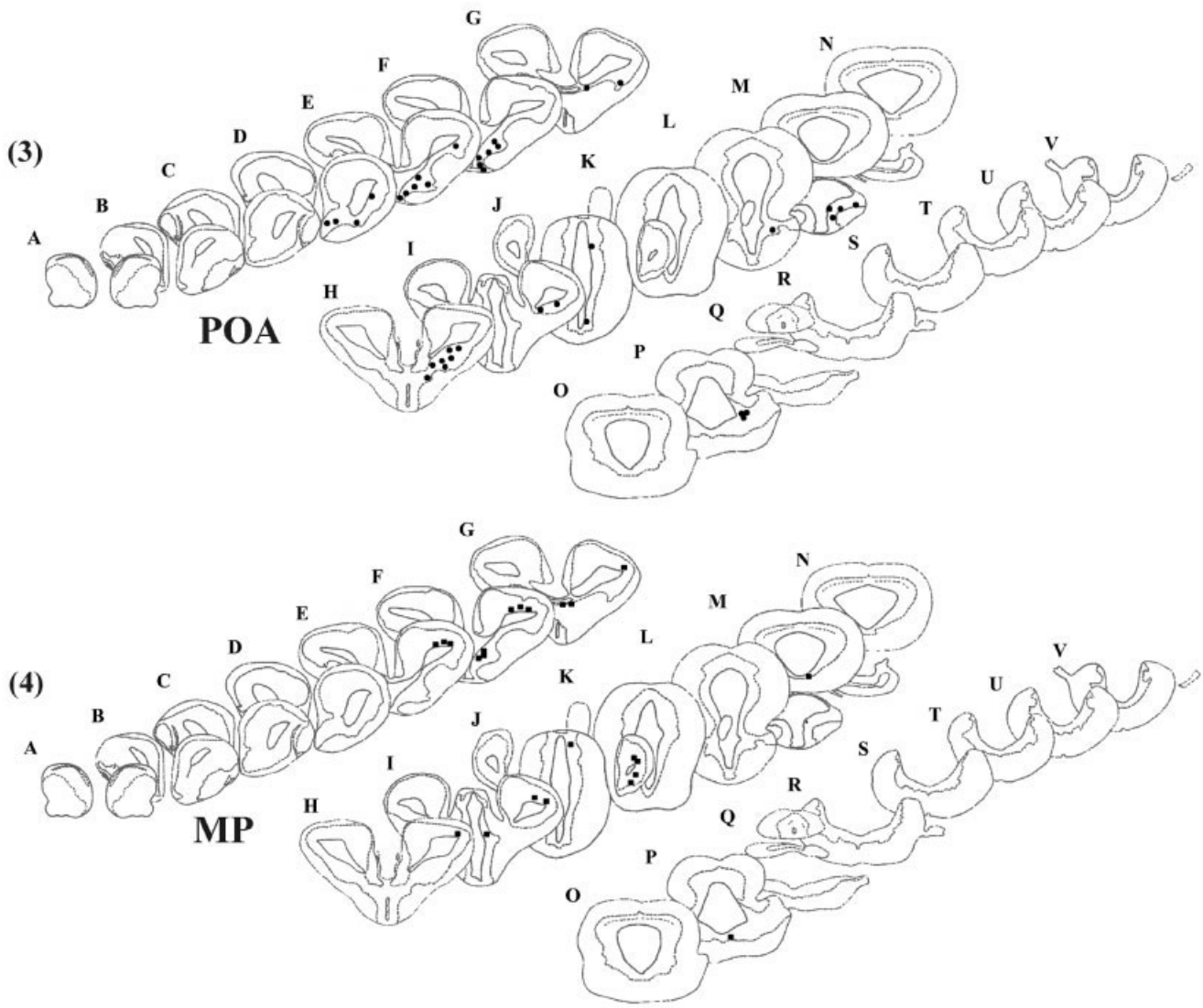

Figure 8 (Continued)

caudal striatum have dendritic trees oriented dorsally, ventrally, and rostrally, respectively; neurons labeled in the intermediate striatum have dendritic trees that extend to all parts of the striatal neuropil. The dendrites of labeled neurons in the intermediate striatum bear a low to moderate amount of spines, whereas those in the lateral caudal striatum carry numerous spines. Neurons in the rostral-most part of the striatum were not studied here. Neurons labeled in the SPTA have dendritic trees that fan out laterally from the somata (Fig. 10F,G). Two neurons labeled in the caudal SPTA have dendrites that bend ventrolaterally and reach as far caudally as the caudal pole. Dendrites of SPTA neurons bear numerous spines. In Figure 10, the morphology of labeled neurons is given by a series of reconstructions of typical neurons found in the regions described above, except for the caudal striatum, which is shown in Figure 11.

Figure 12A–E shows camera-lucida reconstructions of neurons found in the caudal amygdala. These neurons often have large dendritic trees that span a large portion of the telencephalon. In addition to the bulk of the dendritic tree in the white matter overlying the caudal amygdala, 8 of 12 neurons or neuron clusters in this region extend dendrites over the SPTA, 7 over the caudal pole, 4 over the lateral pallium, 1 over the preoptic area, and 1 cluster of neurons has a dendritic branch reaching the striatal neuropil. Many of the neurons situated in the transition zones between the caudal amygdala and the lateral pallium as well as between the caudal amygdala and the preoptic area likewise send dendrites to multiple brain regions (SPTA, striatum, caudal amygdala, caudal pole, preoptic area). The dendrites of neurons in the caudal amygdala bear numerous spines. Among labelings of good quality, three types of neurons can be distinguished in the caudal amygdala at the level where this nucleus is especially thick because of the presence of numerous somata (level H in Fig. 1). Two examples of each type are shown in Figure 12F–H. Type 1 (Fig. 12F) has a small

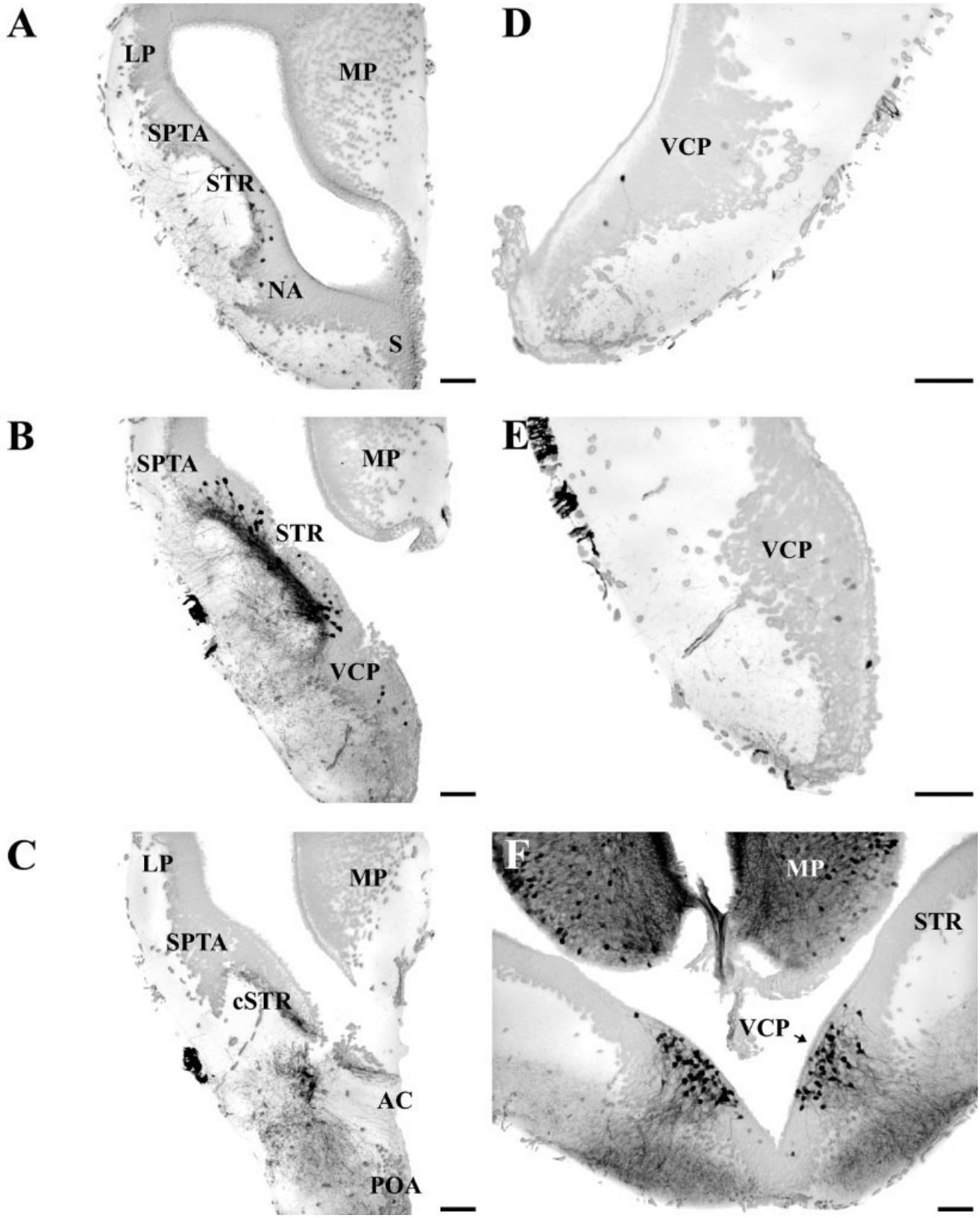

Fig. 9. Examples of retrogradely labeled neurons. **A–C:** Photomicrographs showing the result of biocytin application to the rostral medulla; few neurons are labeled in the rostral third of the striatum (A), many in the intermediate striatum (B), and none in the caudal striatum (C). Retrogradely filled neurons in the nucleus accumbens can be seen in A and in the posterior part of the ventral cellular prominence (VCP) in B. **D:** Photomicrograph showing retrogradely labeled neurons in the anterior part of the VCP after biocytin application to the ventral hypothalamus. **E:** Photomicrograph showing retrogradely labeled neurons in the ventral cellular prominence after biocytin application to the preoptic area. **F:** Photomicrograph showing retrogradely labeled neurons in the posterior part of the VCP after bilateral application of biocytin to the medial pallium. For abbreviations, see list. Scale bars = 100 μm in A–F.

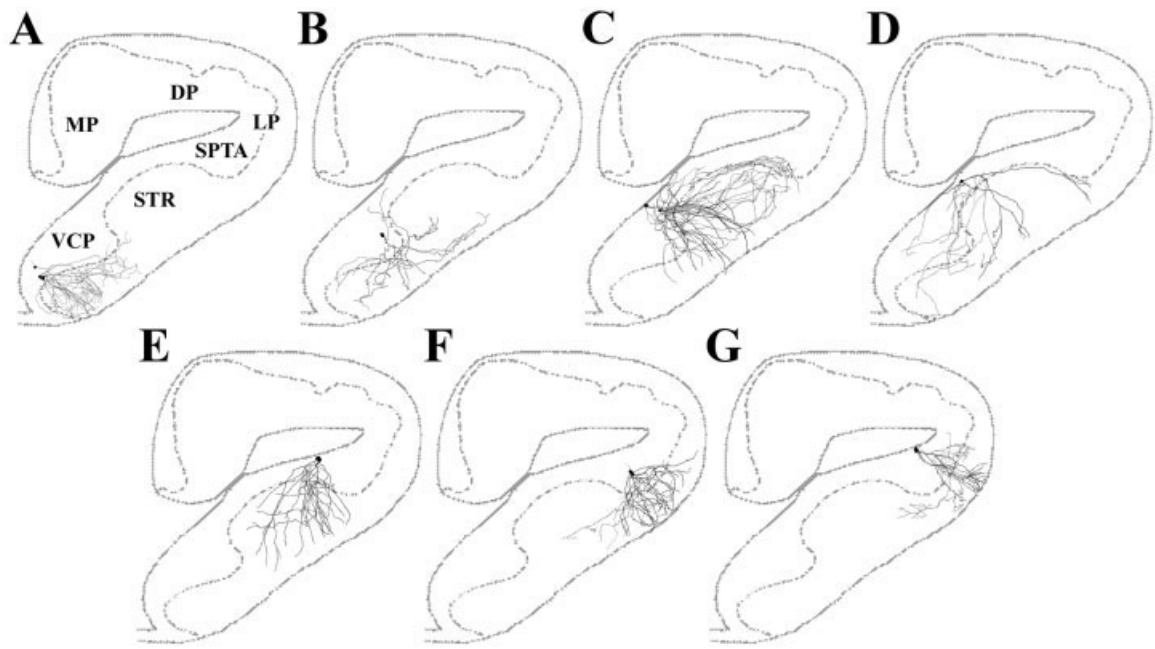

Fig. 10. Reconstruction of neurons situated at rostrocaudal level F in Figure 1. **A–G:** Seven examples of camera lucida reconstruction of neurons or clusters of neurons in the region below the ventral cellular prominence (below VCP, A), VCP (B), striatum (C–E), and SPTA (F,G). All neurons projected to the right side. For abbreviations, see list.

soma, and most dendrites spread ventrally over the caudal amygdala. Type 2 (Fig. 12G) has a medium-sized soma, and most dendrites extend parallel to the lateral ventricle and reach the SPTA and caudal pole, in addition to the caudal amygdala, and sometimes the lateral pallium and the striatum. Type 3 (Fig. 12H) is characterized by a large bipolar soma and large dendritic branches coursing along the SPTA rostrally and to the caudal amygdala, caudal pole, or preoptic area ventrocaudally. Again in the caudal amygdala, just before the level where the caudal pole becomes detached from the diencephalon (level I in Fig. 1), labeled neurons have spiny dendritic trees that fan out ventrally from the somata and occupy the regions overlying the caudal amygdala and the caudal pole (Fig. 13). Note that the neurons with somata situated medially (Fig. 13A,B) distribute dendrites in the region that receives input from the accessory olfactory bulb (Fig. 3), whereas the neurons with somata found more laterally (Fig. 13C,D) extend dendrites laterally, i.e., outside the region of accessory olfactory bulb input.

Axonal projection patterns. Table 3 lists the frequency of all axonal projection sites by regional distribution of intracellularly labeled neurons as well as the number of neurons labeled in each region (n). Figure 14 correlates the axonal projection sites with the location of labeled neuron somata for 13 selected projection sites. Figure 14B demonstrates that neurons projecting to the region just caudal to the accessory olfactory bulb are found mostly in the caudal amygdala and in the SPTA, whereas most of the labeled neurons projecting to the anterior commissure are found in the striatum or in the region close to the lateral pallium (Fig. 14C). Neurons labeled in the VCP/below VCP do not project to these brain regions. Figure 14D,E indicates that all labeled neurons projecting to the medial pallium and most of the labeled neurons projecting to the septum–nucleus accumbens region are found in the VCP/below VCP. Figure 14F shows that labeled neurons projecting to the ventral tegmentum are found in the striatum, VCP/below VCP, and caudal amygdala, whereas those projecting to the medulla are located in the rostral two thirds of the striatum and the VCP (Fig. 14G). Figure 14H shows that labeled neurons projecting to the dorsal thalamus/habenular region are found in the VCP/below VCP and caudal amygdala only, whereas those projecting to the ventral thalamus are widely distributed throughout the telencephalon and preoptic area (Fig. 14I). Of interest, type 3 neurons labeled in the caudal amygdala exhibit the only projections of that region to the dorsal thalamus/habenular region and main olfactory bulb found in the present study and two of the three neurons labeled in the caudal amygdala that project to the ventral thalamus. Figure 14J indicates that labeled neurons projecting to the striatum are found in the VCP/below VCP, striatum, and caudal amygdala. A peculiar striatal projection pattern is shown by the neurons labeled in the caudal striatum and the caudal amygdala, viz., a projection to a narrow lateral band or layer of the striatum neuropil. All neurons labeled in the middle or dorsal portion of the caudal striatum, as well as an application of biocytin crystals restricted to the caudal striatum, display a strong axonal input to the middle layer of the striatal neuropil (Figs. 7E, 11C). On the other hand, five of the six neurons situated in the caudal amygdala that project to the striatum form an input to the outer-most portion of the neuropil. Neurons projecting to the striatum that are found in other regions of the telencephalon do not exhibit such distinct axonal projection patterns. Figure 14K shows that labeled neurons projecting to the SPTA are found mostly

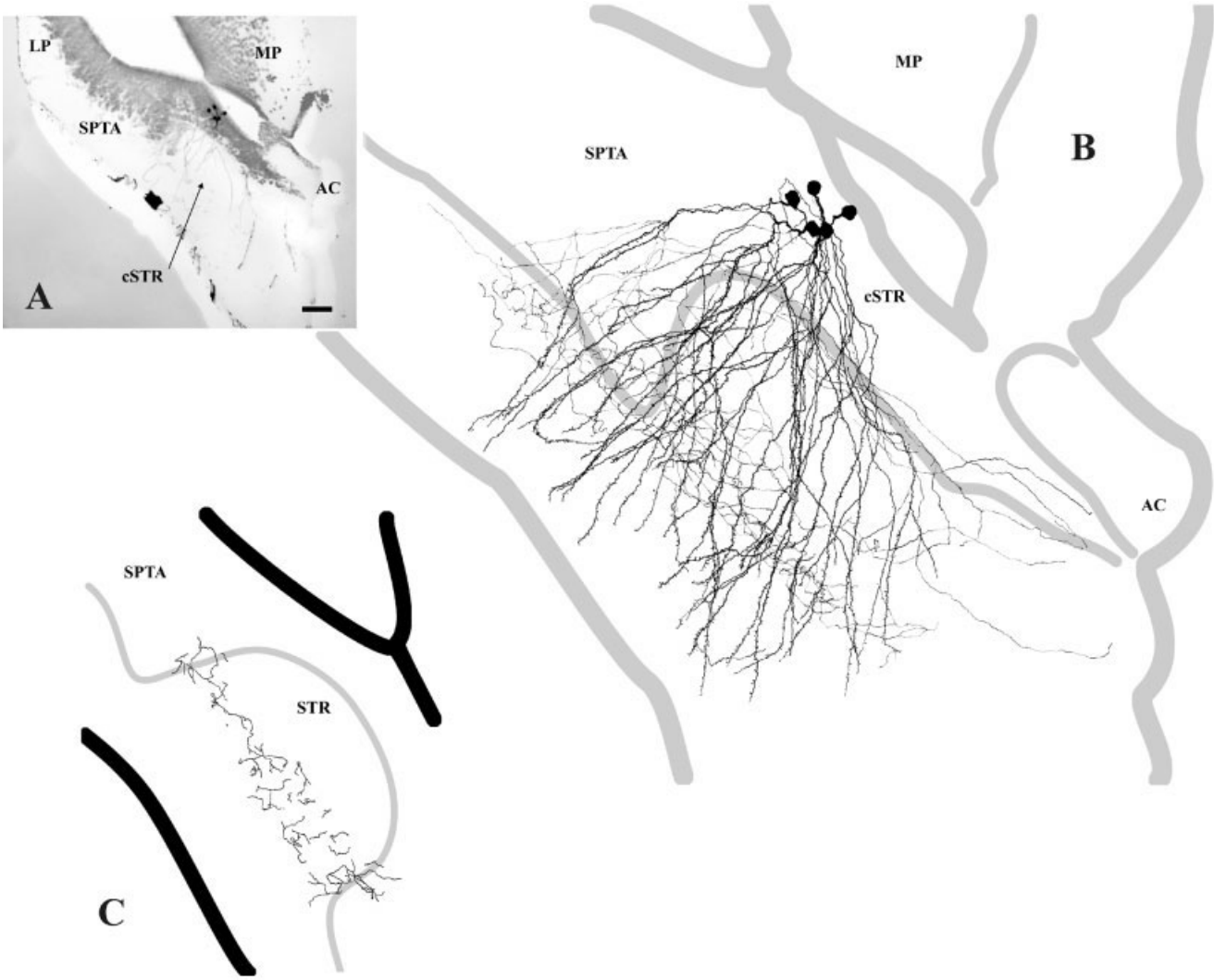

Fig. 11. Caudal striatum of *Plethodon shermani*. **A:** Photomicrograph of a section through of the caudal striatum containing labeled somata of a cluster of five neurons located in the dorsal part. **B:** Camera lucida reconstruction of the labeled cluster of neurons seen in A. The dendrites are directed rostrally into the striatum neuropil. **C:** Camera lucida reconstruction of the same cluster of neurons with projection to the middle layer of the rostral striatum neuropil (three sections are projected on top of each other). For abbreviations, see list. Scale bar = 100 μm in A.

in the ventral striatum and caudal amygdala. Figure 14L–N demonstrates that labeled neurons projecting to the hypothalamus and preoptic area are found abundantly in the VCP/below VCP and caudal amygdala. Labeled neurons projecting to the ventral hypothalamus are found in all regions studied except the posterior part of the VCP/below VCP. Intracellular labeling demonstrates that all neurons in the caudal amygdala projecting to the ventral hypothalamus (n = 9) target the medial neuropil of the ventral hypothalamus, and three of these neurons target the lateral ventral hypothalamus neuropil in addition.

DISCUSSION

Olfactory projections

After biocytin application to the main olfactory bulb, some fibers carrying varicosities can be seen in the rostral pallium, which was named the nucleus olfactorius anterior by Herrick (1948). Therefore, it cannot be excluded that the whole rostral pallium receives input from the main olfactory bulb. However, fibers found there appear to be part of a dense tract not specialized for synaptic contacts, unlike other regions targeted by the main olfactory bulb. Ipsilateral input from the main olfactory bulb to the septum and the lateral pallium rostral to the habenula appears to be less abundant than previously described in salamander; an input to the medial pallium, striatum, VCP, and habenula was not found in the present study (cf. Northcutt and Kicliter, 1980; Schmidt and Roth, 1990). The termination site of the contralateral projection of the main olfactory bulb is problematic. Some fibers carrying varicosities are observed in the contralateral rostral pallium. However, they do not form a clear terminal neuropil and appear to merely course dorsally to form the descend-

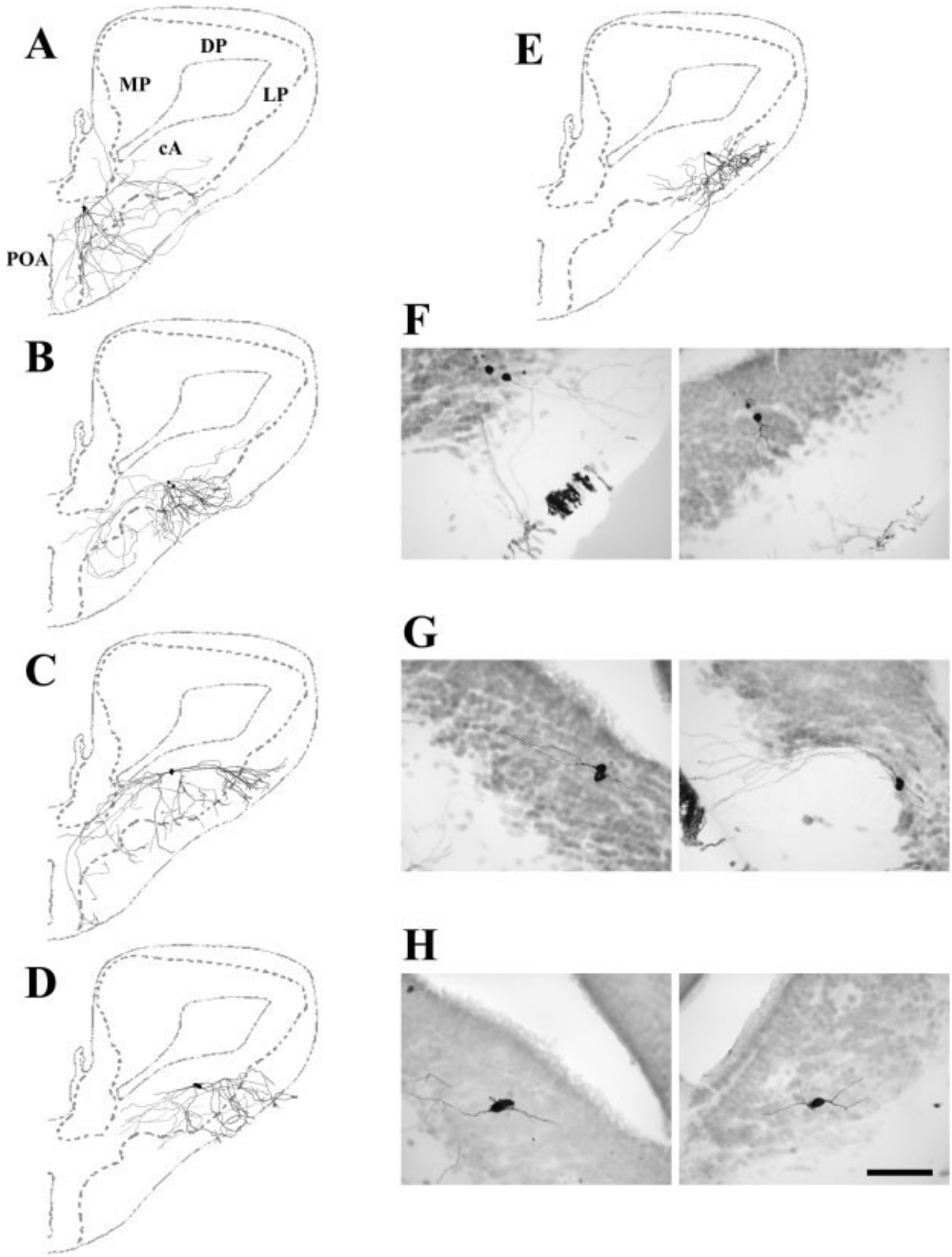

Fig. 12. Reconstruction of neurons and neuronal types in the caudal amygdala. **A–E:** Five examples of camera lucida reconstructions of neurons or clusters of neurons. One is situated in the transition region between the preoptic area and the caudal amygdala (A), three in the caudal amygdala (B–D), and another one in the caudal amygdala–lateral pallium region (E). All neurons are projected to the right side of the brain. **F–H:** Photomicrographs showing two examples of each neuronal type found in the caudal amygdala, as described in the text: type 1 (F), type 2 (G), and type 3 (H). The photomicrographs in the left column of F–H show the same neurons reconstructed in B–D. For abbreviations, see list. Scale bar = 100 μm in H (applies to F–H).

ing lateral corticohabenular tract. Unfortunately, the quality of labeling gradually decreased with the length of these very long axons. Accordingly, the site of termination of the lateral corticohabenular tract or the proportion of fibers from the anterior olfactohabenular tract composing it could not be ascertained. The present study does not support the previously held notion that contralateral main olfactory bulb efferents connect the left and right main olfactory bulb, because the ascending olfactohabenular tract does not extend beyond the rostral pallium to enter the olfactory bulb (Schmidt and Roth, 1990).

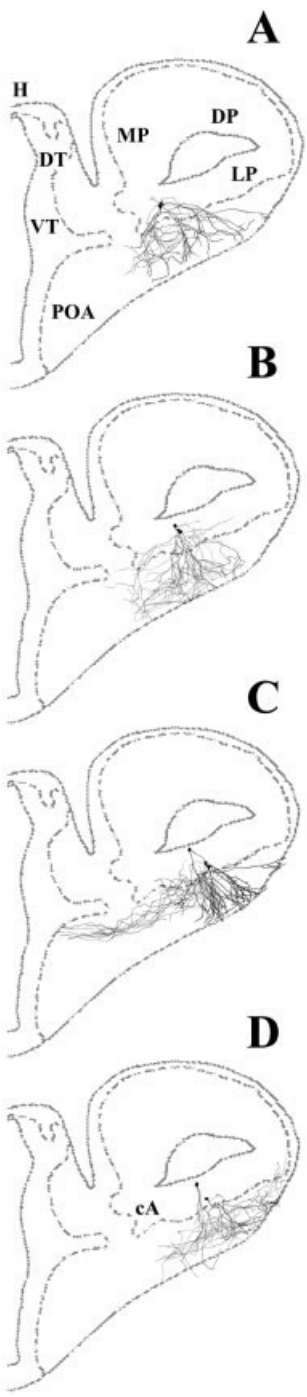

Fig. 13. Reconstruction of neurons at rostrocaudal level I of Figure 1. **A–D:** Four examples of camera lucida reconstruction of a cluster of neurons in the posterior part of the caudal amygdala (A,B) and the lateral pallium (C,D). For abbreviations, see list.

There appears to be no significant projection of the main olfactory bulb to the rostral region of the amygdala in *P. shermani*, as was suggested in *Ambystoma* (Northcutt and Kicliter, 1980). However, by comparing the positions of the "cortical amygdaloid nucleus" of Scalia and collaborators (1991) or the "lateral amygdala" of Moreno and González (2003) in anurans with the same region in the salamander brain, one can infer the possibility that a division of the amygdala receiving main olfactory bulb input exists lateral to the vomeronasal amygdala in the caudal lateral pallium. This portion of the lateral pallium possibly homologous to the cortical olfactory amygdala is inconspicuous for all but its projection to the ventral hypothalamus, which is restricted to the caudal lateral pallium. A hypothalamic projection from the caudal lateral pallium was also demonstrated in another urodele, the crested newt *Triturus carnifex* (Sassoè-Pognetto et al., 1995). Neurons in the caudal part of the amygdala and lateral pallium extend their dendrites into segregated dendritic fields that could correspond to the terminal fields of the accessory and main olfactory bulbs, respectively (Figs. 2I, 3I, 13). However, if present, main olfactory bulb input in this region would be weak, because projections of the main olfactory bulb show only a small amount of varicosities in and around the olfactohabenular tract coursing in the caudal lateral pallium, where the neurons projecting to the ventral hypothalamus are found. In contrast, the projection of the accessory olfactory bulb clearly identifies the portion of the caudal amygdala receiving accessory olfactory bulb input and suggests a homology of that region to the mammalian vomeronasal amygdala. However, in light of recent results in the frog, it appears that the anamniote vomeronasal amygdala is solely of subpallial origin, whereas the main olfactory amygdala is of pallial origin (Brox et al., 2002). Thus, the projection of the accessory olfactory bulb to the pallial posteromedial cortical amygdala and the projection of the main olfactory bulb to the subpallial anteroventral medial amygdala observed in mammals could represent amniote innovations (Scalia and Winans, 1975).

Methodological considerations

A new method of tracer application from the medial surface in longitudinally split isolated brains was used in the present study. This method appears especially appropriate for the study of brain connections in urodeles, where somata of neurons are found almost exclusively around the ventricle and fiber tracts in a superficial position. Only a minimal degree of inadvertent retrograde labeling of somata was observed in our tract-tracing studies compared with other studies of amphibian telencephalic connections (Marín et al., 1997c; Moreno and González, 2003).

The results obtained with crystalline and intracellular biocytin application show the same major projection sites for the regions investigated, and an inspection of Tables 2 and 3 reveals only minor differences between the results obtained with the two methods. Additional projection sites are sometimes observed after application of crystalline biocytin, because a much larger number of neurons are sampled with this method compared with the limited sample sizes reached by intracellular injection. On the other hand, projection sites can sometimes be hard to ascertain with crystalline applications, because the application site itself or a dense fiber tract can obscure the visibility of axonal terminals. This finding is the case with intrastriatal projections and the projections of the caudal amygdala to the SPTA and the dorsal hypothalamus. The abundance of dendrites of caudal amygdala neurons along the SPTA obscures axonal terminals in the SPTA that can be seen when a single neuron or few neurons are labeled in

TABLE 3. Intracellular Labeling: Frequency Distribution of Axonal Projection Sites of Different Regions of the Telencephalon[1]

	Regions of the telencephalon									
Axonal projection sites	VCP (n = 10)	Below VCP (n = 11)	Ventral STR (n = 13)[2]	Dorsal STR (n = 3)	Caudal STR (n = 5)[2]	SPTA (n = 4)	SPTA-LP (n = 3)	cA (n = 12)	cA-LP (n = 3)	cA-POA (n = 3)
MOB	I	I	-	-	-	-	I	II	-	-
AOB	I	-	-	-	-	-	II	-	-	-
Below AOB	II	-	-	-	-	I	I	I	-	-
Caudal to AOB	-	-	I	-	-	IIII	III	IIIIII	III	-
S/NA	IIII	IIIIII	I	-	-	I	-	I	-	I
MP	III	I	-	-	-	-	-	-	-	-
STR	III	II	IIIII	II	IIIII[3]	I	-	IIIII[4]	I	-
VCP/below VCP	II	I	II	II	-	I	I	III	I	II
SPTA	I	-	III	-	I	I	-	IIIIII	I	I
AC	-	-	III	II	IIII	-	II	I	II	-
POA	IIIII	IIIIIIII	IIIII	-	-	II	II	IIIIII	-	I
cA	-	I	-	I	I	-	II	I	-	I
LP	-	-	-	-	II	-	-	II	-	-
Caudal pole	I	I	II	-	-	III	III	IIIII	I	I
VT	IIIIIIII	IIIII	IIIII	I	-	I	II	III	-	III
DT/H	II	IIII	-	-	-	-	-	II	-	-
PT	I	II	-	-	-	-	-	-	-	-
OT	-	I	-	-	-	-	-	-	-	-
POC	-	-	-	-	-	-	-	I	I	-
DH	III	IIIIII	I	-	I	-	-	IIIIIIII	II	II
TP	IIII	II	-	-	-	I	-	-	-	-
VH	-	IIIIIII	III	I	IIIII	II	I	IIIIIIII	II	I
VTEG	IIIII	IIIIII	III	-	-	-	-	I	-	I
DTEG	I	I	IIIII	I	-	-	-	-	-	-
CB	-	I	-	-	-	-	-	-	-	-
Nucleus isthmi	-	-	-	-	-	-	-	I	-	-
MO	I	I	IIIIII	I	-	-	-	-	-	-

[1]For abbreviations, see list.
[2]Four neurons in the ventral part of the caudal STR were included in the ventral STR group, because they do not display the typical projections of the middle and dorsal part of the caudal STR.
[3]Middle layer of STR neuropil.
[4]Outer layer of STR neuropil, except one neuron in middle layer.

the caudal amygdala. In the dorsal hypothalamus, a dense fiber tract passes over and appears to constitute only weak input to this region on its way to the ventral hypothalamus. However, intracellular labeling reveals that most neurons projecting to the ventral hypothalamus also display varicosities while passing through the dorsal hypothalamus. Neurons found at a more superficial level are not necessarily labeled by a periventricular biocytin application. The case of the type 3 neuron of the superficial caudal amygdala is telling. These neurons reveal characteristic projections to the main olfactory bulb, dorsal thalamus, and ventral thalamus that are not found in neurons situated in the periventricular region of the caudal amygdala. Therefore, the absence of projections to the main olfactory bulb, dorsal thalamus, and ventral thalamus is not surprising in cases of crystalline biocytin application that label the caudal amygdala from the medial surface without reaching its superficial portion. Finally, the border between the VCP and the region below it is arbitrary, and neurons located at the junction between these two regions sometimes reveal characteristics of one region or the other. Overall, it is easier to attribute a projection site with intracellularly filled neurons compared with large biocytin applications.

Comparison of connection patterns

The telencephalic systems investigated in the present study can be broadly divided into an amygdaloid and a striatopallidal complex. Four major divisions of the amygdala are expected from the situation described in amniote vertebrates (Bruce and Neary, 1995; Swanson and Petrovich, 1998): (1) a main olfactory amygdala that receives main olfactory bulb input and is characterized by a projection to the ventromedial hypothalamus, corresponding to the mammalian posterolateral cortical and anteroventral medial amygdala; (2) an accessory olfactory, or vomeronasal, amygdala that receives accessory olfactory bulb input and is characterized by projections to the medial and lateral hypothalamus, corresponding to the mammalian anterodorsal and posterior medial amygdala and parts of the bed nucleus of the stria terminalis; (3) a visceral–autonomic amygdala with descending projections to the lateral hypothalamus and brainstem, corresponding to the mammalian central amygdala and portions of the bed nucleus of the stria terminalis; and (4) a portion of the amygdala with output to the limbic cortex, the striatum, and the nucleus accumbens but with little projection to the hypothalamus, corresponding to the mammalian basal, basolateral, and basomedial amygdala. The view adopted here is that extant amphibians, whose ancestors gave rise to early amniotes, could help elucidate the vertebrate ancestral condition of the amygdala complex.

Four major striatopallidal divisions can be expected from the situation described in amniotes (Medina and Reiner, 1995; Butler and Hodos, 1996; Reiner et al., 1998): a dorsal striatum (or striatum proper) and ventral striatum (nucleus accumbens and olfactory tubercle) along with a dorsal and ventral pallidum. The dorsal striatum is characterized by projections to the dorsal pallidum and tegmentum, whereas the ventral striatum (nucleus accumbens) has projections to the ventral pallidum, tegmentum, and rostral medulla. Note that the amphibian striatum and nucleus accumbens send numerous long descending projections to the medulla (Marín et al., 1997c). The dorsal pallidum is characterized by projections to the dorsal striatum, subthalamus, tegmentum,

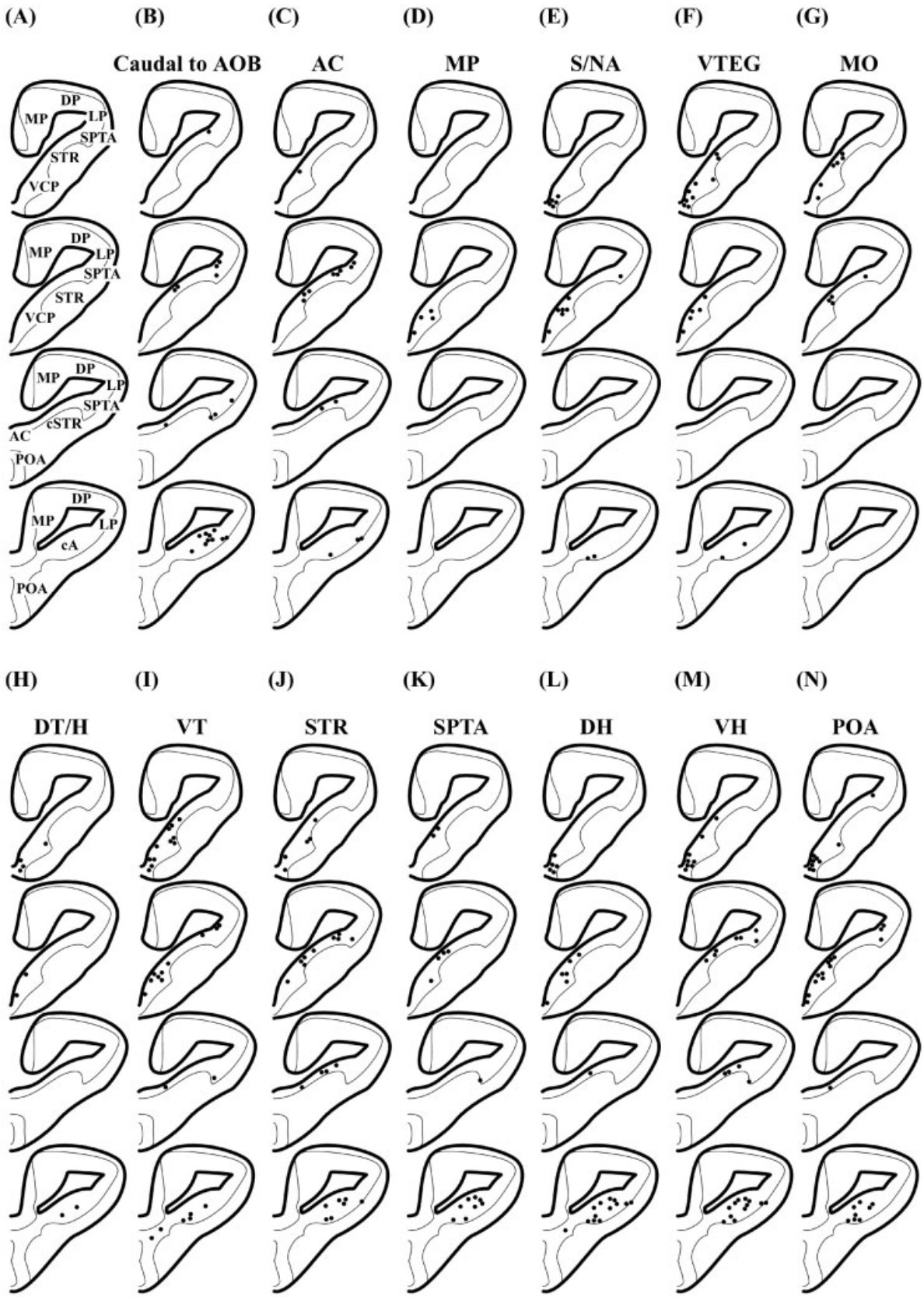

Fig. 14. Schematic representation of axonal projections of intracellularly labeled neurons. **A:** Site of major telencephalic structures on schematic brain sections. **B–N:** Location of labeled neurons or cluster of neurons (black dots) projecting to the following regions: region just caudal to the accessory olfactory bulb (B), anterior commissure (C), medial pallium (D), septum–nucleus accumbens region (E), ventral tegmentum (F), medulla oblongata (G), dorsal thalamus–habenular region (H), ventral thalamus (I), striatum (J), striatopallial transition area (K), dorsal hypothalamus (L), ventral hypothalamus (M), and preoptic area (N). Sites of somata were charted on the right side of the brain on the closest schematic transverse section to their actual brain locations. The schematic brain sections shown are from rostrocaudal levels E, F, G, and H of Figure 1. Note that the same neuron or cluster of neurons can project to multiple brain sites. For abbreviations, see list.

and pretectum (reptiles and birds) or dorsal thalamus (mammals). An input from the basal ganglia to the pretectum is present in amphibians (Lazar et al., 1990; Reiner et al., 1998). However, the cellular origin of this projection to the pretectum could not be attributed to either the striatum or the pallidum. Recently, Endepols and coworkers (2004) proposed that the anuran dorsal pallidum is found in the caudal part of the striatal region and projects to the caudal thalamus. They also highlighted that the region homologous to the mammalian subthalamus has yet to be identified in amphibians. In the rat brain, the ventral pallidum projects to limbic cortical areas, septum, ventral striatum, lateral and central amygdala, lateral hypothalamus, dorsal thalamus, subthalamus, habenula, tegmentum, and medulla (Groenewegen et al., 1993). Ventral pallidal projections to the hypothalamus, habenula, and tegmentum were suggested from observations in lizard, along with a projection to the ventral thalamus (Russchen and Jonker, 1988).

Figure 15 summarizes important characteristics of the projection pattern of the groups of neurons under study. According to the expected characteristics described above and our present results, basic nuclear divisions of the salamander telencephalon are proposed in Figure 16. The anterior VCP is characterized by projections to the dorsal pallium, septum–nucleus accumbens, and ventral hypothalamus. The posterior VCP is the only telencephalic neuron group outside the striatum that sends projections to autonomic and visceral centers in the mesencephalic tegmentum and medulla. It also projects to the medial pallium and septum–nucleus accumbens and has little projections to the ventral hypothalamus. Thus, each part of the VCP shares characteristics of the mammalian basolateral and central amygdala. Therefore, the VCP could be considered a mixed nucleus that is functionally equivalent to the mammalian basolateral amygdala and central extended amygdala (including the lateral portion of the bed nucleus of the stria terminalis).

The region below the VCP could be pallidal in nature because of its projections to the dorsal thalamus/habenular region and pretectum; its proximity to the nucleus accumbens suggests that it corresponds to the ventral pallidum. The position of the dorsal pallidum in anurans as described by Endepols and coworkers (2004) corresponds well to the large intermediate striatum of salamanders, which, in addition to an abundant output to the medulla, sends projections to pretectal, thalamic, and tegmental targets, which are typical of the amniote dorsal pallidum. An alternative is that functional equivalents to both the ventral and dorsal pallidum are found in the region below the VCP in salamander.

The population of neurons located in the very caudal striatum has dendrites oriented rostrally that occupy the caudal half of the striatal neuropil. This group of neurons projects to the striatal neuropil, lateral pallium, anterior commissure, and medial ventral hypothalamus and does not have an equivalent in anurans.

The caudal amygdala of salamanders appears to be equivalent to the mammalian medial (vomeronasal) amygdala, because it receives strong input from the accessory olfactory bulb and has abundant projections to the medial ventral hypothalamus. It is definitely homologous to the anuran "medial amygdala" of Moreno and González (2003), because it shares with that structure an input from the accessory olfactory bulb and projections to the septum–nucleus accumbens, VCP (their bed nucleus of the stria terminalis), striatum, lateral pallium, SPTA (their anterior amygdala), preoptic area, medial hypothalamus, and a weak output to the tegmentum. Of interest, the SPTA displays a similar connectivity to the caudal amygdala and receives a strong ascending input from it. This finding suggests that the SPTA and the caudal amygdala represent an extended vomeronasal amygdala. A nearby neuron group found in the caudal lateral pallium projects to the medial ventral hypothalamus, accessory olfactory bulb, and striatum. It possibly receives main olfactory bulb input and could be considered homologous to the mammalian cortical (main olfactory) amygdala.

Evolutionary implications

Our study shows that the telencephalon of salamanders possesses centers that correspond and probably are homologous to the medial (vomeronasal) and possibly to the cortical (main olfactory) amygdala of mammals. In addition, the VCP has a projection pattern that resembles those of the mammalian basolateral and of the central amygdala, i.e., projections to the septum–nucleus accumbens, medial pallium, and dorsal pallium, as well as sensory afferents from the dorsal thalamus (Roth and Grunwald, 2000) typical of the basolateral amygdala, and projections to visceral centers in the ventral hypothalamus, tegmentum, and medulla characteristic of the central amygdala. A portion of the amygdala corresponding functionally to the mammalian basolateral amygdala has likewise been found in the toad *Bombina orientalis* (Roth et al., 2004), but there it is situated in the medial portion of the caudal telencephalon. The salamander *P. jordani/shermani* as well as the toad *Bombina orientalis* can be fear-conditioned in a context-dependent manner (Dicke, personal communication; Mühlenbrock-Lenter, Roth, Heidorn, unpublished results), which in mammals is typically related to the activity of the basolateral amygdala (LeDoux, 2000). There is also evidence that the medial pallium of frogs and salamanders is involved in conditioning and learning (Wenz and Himstedt, 1990; Muzio et al. 1993; Ewert et al., 1994; Papini et al., 1995; González and Lopez, 2002).

The main problem arising with this interpretation is that the VCP of salamanders, as well as the medial portion of the amygdala of anurans, clearly are of subpallial origin, whereas the basolateral amygdala complex of amniotes is considered to be a pallial structure (Swanson and Petrovich, 1998; Puelles et al., 2000; Brox et al., 2002; Martínez-García et al., 2002). It has been suggested that the mammalian basolateral complex is a phylogenetically new structure formed by expansion and differentiation of the piriform lobe in mammals (Johnston, 1923). Thus, whereas the subpallial central-like component of the amphibian amygdala appears to be homologous to its mammalian counterpart, the basolateral components of the amygdala in amphibians and mammals would be a case of homoplasy, where neuron populations of different origin have similar limbic functions in both groups. The apparent absence of a direct projection of the basolateral amygdala to the limbic cortex in reptiles (Martínez-García et al., 2002) could indicate that this projection was lost during the anamniote–amniote transition, but reappeared in mammals.

Another problem arises, when we compare the anuran and urodele dorsal striatum. Anurans have been assumed

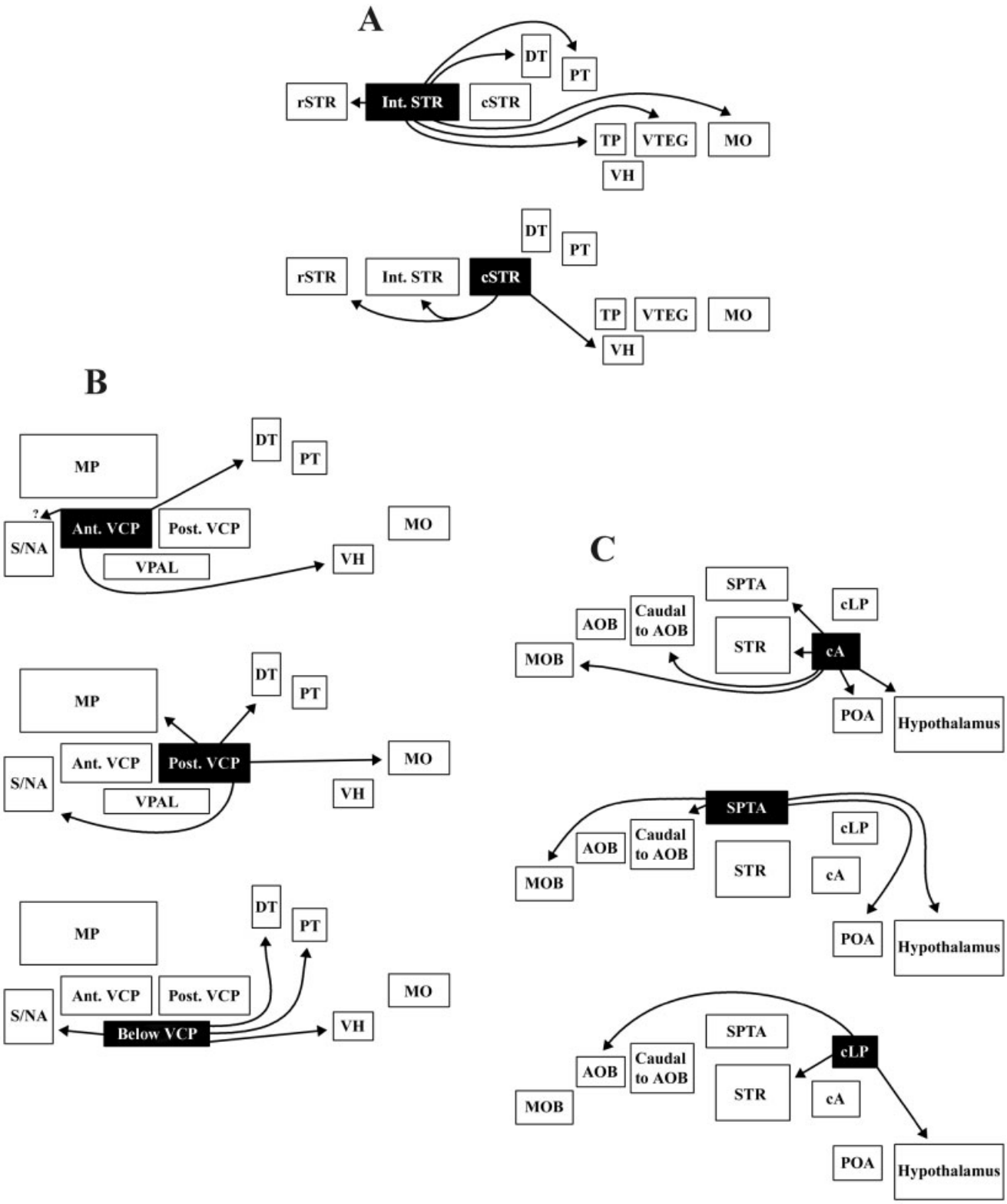

Fig. 15. Summary of major targets of neurons in the salamander telencephalon. **A–C:** Schematic illustrations of projections of the striatum (A), the VCP and below VCP (B), and groups of neurons in the vomeronasal and olfactory amygdala (C). The projection of only one group of neurons is shown in each drawing. For abbreviations, see list.

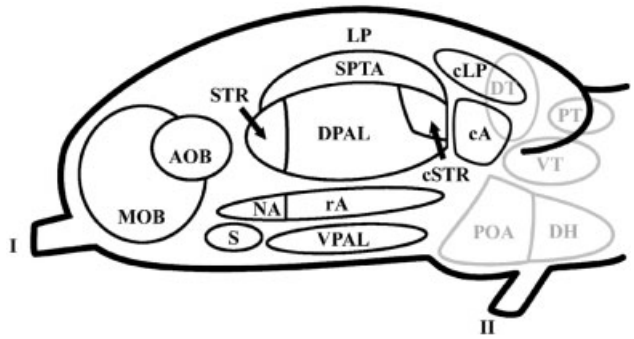

Fig. 16. Schematic lateral overview of the telencephalic (black) and diencephalic (gray) structures of the salamander forebrain. The dorsal thalamus (DT) is found medial to the caudal telencephalon. Rostral is to the left and dorsal on top. The olfactory (I) and optic (II) nerves are shown. For other abbreviations, see list.

to possess a rostral striatum proper that projects to the dorsal pallidum, which occupies the caudal part of the striatum and projects to the caudal thalamus (Endepols et al., 2004). Our own results obtained in the fire-bellied toad *Bombina orientalis* confirm that descending projections to the medulla are mostly found in the caudal striatum in anurans (Roth et al., 2004). The situation in salamander resembles this situation found in anurans in the sense that a small rostral striatal region has only few and a large caudally adjacent region has many descending projections to the medulla but differs in the presence of a small region in the caudal striatum without any long descending projections. Of interest, the entire salamander striatum receives an input from the central dorsal thalamus (Roth and Grunwald, 2000). It appears, thus, that the urodele striatum has gained a functional division in its caudal part not found in anurans. On the other hand, in *P. shermani*, the striatum does not appear to be divided into a dorsal and ventral part as is seen in anurans (Marín et al., 1997c; Roth et al., 2004).

Some features of the urodele brain described in the present report warrant further interest. The function of the caudal striatum with its strong projections to the rostral striatum and medial ventral hypothalamus is unknown. Furthermore, its ascending projection to the striatum is restricted to a narrow band in the middle of the neuropil, whereas the ascending striatal projection of the vomeronasal amygdala terminates mostly in the outer striatal neuropil. These projections to different parts of the striatum suggest that they target different dendritic compartments of the same neurons and have different effects on the generation of action potentials, as can be seen in mammalian pyramidal neurons (Williams and Stuart, 2003). Another feature of the salamander brain is the existence of distinct cell types in the vomeronasal amygdala. Future experiments could be designed to determine, if these types of neurons mediate specific behavioral or physiological functions, because the vomeronasal system of *P. shermani* is known to detect specialized chemical signals involved in reproduction (Wirsig-Wiechmann et al., 2002). Lastly, it appears that the region just caudal to the accessory olfactory bulb is a distinct structure involved in the modulation of accessory olfactory information, because it receives most of its input from the vomeronasal amygdala and SPTA.

ACKNOWLEDGMENT

The authors thank Dr. Wolfgang Grunwald (University of Bremen) for his help with the reconstruction of intracellularly labeled neurons and the preparation of figures. We also thank Dr. Lynne Houck (Oregon State University) for funding animal capture and transport.

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