Organization of the Pallium in the Fire-Bellied Toad *Bombina orientalis*. I: Morphology and Axonal Projection Pattern of Neurons Revealed by Intracellular Biocytin Labeling

GERHARD ROTH,* FRÉDÉRIC LABERGE, SABINE MÜHLENBROCK-LENTER,
AND WOLFGANG GRUNWALD
Brain Research Institute, University of Bremen, D-28334 Bremen, Germany

ABSTRACT

The cytoarchitecture and axonal projection pattern of pallial areas was studied in the fire-bellied toad *Bombina orientalis* by intracellular injection of biocytin into a total of 326 neurons forming 204 clusters. Five pallial regions were identified, differing in morphology and projection pattern of neurons. The *rostral pallium* receiving the bulk of dorsal thalamic afferents has reciprocal connections with all other pallial areas and projects to the septum, nucleus accumbens, and anterior dorsal striatum. The *medial pallium* projects bilaterally to the medial pallium, septum, nucleus accumbens, mediocentral amygdala, and hypothalamus and ipsilaterally to the rostral, dorsal, and lateral pallium. The ventral part of the medial pallium is distinguished by efferents to the eminentia thalami and the absence of contralateral projections. The *dorsal pallium* has only ipsilateral projections running to the rostral, medial, and lateral pallium; septum; nucleus accumbens; and eminentia thalami. The *lateral pallium* has ipsilateral projections to the olfactory bulbs and to the rostral, medial, dorsal, and ventral pallium. The *ventral pallium* including the striatopallial transition area (SPTA) has ipsilateral projections to the olfactory bulbs, rostral and lateral pallium, dorsal striatopallidum, vomeronasal amygdala, and hypothalamus. The medial pallium can be tentatively homologized with the mammalian hippocampal formation, the dorsal pallium with allocortical areas, the lateral pallium rostrally with the piriform and caudally with the entorhinal cortex, the ventral pallium with the accessory olfactory amygdala. The rostral pallium, with its projections to the dorsal and ventral striatopallidum, resembles the mammalian frontal cortex. J. Comp. Neurol. 501:443–464, 2007. © 2007 Wiley-Liss, Inc.

Indexing terms: amphibians; rostral pallium; medial pallium; dorsal pallium; lateral pallium; ventral pallium

Amphibians have a large telencephalon making up about half of the total brain volume, and more than half of the telencephalon is formed by the pallium. However, basic structural and functional aspects are not well studied. Medial, dorsal, and lateral pallium are traditionally distinguished, but the boundaries between these pallial regions are disputed. This is true for the border between medial and dorsal pallium (Hoffman, 1963; Northcutt, 1974; Scalia, 1976; Northcutt and Kicliter, 1980; Neary, 1990; Scalia et al., 1991; Northcutt and Ronan, 1992; Westhoff and Roth, 2002). Also, it is disputed whether a dorsal pallium exists at all, what its borders are, and whether a distinction between a dorsal and a lateral pallium is justified (Gaupp, 1899; Herrick, 1933; Ariens Kap-pers et al., 1936; Hoffman, 1963; Kicliter and Ebbesson, 1976; Northcutt, 1974; Northcutt and Kicliter, 1980; Scalia et al., 1991; Northcutt and Ronan, 1992; Westhoff and

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Roth, 2002). Recently, based on gene expression studies, a ventral pallium has been proposed (Puelles et al., 2000; Brox et al., 2003), including what has been called the "striatopallial transition area" (Marín et al., 1998).

Besides the question of the exact number and boundaries of different pallial areas in anurans, detailed studies on the morphology of pallial neurons and their projection pattern are lacking, except for a study from our laboratory on the medial and dorsal pallium in the frog *Discoglossus pictus*, based on a limited number of labeled neurons (Westhoff and Roth, 2002). Several studies have consistently shown that the anterior dorsal thalamic nucleus projects unilaterally or bilaterally to the medial and partially to the dorsal and lateral pallium, terminating predominantly in their rostral parts (Vesselkin et al., 1971; Kicliter, 1979; Mudry and Capranica, 1980; Neary, 1984, 1990; Roth et al., 2003). There are additional projections to the medial pallium ascending from the hypothalamus, nucleus visceralis secundarius, locus coeruleus, and raphe nuclei (Neary, 1990; Northcutt and Ronan, 1992; Roth et al., 2004).

Finally, little is known about the function of the amphibian pallium. Originally it was assumed that this tissue, like the pallium of all anamniote vertebrates, was involved exclusively in olfactory processing, given the presence of large olfactory bulbs and their massive projections to the entire pallium (cf. Kicliter and Ebbesson, 1976). However, it was demonstrated later that the amphibian pallium receives additional visual, auditory, and somatosensory input (Supin and Guselnikov, 1965; Karamian et al., 1966; Mudry and Capranica, 1980). Veenman and coworkers (1989) conceived the anuran pallium as a "selection system" interacting with the striatum as a sensorimotor system. The medial pallium is considered homologous to the hippocampal area of mammals, at least Ammon's horn and subiculum, whereas a dentate gyrus is believed to be absent (for discussion see Westhoff and Roth, 2002). The lateral pallium is generally considered an olfactory cortex, but precise evidence is lacking, and the function of the ventral pallium/striatopallial transition area (SPTA) is disputed. Some authors consider it to represent the amphibian equivalent of the anterior/basolateral amygdala (Moreno and González, 2003, 2004), but others conceive it to be part of the accessory (vomeronasal) olfactory amygdala (Roth et al., 2004; Laberge et al., 2006).

The present paper is the first in a series of articles about the structural and functional organization of the pallium of *Bombina orientalis* as an example of the anuran pallium. It is a continuation of studies on the diencephalon and subpallial telencephalon in *Bombina* (Roth et al., 2003, 2004; Mühlenbrock-Lenter et al., 2005; Endepols et al., 2006). One important goal of our study is to lay the groundwork for behavioral experiments on emotional and cognitive learning in amphibians and another is to contribute to a better understanding of the evolution of the pallium/cortex of vertebrates.

MATERIALS AND METHODS

For these experiments, 140 specimens of the fire-bellied toad *Bombina orientalis* were used. The animals were mostly taken from a breeding colony at our institute; some were obtained from a professional animal dealer (Tropenhaus, Hamburg, Germany). For the experiments, animals were deeply anesthetized in 0.5% tricaine methane sulfonate before quick decapitation. Brains were removed from the skull by a ventral approach and stored in Ringer's solution consisting of Na^+ 100 mM, K^+ 2 mM, Ca^{2+} 2 mM, Mg^{2+} 0.5 mM, Cl^- 82 mM, HCO_3^- 25 mM, glucose 11 mM, buffered to a final pH of 7.3 through continuous perfusion of 95% O_2 + 5% CO_2 (Straka and Dieringer, 1993). For the

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Abbreviations

aEN	anterior entopeduncular nucleus
AOB	accessory olfactory bulb
cA	caudal amygdala
cMS	caudal medial septum
COA	cortical amygdala
Comm	telencephalic commissures
dRP	dorsal rostral pallium
dMP	dorsal medial pallium
DP	dorsal pallium
DPAL	dorsal pallidum
DPl	lateral portion of the dorsal pallium
DPm	medial portion of the dorsal pallium
dVP	dorsal ventral pallium
ET	eminentia thalami
HYP	hypothalamus
lfb	lateral forebrain bundle
LA	lateral (vomeronasal) amygdala
lDP	lateral dorsal pallium
lMP	lateral medial pallium
LP	lateral pallium
lRP	lateral rostral pallium
LS	lateral septum
MA	medial amygdala
MA/BNST/CA	mediocentral amygdala/bed nucleus of the stria terminalis
mDP	medial dorsal pallium
MOB	main olfactory bulb
MOB-gcl	main olfactory bulb, granule cell layer
MOB-mcl	main olfactory bulb, mitral cell layer
MP	medial pallium
mRP	medial rostral pallium

MS	medial septum
NA	nucleus accumbens
NA/VPAL	nucleus accumbens/ventral pallidum
NB	nucleus of the diagonal band
POA	preoptic area
rP	rostral pallium
S	septum
sd	sulcus dorsalis
sdm	sulcus dorsomedialis
ser	sulcus entorhinalis
srh	sulcus rhinalis
STR/DPAL	dorsal striatum/dorsal pallidum
vMP	ventral medial pallium
VP	ventral pallium
VPAL	ventral pallidum
VPd	dorsal portion of the ventral pallium
VPv	ventral portion of the ventral pallium
vRP	ventral rostral pallium
VT	ventral thalamus
vVP	ventral ventral pallium
I	olfactory nerve
II	optic nerve
III	oculomotor nerve
IV	trochlear nerve
V	trigeminal nerve
VIII	statoacoustic nerve
IX/X/XI	glossopharyngeus/vagus/accessories nerve complex
XII	hypoglossal nerve
2Sp	second spinal nerve

majority of intracellular labeling experiments (104), brains were split longitudinally, and one-half of the brain was fixed with stainless-steel insect pins on the floor of a recording chamber (modified from Schaffer, 1982) with the cut medial or the outer surface pointing upward, and the electrode was advanced from that side. In this way, neurons in the medial as well as the lateral and ventral pallium could be aimed at and penetrated more easily. However, in these cases, contralateral projections known to occur in medial pallial neurons could be identified only by their axons or axon collaterals entering the commissures, and their exact contralateral projection pattern remained undetermined. Therefore, in a minority of cases (36), intact (nonsplit) brains were used with the dorsal surface pointing upward, but here injection into medial pallial neurons was more difficult because of massive bundles of densely packed fibers beneath the surface. The brain was continuously perfused with oxygenated Ringer's solution (6 ml/minute) at 14–18°C. For intracellular labeling, micropipettes were filled with a 2% solution of biocytin (Sigma) dissolved in 0.3 M potassium chloride. The impedance of the electrodes was 40–100 MΩ. The electrodes were advanced in steps of 1 or 2 μm, and a 200-msec 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 ±0.5 nA was applied for 4 minutes. In most cases, only one injection was made in each half of the brain. An additional injection was made if the first one appeared unstable. After the injection, brains were stored in oxygenated Ringer's solution at room temperature for 3 hours and at 4°C overnight. Brains were fixed in 2% paraformaldehyde and 2% glutaraldehyde; 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 Laboratories, Burlingame, CA) with 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. Reconstructions of labeled neurons were made by hand with the aid of a camera lucida. The photomicrographs were scanned with a digital camera (AxioCam HR; Carl Zeiss Inc.) at a resolution of 3,900 × 3,090 pixels and graphically processed for optimal contrast in CorelPaint 10 (Corel Corporation). For statistics regarding the intrapallial rostrocaudal extension of dendritic trees and axonal projections, Student's *t*-test (two-tailed) was used.

RESULTS

The pallium of *Bombina orientalis* was divided rostrocaudally into a rostral zone covering the rostral pole of the pallium up to the level of the accessory olfactory bulb, an anterior zone extending between the rostral pallium and the level of the commissures, and a posterior zone extending between the level of the commissures and the caudal pole of the telencephalic hemispheres. From medial to lateral, the pallium was divided into a medial, dorsal, lateral, and ventral pallium (cf. Fig. 1). The medial pallium is separated ventrally from the septal region by the cell-free zona limitans medialis, and its dorsal border was defined as a straight line extending from the sulcus dorsomedialis about 60° dorsomedially and roughly coincid-

ing with a distinct reduction in the number of migrated cells, which is generally higher in the medial compared with the dorsal pallium. The dorsal pallium was defined as the area between the sulcus dorsomedialis and a line that extends from the sulcus rhinalis about 10° dorsolaterally. It was divided into a medial and a lateral portion separated by the sulcus dorsalis. The lateral pallium was defined as the area between the sulcus rhinalis and the sulcus entorhinalis/lateral cellular prominence. The ventral pallium was defined as the area between the lateral cellular prominence and the dorsal edge of the dorsal striatopallidum, which corresponds to the extent of the developmental territory called the "ventral pallium," as defined on the basis of gene expression pattern in *Xenopus* (Brox et al., 2004). Therefore, it includes both regions previously identified as ventral part of the lateral pallium and SPTA (Neary, 1990; Marín et al., 1998). Because the SPTA was proposed to be homologous to the mammalian anterior amygdala and to differ from the immediately adjacent dorsal portion of the pallium (Marín et al., 1998), we divided the ventral pallium into a dorsal and a ventral area, the latter occupying a region roughly 150 μm dorsal to the dorsal striatopallidum, which corresponds to the SPTA. Rostrally, up to the level of the accessory olfactory bulb, the ventral portion of the ventral pallium extends ventrolaterally as a cellular band within the white matter and appears to encapsulate the neuropil of the striatum.

In the following paragraphs, the morphology and axonal projection pattern of 326 pallial neurons forming 204 clusters are described. We included 12 ventral pallial clusters/neurons, which had been described briefly before (Roth et al., 2004) and were reexamined. Usually, one to three neurons were darkly labeled at a single injection of biocytin. The reason for multiple labeling is still unclear; it might be due to simultaneous penetration of two cells and leakage of biocytin or to transcellular biocytin transport via dendritic or axonal gap junctions (Dermietzel and Spray, 1993) or via chemical synapses (Luo and Dessem, 1996). In the case of clusters, only the targets per cluster, not per single neurons, are given, because it is difficult or impossible to determine with precision the axonal projections of simultaneously labeled neurons.

Rostral pallium

In the rostral pallium covering the rostral pole of the telencephalic hemispheres up to the level of the accessory olfactory bulb, 23 clusters containing a total of 36 neurons were labeled. Ten clusters were situated medially, nine clusters dorsally, three clusters dorsolaterally, and one cluster ventrolaterally (Figs. 2A,B, 7A, 8, solid circles). All clusters extended dendritic trees and axons into the pallial region covering the main olfactory bulb dorsally. The medial clusters projected to the dorsal pallium (6), lateral pallium (3), septum (5), and nucleus accumbens/ventral striatopallidum (4), and four clusters sent axons to the contralateral side, where they terminated at a location symmetrical to the site of origin. The dorsal clusters (Figs. 2A, 7A) projected to the medial pallium (9), lateral pallium (8), ventral pallium and rostral pole of the dorsal striatum (3), septum (5), and nucleus accumbens/ventral striatopallidum (9). The lateral clusters projected to the rostral dorsal edge of the striatum (2) and nucleus accumbens/ventral striatopallidum (2). The ventrally situated cluster projected to the dorsal pallium, lateral pallium, and dorsal edge of the striatum. No extratelencephalic projections

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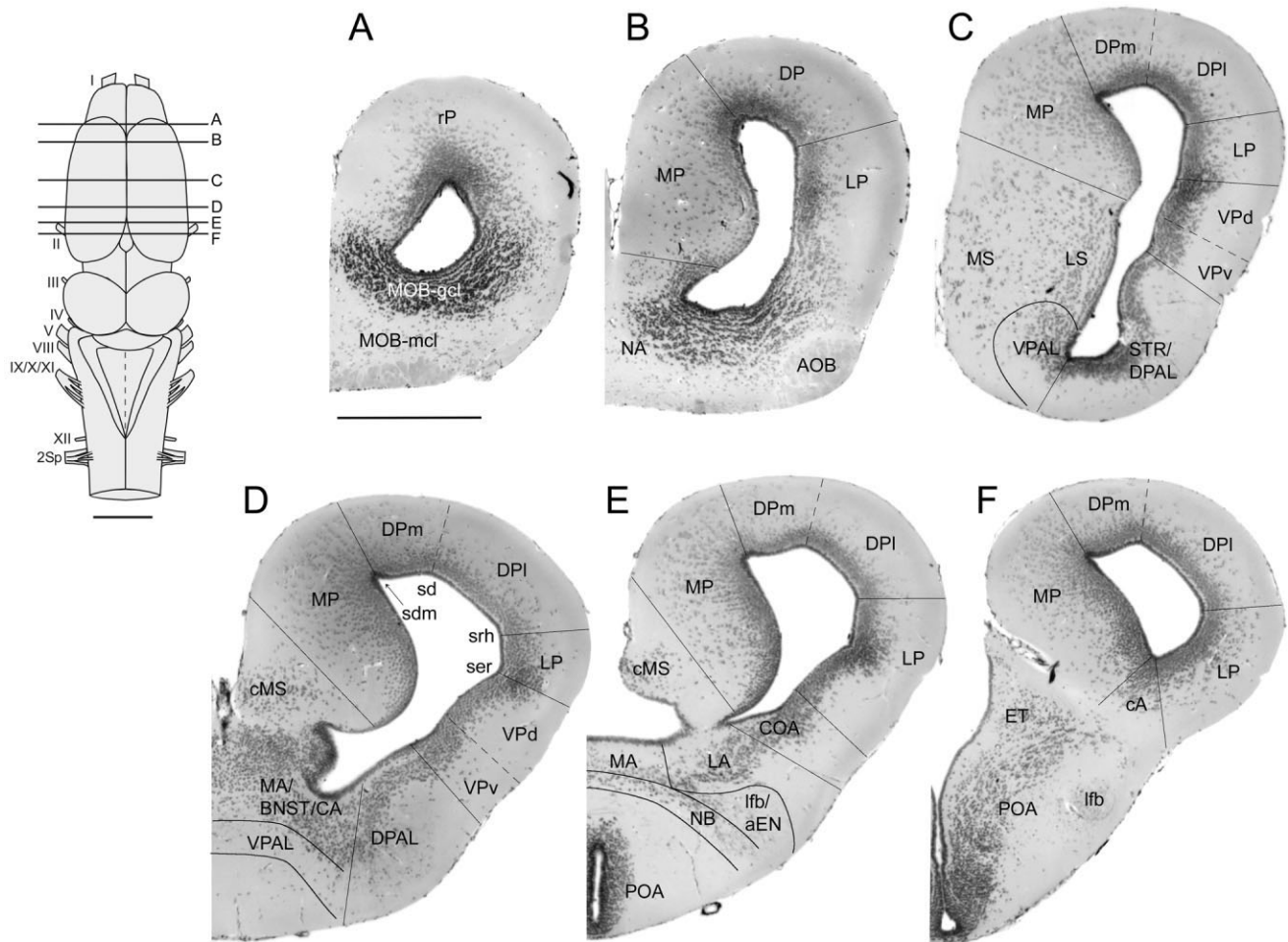

Fig. 1. Transverse sections through the telencephalon and rostral diencephalon of *Bombina orientalis* indicating the boundaries of major pallial and subpallial telencephalic areas at levels indicated in the **inset**. **A:** Level of the main olfactory bulb. **B:** Level of the accessory olfactory bulb and nucleus accumbens. **C:** Midtelencephalic level.

D: Level of the mediocentral amygdala-BNST. **E:** Level of the telencephalic commissures and the beginning of the preoptic area. **F:** Caudal telencephalon and rostral diencephalon with the eminentia thalami. For abbreviations see list. Scale bars = 500 μm in A–F; 1,000 μm in inset.

were observed. The rostrocaudal extension of intrapallial axonal projections is given in Figure 9A.

Usually two or three primary dendrites originated from the soma, sparsely dividing into secondary and tertiary dendrites. Generally, the arborization of the dendritic tree was more widespread compared with neurons in the other pallial areas, especially in the case of neurons located at the very rostral pole of the telencephalic hemispheres (cf. Figs. 2A, 7A). Most dendrites or at least their distal parts were densely covered with spines. The rostrocaudal extension of dendritic trees is given in Figure 9A.

Medial pallium

In the medial pallium, 67 clusters containing a total of 95 neurons were labeled, 35 clusters in the dorsal, 22 clusters in the intermediate, and 10 clusters in the ventral portion of the medial pallium (cf. Fig. 8, triangles). Among the 35 clusters in the dorsal portion (Figs. 3A,B, 7B), ipsilateral projections terminated in or close to the accessory olfactory bulb (9), rostral pallium (11), septum (all), dorsal pallium (29), lateral pallium (16), nucleus

accumbens/ventral striatopallidum (14), mediocentral amygdala (14), ventral thalamus (3), dorsal hypothalamus (10), and ventral hypothalamus (5). No projections to the ventral pallium or to the dorsal striatopallidum were observed. All clusters except for one showed projections to the contralateral side via the commissura hippocampi. When studied (in whole-brain preparations), these projections terminated in the contralateral septum (1), in nucleus accumbens (1), or in dorsal medial pallium at a position roughly symmetrical to the site of origin (4); no projections to the contralateral dorsal, lateral, or ventral pallium were observed. Two clusters showed bilateral projections to the preoptic-hypothalamic region. There was no clear spatial segregation between clusters with and without extratelencephalic projections. The rostrocaudal extension of intrapallial axonal projections is given in Figure 9B.

Among the 22 clusters in the intermediate portion of the medial pallium (Fig. 3C,D), ipsilateral projections terminated in the accessory olfactory bulb or close to it (5), rostral pallium (5), dorsal pallium (10), lateral pallium (2),
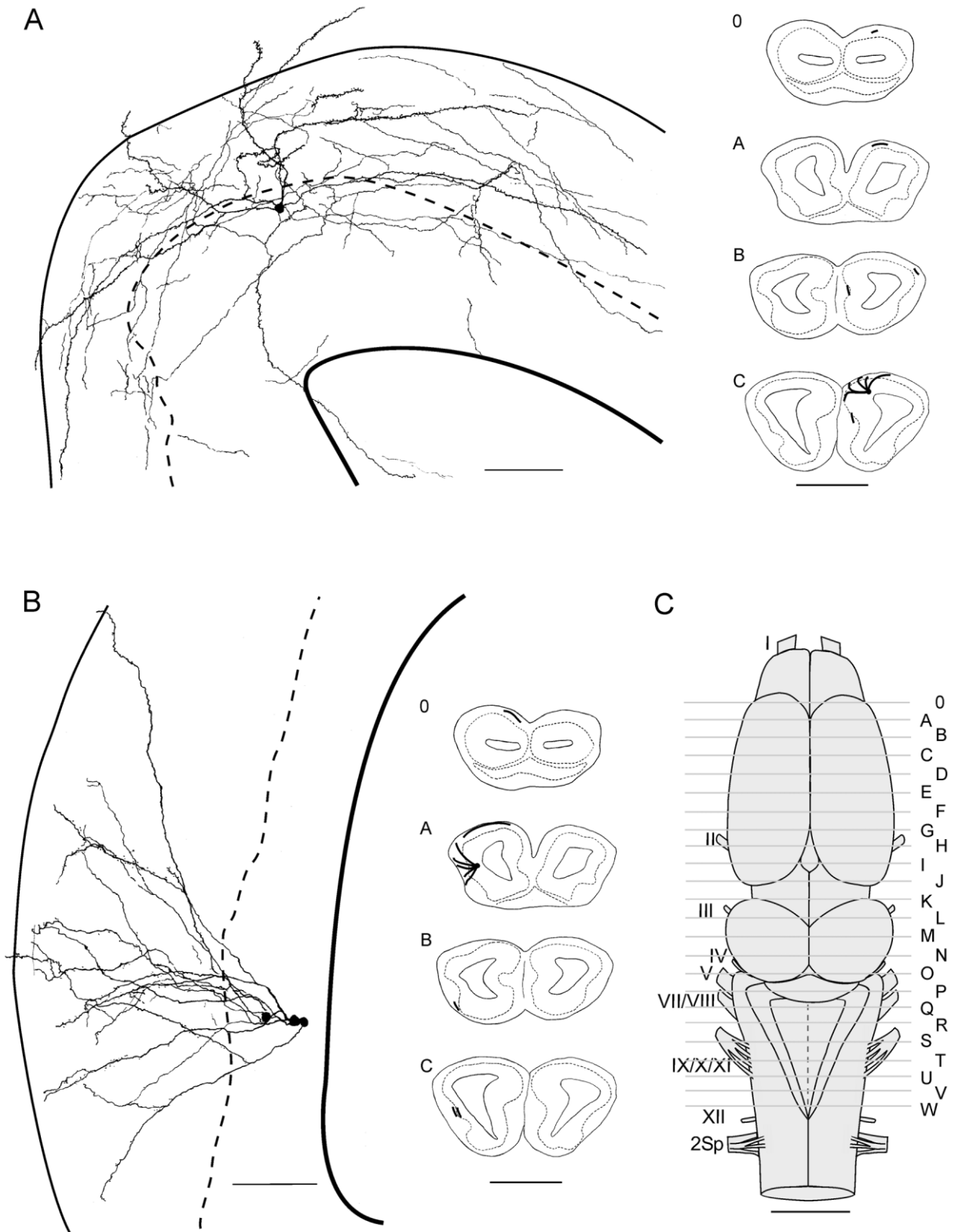
Fig. 2. **A,B:** Camera lucida reconstructions of neurons/neuron clusters located in the rostral pallium of *Bombina orientalis*. A (left): Neuron in the dorsomedial rostral pallium projecting to the medial, dorsal, and lateral pallium. Note the very widespread and "chaotic" dendritic arborization of the neuron. A (right): Reconstruction of the axonal projection pattern of the neuron. The figures show transverse sections through the brain at levels indicated in C. B (left): Cluster of three neurons in the lateral rostral pallium projecting to the dorsal pallium and the rostral dorsal edge of the dorsal striatopallidum. B (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in C. Thin solid line: surface of the rostral pallium; thick solid line: border of the ventricle; dashed line: border of the periventricular gray matter. **C:** Diagram of levels of transverse sections through the brain of *Bombina orientalis* at distances of 250 μm used for reconstruction of axonal projection patterns. For abbreviations see list. Scale bars = 100 μm in A,B (left); 1,000 μm in A,B (right),C.

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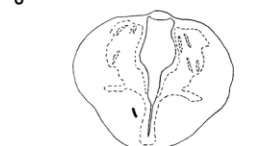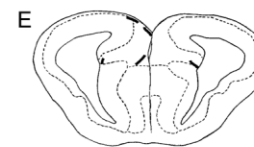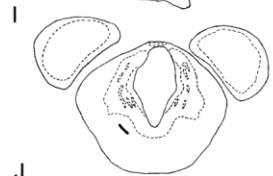
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Figure 3

Figure 3 (Continued)

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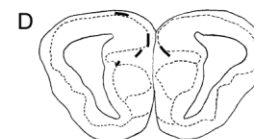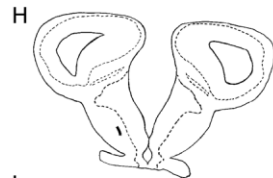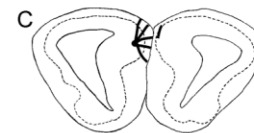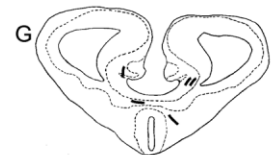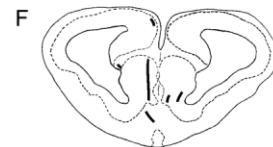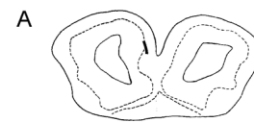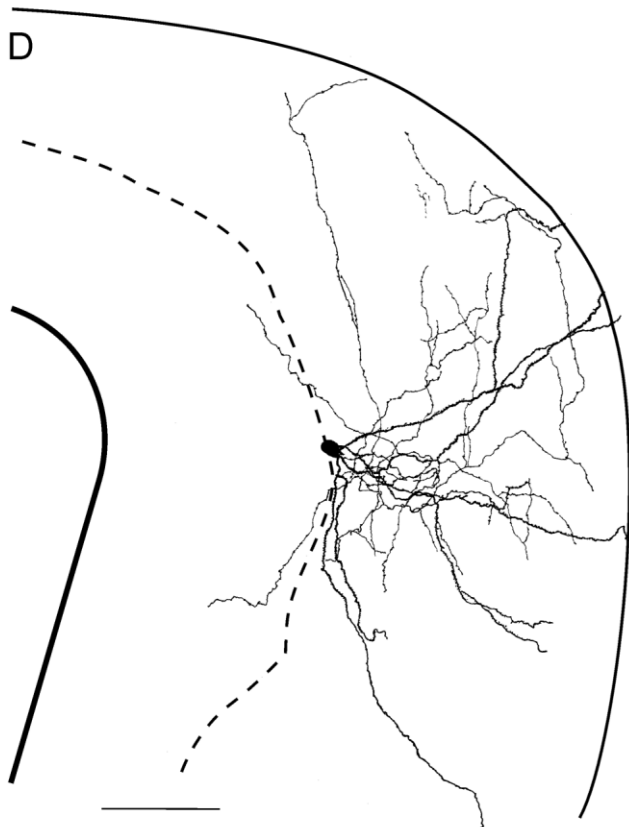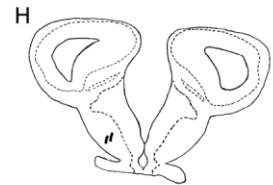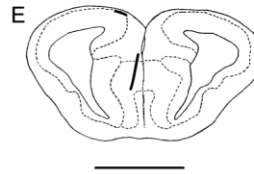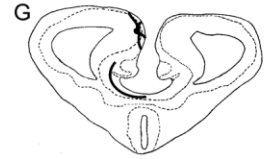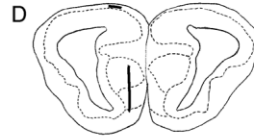
Fig. 3. **A–E:** Camera lucida reconstruction of neurons/neuron clusters situated in the medial pallium of *Bombina orientalis*. A,B (left): Neuron and cluster of two neurons situated in the dorsal portion of the medial pallium both projecting to the dorsal and lateral pallium, septum, and hypothalamus and to the contralateral side. Note the extensive arborization of axon collaterals inside the septal region. A,B (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in Figure 2C. C (left): Neuron situated in the intermediate portion of the medial pallium projecting to the ipsilateral dorsal pallium, nucleus accumbens, and ventral thalamus and to the contralateral side. C (right): Reconstruction of the axonal projection pattern of the neuron at levels indicated in Figure 2C. D (left): Neuron studied in a nonsplit brain situated in the intermediate me-
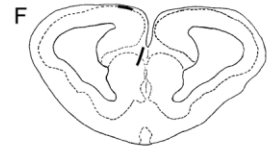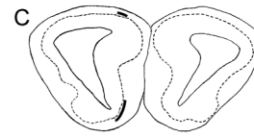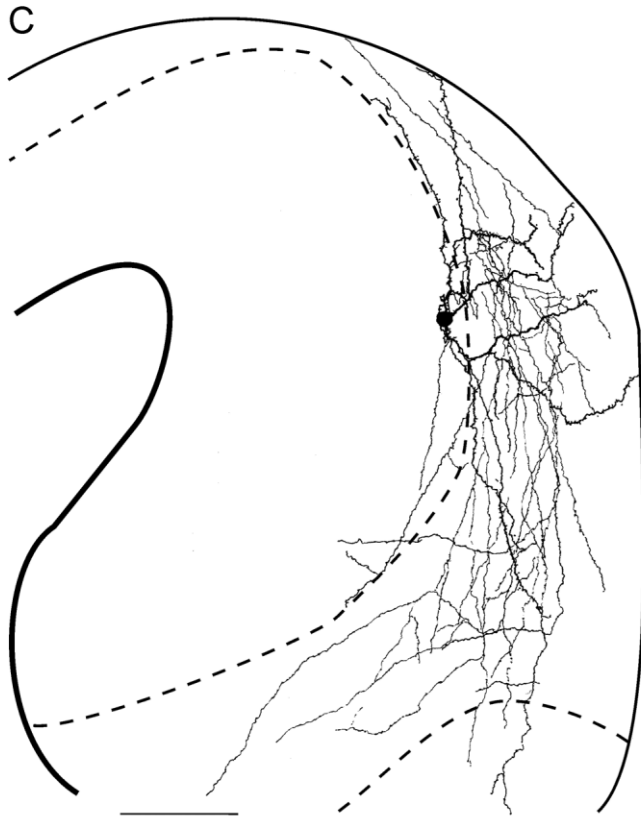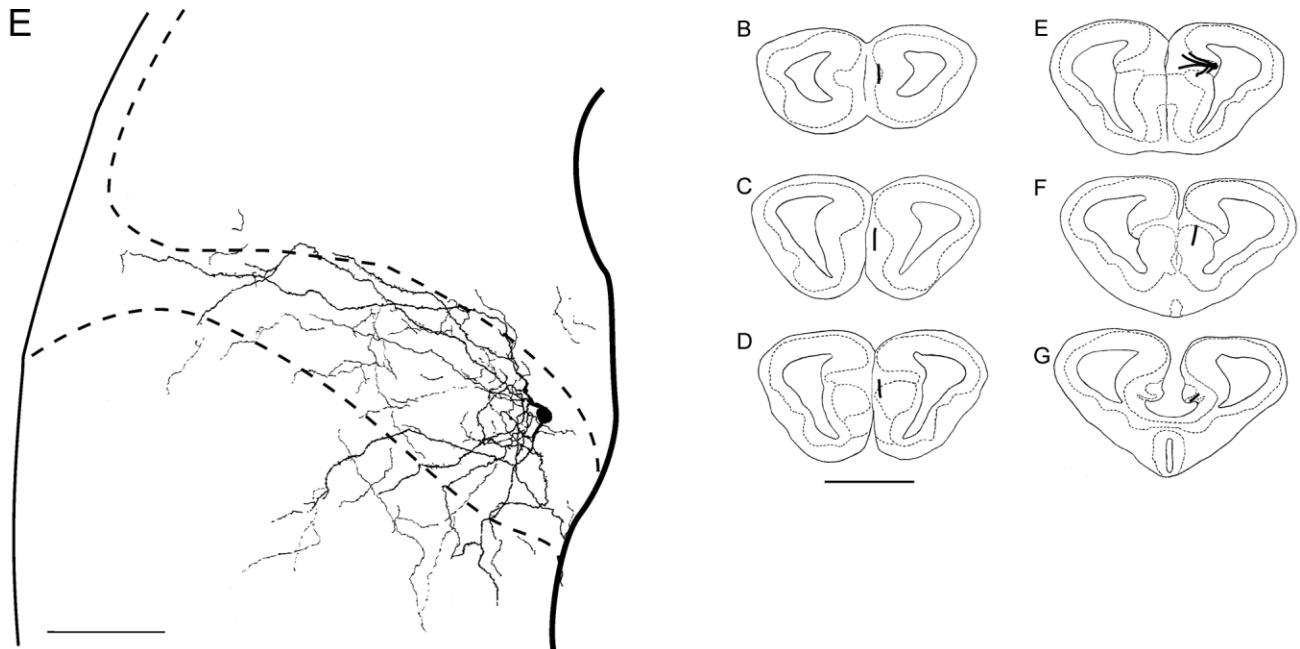
dial pallium projecting to the ipsilateral dorsal pallium, eminentia thalami, and suprachiasmatic nucleus and to the contralateral ventral medial pallium and preoptic area. D (right): Reconstruction of the axonal projection pattern of the neuron at levels indicated in Figure 2C. E (left): Neuron found in the ventral medial portion of the medial pallium with dendrites arborizing the zona intercalaris and the dorsal septum and projecting to the eminentia thalami. The neuron has no contralateral projections. E (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in Figure 2C. Thin solid lines: surface of the medial pallium; thick solid lines: border of the ventricle; dashed lines: border of the periventricular gray matter. Scale bars = 100 μm in A–E (left); 1,000 μm in A–E (right).

septum (all), nucleus accumbens/ventral pallidum (7), ventral thalamus (1), dorsal hypothalamus (2), and ventral hypothalamus (5). No projections to the ventral pallium or to the dorsal striatopallidum occurred. Again, all clusters except for one showed projections to the contralateral side, which terminated in the medial pallium at the same level as the cells of origin; no projections to the contralateral dorsal, lateral, or ventral pallium occurred. Contralaterally descending projections terminated in the mediocentral amygdala and preoptic region. The rostrocaudal extension of intrapallial axonal projections is given in Figure 9B.

Neurons in the dorsal and intermediate zone were very similar in morphology. In most cases, two to four primary dendrites originated from the round or pear-shaped soma, then quickly divided into two or three secondary dendrites and further extended in a wide to very wide fan-like fashion toward the surface. Often, they continued dorsally or ventrally subpially for a certain distance. Usually the dendritic tree widened and flattened with increasing distance of the soma from the ventricle. The most superficial neurons had spindle-shaped somata, and their dendrites extended dorsolaterally and ventromedially parallel to the curvature of the dorsomedial surface of the telencephalon. Most of the dendrites are covered with spines, some of them heavily.

Dendrites of neurons in the dorsal and intermediate medial pallium, except the most dorsal and superficial ones (cf. Fig. 3A), did not cross the border between medial and dorsal pallium. However, heavily beaded axons invaded the white matter of the dorsal and lateral pallium in loose bundles contributing to the "dorsal association tract," part of them reaching the lateral cellular prominence, i.e., the border between the dorsal and the ventral lateral pallium, but did not enter the ventral lateral pallium. Ventromedially, neurons again formed an extensive meshwork of heavily beaded axons that filled the zona limitans medialis and invaded the septal region (cf. Fig. 3A,C). The rostrocaudal extension of dendritic trees is given in Figure 9B

Among the 10 clusters labeled in the ventral medial pallium (Figs. 3E, 7C), two projected to the main and one to the accessory olfactory bulb, all projected to the dorsal septum and to the intermediate medial pallium, three to the rostral pallium, two to the dorsal medial and the medial part of the dorsal pallium, one to the nucleus accumbens, and four to the eminentia thalami. None projected to the contralateral side or to any extratelencephalic target except for the eminentia thalami. Their morphology differed from that of the neurons in the dorsal and intermediate zone, in that dendritic trees were less widespread and extended into the zona limitans medialis and

the dorsal septum. Dendrites were variably covered with spines. Only in two cases did vertically oriented dendrites invade the intermediate and dorsal portion of the medial pallium. The rostrocaudal extension of intrapallial axonal projections and dendritic trees is given in Figure 9B.

Dorsal pallium

Among the 55 clusters (96 neurons) labeled in the dorsal pallium, 24 were situated more medially and 31 more laterally (Fig. 8, open squares). None had projections to the contralateral telencephalon or to extratelencephalic targets, except to the eminentia thalami.

The 24 medial clusters (Figs. 4A, 7D) sent projections to the rostral pallium (5), medial pallium (22), lateral pallium (17), septum (14, mostly to the dorsal part), nucleus accumbens/ventral striatopallidum (3), and eminentia thalami or its vicinity (4). Many of them formed a dense meshwork of axon terminals close to or directly above the lateral cellular prominence. None projected to the ventral pallium. The 31 more laterally situated clusters (Fig. 4B) sent projections to the medial pallium (13), lateral pallium (26), dorsal septum (7), nucleus accumbens/ventral striatopallidum (3, mostly rostrally situated clusters), and eminentia thalami or close to it (5, mostly caudally situated clusters). The rostrocaudal extension of intrapallial axonal projections is given in Figure 9C.

The morphology of dorsal pallial neurons was similar to that of medial pallial ones; i.e., neurons with somata situated closer to the ventricle had more fan-like dendritic trees (cf. Fig. 4A), whereas those with somata in a migrated position had wide to very wide dendritic trees (cf. Figs. 4B, 7D). A few interneurons with relatively narrow, fan-like dendritic trees could be labeled (Fig. 4C). Three clusters contained spindle-shaped neurons with horizontally extending dendrites. Most dendritic trees were densely covered with spines. Axons were generally thin to very thin and extended medially as well as laterally as far as the lateral cellular prominence within the superficial fiber layer contributing to the "dorsal association tract." Many of them formed axonal clouds in the medial pallium and in the zona limitans medialis. The rostrocaudal extension of dendritic trees is given in Figure 9C.

Lateral pallium

Among the 28 clusters (46 neurons) labeled in the lateral pallium, nine were located in the anterior (precommissural and commissural) and 19 in the posterior (postcommissural) part (Fig. 8, inverted triangles). Among the clusters situated in the anterior part (Figs. 5A, 7E), two projected to the level of the main olfactory bulb, four to the rostral, one to the medial, four to the dorsal, and five to the ventral pallium. Three clusters sent axons to the vomeronasal amygdala and preoptic area. The others had axons that did not leave the lateral pallium. Among the clusters situated in the postcommissural part (Fig. 5B), eight projected via a ventromedial route to the medial pallium, where they arborized extensively in its intermediate and ventral portion, eight to the dorsal pallium via the dorsal association tract, and six to the ventral pallium via a ventrolateral route. Thirteen clusters sent one axon per neuron ventromedially and then rostrally at a subpial position inside the septum/diagonal band of Broca, terminating in the rostromedial pole of the telencephalon. This tract was called the "fimbrial tract" by Herrick (1934). Neurons in the caudal pole of the telencephalic hemi-

sphere sent dendrites and axonal bundles ventrally accompanying the lateral olfactory tract on its course toward the habenular commissure. Sometimes, their dendrites and axons covered the entire caudal pole of the telencephalic hemisphere. No contralateral projections were observed. The rostrocaudal extension of intrapallial axonal projections is given in Figure 9D.

The dendritic trees of neurons in the lateral pallium were generally fan-like, and no cells with spindle-shaped somata were seen. Mostly the distal parts of dendrites were covered with spines. The rostrocaudal extension of dendritic trees is given in Figure 9D.

Ventral pallium

In total, 31 clusters (53 neurons) were labeled in the ventral pallium (Fig. 8, open circles). Among the 14 clusters (22 neurons) situated in the dorsal portion (Figs. 6A, 7F), all projected to the immediately adjacent ventral portion, 13 projected to the rostral pallium, eight to the lateral pallium, and none to the dorsal or medial pallium. One cluster projected to the main olfactory bulb and four clusters projected to or in close proximity to the accessory olfactory bulb. Axons of 12 clusters displayed fiber varicosities in the lateral subpial portion of the dorsal striatopallidum; 12 projected to the neuropil adjacent to the lateral (vomeronasal) amygdala/preoptic area, four to the dorsal and one to the ventral hypothalamus, and one to the caudal medial tegmentum/nucleus interpeduncularis.

Among the 17 clusters (31 neurons) labeled in the ventral portion (Fig. 6B), two projected to the rostral, two to the lateral and none to the dorsal or medial pallium, two to the main olfactory bulb, and six to the accessory olfactory bulb. Eleven clusters had axons that displayed fiber varicosities in the subpial portion of the dorsal striatopallidum, 15 to the neuropil of the vomeronasal amygdala, three to the ventral thalamus, seven to the dorsal, and one to the ventral hypothalamus. The rostrocaudal extension of intrapallial axonal projections is given in Figure 9E.

The morphology of neurons in the ventral pallium differed from that of neurons in the medial, dorsal, and lateral pallium in that the dendritic trees were mostly slender. In half of cases, only one primary dendrite originated from the pear-shaped soma and divided into secondary and tertiary dendrites. They did not or only sparsely reach into the white matter of the lateral pallium; rather, they extended their dendrites laterally or ventrolaterally, curving around the outer striatal neuropil, where they often formed dense meshworks (cf. Fig. 6B). In the rostral lateral telencephalon, these dendrites are separated from the striatal neuropil by a cellular protrusion that extends ventrally or ventrolaterally almost to the level of the ventral border of the dorsal striatum.

The density of dendritic spines varied strongly; dendrites of some neurons were heavily covered with spines, others were covered only sparsely, and some dendritic trees carried no spines. The rostrocaudal extension of dendritic trees is given in Figure 9E.

Rostrocaudal extension of intrapallial axon collaterals and dendritic trees

In Figures 8–9, the sites of the somata of pallial neurons/neuronal clusters and the rostrocaudal extension of their intrapallial projections are shown. The average rostrocaudal extension of intrapallial axonal projections of clusters in the rostral pallium is 1,046 μm and that of

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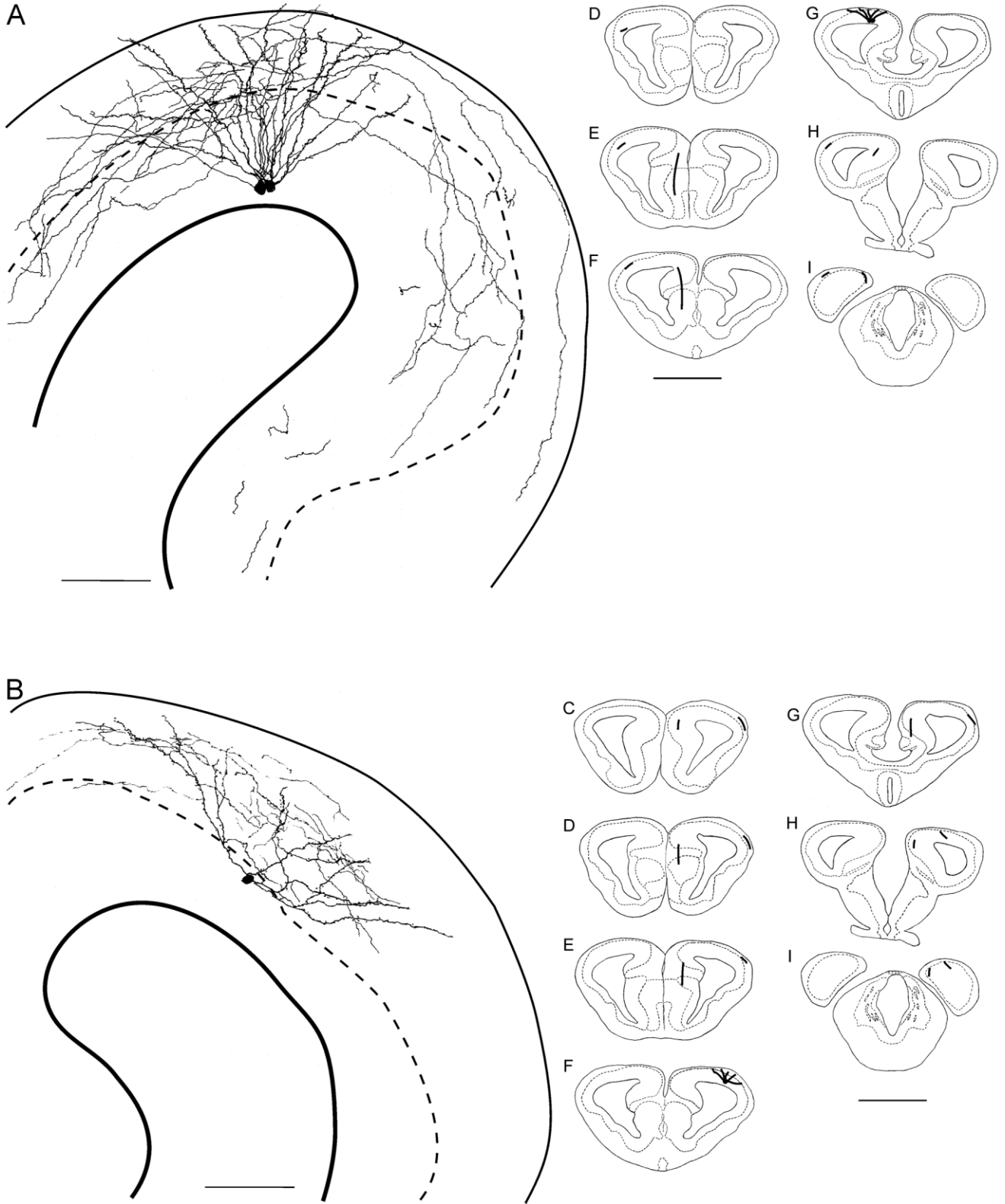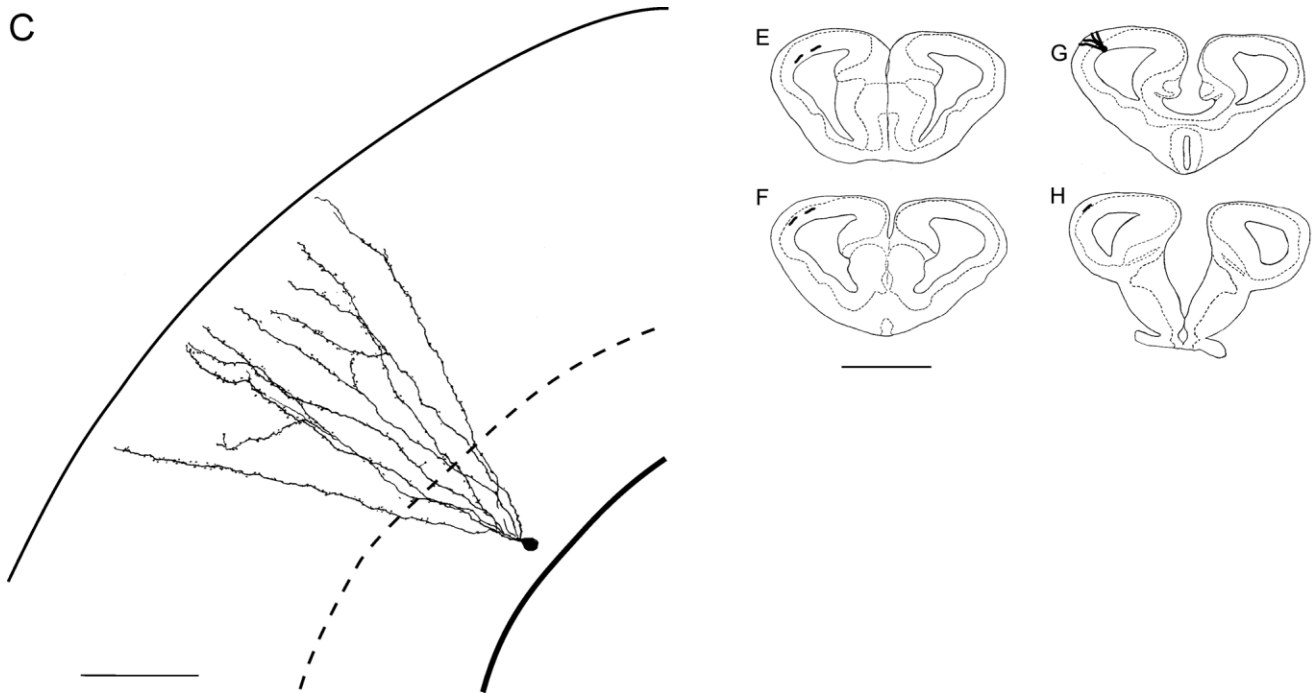

Figure 4   (Continued)

Fig. 4. **A–C:** Camera lucida reconstruction of neurons/clusters of neurons situated in the dorsal pallium of *Bombina orientalis*. A (left): Cluster of two neurons situated in the rostral caudal medial portion of the dorsal pallium projecting to the caudal lateral and medial pallium and caudal septal area. Note the extensive arborization of axon collaterals. A (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in Figure 2C. B (left): Neuron situated in the lateral portion of the dorsal pallium projecting to the lateral and medial pallium, to dorsal septum, and to the vicinity of the eminentia thalami. Note the very wide dendritic tree. B (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in Figure 2C. C (left): Interneuron situated in the commissural lateral portion of the dorsal pallium, with axons confined to a narrow region within the dorsal pallium, as shown in C (right). Thin solid lines: surface of the dorsal pallium; thick solid lines: border of the ventricle; dashed lines: border of the periventricular gray matter. Scale bars = 100 μm in A–C (left); 1,000 μm in A–C (right).

dendritic trees 376 μm (Fig. 9A). Average intrapallial rostrocaudal extension of axon collaterals of the 35 somata labeled in the dorsal portion of the medial pallium is 1,057 μm and that of dendritic trees 377 μm (Fig. 9B). The axonal projection of one neuron covers the entire rostrocaudal length of one hemisphere. The intrapallial rostrocaudal extension of axon collaterals of the 22 clusters located in the intermediate portion of the medial pallium is 955 μm and that of dendritic trees 364 μm. The intrapallial rostrocaudal extension of axon collaterals of the 10 clusters found in the ventral portion of the medial pallium is 1,025 μm and that of dendritic trees 485 μm. The dorsal pallium was divided into a medial and a lateral portion. The intrapallial rostrocaudal extension of axon collaterals of the 24 clusters in the medial portion is 1,000 μm, that of the 31 clusters in the lateral portion is 976 μm, and that of dendritic trees is 329 and 348 μm, respectively (Fig. 9C). The 28 clusters labeled in the lateral pallium are found mostly in the postcommissural part, because this region was of specific interest. Intrapallial rostrocaudal extension of axon collaterals is 955 μm and that of dendritic trees 384 μm (Fig. 9D). The ventral pallium was divided into a dorsal and a ventral portion, the latter corresponding to the SPTA. Among the 14 clusters in the dorsal portion, intrapallial rostrocaudal extension of axon collaterals is 1,468 μm and that of dendritic trees 364 μm; for the 17 clusters in the ventral portion, it is 1,456 μm and 485 μm for dendritic trees.

The differences in intrapallial rostrocaudal extension of axon collaterals and of dendritic trees among rostral, medial, dorsal, and dorsal lateral pallium; among the three portions of the medial pallium between the medial and the lateral portion of the dorsal pallium; and between the dorsal and ventral portion of the ventral pallium are not statistically significant. In contrast, differences in the rostrocaudal extension of axon collaterals, but not of dendritic trees, in the ventral pallium vs. all other pallial areas are significant ($P < 0.002$).

Projection neurons—interneurons

The vast majority of labeled pallial neurons are projection neurons; i.e., they have axons that project to other pallial and extrapallial areas. The number of well-stained interneurons, i.e., with axons remaining in the close vicinity of the neuron (cf. Fig. 4C), was generally low. The number of interneurons does not exceed 5% of labeled neurons.

DISCUSSION

Based on the data presented here, the pallium of *Bombina orientalis* can be divided into five major parts, rostral, medial, dorsal, lateral, and ventral pallium, which exhibit clear differences in the projection pattern of neurons (Fig. 10). The rostral pallium occupies the rostral pole of the telencephalic hemispheres dorsal to the caudal

Fig. 5. **A,B:** Camera lucida reconstruction of clusters of neurons situated in the lateral pallium of *Bombina orientalis*. A (left): Cluster of two neurons situated in the lateral pallium projecting to the medial, dorsal and caudal (olfactory) pallium. A (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in Figure 2C. B (left): Cluster of five neurons situated in the caudal (olfactory) portion of the lateral pallium projecting to the caudal medial pallium and via the "fimbria tract" to the rostral medial pallium and nucleus accumbens. B (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in Figure 2C. Thin solid lines: surface of the lateral pallium; thick solid lines: border of the ventricle; dashed lines: border of the periventricular gray matter. Scale bars = 100 μm in A,B (left); 1,000 μm in A,B (right).
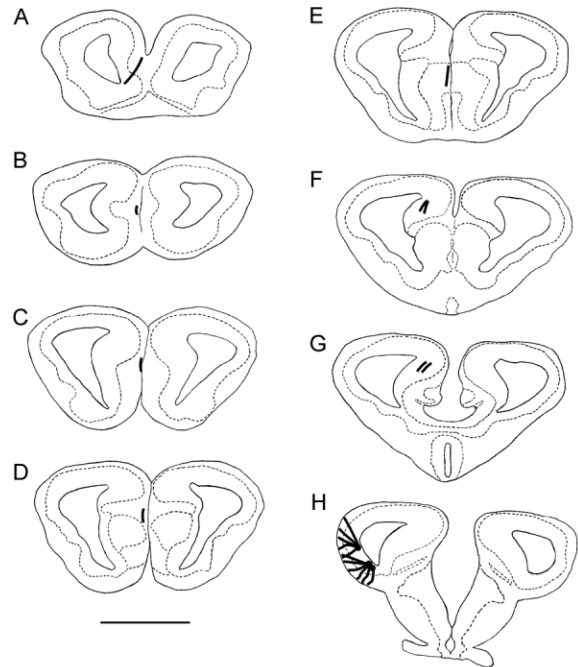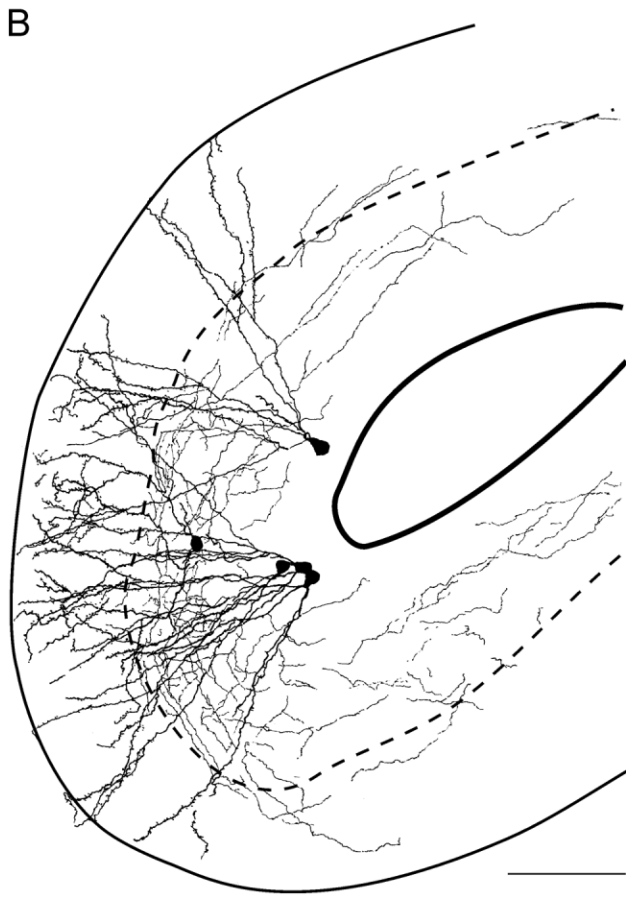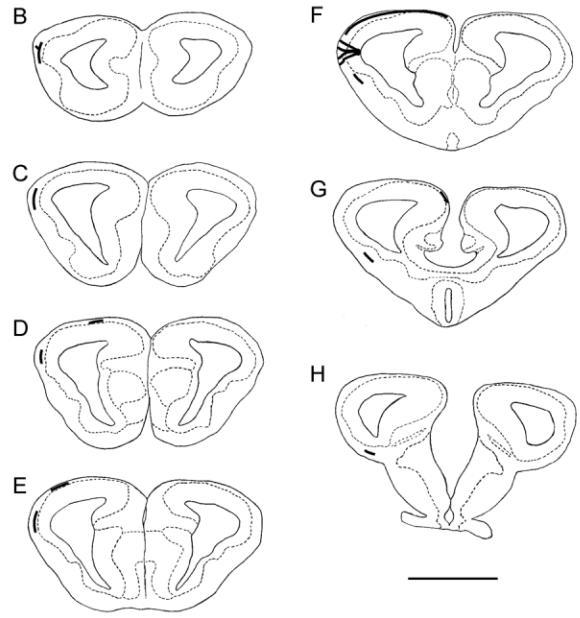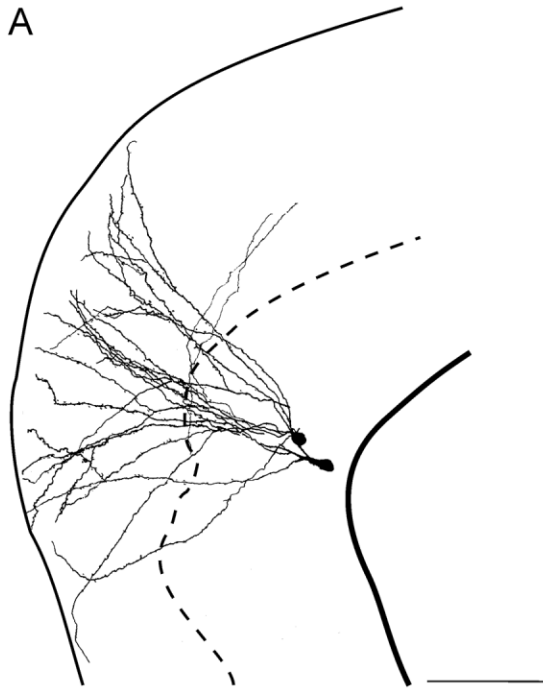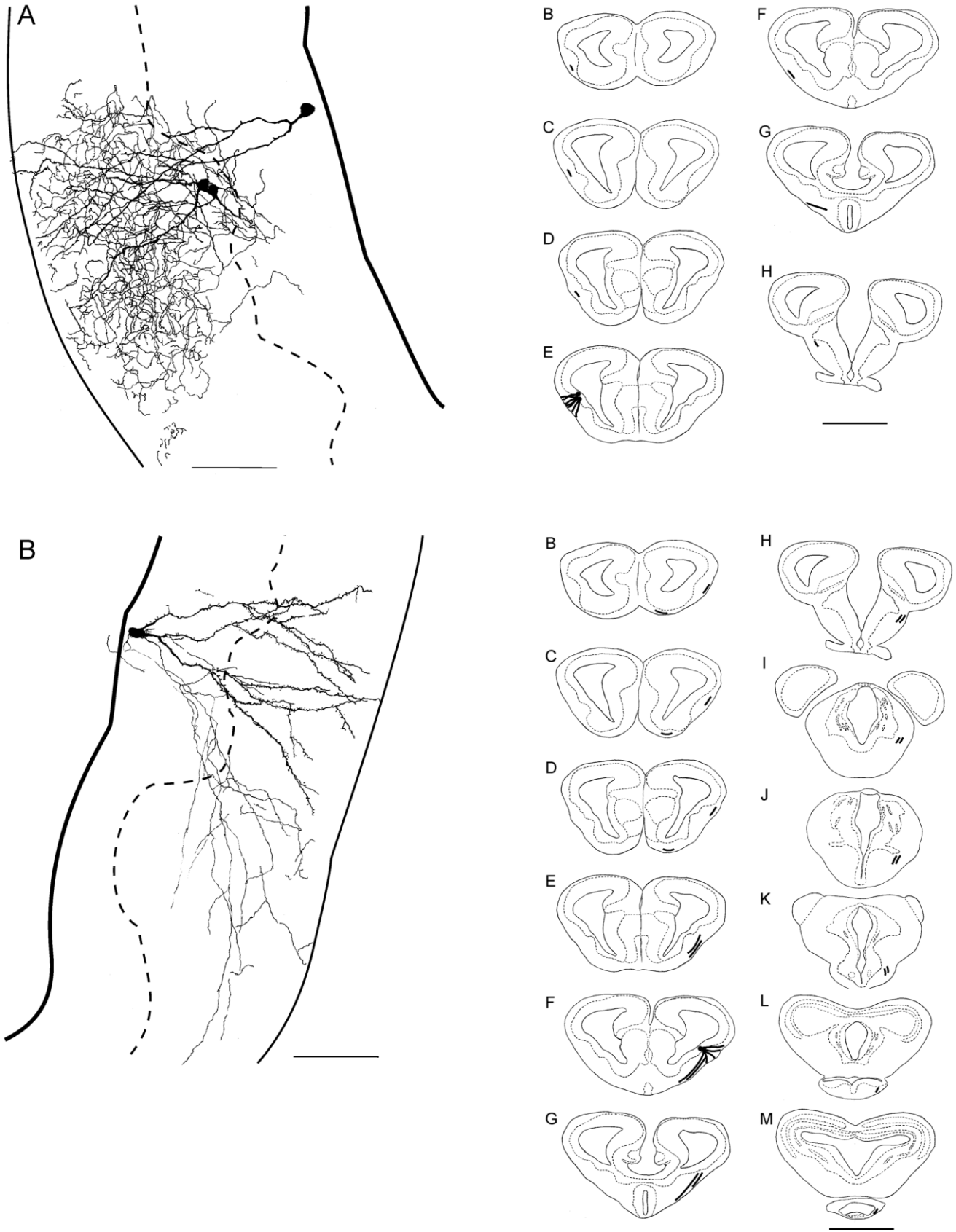
Fig. 6. **A,B:** Camera lucida reconstruction of neurons/clusters of neurons situated in the ventral pallium. A (left): Cluster of three neurons situated in the dorsal portion of the ventral pallium projecting to the lateral (vomeronasal) amygdala, preoptic area and rostral thalamus. Note the extensive arborization of tertiary dendrites and local axon collaterals. A (right): Reconstruction of the axonal projection pattern of the cluster at levels indicated in Figure 2C. B (left): Neuron situated in the ventral portion/SPTA of the ventral pallium projecting to the lateral region of the dorsal striatopallidum, ventral striatum, lateral amygdala, and ventral hypothalamus/infundibulum. Note the tertiary dendrites and axon collaterals curving laterally around the striatal neuropil. Thin solid lines: surface of the ventral pallium; thick solid lines: border of the ventricle; dashed lines: border of the periventricular gray matter. Scale bars = 100 μm in A,B (left); 1,000 μm in A,B (right).
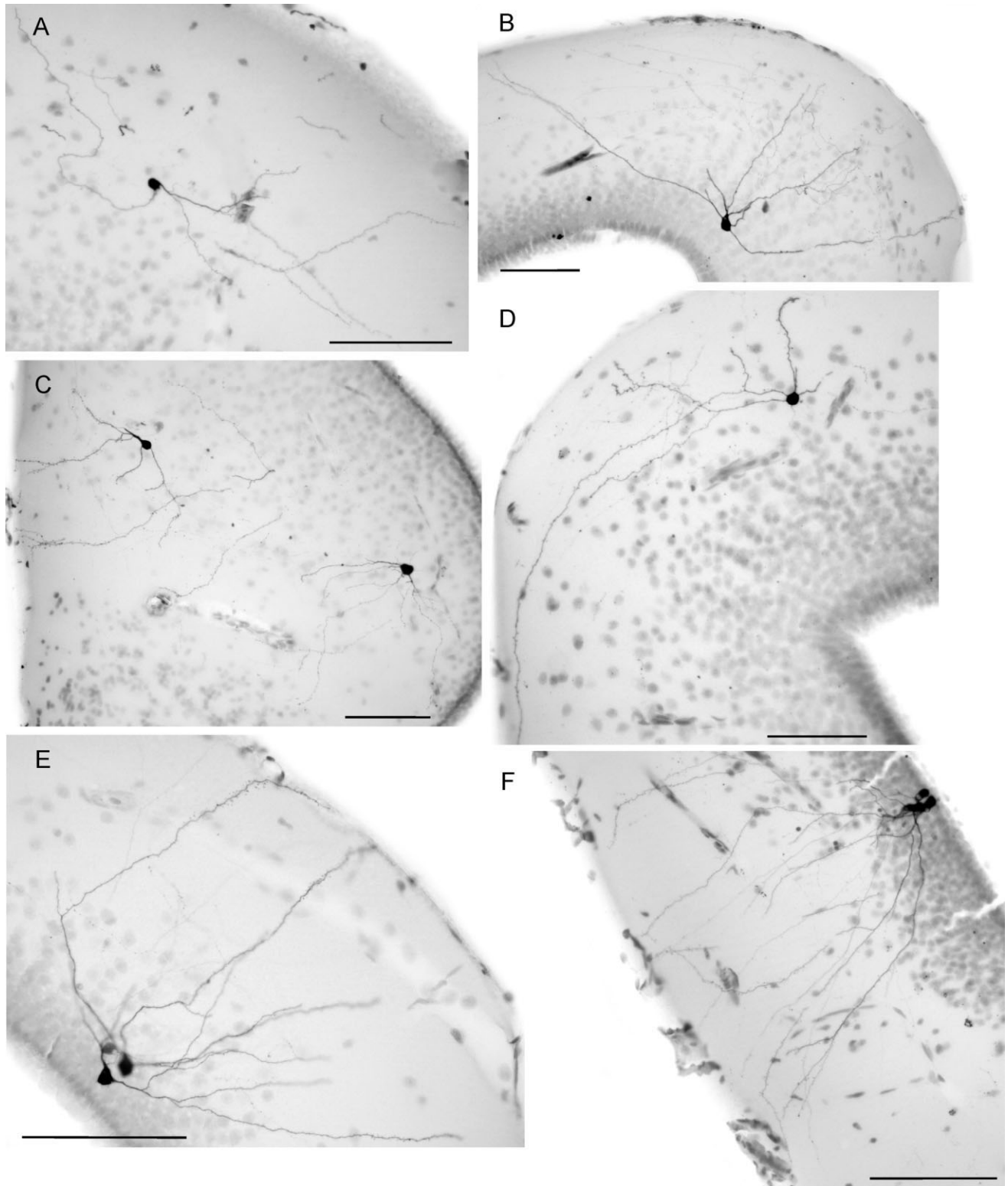
Fig. 7. **A–F:** Photomicrographs of pallial neurons. A: Neuron situated in the dorsal portion of the right rostral pallium projecting to the nucleus accumbens and rostral dorsal edge of the dorsal striatopallidum. Note the very widespread dendritic tree and the "upsidedown" position of the neuron. B: Neuron situated in the dorsal portion of the left medial pallium projecting to the hypothalamus. Note the very widespread dendritic tree. C: Two neurons situated in the ventral portion of the left medial pallium projecting to the eminentia thalami. D: Neuron situated in the medial portion of the right dorsal pallium. Note the thick medially descending axon. E: Cluster of two strongly labeled neurons plus one weakly labeled one situated in the right lateral pallium projecting to the dorsal and dorsal medial pallium. F: Cluster of four neurons situated in the dorsal portion of the left ventral pallium projecting to the dorsal hypothalamus. Scale bars = 100 μm.
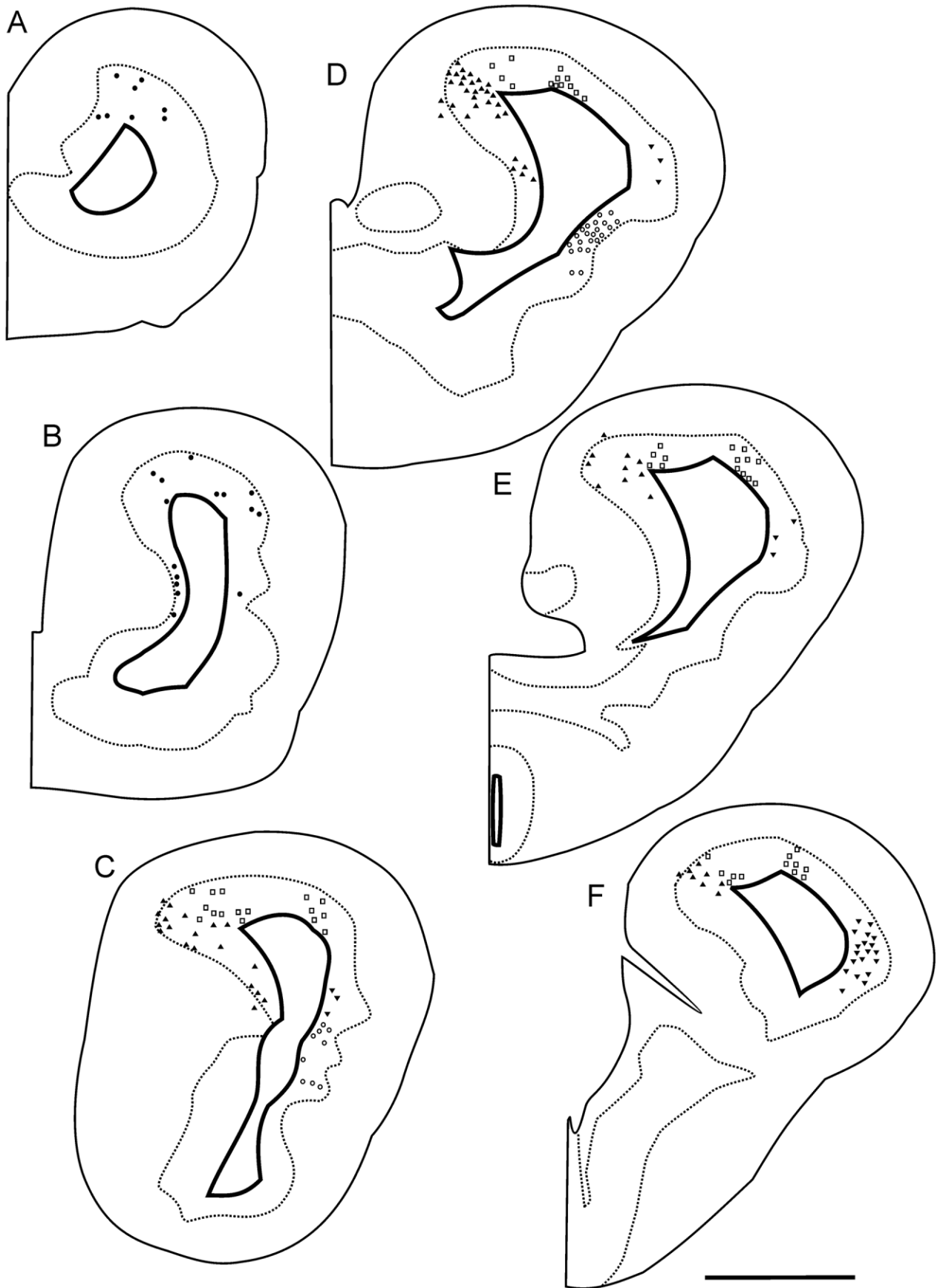
Fig. 8.   **A–F:** Distribution of labeled pallial neurons. Sites of labeled neurons were projected onto the five transverse sections (A–F) shown in Figure 1. Solid circles: rostral pallial neurons; triangles: medial pallial neurons; squares: dorsal pallial neurons; inverted triangles: lateral pallial neurons; open circles: ventral pallial neurons. Scale bar = 500 μm.
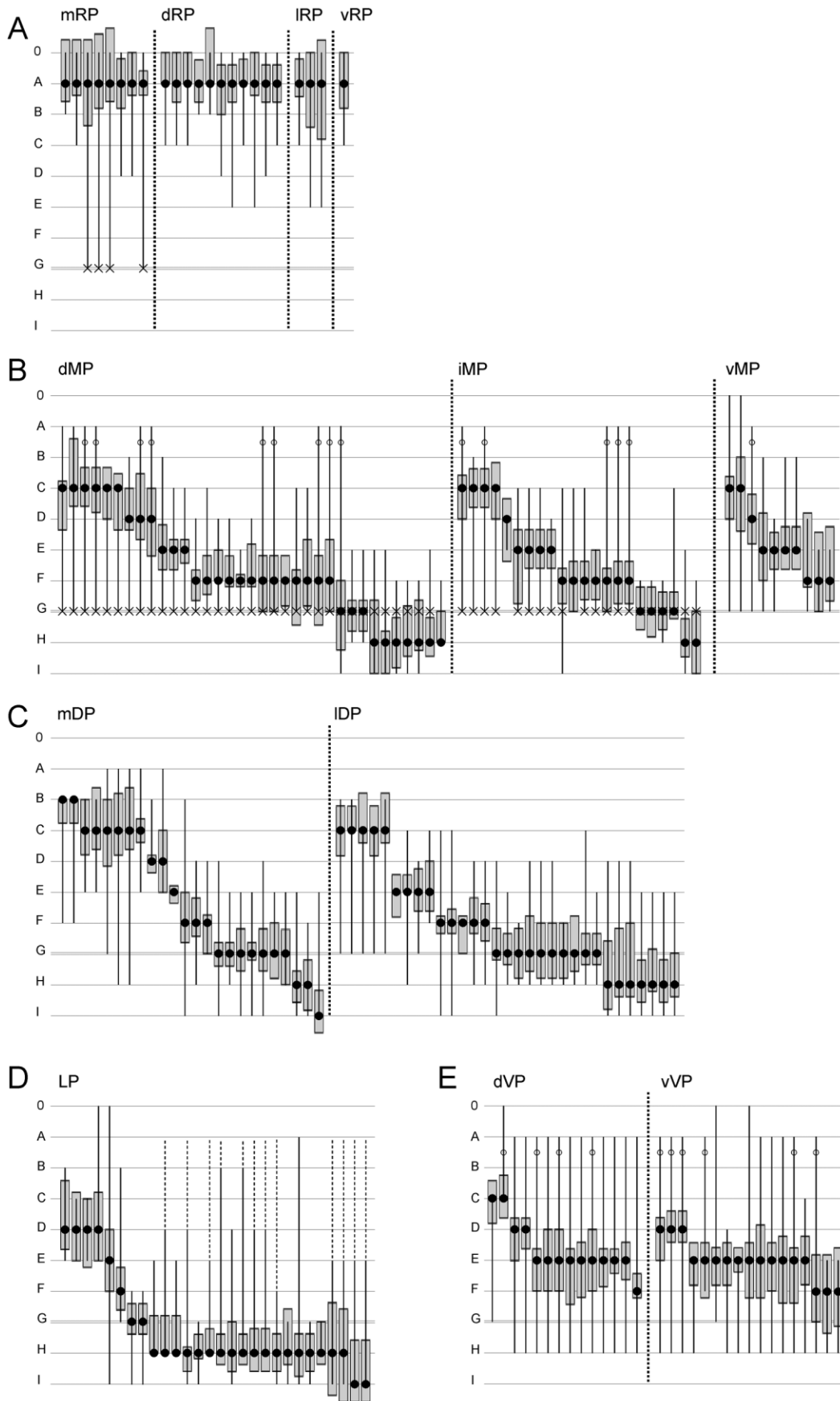
Fig. 9. Diagrams of the rostrocaudal extension of intrapallial ax-
onal projections (vertical lines) and dendritic trees (gray vertical
rectangles) of neurons in the medial, dorsal, lateral, and ventral
rostral pallium (**A**); dorsal, intermediate, and ventral medial pallium
(**B**); medial and lateral dorsal pallium (**C**); lateral pallium (**D**); and
dorsal and ventral ventral pallium (**E**). Rostrocaudal levels indicated
by Roman letters refer to Figure 2C. O indicates the level of the main
olfactory bulb, G and double lines the level of the telencephalic com-
missures. Solid circles: sites of somata; X: axons crossing to the
contralateral side; open circles: contacts with the accessory olfactory
bulb. For abbreviations see list.

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Fig. 10. Diagrams of projection patterns of neurons and neuron clusters in pallial regions of *Bombina orientalis*. **A:** Rostral pallium. **B:** Medial pallium (only projections of the dorsal and intermediate portion are shown). **C:** Dorsal pallium. **D:** Lateral pallium. **E:** Ventral pallium. Origins of projections are indicated by thick rectangles, projections by thick lines. For abbreviations see list.

main olfactory bulb, the accessory olfactory bulb, and the rostral dorsal striatum. All neurons extend both dendrites and axons into the rostralmost portion of the pallium above the main olfactory bulb. This region is reciprocally connected with the caudally adjacent anterior medial, dorsal, lateral, and ventral pallium. The rostral pallium is distinguished from the other pallial regions by rostrally oriented and rather "chaotically" arborizing dendritic trees and also by receiving the bulk of input from the anterior dorsal thalamus. It likewise differs from the medial, dorsal, and lateral pallium by projections to the rostral dorsal striatum. Thus, it seems justified to consider the rostral pallium a pallial region of its own, but this hypothesis requires futher investigation.

The medial pallium can clearly be distinguished from the adjacent dorsal pallium by the presence of contralateral and extratelencephalic projections (except to the eminentia thalami). The border between medial and dorsal pallium roughly coincides with the sulcus dorsomedialis

and a reduction in cell migration, which in many anuran species (e.g., *Hyla*, *Afrixalus*, *Bufo*, *Eleutherodactylus*) is even more pronounced than in *Bombina* (W. Walkowiak and G. Roth, unpublished observations). In contrast to previous findings in *Discoglossus pictus* (Westhoff and Roth, 2002), neurons located in the dorsal and intermediate portion of the medial pallium in *Bombina* do not differ substantially in their projection pattern (in *Discoglossus*, the border between medial and dorsal pallium is found more dorsolaterally compared with *Bombina*). However, neurons in the dorsal portion project more heavily to the dorsal and lateral pallium and to the nucleus accumbens than neurons in the intermediate portion. Neurons in the ventral portion show no contralateral projections and restrict their axonal arborizations mostly to the medial pallium. However, four clusters in the ventral portion projected to the eminentia thalami or close to it. The dorsal pallium differs from the medial pallium by the absence of contralateral and extratelencephalic projections, as was

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found previously by a number investigators (e.g., Neary, 1990; Northcutt and Ronan, 1992; for an extended discussion see Westhoff and Roth, 2002), except to the eminentia thalami, which is believed to be part of the diencephalon. The septum and the nucleus accumbens/ventral striato-pallidum are the only extrapallial telencephalic targets. Inside the dorsal pallium, medially situated neurons project more heavily to the medial pallium and septum, whereas laterally situated neurons are more heavily connected with the lateral pallium. The lateral pallium differs from the dorsal pallium by projections to the ventral pallium. There are clear differences between the anterior and the posterior part of the lateral pallium. The anterior part has only weak connections with the medial pallium but is heavily connected with the ventral pallium, whereas the posterior part is heavily connected with the medial pallium, and most clusters situated there send a narrow projection, the fimbria tract, to the rostral medial pallium running in a subpial position through the diagonal band of Broca. Also, their dendrites and axons accompany the lateral olfactory tract on its course around the caudal pole of the telencephalic hemispheres toward the habenular commissure. The ventral pallium comprises the ventralmost pallial zone between lateral pallium and dorsal striatopallidum. Its intrapallial projections are restricted to the olfactory bulbs and lateral pallium, and its extrapallial projections terminate in the neuropil of the lateral (vomeronasal) amygdala, the preoptic region and the hypothalamus, and in the subpial part of the dorsal striatopallidum.

Comparison with other studies on the anuran pallium and functional considerations

Rostral pallium. The rostral pallium has been known to receive substantial afferents from the anterior dorsal thalamic nucleus (Vesselkin et al., 1971; Kicliter, 1979; Neary, 1984; Roth et al., 2003; and unpublished data). The anterior dorsal thalamic nucleus possibly represents a mixture of traits found in various "limbic" thalamic nuclei of mammals (e.g., the anterior, dorsomedial, intralaminar, and midline nuclei), especially with respect to their multimodal-limbic projection to the orbital and medial frontal cortex (Conde et al., 1990; Nakano et al., 1999; Groenewegen and Uylings, 2000). Aside from the data on its connectivity presented here, electrophysiological and preliminary c-Fos data from our laboratory (F. Laberge, S. Mühlenbrock-Lenter, and G. Roth, unpublished data) suggest that the rostral pallium of anurans is a multimodal sensory integration center possibly with emotional-limbic and cognitive evaluatory functions. The rostral pallium of *Bombina* as well as that of the salamander *Triturus* (Sassoe-Pognetto et al., 1991) projects to the dorsal striatopallidum laterally and the nucleus accumbens/ventral striatopallidum medially. In amphibians, these regions project to the dorsal and ventral thalamus with back-projections, via anterior and central dorsal thalamus, to the pallium and dorsal striatopallidum, respectively (Roth et al., 2003). Thus, as in mammals, a dorsal and a ventral "executive loop" may exist in anuran amphibians, but this speculation requires further investigation.

Medial pallium. Our findings support the assumption that the dorsomedial telencephalic sulcus roughly marks the border between medial and dorsal pallium (Hoffman,

1963; Northcutt, 1974; Scalia et al., 1991). After application of tracer to the medial pallium, labeled neurons form a rather sharp border at the level of the dorsomedial sulcus (S. Mühlenbrock-Lenter, unpublished observation). For the anuran medial pallium, authors distinguish either two subdivisions, viz. a ventral small-celled part and a dorsal large-celled part (Röthig, 1912; Hoffman, 1963), or three subdivisions, viz. a small-celled part, a large-celled part, and a transitional part (Neary, 1990). The medial pallium of *Bombina* does not reveal such differences in cell size between a dorsal and a ventral region, as is the case in the frog species *Ascaphus* (Neary, 1990) and *Discoglossus pictus* (Westhoff and Roth, 2002). In the frog *Discoglossus pictus*, three types of medial pallial neurons were identified (Westhoff and Roth, 2002): 1) neurons in the ventral medial pallium with bilateral projections to telencephalic areas, including septum, amygdala, and striatum, and areas in the secondary prosencephalon and diencephalon, including the preoptic area, hypothalamus, and anterior dorsal and ventral thalamus; 2) neurons in the dorsal medial pallium with projections to the contralateral medial pallium and only ipsilateral projections to the dorsal and lateral pallium, septum, nucleus accumbens, amygdala, preoptic area, hypothalamus, and anterior dorsal and ventral thalamic nucleus; and 3) neurons at the border between medial and dorsal pallium with ipsilateral and contralateral projections to the medial and dorsal pallium, ipsilateral projections to the septum, and no extratelencephalic projections. A single neuron was found in the ventralmost portion of the medial pallium that exhibited no contralateral projections. A spatial separation of neurons with different bilateral projection patterns was not found in *Bombina*, but we confirm that neurons in the ventral portion of the medial pallium lack contralateral as well as extratelencephalic projections. Axons of most medial pallial neurons and of some dorsal pallial ones heavily arborize in the zona intercalaris and the dorsal portion of the septal region. Recent gene marker labeling suggests that a small dorsal part of the avian and mammalian septum is of pallial rather than subpallial origin (Puelles et al., 2000), and this seems to apply to the septum of amphibians as well (Moreno et al., 2004).

Most authors interpret the medial pallium as homologous to Ammon's horn and subiculum of the mammalian hippocampus (for a detailed discussion see Westhoff and Roth, 2002). Strong reciprocal connections of the medial pallium with the septal nuclei (cf. Endepols et al., 2005; Roden et al., 2005) resemble the mammalian septohippocampal system, especially insofar as it has been shown that anurans possess a cholinergic basal forebrain system (González and Lopez, 2002). There is little doubt that at least the dorsal and intermediate portions of the anuran medial pallium correspond to Ammon's horn of the mammalian hippocampus, but it must remain undecided whether the ventral portion of the anuran medial pallium corresponds more to the subiculum (more likely) or more to the dentate gyrus of the mammalian hippocampus.

The amphibian medial pallium, apparently mostly its rostral part, appears to be involved in memory extinction, behavioral flexibility, and inhibition of behavior (Finkenstädt and Ewert, 1988; Wenz and Himstedt, 1990; Papini et al., 1995; Ewert et al., 2001), although convincing evidence for an involvement in learning and memory formation is lacking. Extra- and intracellular recordings from

neurons of the medial pallium in frogs after stimulation of visual, somatosensory, and olfactory afferent pathways reveal only multimodal response properties (Supin and Guselnikov, 1965; Karamian et al., 1966; Mudry and Capranica, 1980; Laberge and Roth, in press).

Dorsal pallium. Our cytoarchitectural and connectional data suggest that the border between dorsal and lateral pallium is marked by the rhinal sulcus. In *Discoglossus pictus* (Westhoff and Roth, 2002) as well as in *Bombina*, intracellular biocytin injections revealed no extratelencephalic and only ipsilateral intratelencephalic-intrapallial projections of dorsal pallial neurons. Axons form a dense meshwork confined to the central layer of the dorsal pallium and adjacent pallial regions forming a massive intrapallial associative network. The function of the dorsal pallium is unclear. It receives, especially in its rostral part, afferents from the anterior dorsal thalamic nucleus but lacks direct extratelencephalic efferents, except to the eminentia thalami. Thus, we interpret the dorsal pallium as exerting associative functions similar to allocortical areas of the mammalian brain (e.g., cingulate gyrus). Here, further research is needed.

Lateral pallium. Traditionally, the lateral pallium was divided into a dorsal and a lateral portion (cf. Kicliter and Ebbesson, 1976) separated by the sulcus entorhinalis/lateral cellular prominence. However, recent gene expression data corroborate the view that these parts have to be considered separate types of pallium called *lateral* and *ventral pallium* (cf. Fernandez et al., 1998; Puelles et al., 2000; Puelles, 2001; Brox et al., 2004). Our data are in line with this view, insofar as the lateral and ventral pallium exhibit different cytoarchitectures and projection patterns: the lateral, but not the ventral, pallium is closely connected to the septum and medial and dorsal pallium. Also, the lateral pallium appears to continue caudally into the caudal lateral pallium containing the main olfactory amygdala and "entorhinal pallium," whereas the ventral pallium merges with the lateral (vomeronasal) amygdala.

The anterior lateral pallium is crossed by the lateral olfactory tract, which gives off numerous collaterals. Accordingly, it can be considered an olfactory pallium. The caudal lateral pallium has to be considered a pallial zone of its own, because in its anterior part it contains the main olfactory amygdala (of lateral pallial origin) and in its posterior part a primary olfactory region probably corresponding to the mammalian entorhinal cortex. This latter region has a particularly dense projection to the ventral and intermediate medial pallium. Also, the medial pallium projects back to the lateral pallium. Furthermore, it distinctly projects through the diagonal band of Broca to the rostral medial pallium called fimbrial tract by Herrick (1934), which may be homologous to the angular bundle in mammals. During embryogenesis, a robust direct projection (the angular bundle) between the presumptive entorhinal and the hippocampal cortex develops (and partially disappears later) long before the perforant pathway appears (Snyder et al., 1991).

Ventral pallium. The accessory (vomeronasal) olfactory tract runs through the cellular layer of the ventral pallium, giving off collaterals on its way to the vomeronasal amygdala, where it forms a dense terminal neuropil. The ventral pallium terminates at the level of the lamina terminalis and the pallial commissures and merges with the vomeronasal amygdala (cf. Roth et al., 2004). Ventral pallial neurons project massively to the main and acces-

sory olfactory bulb, to the lateral subpial part of the dorsal striatopallidum, and to the neuropil adjacent to the lateral (vomeronasal) amygdala/preoptic area as well as to the hypothalamus. It appears that this region forms the rostral extension of an extended vomeronasal amygdala as discussed previously (Marín et al., 1998; Laberge et al., 2006). Extratelencephalic afferents are proposed to originate from the preoptic area, hypothalamus, anterior and central dorsal thalamus (weak), and tegmental parabrachial nucleus (Moreno and González, 2004). Moreno and González (2004) consider the rostral ventral pallium/SPTA homologous to the mammalian anterior amygdala, the caudal ventral pallium homologous to the medial amygdala, and the dorsal part of the ventral pallium homologous to parts of the mammalian basolateral complex. In the present study, however, we were unable to detect major differences in morphology and projection pattern between the dorsal and the ventral parts of the ventral pallium, which speaks against this assumption.

Cytoarchitecture and intrapallial projection pattern of pallial neurons

The morphology of neurons situated in the medial, dorsal, and lateral pallium is very homogeneous, as opposed to the morphological variability of neurons in other parts of the brain such as the tectum, the torus, and the thalamus. As mentioned above, usually three or four primary dendrites originate from the round or pear-shaped soma and quickly divide into two or three secondary dendrites that extend toward the surface in a fan-like fashion (cf. Figs. 3, 4A,B, 5, 6B, 7). The only major variation on this type consists of the dendritic trees widen and flatten with increasing distance of the soma from the ventricle such that migrated neurons often have spindle-shaped somata and two primary dendrites that extend parallel to the surface of the telencephalon. Only the morphology of some neurons in the rostral pallium somewhat deviates from this type in the sense that neurons found at the very rostral pole of the telencephalon exhibit very widespread and rather "disorganized" dendritic trees (cf. Fig. 2A). Pallial dendrites are usually covered with spines, but the degree of coverage varies strongly even within one pallial area. On average, neurons in the medial and dorsal pallium are more strongly covered with spines than in the other pallial areas.

As can be seen in Figure 9, the overlap of dendritic trees (rectangle) is enormous in all pallial areas. The dendritic tree of one pallial neuron exhibits on average about 400 μm of rostrocaudal extension, which is roughly 17% of the total length of the pallium. Neurons with small dendritic fields are very rare in the pallium of *Bombina orientalis*. The intrapallial rostrocaudal extension of axonal projections of pallial neurons (Fig. 9, vertical lines) is on average about 1,000 μm in the rostral, medial, dorsal, and dorsal lateral pallium and about 1,500 μm in the ventral lateral and ventral pallium, such that one neuron covers about one-half of the length of the pallium. Axon collaterals of some pallial neurons even cover the entire length of one hemisphere. All this gives the impression that the amphibian pallium is a large network of great cellular homogeneity—at least at a gross level—and a high degree of internal connectivity.

One astonishing fact is that the large pallial regions receive relatively little input and send relatively little output to the rest of the brain. In amphibians, two tha-

lamic nuclei have been identified that project to the pallium, viz. the anterior dorsal thalamic nucleus (Vesselkin et al., 1971; Kicliter, 1979; Neary, 1984, 1990; Roth et al., 2003) and the eminentia thalami/bed nucleus of the pallial commissure (Roden et al., 2005). The rostral medial pallium is the main target of the projections of the anterior dorsal thalamic nucleus, followed by the rostral dorsal pallium. In *Bombina*, the lateral pallium receives a weaker thalamic input. In most cases, one thalamic neuron sends several axon collaterals to the pallium such that the area of thalamic input to the pallium usually is very large (Roth et al., 2003). In addition, the hypothalamus projects to the caudal medial pallium (Neary, 1990), and there are projections from the locus coeruleus and the raphe nuclei to the medial pallium (Northcutt and Ronan, 1992). Among the pallial regions receiving thalamic input, only the medial pallium sends substantial projections outside the telencephalon. Dorsal and lateral pallium have only indirect extratelencephalic efferents via their projections to the medial pallium, septum, nucleus accumbens, and ventral striatopallidum as well as via their projections to the ventral pallium. The latter send projections to the preoptic area and hypothalamus. A minor number of pallial neurons projects to the eminentia thalami/bed nucleus of the pallial commissure, which in turn projects to the habenula, to the dorsal and ventral thalamus, and back to the medial pallium and septum (Roden et al., 2005).

The dorsal striatopallidum is the origin of the most massive descending telencephalic pathway terminating in the tegmentum, medulla oblongata, and rostral spinal cord (Marín et al., 1997; Roth et al., 2004). It receives input from central dorsal thalamic nucleus via the lateral forebrain bundle, which is more massive than the projections of the anterior dorsal thalamic nucleus to the pallium (Roth et al., 2003). The connection between pallium and striatopallidum is remarkably limited. Neurons in the dorsal and lateral parts of the rostral pallium project to the rostral dorsal edge of the striatum, and neurons in the ventral pallium send dendrites and axons into the region between the striatal neuropil and the lateral surface of the telencephalon, where the latter probably contact the distal dendrites of striatal neurons. This should be studied in greater detail.

Thus, we find three direct pallial pathways to targets outside the telencephalon: 1) of the medial pallium to the preoptic region, ventral thalamus, and hypothalamus; 2) of the ventral pallium to the same targets; and 3) of the medial and dorsal pallium to the eminentia thalami/bed nucleus of the pallial commissure, which in turn projects mostly to the habenula and back to the medial telencephalon. All these targets are limbic centers, the preoptic area and hypothalamus being implicated in behavioral and neuroendocrine control. This—in addition to the massive projections of the medial, dorsal, and lateral pallium to the septum, nucleus accumbens, and amygdala—corroborates the concept that the amphibian pallium has predominantly limbic-evaluatory and associative functions. However, it is in a position to influence directly via the rostral and ventral pallium and indirectly, via the amygdala, the telencephalic sensorimotor (or executive) output system in the dorsal striatopallidum. The dorsal striatopallidum projects to, among other areas, the ventral and, although sparsely, the dorsal thalamus such that something like a "dorsal sensorimotor loop" may exist in

the amphibian forebrain connecting pallium, striatopallidum, and thalamus in a reentrant fashion. This hypothesis is being studied in greater detail in our laboratory

The amphibian pallium generally reveals a two-layered structure, i.e., a periventricular cellular layer of varying thickness and a superficial fiber layer. The pallium of many anuran species, especially those with relatively small cells such as ranid, bufonid, or leptodactylid frogs, exhibits extensive cell migration and the formation of single-cell laminae in the medial pallium, whereas the—mostly small—dorsal pallium and the larger lateral pallium show either no or only weak lamination, which in no way is comparable to the situation found in the mammalian isocortex. Not surprisingly, therefore, this lack of multiple lamination in the dorsal pallium has been traditionally interpreted as the most primitive state of the pallium among vertebrates (cf. Herrick, 1948; Leghissa, 1962; Northcutt and Kicliter, 1980).

However, at the same time, it is assumed that many traits of the amphibian brain have undergone secondary simplification (for detailed discussion see Roth et al., 1993, 1997; Dicke and Roth, 2006). Therefore, an important question is whether the amphibian pallial regions represent the ancestral condition or a secondary simplification. This question can be answered only by a phylogenetic comparative analysis. Such an analysis is hindered by the fact that the dipnoans, forming the outgroup of tetrapods, themselves are secondarily simplified (Northcutt, 1987). The pallium of *Latimeria*, representing the sister group of dipnoans, is assumed not to be secondarily simplified but likewise gives a primitive appearance (cf. Nieuwenhuys, 1998). This could mean that the pallium of amphibians is primitive and not secondarily simplified or pedomorphic. However, for the pallium of dipnoans, we find a lamination similar to the situation found in the medial dorsal cortex of turtles and lizards (Nieuwenhuys et al., 1998). Therefore, we can speculate that some pattern of lamination in the medial and dorsal pallium is a plesiomorphic feature of sarcopterygians and all tetrapods and was lost in amphibians (cf. Dicke and Roth, 2006).

If we consider the amphibian pallium as having evaluatory-limbic functions, it should be compared with the mammalian limbic allocortex and not with the isocortex. The allo- or mesocortex of amniotes, as found in turtles or in the mammalian hippocampus, is three-layered, with a superficial fiber (plexiform or molecular) layer receiving—as in amphibians—afferents from the olfactory tracts and from subcortical limbic centers and containing the apical dendrites of allocortical neurons, a cellular layer containing mostly pyramidal cells and a periventricular fiber layer formed mostly by the basal dendrites of allocortical neurons. An isocortical layer III or IV receiving thalamic afferents is missing (Valverde et al., 2002; Miller and Maitra, 2002). The two-layered amphibian pallium can be conceived as developing into the amniote three-layered allo- or mesocortex by the addition of a periventricular fiber layer, which already has happened in parts of the amphibian brain, e.g., in the thalamus or torus semicircularis.

ACKNOWLEDGMENTS

The Journal of Comparative Neurology. DOI 10.1002/cne

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