NEURONAL REGENERATION IN FROG OLFACTORY SYSTEM

P. P. C. GRAZIADEI and R. S. DeHAN. From the Department of Biological Science, The Florida State University, Tallahassee, Florida 32306

INTRODUCTION

There is increasing evidence that olfactory neurons can differentiate from the basal cells of the olfactory neuroepithelium in normal adult vertebrates (Nagahara, 1940; Andres, 1965; Thornhill, 1970; Takagi, 1971; Moulton, 1971; Graziadei, 1971, 1973; Graziadei et al., 1971; Graziadei et al., 1972). Autoradiography at both the light microscope and the transmission electron microscope levels using [H³]thymidine has been used in our laboratory and we have positively identified the labeled cells as olfactory neurons (Graziadei et al., 1971, and Graziadei, 1973).

It has been shown further that cutting of the olfactory nerve is followed by complete degeneration of all mature neurons in the olfactory mucosa (Graziadei, 1973). This degeneration, which is complete in a period of 2 wk in frogs and follows a different time schedule in other animals, is followed by an outburst of mitoses in the basal cells which eventually differentiate into fully mature neurons (Graziadei, 1973). The maturation of new neurons from basal cells occurs in frogs in a period of 6–8 wk after severing of the olfactory nerve. However, in mammals the process is more rapid and depends on many parameters such as the species of the animal, age, and environmental conditions (unpublished results).

The present experiments are concerned with the growth of axons from new olfactory neurons and the possibility that these axons cross the surgical gap resulting from the cut of the olfactory nerve and reach the olfactory bulb where they reestablish "normal" synaptic contacts within the glomeruli.

MATERIALS AND METHODS

A group of frogs (Rana pipiens) in normal adult stage of development were operated on in such a way that the left olfactory nerve was completely severed mid-way between the olfactory mucosa and the olfactory bulb. The distance between the olfactory mucosa and the front end of the olfactory bulb is approximately 2 mm in a 50-g frog. The olfactory bulb is 1.5 mm long and 0.8 mm in diameter. The section left a gap of some 300 μm between the two nerve stumps. The right olfactory nerve was left intact to serve as a control. The frogs were sacrificed over a period of time starting at 2 days postoperative survival time and then as follows: 4, 7, 12, 15, 18, 20, 25, 30, 40, 60, 80 and 100 days and 5 and 8 mo. Two frogs were operated on each time and sacrificed at the above established intervals. Appropriate specimens of tissue were obtained from the left olfactory mucosa, bulb, and proximal and distal nerve stumps. Similar specimens were also taken from the right side of each frog for control purposes. Specimens were prepared for electron microscopy by initial fixation in a mixture of 2% glutaraldehyde and 0.6% paraformaldehyde in 0.06 M sodium cacodylate buffer. This was followed by postfixation in 2% osmium tetroxide in the same buffer. Pieces of tissue not larger than 1 mm were embedded in Araldite and sectioned to 600-Å thick sections for examination with the Philips 300 electron microscope. This procedure has been described in more detail in a previous paper (Graziadei, 1973).

RESULTS AND DISCUSSION

Gross examination of frogs of periods 2–20 days showed the two nerve stumps to be still separated. A small amount of scar tissue covered the area of nerve section. Both nerve stumps and bulb showed some 20–30% decrease in size as compared to the unoperated side.

Electron microscope examination of the mucosa of 1–12-day groups showed the gradual complete disappearance of the olfactory receptors. The nerve profiles underneath the basal lamina and running in the tunica propria mucosae exhibited extensive degeneration (Figs. 1 and 2) which
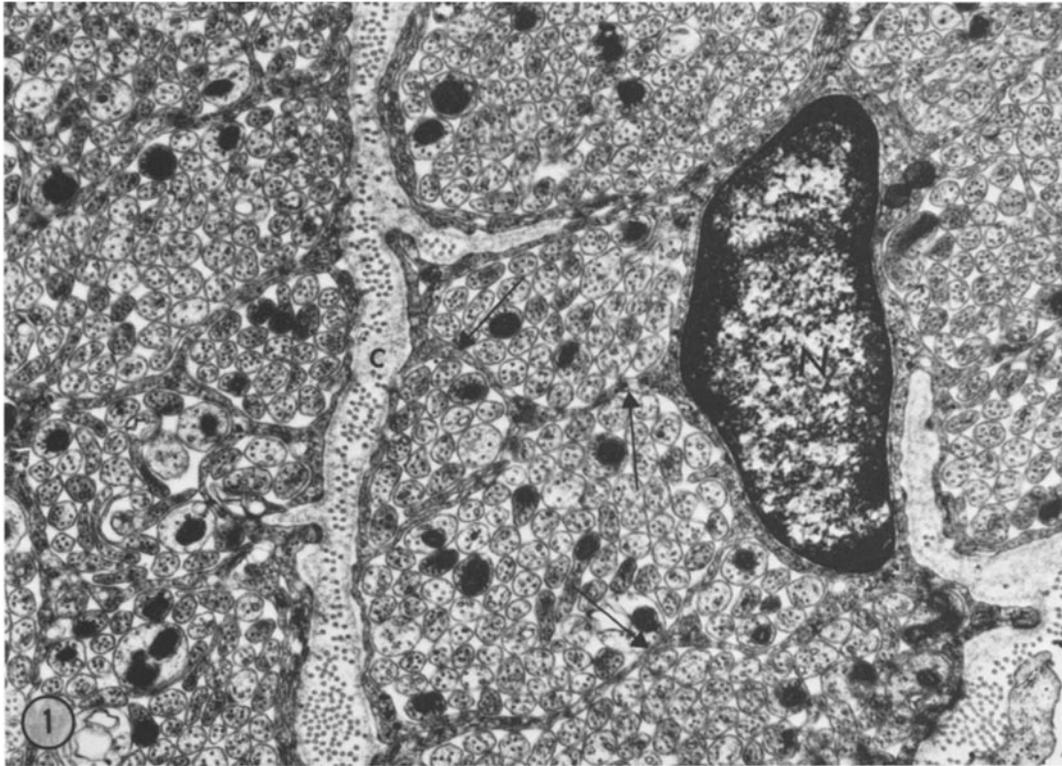
FIGURE 1   Electron micrograph of a portion of a normal olfactory nerve bundle. The olfactory axons are enclosed in pocket-like arrangements of the Schwann cells (*N*) cytoplasm. Their profiles are indicated in this picture by arrows. Between one Schwann cell domain and the other, narrow spaces are filled with collagen fibers (*c*) cut in cross section in this preparation. Magnification × 48,500.
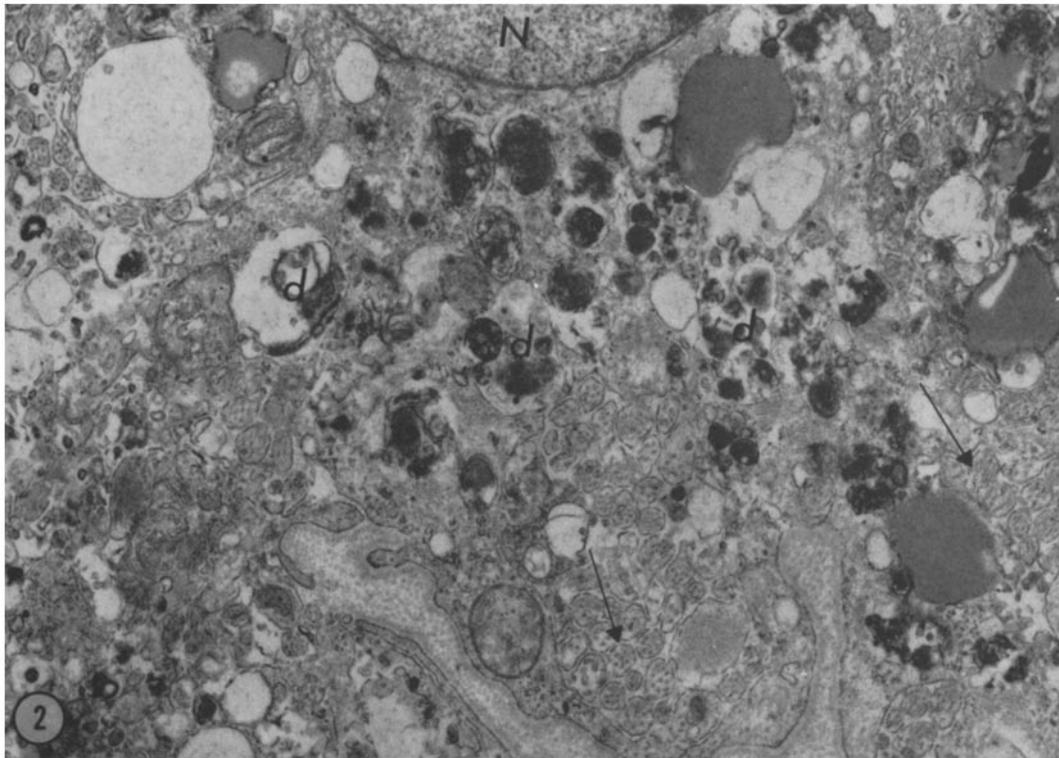

FIGURE 2   Electron micrograph of a portion of a degenerated olfactory nerve bundle, 7 days after nerve section. Notice the abundance of degenerating nerve profiles (*d*). Some axons, however, still retain an intact membrane (arrows). Compare with Figs. 1 and 5. Magnification × 38,500.
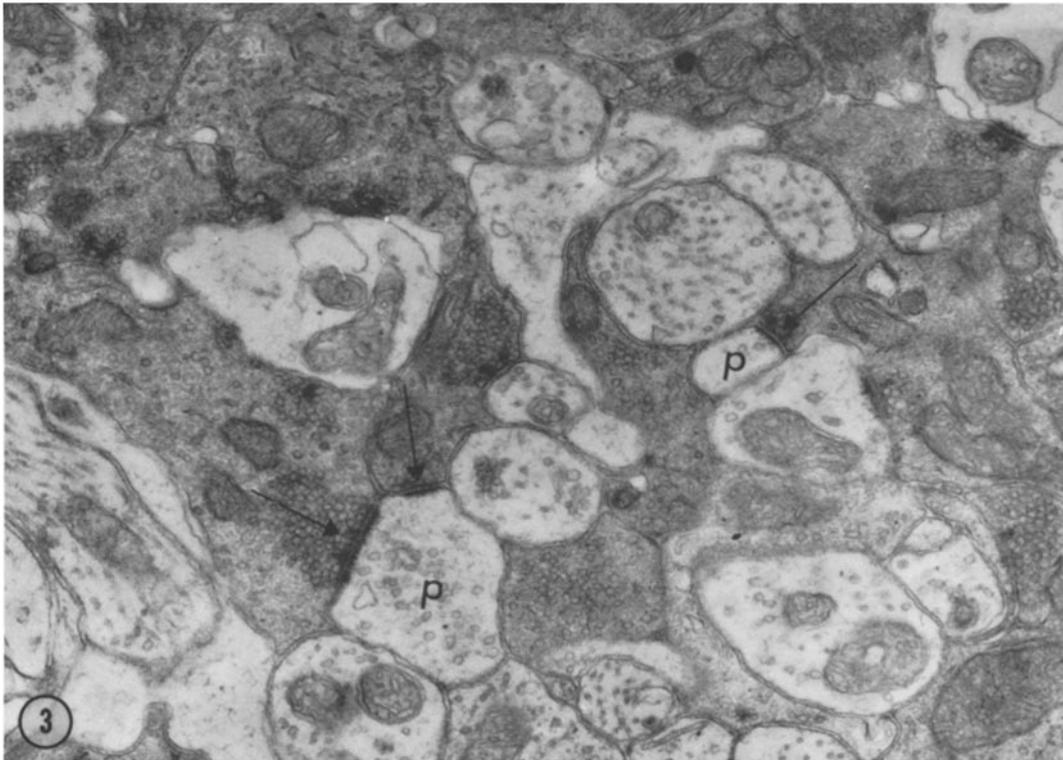
FIGURE 3 Electron micrograph of a detail of a normal olfactory glomerulus. Dense profiles filled with vesicles are the terminals of the olfactory axons and three of them are indicated by arrows. The post-synaptic profile is indicated at (p). Magnification × 48,500.
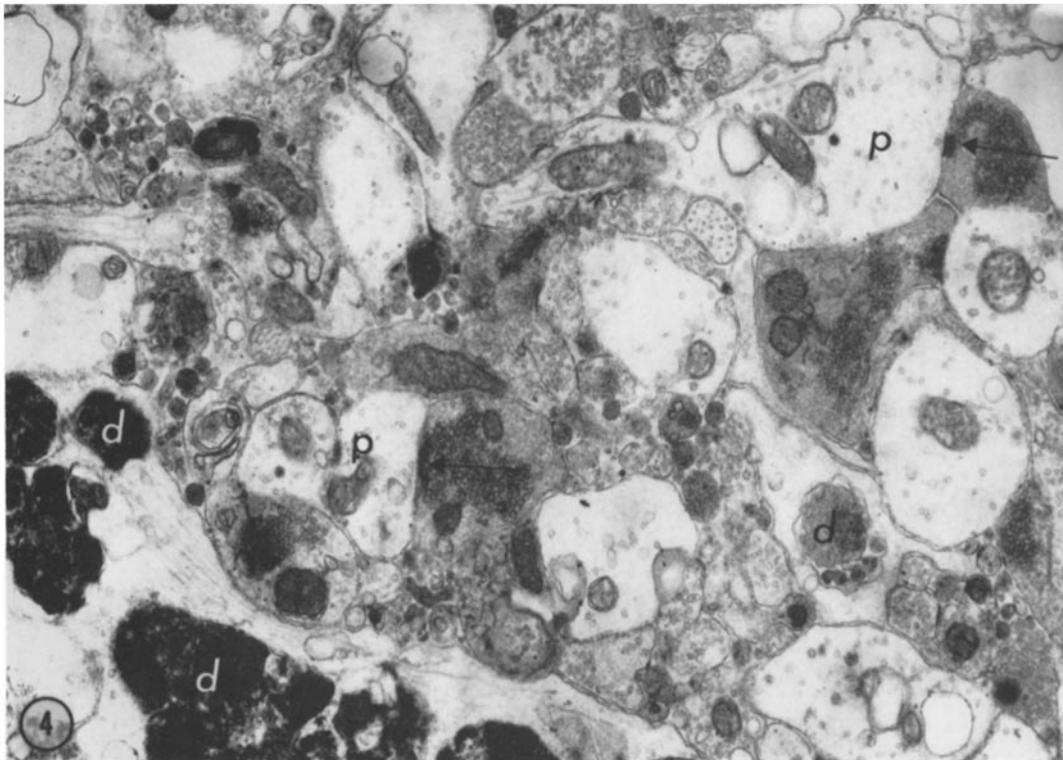

FIGURE 4 Electron micrograph of a portion of an olfactory glomerulus 7 days after the olfactory nerve has been sectioned. Some dense profiles with vesicles still maintain synaptic relations (arrows) with post-synaptic elements (p). However, degenerative phenomena (d) have altered most of the glomerular pattern. Compare with Figs. 2 and 6. Magnification × 38,500.
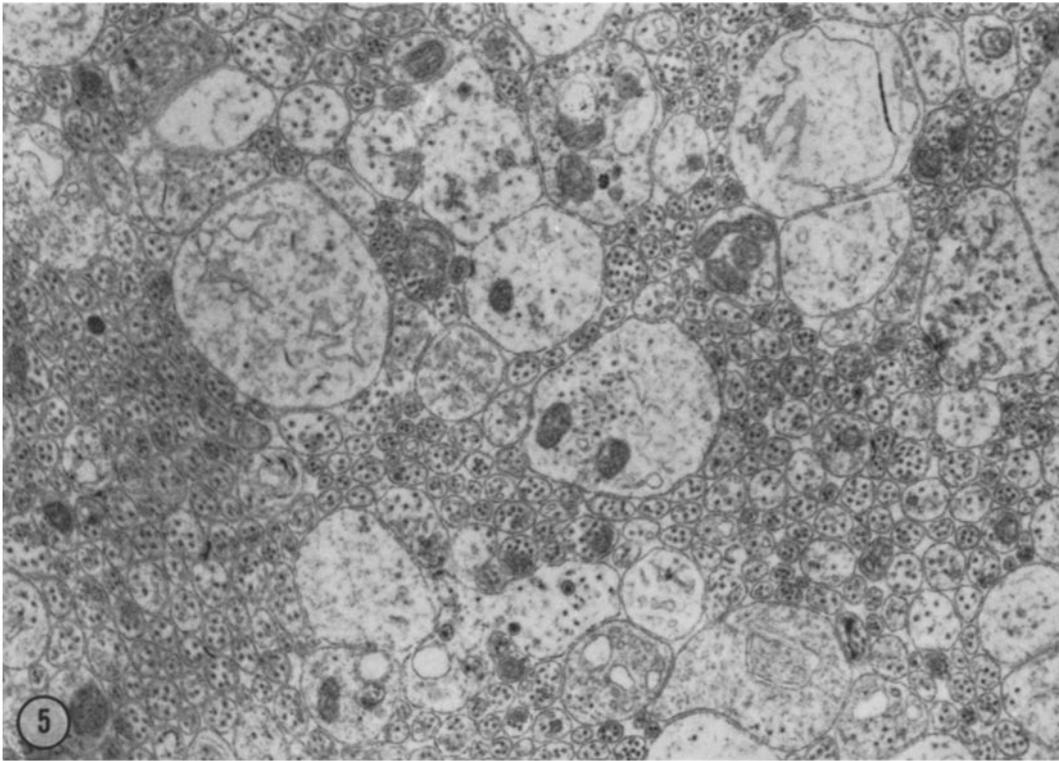
FIGURE 5   Electron micrograph of a regenerated olfactory nerve 60 days after nerve section. Notice the variation in diameter of the olfactory axons as compared with Fig. 1. The content of the axons is represented by microtubules and mitochondria and not dissimilar from the axons of the control side. Magnification × 48,500.
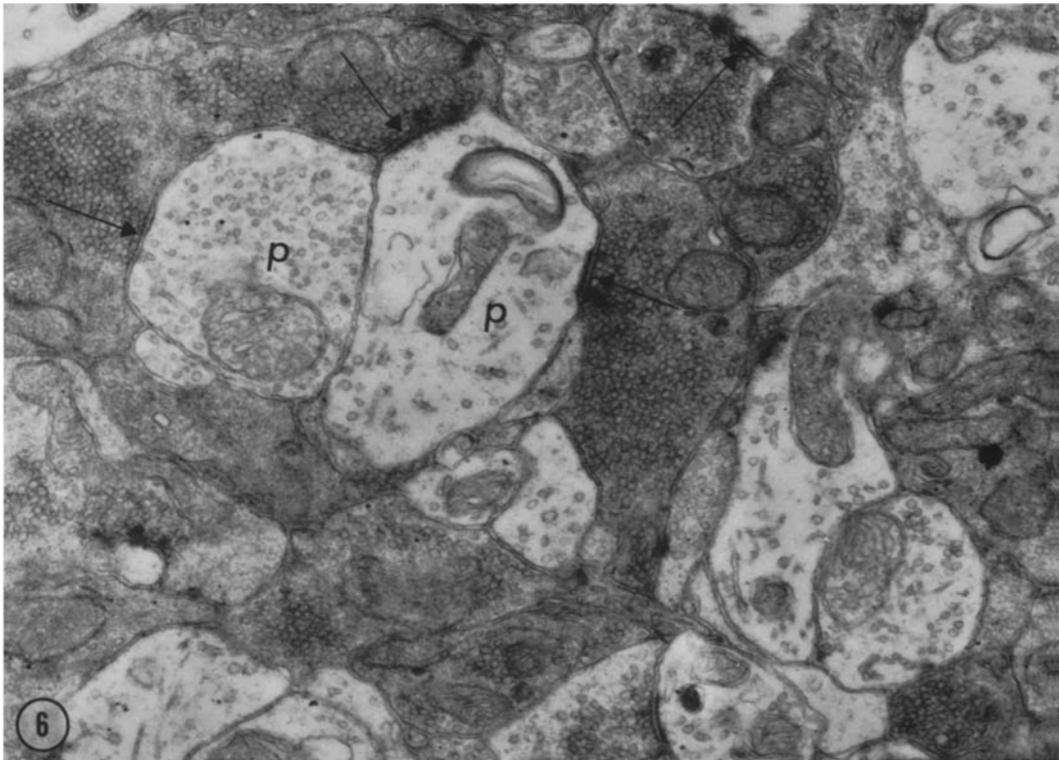

FIGURE 6   Electron micrograph of a portion of a reinnervated glomerulus 60 days after nerve section. The terminals of the olfactory axons are filled with vesicles and have reestablished normal synaptic contacts (at arrows). The postsynaptic is indicated at p. Compare with Figs. 2 and 4. Magnification × 48,500.

extended to the bulb in the 7–12-day groups (Figs. 3 and 4). The first sign of differentiation of new neurons in the olfactory mucosa was noticed in the 18–20-day groups. The number of newly differentiated receptors reached a close to normal density in the frogs sacrificed after 40 days. The degenerating profiles in the peripheral nerve stumps were substituted by new axons already from the 20-day groups on. The new axons are generally smaller than the average normal olfactory axons, however larger than normal axons can also be observed. Their content of microtubules and scanty mitochondria compares to the normal axons. Clear regeneration of the nerve fibers all along the olfactory nerve was observed in the 40-day groups (Fig. 5). Macroscopic observation of the operated side showed the regenerated nerve fibers to have bridged the surgical gap and re-established contact with the bulb. Both nerve and bulb exhibited some 20–30% decrease in size as compared to the unoperated side. At the bulbar level the number of glomeruli was less than normal while the synaptic contacts and the general pattern of nerve of profiles the glomerulus itself was comparable to the normal glomeruli (Fig. 6). From 60 days through the 8-mo period of the experiment the olfactory mucosa, nerve, and olfactory bulb persisted with the characteristics just mentioned.

Examination of the above data indicate that both degenerative and regenerative processes proceed slowly from the receptor cell bodies at the periphery towards their synaptic terminals in the glomeruli of the olfactory bulb. The cell bodies of the receptors are the first to disappear after their axons have been cut, followed by the axons under the epithelium and along the course of the nerve The centripetal parts of the axons ending in the bulb degenerate as well, and the degenerative process persists for a long time even while the newly formed receptors begin to appear in the peripheral sensory epithelium. The reappearance of normal synaptic contacts in the olfactory glomeruli follows in time the regeneration of the new olfactory neurons and the growth of their axons across the surgical gap and their penetration into the olfactory bulb. The microscopic process of degeneration at the glomerular level is also macroscopically confirmed by a reduction of the overall diameters of the entire bulb, as compared to the normal contralateral one. We have observed that even 8 mo after surgery the olfactory bulb has not regained its original volume. We interpret

this as a sign that at least part of the denervated glomeruli have not been reinnervated, or have been only partially so. Similar experiments to the ones carried out in frogs have been performed in pigeons with similar results. In pigeons electrophysiological and behavioral evidence points to a normal reestablishment of functional contacts between the olfactory mucosa and the olfactory bulb (Graziadei, Smith, and Tucker, 1973, unpublished data).

We conclude that the severance of the olfactory nerve in frogs results in the degeneration of the mature olfactory neurons of the olfactory mucosa. The basal cells of the olfactory mucosa, triggered by this degenerative process, undergo extensive mitotic activity and supply a new set of olfactory neurons. The new neurons send new axons which cross the surgical gap and reestablish normal synaptic contacts in the olfactory glomeruli of the bulb. The anatomical reestablishment of these synapses demonstrates a unique feature of the olfactory neurons among the vertebrate neural elements, namely their capacity of differentiation in adult animals from the so-called basal cells. Their axons, following the path of their predecessors, reestablish contact within the bulb. The unique plasticity of these nerve cells pave the way for experiments which may further clarify problems connected with nerve growth and neuronal specificity.

SUMMARY

Experimental olfactory nerve section in the frog induces a rapid, total degeneration of the olfactory neurons. These cells disappear from the neuroepithelium within 15 days after surgery. In a period of some 40 days of postoperative survival time new neurons differentiate from the basal cells of the olfactory epithelium develop new axons which cross the experimental gap, and establish new contacts with the glomeruli in the olfactory bulb. The new neurons are morphologically similar to the original mature receptors in every respect. The regeneration of new neurons capable of following the precise pattern of their predecessors in sending axons to the olfactory glomeruli offers a unique tool for the study of neuronal growth and specificity in an adult vertebrate.

REFERENCES

ANDRES, K. H. 1965. Differenzierung und regeneration von sinnezellen in der regio olfactoria. *Naturwissenschaften.* 17:500.

GRAZIADEI, P. P. C. 1971. The olfactory mucosa of vertebrates. *In* Handbook of Sensory Physiology. Vol. IV. L. M. Beidler, editor. Springer-Verlag, New York Inc.

GRAZIADEI, P. P. C. 1973. Cell dynamics in the olfactory mucosa. *Tissue Cell.* 5:113–131.

GRAZIADEI, P. P. C., and J. F. METCALF. 1971. Autoradiographic and ultrastructural observations of the frog's olfactory mucosa. *Z. Zellforsch. Mikrosk. Anat.* 116:305.

GRAZIADEI, P. P. C., J. F. METCALF, and R. S. DE-HAN. 1972. Cell dynamics in the olfactory mucosa of vertebrates. Society for Neurosciences Second Annual meeting, Houston, Texas. 2:77.

NAGAHARA, Y. 1940. Experimentelle studien uber die histologischen veranderungen des Geruchsorgans nach der olfactoriusdurchschneidung. *Jap. J. Med. Sci.* 5:165.

MOULTON, D. G. 1971. Detection and recognition of odor molecules. *In* Gustation and Olfaction. G. Ohloff and A. F. Thomas, editors. Academic Press, Inc., London. 1–26.

TAKAGI, S. F. 1971. Degeneration and regeneration of the olfactory epithelium. *In* Handbook of Sensory Physiology. Vol. IV. L. M. Beidler, editor. Springer-Verlag New York Inc.

THORNHILL, R. A. 1970. Cell division in the olfactory epithelium of the lamprey, *Lampetra fluviatilis. Z. Zellforsch Mikrosk. Anat.* 109:147.