

## A Forebrain Visual Projection in the Frog (*Rana pipiens*)

EDWARD R. GRUBERG AND VICTOR R. AMBROS<sup>1</sup>

*Research Laboratory of Electronics and Department of Biology,  
Massachusetts Institute of Technology,  
Cambridge, Massachusetts 02139*

*Received March 8, 1974*

In the frog (*Rana pipiens*) using a modification of the Fink-Heimer degeneration stain, a projection was traced from the lateral anterior thalamus via the lateral forebrain bundle to the ipsilateral striatum in the ventrolateral area of the forebrain. Striatal degeneration extended from the anterior commissure to the olfactory bulb. Single-unit microelectrode recording revealed that this area contained visual units that responded to the on and off of light.

### INTRODUCTION

It has been generally accepted that in vertebrates below the reptiles, the cerebral hemispheres are under the domain of the olfactory system (2, 8). The main thrust of the argument is that other than olfaction, no exteroceptive system has a localized presence in the forebrain. However, this view has been questioned recently by Ebbesson and Schroeder (5), who have shown in the nurse shark the presence of an ascending thalamotelencephalic tract which terminates in a "large well-defined region," a region which has almost no direct olfactory bulb contribution.

In this paper we trace a thalamotelencephalic projection in the frog *Rana pipiens*, using a modification of the Fink-Heimer stain for degenerating fibers, and we show by microelectrode recordings that units in this region of the forebrain are driven by visual stimuli.

<sup>1</sup> This work was supported in part by a grant from the Bell Telephone Laboratories, Inc., in part by the National Institutes of Health (Grant 5 P01 GM14940-06) and in part by NIGMS (Grant 5 T01 GM01555-06). We thank Dr. J. Y. Lettvin and Eric Newman for critical reading of the text, Mr. Joel Rosenberg for technical assistance, Ms. Janet Faulkner for histological assistance, and Ms. A. P. Chase for help in preparing the manuscript.

## METHODS AND MATERIALS

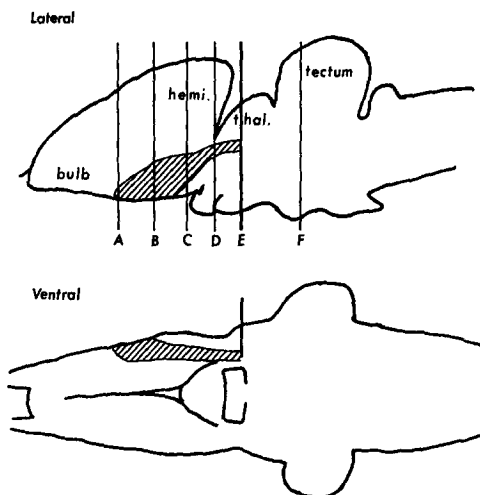
*Scalpel Lesions.* Leopard frogs (*Rana pipiens*), 48–60 mm (20–30 g), were obtained from Vermont. Under Finquel (Ayerst) anesthetic, the diencephalon was exposed by cutting through a portion of the frontoparietal bone using a dental drill. Unilateral scalpel lesions were made in the anterior thalamus in a set of frogs which were then maintained at 20–21 C postoperatively for 5, 6, 7, 8, 9, 10, and 14 days. The brains were then removed after decapitation and fixed for a day in 4% buffered neutral formalin. The brains were prepared, sectioned, and stained, using a modification of the Fink–Heimer stain (7).

In order to determine the caudal extent of fibers contributing to the forebrain projection, unilateral scalpel lesions were made in the posterior thalamus and in the anterior mesencephalon.

*Electrolytic Lesions.* Smaller electrolytic lesions were made with alloy-filled, platinum-tipped electrodes (3) in various regions of the anterior dorsal thalamus from which visual responses could be elicited. Direct current of 10  $\mu$ a was passed through the recording electrodes (electrode negative) for 40 sec. Nine animals were used, and they were maintained postoperatively for periods of 6–9 days. Their brains were processed and stained as described above.

*Forebrain Recordings.* Animals were placed under Finquel anesthetic, and using a small dental drill, a part of the frontoparietal bone was cut out exposing the cerebral hemispheres. The animals recovered from the anesthetic in 30–60 min sufficiently for them to have breathing movements, optokinetic responses, and an “awake” posture. The animals were then lightly paralyzed with tubocurarine chloride (3 mg/cc, Squibb), usually 0.15 mg for a 25-gm frog. We have found it critical to keep the curare level to a minimum; that is, the animals should be passive but still have breathing movements, otherwise few forebrain units are recordable. With forelegs at their sides and hind legs extended, the animals were then “swaddled” in a damp gauze covering the whole extent of the animal caudal to the ears. Tape covering the snout (with holes for the nostrils) held the animal in position. This procedure was sufficient to keep the frog passive for several hours. Animals then unswaddled after an experiment had sufficient tone to jump away. As viewed through a dissecting microscope, circulation over and into the brain was vigorous.

We used both platinum-plated, alloy-filled microelectrodes and insulated stainless-steel microelectrodes (6) connected to a high-input impedance a-c-coupled amplifier to record single units. For the purpose of these experiments the units were considered “visual” if they responded directly to the on and off of light (room lights, lamps, flashlights).



The following abbreviations are used in this and succeeding Figures: a.v., angulus ventralis; b., nucleus Bellonci; c.g., corpus geniculatum; h., hippocampus; hemi., cerebral hemisphere; l.f.b., lateral forebrain bundle; l.g., lateral geniculate nucleus; p., pallium; p-c, posterocentral nucleus; p-l, posterolateral nucleus; r., nucleus rotundus; s., septum; s.l.h., sulcus limitans hippocampi; s.p.d., dorsal striatum; s.p.v., ventral striatum; st., striatum; thal., thalamus; v-l, venterolateral area; z.l.l., zona limitans lateralis.

FIG. 1. The distribution of the rostral degeneration (shading) caused by a unilateral lesion at the plane of line E. Degeneration is projected onto lateral and ventral views. Alphabetical lines show the level of the transverse sections of Fig. 2. Line A is immediately caudal to the olfactory bulb.

The locations of forebrain visual units that had spike height-to-background noise ratio of at least two, were determined from electrolytic lesions made with the recording electrodes. For platinum-tipped electrodes a direct current (electrode negative) of  $10 \mu\text{a}$  was passed for 20 sec. For steel electrodes a direct current (electrode positive) of  $10 \mu\text{a}$  was passed for 5 sec. The brains were removed immediately after the experiment and fixed overnight in 4% buffered neutral formalin, dehydrated, embedded in paraffin, and sectioned at  $15 \mu\text{m}$ . Brains with steel-electrode lesions were first stained using Mallory's (Prussian blue) method for iron (13). All brains were stained in 0.1% cresyl violet for 10 min. Diameter of lesions from both types of electrodes average  $60\text{--}100 \mu\text{m}$ .

To check for possible retinotopic projection from this visual area, an opaque hemisphere of 17 cm radius was centered on one eye. Small visual stimuli could be manipulated from behind the hemisphere by magnets (14).

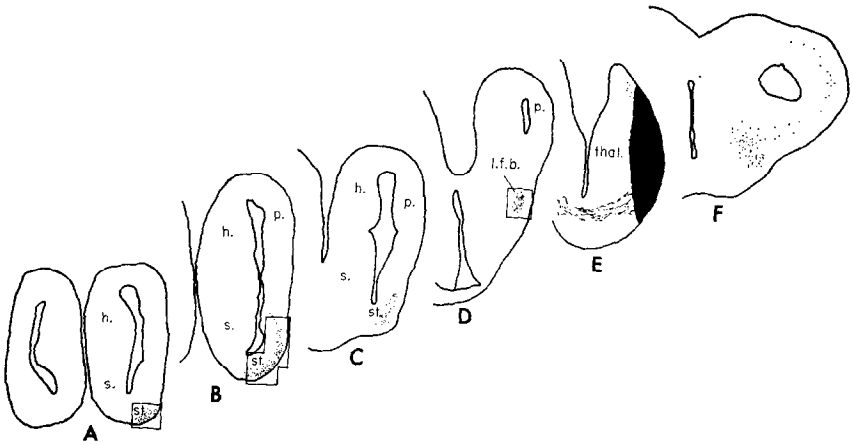


FIG. 2. Selected camera-lucida drawings of transverse sections through the brain showing 6-day degeneration (stippling) caused by a unilateral scalpel lesion in the anterior thalamus. Each section corresponds to the plane of the alphabetical lines of Fig. 1. Extent of lesion is shown in black in Section E. The boxed areas of sections A, B, and D correspond to the photomicrographs of Figs. 6, 7, and 8, respectively. A photomicrograph of the lesion is shown in Fig. 9.

## RESULTS

Unilateral scalpel lesions in the anterior lateral thalamus led to circumscribed degeneration in the ipsilateral striatal (ventrolateral) area of the cerebral hemispheres. The extent of the lesions which produced striatal degeneration is shown in Fig. 3A. In addition, all electrolytic lesions that included some part of the lateral forebrain bundle showed striatal degeneration

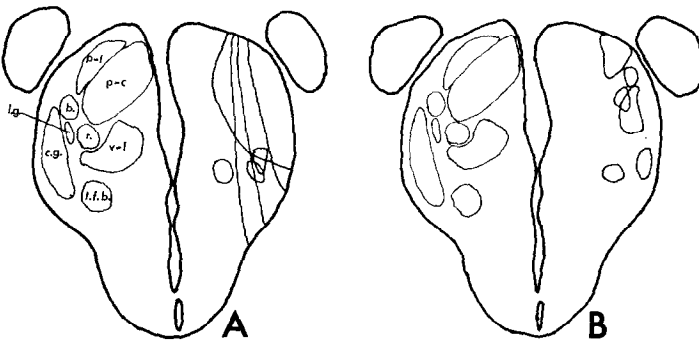


FIG. 3. A. Transverse section of the anterior thalamus showing on the left side the normal thalamic divisions and on the right side a montage of the distribution of lesions causing degeneration in the striatum. B. The same transverse section as A, showing on the right side a montage of the set of lesions that did not cause degeneration in the striatum.

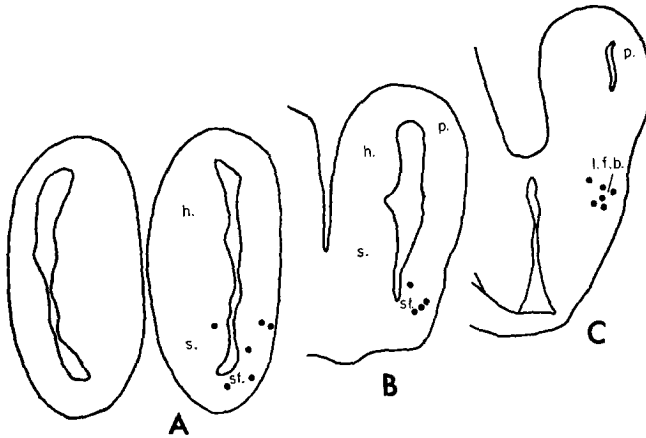


FIG. 4. The distribution of forebrain electrolytic lesions (dots) normalized into three sections. Section A is in a plane through the anterior striatum, section B is through the posterior striatum, and Section C is through the caudal pole of the forebrain. Lesions were made with microelectrodes after recording light-on/off units. Fifteen of the 16 lesions were found in either the striatum or the lateral forebrain bundle.

tion. Smaller electrolytic lesions in the nucleus Bellonci and geniculate body do not of themselves lead to degeneration in the striatum.

Dense, uniform degeneration made up of fine particles is first seen in the striatum at 6 days postoperatively and is presumed to show mostly terminal boutons (Figs. 6, 8). Degeneration can be traced from the anterior lateral thalamus via the lateral forebrain bundle. This shows that even at earliest times some fibers of passage also stain. At approximately the level of the anterior commissure the degeneration begins to spread out, and slightly

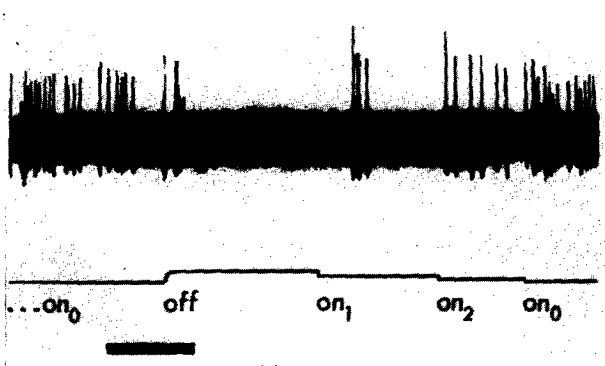


FIG. 5. Multiunit recording from the striatum in which the room lights have been on for several minutes ( $on_0$ ). The room is darkened at off causing an inhibition. The light is turned on in steps ( $on_1$ ,  $on_2$ ,  $on_0$ ) back to the original illumination. Light level monitored by silicon solar cell (lower trace). Scale 1 sec.

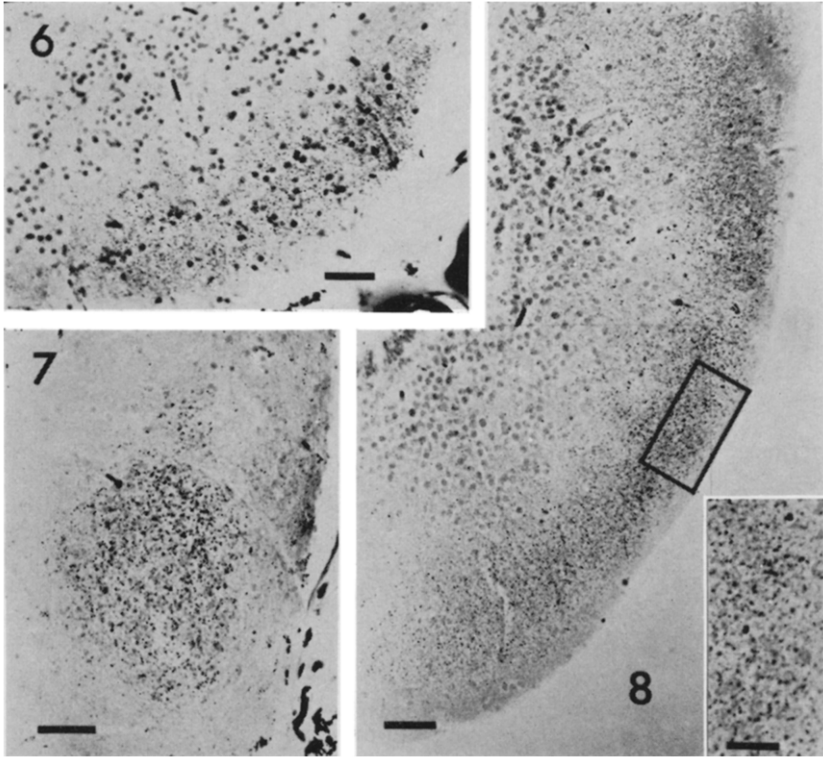


FIG. 6. Photomicrograph of 6-day striatal degeneration of Fig. 2A at the extreme rostral end of the striatum. Scale 50  $\mu$ m.

FIG. 7. Photomicrograph of 6-day lateral forebrain bundle degeneration of Fig. 2D. Scale 50  $\mu$ m.

FIG. 8. Photomicrograph of 6-day striatal degeneration of Fig. 2B. Scale 50  $\mu$ m. Inset: Higher magnification of boxed area. Scale 25  $\mu$ m.

more rostral it is seen over most of the neuropil of both and the dorsal and ventral striatum (Figs. 1, 2, 6, 7, 8, 9). It extends medially to the level of the angulus ventralis and dorsally as high as the lateral limiting zone. In the most dorsal part of the area, degeneration is restricted to the inner part of the neuropil and does not extend to the surface. The area of degeneration extends rostrally to the caudal border of the olfactory bulb. Little degeneration is seen earlier than 6 days. By 14 days postoperatively, fine-particle degeneration cannot be seen, but coarser particles, presumably mostly fibers of passage, can still be traced from the anterior thalamus via the lateral forebrain bundle to the striatum (Fig. 10).

No lesions caudal of the anterior thalamus produce the striatal degeneration described above. A hemisection of either the posterior thalamus or the anterior mesencephalon leads to degeneration in a thin ventrolateral lamina

which extends no further rostral than the extreme caudal striatum. This pathway is via the ventromedial margin of the lateral forebrain bundle in the thalamus and shifts to the ventromedial of the bundle as it enters the forebrain (Fig. 11). Thus, we can conclude that the anterior thalamus is the origin of the described striatal degeneration. From the lesions in the thalamus, both those that lead to striatal degeneration and those that do not (Fig. 3), it is seen that the fibers arise from a diffuse part of this region.

In two specimens in which more dorsal areas of the thalamus were included in the unilateral lesions, degeneration was seen in the medial forebrain bundle. Some fibers of the medial forebrain bundle decussate in the anterior commissure and thence terminate in a bilateral projection in the septum.

Caudal to the thalamic lesions, ipsilateral degeneration was seen in the tectum in the same layers as retinotectal fibers, and, indeed, some optic tract fibers were cut in most scalpel lesions. Ipsilateral degeneration was also seen in the ventrolateral area of the tegmentum. Several of our specimens showed degenerating fibers running into the optic nerve. But in other work (unpublished), we have not been able to trace these fibers into the retina.

Our light-on/off criterion for visual units was sufficient to provide an unequivocal indication that we were recording from the striatal area. Of 16 electrolytic lesions made to mark recording sites, 15 were found in the striatum (Figs. 3, 12). The lone lesion not found in the striatum was obtained from an animal that moved while current was being passed. A more detailed characterization of these visual units will be discussed in a later paper, but in general the units we have recorded responded to the *on* of light and were inhibited transiently by the *off* of light (Fig. 5). Figure 12 shows a lesion made during the ventral descent of an electrode on first encountering units that responded to light-on/off. This lesion is coincident with the dorsal boundary seen in the degeneration described above. In addition, we recorded from the hippocampus and the septum but were unable to drive any units by light-on/off or any other visual stimulus that we tried (moving edges, spots, etc.).

No obvious retinotopic projection was found in the striatal area. The receptive fields of the units were all quite large, at least 50° to 60° and some much wider. In multiunit recordings responses could be elicited over most of the monocular visual field. Movement of small objects in successive, different directions and locations seemed best for eliciting vigorous responses. Repeated stimuli either through movement of objects of light-on/off led to rapid habituation. We must emphasize again that, although our criterion of light-on/off was sufficient to identify the striatal area, the units were much more complex and intriguing. No units in this area showed any obvious responses to touch or to auditory stimuli.

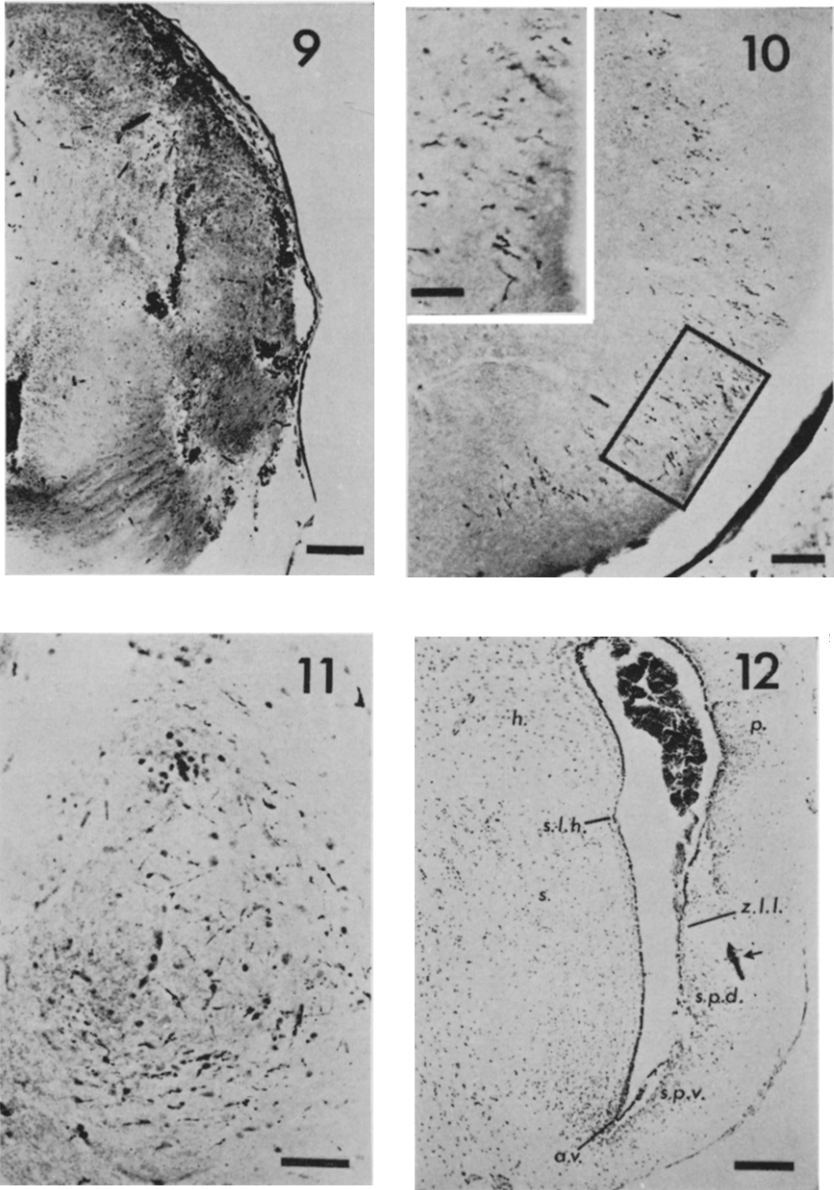


FIG. 9. Photomicrograph of 6-day anterior thalamic lesion of Fig. 2E. Scale 200  $\mu$ m.

FIG. 10. Photomicrograph of 14-day striatal degeneration of an area on a level with that of Fig. 8. Scale 50  $\mu$ m. Inset: Higher magnification of boxed area. Note coarser degeneration than in Fig. 8. Scale 25  $\mu$ m.

FIG. 11. Photomicrograph of 8-day lateral forebrain bundle degeneration at the diencephalon/forebrain boundary. Ipsilateral rostral mesencephalic scalpel lesion. Degeneration is restricted primarily to ventral margin. Scale 50  $\mu$ m.



## DISCUSSION

Visual activity in the frog forebrain has been mentioned by previous investigators. Liege and Galand (12) briefly described several units in the forebrain at the posterior pole of the telencephalon. Six of the seven units they studied were at a depth of 2000  $\mu\text{m}$ , as measured by a scale on their micromanipulator. The units had large receptive fields, were monomodal, were inhibited by dark and excited by light and, in general, seemed in congruence with the units we recorded. Boldyreva and Grindel (1) recorded slow waves in the forebrain driveable by photic stimuli. Karamian *et al.* (10) stimulated the optic nerve and thalamus and found slow-wave responses in the hippocampus, but they were "extremely inconstant, their amplitude was variable and considerable fatigue was displayed on rhythmical stimulation." Zagorulko (18) found slow-wave responses in the contralateral forebrain after photic stimulation.

Vesselkin *et al.* (17) described making "simultaneous unilateral lesions of the caudal region of postcentral and posterolateral nuclei and posterior thalamic nucleus" and found degenerating fibers bilaterally in the primordium hippocampus. From similar but more rostral lesions in the dorsal thalamus, we found on two occasions degeneration going via the medial forebrain bundle and projecting bilaterally not to the hippocampus, but to the septum just below it. Scalia and Gregory (16) have vividly shown the complexity of the distribution of dendrites of the frog thalamic nuclei. They described, for instance, that cells from the lateral geniculate, nucleus rotundus, posterocentral nucleus, posterolateral nucleus, and ventrolateral area all have dendrites located in regions where retinal fibers could terminate. Thus, from our lesions, we cannot say more than that the lateral anterior thalamus is the origin of most of the striatal projection.

Rubison and Colman (15), in a preliminary paper, described bilateral degeneration in the frog striatum after making one lesion in the mid-mesencephalon at the lateral edge of the central gray. We find that hemisections of the rostral mesencephalon lead only to degeneration in a ventrolateral lamina in the ipsilateral caudal striatum. It is interesting to note that, in the alligator, after lesions of the entire general cortex and rostral portion of the lateral forebrain bundle, retrograde degeneration was seen in the nucleus rotundus and medialis anterior, but no degeneration was observed in the lateral geniculate body (11).

At this point, however, it is too early to seek homologies between thalamo-forebrain projections in the frog and those in higher animals. It

---

FIG. 12. Site of an electrolytic lesion (arrow) made with steel microelectrode, using Mallory's (Prussian blue) stain. The lesion was made upon first encountering light-on/off units as the electrode was advanced ventrally. The position of the lesion corresponds to the dorsal border of the degeneration seen in Fig. 8. Scale 200  $\mu\text{m}$ .

is striking that a circumscribed area of the forebrain, the striatum, with an input from the thalamus responds in a unique way to visual stimuli. It brings into question the notion of the mammalian "neocortex," which is predicated on the assumption that it is a new area of the brain, containing separate sensory areas unknown in lower forms. "Neocortex" perhaps should have utility only as a cytoarchitectonic description. This, too, seems inappropriate, for by analogy, it would seem curious on the basis of layering to call the frog superior colliculus a "neotectum" to distinguish it, say, from the salamander tectum.

Ebbeson *et al.* (4) have argued that the "olfactory representation in the diencephalon and telencephalon is probably no more extensive in non-mammalia than in mammalia." It is striking to see throughout the vertebrate subphylum the same five primary projections of the retina. With a visual projection shown in the forebrain in the frog (and others previously shown in the isthmus and rotundus), there is a possibility that secondary visual projections are also the same.

#### REFERENCES

1. BOLDYREVA, G. N., and O. M. GRINDEL. 1959. Investigation of the electrical activity of different areas of the brain in the frog. *Fiziol. Zh. SSSR. I. M. Sechenov* **45**: 1035-1044.
2. DIAMOND, I. T., and W. C. HALL. 1969. Evolution of neocortex. *Science* **164**: 251-262.
3. DOWBEN, R. M., and J. E. ROSE. 1953. A metal-filled microelectrode. *Science* **118**: 22-24.
4. EBBESON, S. O. E., J. A. JANE, and D. M. SCHROEDER. 1972. A general overview of major interspecific variations in thalamic organization. *Brain Behav. Evol.* **6**: 93-130.
5. EBBESON, S. O. E., and D. M. SCHROEDER. 1971. Connections of the nurse shark's telencephalon. *Science* **173**: 254-256.
6. GREEN, J. D. 1958. A simple microelectrode for recording from the central nervous system. *Nature (London)* **182**: 962.
7. GRUBERG, E. R. 1973. Optic fiber projections of the tiger salamander *Ambystoma tigrinum*. *J. Hirnforsch.* **14**: 399-411.
8. HERRICK, C. J. 1933. The functions of the olfactory parts of the cerebral cortex. *Proc. Nat. Acad. Sci. USA.* **19**: 7-14.
9. HOFFMAN, H. H. 1963. The olfactory bulb, accessory olfactory bulb and hemisphere of some anurans. *J. Comp. Neurol.* **120**: 317-368.
10. KARAMIAN, A. I., N. P. VESSELKIN, M. G. BELEKHOVA, and T. M. ZAGORULKO. 1966. Electrophysiological characteristics of tectal and thalamo-cortical division of the visual system in lower vertebrates. *J. Comp. Neurol.* **127**: 559-576.
11. KRUGER, L., and E. BERKOWITZ. 1960. The main efferent connections of the reptilian telencephalon as determined by degeneration of electrophysiological methods. *J. Comp. Neurol.* **115**: 125-142.
12. LIEGE, B., and G. GALAND, 1972. Single-unit visual responses in the frog's brain. *Vision Res.* **12**: 609-622.

13. LUNA, L. G. [Ed.]. 1968. "Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology." McGraw-Hill, New York.
14. MATURANA, H., J. Y. LETTVIN, W. McCULLOCH, and W. PITTS. 1960. Anatomy and physiology of vision in the frog (*Rana pipiens*). *J. Gen. Physiol.* **43**: 129-175.
15. RUBISON, K., and D. R. COLMAN. 1972. A preliminary report on ascending thalamic afferents in *Rana pipiens*. *Brain Behav. Evol.* **6**: 69-74.
16. SCALIA, F., and K. GREGORY. 1970. Retinofugal projections in the frog: Location of the postsynaptic neurons. *Brain Behav. Evol.* **3**: 17-30.
17. VESSELKIN, N. P., A. L. AGAYAN, and NOMOKONOVA, L. M. 1971. A study of thalamotelencephalic afferent systems in the frog. *Brain Behav. Evol.* **4**: 295-306.
18. ZAGORULKO, T. M. 1957. On the localization of cerebral centers of the visual analyser in the frog. *Fisiol. Zh. SSSR. I. II. Sechenova* **43**: 1156-1165.