THE JOURNAL OF COMPARATIVE NEUROLOGY 228:217-225 (1984)

Catecholamine- and Indoleamine-Containing Neurons in the Turtle Retina

P. WITKOVSKY, W. ELDRED, AND H.J. KARTEN
Daniel B. Kirby Eye Institute, Department of Ophthalmology and Department of Physiology and Biophysics, New York University Medical Center, New York, New York 10016 (P.W.), Department of Biology, Boston University, Boston, Massachusetts 02215 (W.E.), and Department of Neurobiology and Behavior, SUNY-Stony Brook, New York 11794 (H.J.K.)

ABSTRACT

We identified a population of presumed dopaminergic amacrine cells and populations of presumed serotonergic bipolar and amacrine cells in the retina of the turtle *Pseudemys scripta elegans* by a combination of autoradiographic, fluorescence, and immunocytochemical techniques. Antisera directed against the dopamine-synthesizing enzyme, tyrosine hydroxylase (TOH), stained perikarya located at the border of inner nuclear (INL) and inner plexiform (IPL) layers. Processes emitted by these cells arborized in sublaminae 1, 3, and 5 of the IPL. Incubation of retinas in 10^{-6} M ^{3}H-dopamine yielded a labeling pattern identical to the staining pattern achieved with TOH antisera, but when the concentration of ^{3}H-dopamine was increased 25-fold, both amacrine and bipolar cells are labeled. Following intraocular injection of dopamine, fluorescence micrography revealed both stained amacrine and bipolar cells. The bipolar cells had Landolt's clubs, pyriform perikarya located in the distal portion of the INL, and axons that coursed horizontally in the INL, then entered the IPL, and ramified in both its superficial and deeper layers. Although no fluorescent neuronal profiles were revealed following injection of serotonin (5HT), bipolar cells identical to those described were visualized with 5HT antisera. The intensity of bipolar cell staining with 5HT antisera was improved by preinjection of the eye with exogenous 5HT. We suggest that the bipolar cell is serotonergic, but that it also can actively accumulate dopamine. The 5HT antisera also stained a population of large amacrine cells whose processes ramified in IPL sublaminae 1, 4, and 5. The same populations of presumed serotonergic bipolar and amacrine cells were labeled following incubation of the eyecup in 10^{-6} M ^{3}H-5HT.

Key words: dopamine, serotonin, autoradiography, immunocytochemistry, fluorescence

A variety of techniques now permits the identification of various candidate neurotransmitter substances in individual neurons. These methods include (1) formaldehyde-induced fluorescence for the localization of catecholamines and indoleamines (Falck and Owman, '65), (2) direct immunocytochemical identification of a transmitter substance (Steinbusch et al., '78), (3) immunocytochemical identification of an enzyme involved in the synthesis of the transmitter (Joh et al., '73) and (4) autoradiographic localization of isotopically labelled transmitters and/or their precursor substances (Brecha et al., 1979; Osborne et al., 1982; Marc and Lam, '81). In recent years these methods have been applied extensively to the vertebrate retina (Reviewed in Brecha, '83; Ehinger, '82).

Precise morphological localization of transmitter-related substances provides a framework for the correlation of synaptic circuitry with the light-evoked responses of retinal neurons. In that regard, recent studies of horizontal cells in turtle retina (Piccolino et al., '82; Neyton et al., '82; Gerschenfeld et al., '83) have shown that the permeability of gap junctions can be modified by the conventional neurotransmitters GABA and dopamine. In the teleost retina, Dowling and Ehinger ('75) demonstrated that a dopaminergic interplexiform cell made synapses onto horizontal cells. Moreover, in carp retina dopamine appears to modify the spatial integration of the horizontal cell network (Neg-

Accepted April 6, 1984.

ishi and Drujan, '79; Teranishi et al., '83; Cohen and Dowling, '83) in a manner analogous to that found in turtle retina. We wondered, therefore, whether the turtle retina might also possess a catecholaminergic interplexiform cell. However, since both catecholaminergic and indoleaminergic neurons can actively take up the same precursors (Iversen, '75) and be inhibited by some of the same blockers (Seeman, '81), we thought it important to identify as well as to distinguish between these two neuronal populations in the turtle retina.

MATERIALS AND METHODS

Experiments were performed on the red-eared swamp turtle (*Psuedemys scripta elegans*) obtained from a commercial supplier and maintained in aquaria on a diet of goldfish until used. Room lighting was programmed to be on 12 hours/day.

Three different histological procedures were utilized:

Autoradiography

^{3}H-dopamine (NET-131 24.9 Ci/mmol) and ^{3}H-serotonin (NET-398 26.3 Ci/mmol) were obtained from New England Nuclear Corp., Boston, MA. The animal was decapitated, the eyes enucleated, and the posterior pole separated by a razor cut. Excess vitreous was drained and the eyecup was incubated in normal Ringer's solution, bubbled continuously with 95% O_2 5% CO_2 for 30 minutes. Thereafter, the tissue was transferred to a fresh solution containing the radioactive substance diluted with Ringer to a final concentration, in different experiments, of 1×10^{-7} to 2.5×10^{-5} M for 30–45 minutes. In addition, the solution contained 0.5 mM Na ascorbate and 10 μM each of EDTA and pargyline. The tissue was fixed for 2 hours at 4°C in a mixture of freshly prepared 1% paraformaldehyde, 2% purified glutaraldehyde, 100mM cacodylate buffer, 1.8 mM $CaCl_2$ and 3% sucrose, then postfixed 1 hour at 4°C in 1% OsO_4 + 100 mM cacodylate buffer, dehydrated, and embedded in Epon. Sections approximately 1.0 μm thick were collected on subbed slides and coated with Kodak NTB-2 emulsion, incubated 2–4 weeks, and then developed and counterstained with Toluidine blue. Photographs were taken with 40× or 100× oil-immersion objectives

Fluorescence

The so-called FAGLU (Formaldehyde-Glutaraldehyde) method (Furness et al., 1977; Nakamura, 1979) was employed. Five microliters of 2 μg/μl nonradioactive dopamine or serotonin was injected into one or both eyes. Two hours later the animal was decapitated and the posterior pole isolated as described above. The tissue was fixed in a mixture of 4% freshly prepared formaldehyde, 0.5% glutaraldehyde, 20% sucrose, and 100 mM phosphate buffer, pH 7.4, at 4°C for 12–24 hours. Thereafter, the tissue was blocked in frozen tissue embedding media and frozen sections cut at 14 μm. Sections were collected on subbed slides and dried overnight, then mounted with immersion oil, and examined in a fluorescence microscope.

Immunocytochemistry

In some experiments 10 μg serotonin in 5 μl Ringer solution was injected into the eye and the animal killed 2 hours later. Alternatively, 100 μg colchicine in 4 μl Ringer was injected into the eye and the animal killed 24 hours later. The posterior pole of the eye was prepared as above, then fixed in a mixture of freshly prepared 4% paraformaldehyde and 100 mM phosphate buffer, pH 7.4, for 1 hour at room temperature, followed by 12 hours at 4°C in 4% paraformaldehyde + 100 mM disodium hydrogen phosphate buffer at pH 10.0 (Na_2HPO_4 alone). The tissue then was cryoprotected in 20% sucrose/Ringer, blocked in frozen tissue embedding media, and the frozen sections cut at 10 μm were mounted on subbed slides. The sections first were incubated with 10% normal goat serum for 1 hour, then incubated overnight in a moist chamber with 1:250, 1:500 or 1:1,000 tyrosine hydroxylase antiserum (Joh et al., 1973) or 1:1,000 serotonin antiserum (sera-lab clone C5/45, Accurate Chemical and Scientific Corporation, Westburg, NY). The preparation characterization and specificity of the antisera used in these studies have been described previously (antiTOH, Joh et al., '73; antiserotonin, Consolazione et al., '81). When either of the primary antisera were omitted from our preparations, no specific labeling of retinal neurons was obtained. The tissue was washed in 2 × 15-minutes rinses in phosphate buffer and thereafter incubated for 1 hour at 37°C in FITC-conjugated antirabbit Ig G (Accurate Chemical and Scientific Corporation, Westburg, NY). Finally the slides were washed in phosphate buffer and were coverslipped with a 9:1 mixture glycerin/0.05 M carbonate buffer, pH 9.5, for viewing in a fluorescence microscope. Photographs were taken on Tri-X-Pan film with a 40× oil objective.

RESULTS

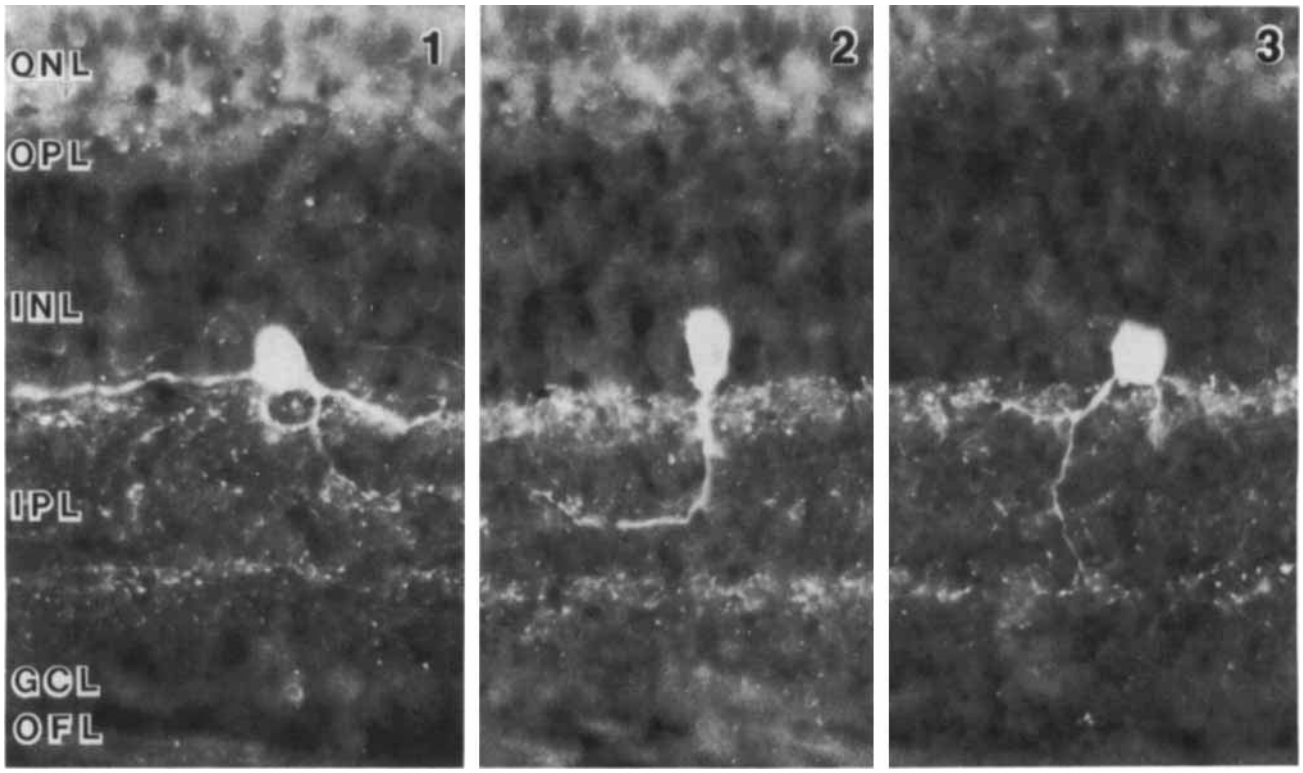
Cellular localization of catecholamines

Immunocytochemistry. The cellular localization of antityrosine-hydroxylase-like immunoreactivity is shown in Figures 1–3. The only retinal neurons stained were the amacrine cells, as judged by the location of stained perikarya at the border of inner nuclear (INL) and inner plexiform layers (IPL), the distribution of cell processes within the inner plexiform layer, and the apparent lack of an axon.

The stained perikarya were about 12 μm in diameter of major axis and approximately round (Figs. 1, 3) or pyriform (Fig. 2) in shape. The cell bodies gave rise to multiple processes which distributed to laminae 1, 3, and 5 of the IPL. As seen in Figures 1 and 3, stained processes from a single cell arborized in more than one sublayer of the IPL. The density of catecholamine neurons was not determined precisely. In one flatmount preparation we observed stained perikarya about 90 μm apart.

Although the perikarya of amacrines stained with TOH antisera occasionally emitted short (< 3 μm) processes that entered the inner nuclear layer, we observed no long ascending processes in an examination of more than 200 stained cells, nor was any staining evident in the distal portion of the INL or in the outer plexiform (OPL) layer of the retina. Thus, on the basis of the immunochemical staining, the turtle retina appeared to lack a catecholaminergic interplexiform cell.

Autoradiography. Autoradiographs prepared from eyecups incubated in 1×10^{-6} to 2.5×10^{-5} M ^{3}H-dopamine are shown in Figures 4–7. A clear dose-dependent variation was found in the pattern of uptake of labeled dopamine by the tissue. When the dosage of dopamine was 1×10^{-6} M or less, and the exposure time 14 days, accumulations of label were seen over presumed amacrine perikarya, as well as in three bands in the IPL corresponding to sublaminae 1, 3, and 5. The size and distribution of the labeled perikarya and processes, respectively, corresponded closely to the data obtained from immunochemical staining with antityrosine hydroxylase antisera as shown in Figures 1–3.



Figs. 1–3. Fluorescence micrographs of turtle retina stained with anti-TOH-like antisera using the FITC technique (see Methods). Note the prominently stained amacrine cell bodies situated at the border of inner nuclear and inner plexiform layer and the amacrine processes which penetrate the inner plexiform layer to various levels. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OFL, optic fiber layer. ×640.

A somewhat different picture, however, was obtained from autoradiographs following incubation in 7×10^{-6} or 2.5×10^{-5} M ^{3}H-dopamine, as illustrated in Figures 5–7. Figure 5 illustrates an autoradiograph after incubation in 7×10^{-6} M dopamine followed by a 14-day exposure. In this case, amacrine perikarya and their processes in the IPL were labeled heavily. In addition, weak labeling of other perikarya in the distal portion of the INL was noted. When the incubation dosage of ^{3}H-dopamine was increased to 2.5×10^{-5} M, additional obliquely oriented processes within the INL were labeled heavily and pockets of label accumulated in the OPL and between photoreceptor bases (Figs. 6, 7). Evidently the higher dose of ^{3}H-dopamine labeled both the dopamine-accumulating amacrine cell as well as a second structure with a lower affinity for the tritiated substance. We considered the possibility that the labeled, oblique structures traversing the INL were the ascending processes of interplexiform cells. However, in detailed examination of many sections we never found a labeled amacrine perikaryon connected to a labeled oblique process.

Fluorescence microscopy. Further insight into the cellular origin of the oblique processes was obtained from examination, by fluorescence microscopy, of tissue prepared by the FAGLU technique (see Methods). Following intraocular administration of unlabeled dopamine, two discrete cell populations were labeled. Figure 8 is a low-magnification view of the visual streak area of the retina viewed in darkfield. Note that most of the fluorescent perikarya are located in the distal portion of the INL, but others are located adjacent to the INL/IPL border. The former cell group appears to be bipolar neurons; the more proximally located cell bodies probably are amacrine cells. The fluorescent bipolar cell profiles were more concentrated in the visual streak area of the turtle retina than in more peripheral retinal regions. The visual streak is a horizontally oriented band extending across the turtle retina which can be distinguished readily in vertical sections of the posterior pole, because of the overall increased thickness of the retina there.

Definitive identification of the distal cells as bipolar neurons is provided by higher-magnification views. Figures 9 and 10 show that distal fluorescent cells give off processes that rise toward the receptor layer, indicating that these cells are bipolar neurons. A further proof is provided by the presence of Landolt's clubs (arrow, Fig. 9), an organelle possessed only by the bipolar cell (Hendrickson, '66). We could not verify, however, that every fluorescent bipolar possessed a Landolt's club.

In Figure 10 it may be seen that the fluorescent process divides at the level of the receptor bases into horizontally coursing dendrites and a vertically directed Landolt's club (short arrow). Axonal processes of fluorescent bipolars (Fig. 10) run diagonally through the INL, then turn vertically to enter the IPL where they appear to arborize in a band of terminals located at the level of sublaminae 1. Other processes continue to more proximal levels of the IPL, but their precise level(s) of termination could not be determined with this method (see below).

Cellular localization of indoleamines

Fluorescence microscopy. In initial experiments we failed to observe any endogenous fluorescence in retinas prepared by the FAGLU method without preinjection of

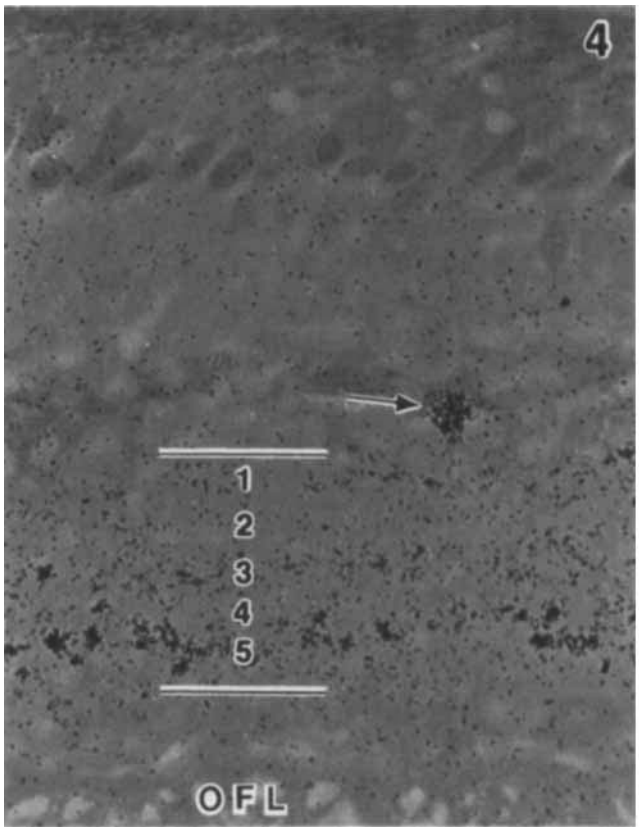
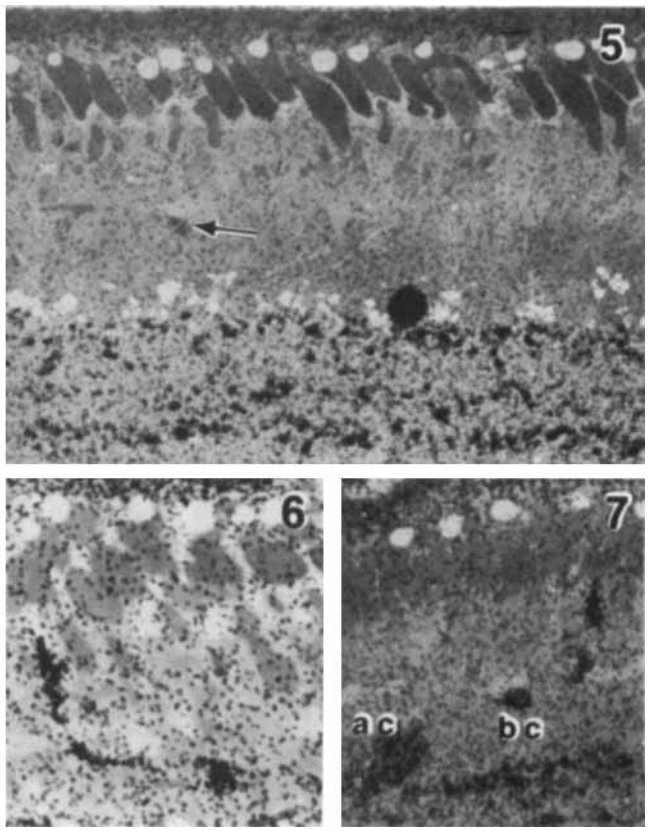


Fig. 4. An autoradiograph of turtle retina incubated in 10^{-6} M ^{3}H-dopamine and exposed for 14 days (see Methods). Label is seen over an amacrine cell body (arrow) and within layers 1, 3, and 5 of the inner plexiform layer. Horizontal bars delimit the extent of the IPL, the numbers indicate its subdivisions. ×560.

Fig. 5. An autoradiograph of turtle retina incubated in 7×10^{-6} M ^{3}H-dopamine and exposed for 14 days. Heavy labeling of an amacrine cell body and layers 1, 3, and 5 of the IPL are evident. In addition, weak labeling of small cell bodies in the inner nuclear layer occurred, one of which is indicated by an arrow. ×560.



Figs. 6, 7. Autoradiographs of turtle retina incubated in 2.5×10^{-5} M ^{3}H-dopamine and exposed for 14 days. Label accumulated over amacrine (ac) and bipolar (bc) cell bodies as well as over oblique processes that traverse the INL and extend into the photoreceptor layer. The clear circles at the top of the figures are cone photoreceptor oil droplets. ×640.

serotonin. Moreover, whether serotonin was injected into the eye (4–8 μg/eye, N = 4) or the eyecup was incubated in Ringer containing 100 μM serotonin, 1 mg/ml ascorbate, and approximately 10 μM pargyline for 20 (N = 4) or 90 minutes (N = 2), no fluorescent cells of any type were visualized.

Autoradiography. In contrast to the negative results obtained with fluorescence microscopy, a consistent labeling pattern was obtained by using ^{3}H-serotonin. Figures 11 and 12 illustrate results obtained following incubation of 1×10^{-6} M ^{3}H-serotonin. Two cell populations accumulated label. One appeared to be an amacrine cell on the basis of the location of the cell body in the proximal portion of the INL (Fig. 11). The presumed amacrine cells had large and heavily labeled perikarya. Although ^{3}H-dopamine administration also resulted in labeled amacrine perikarya (Figs. 4, 5), the location of label within the IPL following ^{3}H-serotonin administration was quite distinct from that seen with ^{3}H-dopamine. Instead of three discrete bands of label (cf. Fig. 5), a patchy pattern of label was observed. Most label was found in sublaminae 1, 4, and 5 of the IPL (Fig. 11).

The other population of cells labeled by ^{3}H-serotonin appeared to be bipolar cells. Labeled bipolar perikarya were located in the distal half of the INL and were smaller and less densely labeled than the amacrine perikarya (Fig. 11). Figure 12 shows a ^{3}H-serotonin-labeled pyriform bipolar cell body whose distal tip extends toward the level of the outer plexiform layer.

Immunocytochemistry. Although both ^{3}H-dopamine and ^{3}H-serotonin labeled amacrine cells, it appeared from the fact that the respective labels had a different pattern of accumulation in the IPL that two different subpopulations of amacrine cells were uncovered. That assumption received further support from the staining pattern of serotonin-like immunoreactivity. Again, two cell populations were stained: amacrine and bipolar cells. Figures 13 and 14 show that the serotonin antisera stained very heavily a population of amacrine cells characterized by large (~10 μm), round or pear-shaped perikarya located at or adjacent to the innermost portion of the INL. The perikarya emitted several processes. Some of these coursed horizontally in sublamina 1 of the IPL; others took a vertical or oblique course through the IPL to form a broad band in sublaminae

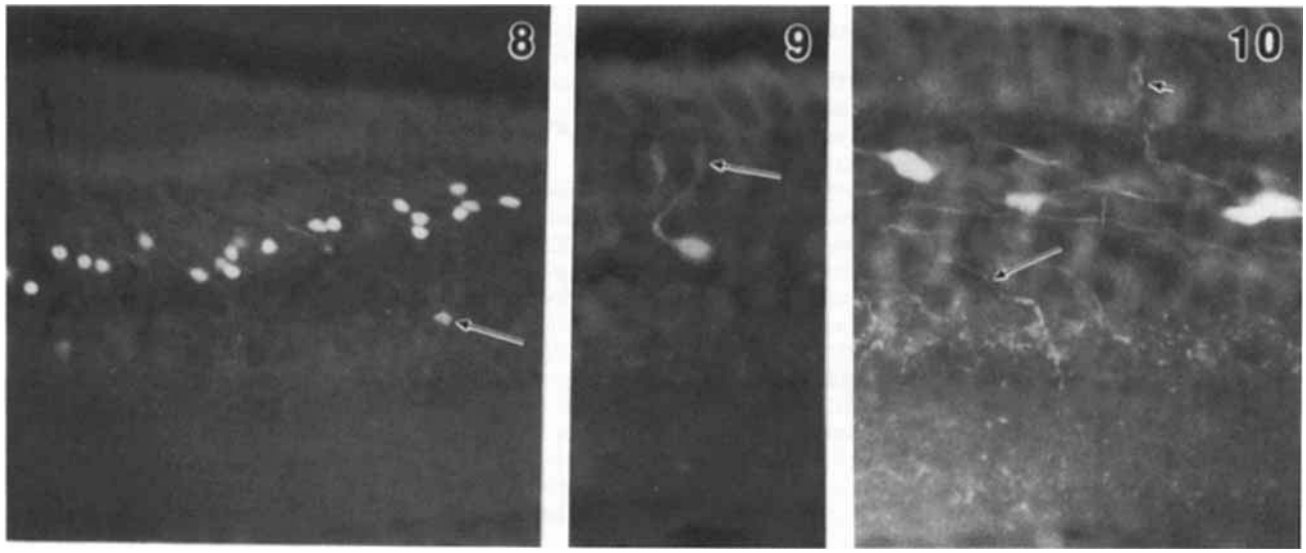


Fig. 8. A fluorescence micrograph of the visual streak area of the turtle retina following intraocular injection of dopamine (see Methods). Primarily bipolar cell bodies are stained, but the arrow indicates a stained amacrine cell. ×300.

Fig. 9. A fluorescence micrograph of the turtle retina following intraocular injection of dopamine. A stained bipolar cell body with its associated Landolt's club (arrow) is visible. ×435.

Fig. 10. A fluorescence micrograph of the turtle retina following intraocular injection of dopamine. A Landolt's club is indicated by a short arrow. The longer arrow points to an obliquely oriented axon which abruptly takes a more vertical course and penetrates the inner plexiform layer. ×470.

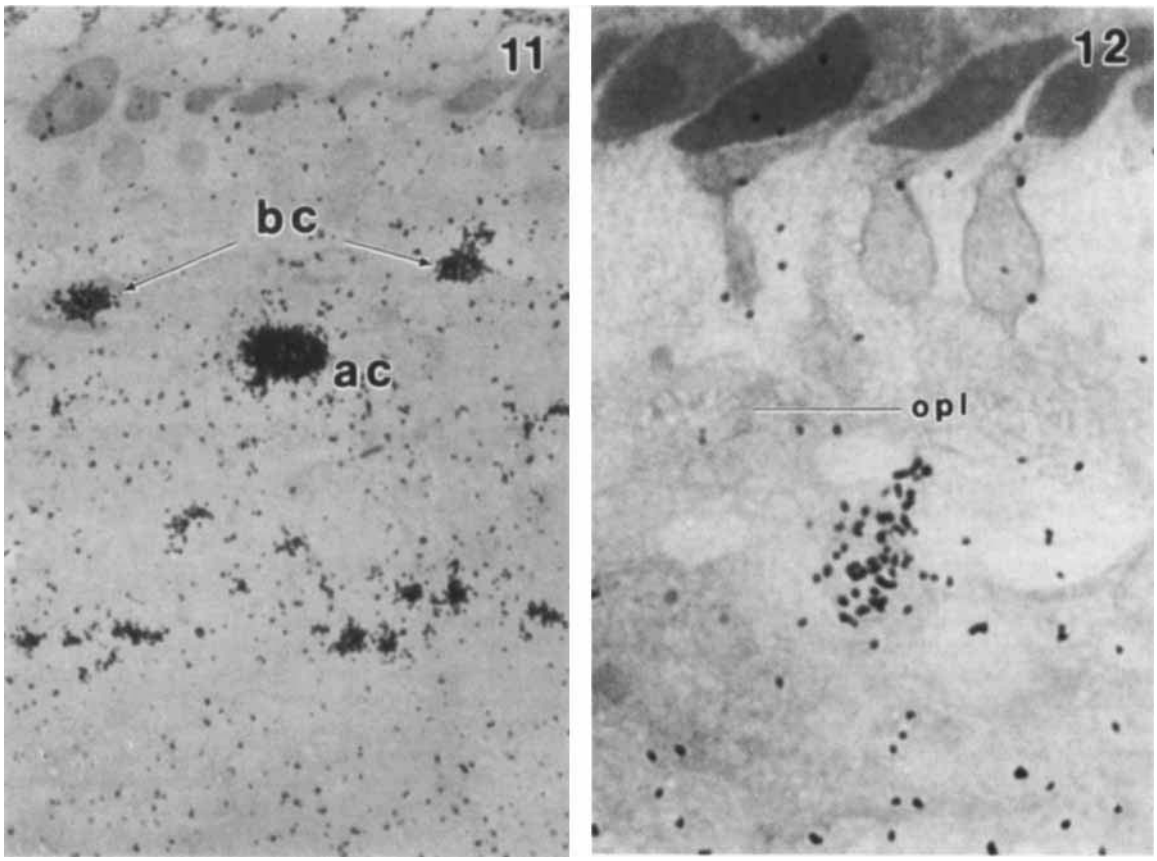


Fig. 11. An autoradiograph of the turtle retina incubated in 10^{-6} M ^{3}H-serotonin and exposed for 14 days. Note two labeled bipolar cells (bc), recognized by their relatively small perikarya and their location in the distal half of the inner nuclear layer. In addition, label has accumulated in an amacrine cell (ac) identified by its relatively large perikaryon located at the border of inner nuclear and inner plexiform layers. The inner plexiform layer itself contains numerous patches of label particularly in sublaminae 4 and 5. ×800.

Fig. 12. An autoradiograph of the turtle retina incubated in 10^{-6} of ^{3}H-serotonin and exposed for 14 days. Label accumulated over a pear-shaped bipolar cell perikaryon whose distal tip extends toward the outer plexiform layer (OPL). ×1,150.

4 and 5. Thus, the sublaminar distribution of stained amacrine cell processes in the IPL resembled that obtained with ^{3}H-serotonin (Fig. 11).

Figure 13 illustrates that, in addition to the stained amacrines, a number of presumed bipolar cells also were weakly stained. In some sections a weakly stained Landolt's club was visible, but the proximal axonal portions of the cell were not discernible. When, however, the eye was injected with serotonin (10 μg serotonin creatinine sulfate in 5-μl solution), allowed to incubate for 2 hours, and then prepared for immunocytochemistry, the cellular morphology of the stained bipolar cell was revealed much more clearly (Figs. 15–17). Figure 15 shows a bipolar perikaryon emitting a Landolt's club and a horizontally coursing axon. Figure 17 shows a similar profile in which the thin dendrites, which run horizontally in the OPL, are visible. To the right of this cell is the presumed bipolar cell axon which is seen to arborize in layers 1 and 4 of the IPL. Another such bistratified axon is illustrated in Figure 16. Its cell body and Landolt's club are in a slightly different focal plane, but are partially visible.

DISCUSSION

Presence of catecholamines in vertebrate retinas

There is abundant evidence that catecholamines are present generally in vertebrate retinas. Ehinger ('82) provided a useful summary of the cellular distribution of these substances in the retinae of representative species. Catecholamine (dopamine in most cases) is found in amacrine cells and, in the retinae of certain teleosts and primates (Ehinger et al., '69; Dowling and Ehinger, '75; Dowling et al., '80), in interplexiform cells.

The perikarya of dopaminergic amacrines invariably are located at the junction of inner nuclear and inner plexiform layers (Ehinger; '82; Fig. 3). The distribution of their processes within the IPL is somewhat variable, but a trilaminar pattern is encountered commonly, e.g., in the retinas of pigeon and chicken (Floren, '79), rabbit (Dowling and Ehinger, '78) and *Cebus* monkey (Ehinger and Falck, '69). The dopaminergic amacrines of turtle retina described in the present report similarly have a trilaminar distribution of processes in layers 1, 3, and 5 of the IPL and large perikarya situated at the INL/IPL border.

Absence of a dopaminergic interplexiform cell in turtle retina

We wish to emphasize that no interplexiform cell was revealed by any of the methods we utilized: immunofluorescence with tyrosine hydroxylase antisera, endogenous or induced fluorescence following administration of dopamine, and autoradiography using ^{3}H-dopamine. The apparent absence of the dopaminergic interplexiform cell in turtle retina is significant, in view of the potent action of dopamine on the physiological response of the large-field horizontal cell of turtle retina (Neyton et al., '82; Gerchenfeld et al., '83). Dopamine, presumably acting through a cAMP-mediated mechanism, was shown to affect the receptive-field properties of the horizontal cell in a manner similar to its effect on the luminosity-type horizontal cell of carp retina (Negishi and Drujan, '79; Teranishi et al., '83; Cohen and Dowling, '83). However, in goldfish retina there is a dopaminergic interplexiform cell (Ehinger et al., '69) which makes synapses onto the external (luminosity type) horizontal cell (Dowling and Ehinger, '75). Van Buskirk and Dowling ('81) demonstrated that accumulation of cAMP by isolated horizontal cells of carp retina was stimulated by dopamine. Thus, although the actions of dopamine on certain horizontal cells are similar in carp, goldfish, and turtle retinas, the underlying circuitry appears to be different.

Presence of indoleamine-accumulating neurons in vertebrate retinae

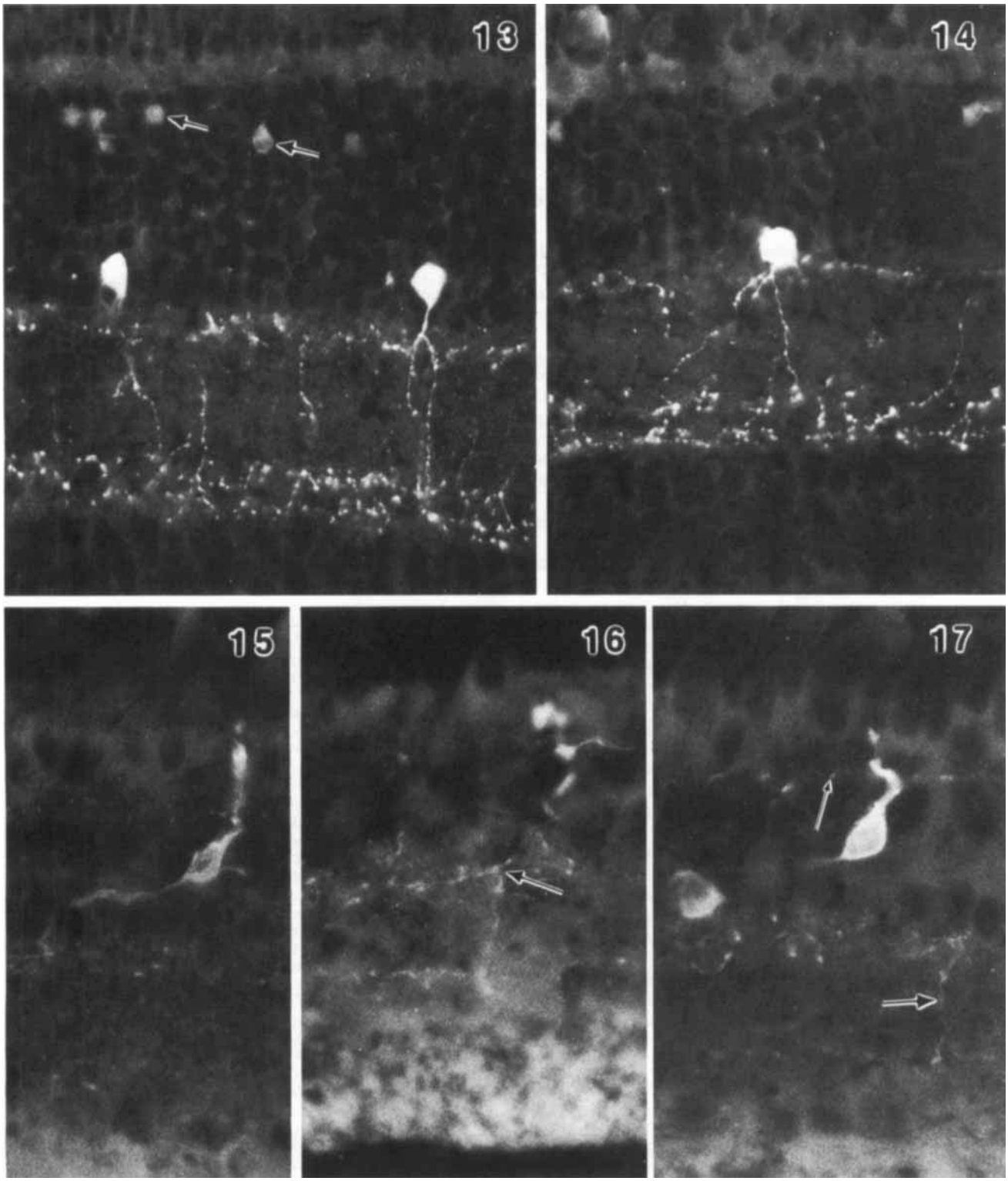
Indoleamine-accumulating amacrine neurons have been demonstrated in most, but not all surveyed retinae (Ehinger, '82). In addition, there is one report of indoleamine uptake by horizontal cells of squirrel monkey retina (Floren and Hendrickson, '80). Furthermore, Floren ('79) found that following intravitreal injection into chick retina of relatively high doses of either a catecholeamine or an indoleamine, bipolar cells are visualized. This same finding has been confirmed and extended to pigeon retina by Tornqvist ('83).

Some controversy exists over the nature and even the presence of indoleamine neurons in at least some vertebrate retinae, a controversy stemming from an inability to demonstrate their presence with each method tried. For example, in bovine retina no neurons are revealed with antisera directed against 5-hydroxytryptamine (Osborne et al., '82) even though in the same species amacrine neurons do label with ^{3}H-5HT and an active uptake system for 5HT is present (Osborne, '81).

Moreover, in all tested retinas (except that of embryonic chick, Hauschild and Laties, '73), no endogenous indoleamine fluorescence is shown with the various Falck-Hallarp methods even when chemical analysis reveals that considerable 5HT may be present, e.g., in frog retina (Osborne et al., '82; Tornqvist, '83). Our data on turtle retina are generally consistent with earlier studies although they differ in details. Thus, our finding of positive staining of bipolar and amacrine cells with a serotoninlike antibody is similar to the results obtained in bird retinae (Floren, '79; Tornqvist, '83). Moreover, uptake of ^{3}H-5HT by a class of amacrine neurons that have large cell bodies at or near the INL/IPL border with heavy staining of terminals deep in the IPL (sublaminae 4 and 5) and lesser staining elsewhere in the IPL is encountered in bird, rabbit, and *Cebus* monkey retinae (Ehinger; '82: Fig. 7). On the other hand, our inability to induce fluorescence by injection of, or incubation with, indoleamines is surprising, particularly in view of the positive staining of the presumed indoleamine neurons achieved with exogenous dopamine. In teleost retina, for example, Negishi et al. ('81) found that both catecholamine- and indoleamine-accumulating neurons are present and may be distinguished on the basis of the wavelength of emitted fluorescence.

In itself, the ability of an indoleaminergic neuron to accumulate a catecholamine is not surprising, since the uptake mechanisms of such neurons generally in the vertebrate CNS is not highly specific (reviewed in Iversen, '75; and Cooper et al., '82). Presumably, this lack of specificity explains the weak labeling, by ^{3}H-dopamine, of cell bodies located in the distal portion of the inner nuclear layer as well as of processes coursing obliquely through the inner nuclear layer (Figs. 6, 7). As discussed in the following section, these labeled cellular components are concluded to belong to indoleaminergic bipolar cells.

Ehinger et al. ('82) have speculated that the indoleamine-accumulating neurons in certain mammalian retinae convert exogenous indoleamines into nonfluorescent moieties. Our results in turtle retina are consistent with this explanation in that the active accumulation of ^{3}H-serotonin (Figs.



Figs. 13, 14. Fluorescence micrographs of turtle retina stained with antiserotonin antisera using the FITC method. Well-stained amacrine cells and their processes are visible. In addition, weakly stained bipolar cell perikarya (arrows, Fig. 13) may be discerned. ×750.

Figs. 15–17. Fluorescence micrographs of turtle retina fixed 2 h following intraocular injection of 10 μg serotonin, then stained with antiserotonin antisera by the FITC method. Figure 15 shows a bipolar cell with a vertically oriented Landolt's club, pyriform cell body and an obliquely oriented axon. Figure 16 illustrates a branching bipolar axon. The arrow points to the branch point which is located at the distal border of the IPL. One branch extends horizontally in layer 1 of the IPL, the other penetrates to layer 4 and 5 of the IPL. Figure 17 shows a bipolar cell whose horizontally oriented dendrites (small arrow) at the level of the OPL are discernible. The larger arrow indicates another branching bipolar axon within the IPL. ×750.

11–13) and the enhanced serotoninlike immunoreactivity following serotonin administration (Figs. 15–17) strongly support the idea that certain retinal neurons do actively accumulate serotonin. On the other hand, inherent serotonin fluorescence might not be expected since the indoleamine concentration of the red-eared swamp turtle employed in this study may be quite low, by reference to a report by Welsh ('64) that the 5HT levels of another turtle (Chelydra) were undetectable.

Presence of an indoleaminergic bipolar cell in turtle retina

Perhaps the most interesting finding of our study is the positive identification of a serotonergic bipolar cell. The fact that it stains positively with serotonin antisera and accumulates ^{3}H-5HT at a concentration too low to induce uptake by the catecholaminergic amacrine cell present indicates that the bipolar cell identified is serotonergic. Bipolar cells with a similar morphology to that of the presumed serotonergic cell also were seen when either the retina was incubated in the relatively high concentration of ^{3}H-dopamine (Figs. 6, 7) or in fluorescence micrographs following intraocular injection of dopamine (Figs. 8–10). Our interpretation is that the bipolar cells labeled with serotonin antisera are the same ones visualized with dopamine, due to the ability of serotonergic uptake systems to take up catecholamines (Iversen, '75).

That the cell in question is truly a bipolar cell is attested to by the presence of a Landolt's club, a structural feature unique to bipolar cells (Hendrickson, '66). Moreover, Kolb ('82) reported that all Golgi-impregnated bipolars of the turtle retina possessed Landolt's clubs. The looping course of the axon in the inner nuclear layer reported in the present study has been noted previously for bipolar cells of the dogfish retina (Witkovsky and Stell, '73) and of the turtle retina (Detwiler and Sarthy, '81). Kolb ('82) distinguished nine types of bipolar in the retina of the red-eared swamp turtle based on the size and position of the perikaryon within the INL and the distribution of the axonal arbor within the IPL. A comparison between our fluorescence profiles (Fig. 3) and her summary diagram (Kolb, '82: Fig. 2) shows that our cell type most resembles her B9 bipolar.

Another interesting feature of the presumed serotonergic bipolar cell is its relatively high concentration in the visual streak area—a horizontal bank of high cellular density (Brown, '69; Peterson and Ulinski, '79; Kolb, '82). We do not know, however, whether all bipolar cell types show an increased density in the visual streak area and thus whether or not the serotonergic bipolar identified in the present study is represented disproportionately in that area.

ACKNOWLEDGMENTS

This work was supported by grants EY03570 to P.W., EY04785 to W.E., and EY04796 to H.K. Additional support was provided by Research Center Award EY01842 and an unrestricted award from Research to Prevent Blindness to the Department of Ophthalmology, NYU. We thank E.D. MacDonald for technical assistance and Dr. Tong Joh for a generous gift of antisera to tyrosine hydroxylase.

LITERATURE CITED

Brecha, N. (1983) Retinal neurotransmitters: Histochemical and biochemical studies. In P.E. Emson (ed): Chemical Neuroanatomy, New York: Raven Press, pp. 85–130.

Brecha, N., H.J. Karten, and C. Laverack (1979) Enkephalin-containing amacrine cells in the avian retina: Immunohistochemical localization. P.N.A.S. *76*:3010–3014.

Brown, K.T. (1969) A linear area centralis extending across the turtle retina and stabilized to the horizon by non-visual clues. Vision Res. *9*:1053–1062.

Cohen, J.L., and J.E. Dowling [1983] The role of the retinal interplexiform cell: Effects of 6-hydroxydopamine on the spatial properties of carp horizontal cells. Brain Res. *264*:307–310.

Consolazione, A., C. Milstein, B. Wright, and A.C. Cuello: (1981) Immunocytochemical detection of serotonin with monoclonal antibodies. J. Histochem. Cytochem. *29*:1425–1430.

Cooper, J.F., F.E. Bloom, and R.H. Roth (1982) The Biochemical Basis of Neuropharmacology, 4th Edition. New York: Oxford Univ. Press.

Detwiler, P.B., and P.V. Sarthy (1981) Selective uptake of lucifer yellow by bipolar cells in the turtle retina. Neurosci. Lett. *22*:227–232.

Dowling, J.E. and B. Ehinger (1975) Synaptic organization of the amine-containing interplexiform cells of the goldfish and Cebus monkey retinas. Science *188*:270–273.

Dowling, J.E., and B. Ehinger (1978) Synaptic organization of the dopaminergic neurons in the rabbit retina. J. Comp. Neurol. *180*:203–220.

Dowling, J.E., B. Ehinger, and I. Floren (1980) Fluorescence and electron microscopical observations on the amine-accumulating neurons of the Cebus monkey retina. J. Comp. Neurol. *192*:665–685.

Ehinger, B. (1982) Neurotransmitter systems in the retina. Retina *2*:305–321.

Ehinger, B., and B. Falck (1969) Morphological and pharmacohistochemical characteristics of adrenergic retinal neurons of some mammals. Graefe's Arch Klin. Exp. Ophthalmol. *178*:295–305.

Ehinger, B., B. Falck, and A.M. Laties (1969) Adrenergic neurons in teleost retina. Z. Zellforsch. *97*:285–297.

Falck, B., and C. Owman (1965) A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta Univ. Lund *Sect. II No. 7*:1–23.

Floren, I. (1979) Indoleamine accumulating neurons in the retina of chicken and pigeons. A comparison with the dopaminergic neurons. Acta Ophthalmol. *57*:198–210.

Floren, I., and A. Hendrickson (1980) Indoleamine accumulating horizontal cells in the squirrel monkey retina. Invest. Ophthalmol. *19(ARVO Suppl.)*:72.

Furness, J.B., M. Costa, and A.L. Wilson (1977) Waterstable fluorophores, produced by reaction with aldehyde solutions, for the histochemical localization of catechol and indoleamines. Histochemistry *52*:159–170.

Gerschenfeld, H.M., J. Neyton, M. Piccolino, and P. Witkovsky (1983) L-horizontal cells of the turtle: Network organization and coupling modulation. Biomed. Res. *3*:21–34.

Hauschild, D.C., and A.M. Laties (1973) An indoleamine containing cell in chick retina. Invest. Ophthalmol. Vis. Sci. *12*:537–540.

Hendrickson, A. (1966) Landolt's club in the amphibian retina: A Golgi and electron microscope study. Invest. Ophthalmol. *5*:484–496.

Iversen, L.L. (1975) Uptake processes for biogenic amines. In L.L. Iversen, S.D. Iversen, and S.H. Snyder (eds): Handbook of Psychopharmacology,New York: Plenum Press: pp. 381–442.

Joh, T.H., C. Geghman, and D. Reis (1973) Immunocytochemical demonstration of increased accumulation of tyrosine hydroxylase protein in sympathetic ganglia and adrenal medulla elicited by reserpine. Proc. Natl. Acad. Sci. USA *70*:2767–2771.

Kolb, H. (1982) The morphology of the bipolar cells, amacrine cells and ganglion cells in the retina of the turtle *Pseudemys scripta elegans*. Philos. Trans. R. Soc. Lond. [Biol.] *298*:355–393.

Marc, R.E., and D.M.K. Lam (1981) Glycinergic pathways in the goldfish retina. J. Neurosci. *1*:152–165.

Nakamura, T. (1979) Application of the Faglu method (Furness et al.) for the histochemical demonstration of catecholamines to the cryostat section method. Acta Histochem. Cytochem. *12*:182.

Negishi, K, and B.D. Drujan (1979) Reciprocal changes in center and surrounding S-potentials of fish retina in response to dopamine. Neurochem. Res. *4*:313–318.

Negishi, K., S. Kato, and T. Teranishi (1981) Indoleamine-accumulating cells and dopaminergic cells are distributed similarly in carp retina. Neurosci. Lett. *25*:1–5.

Neyton, J., M. Piccolino, and H.M. Gerschenfeld (1982) Dopamine (DA) and drugs that increase intracellular cyclic AMP decrease junctional communication between L-horizontal cells. Neurosci. Abstr. *8*:132.

Osborne, N.N. (1981) Noradrenaline, a transmitter candidate in the retina. J. Neurochem. *36*:17–27.

Osborne, N.N. (1982) Evidence for serotonin being a neurotransmitter in the retina. In N.N. Osborn, (ed): Biology of Serotonergic Transmission. New York: John Wiley and Sons, pp 401–429.

Osborne, N.N., T. Nesselhut, D.A. Nicholas, S. Patel, and A.C. Cuello (1982) Serotonin-containing neurones in vertebrate retinas. J. Neurochem. *39*:1519–1528.

Peterson, E.H., and P.S. Ulinski (1979) Quantitative studies of retinal ganglion cells in a turtle *Pseudemys scripta elegans*. I. Number and distribution of ganglion cells. J. Comp. Neurol. *186*:17–42.

Piccolino, M., J. Neyton, P. Witkovsky, and H.M. Gerschenfeld (1981) γ-aminobutyric acid antagonists decrease junctional communication between L-horizontal cells of the retina. P.N.A.S. *79*:3671–3675.

Seeman, P. (1981) Brain dopamine receptors. Pharmacol. Rev. *32*:229–313.

Steinbusch, H.W.M., A.A.J. Verhosfstad, and H.W.J. Joosten (1978) Localization of serotonin in the central nervous system by immunohistochemistry: Description of a specific and sensitive technique and some application. Neuroscience *3*:811–819.

Teranishi, T., K. Negishi, and S. Kato (1983) Dopamine modulates S-potential amplitude and dye-coupling between external horizontal cells in carp retina. Nature *301*:243–246.

Tornqvist, K.S. (1983) 5-hydroxytryptamine and neuropeptides in the retina. Ph.D. Thesis. Univ. Lund: Dept. of Ophthalmology.

Van Buskirk, R., and J.E. Dowling (1981) Isolated horizontal cells from carp retina demonstrate dopamine-dependent accumulation of cyclic AMP. P.N.A.S. *78*:7825–7829.

Welsh, J.H. (1964) The quantitative distribution of 5-hydroxytryptamine in the nervous system, eyes and other organs of some vertebrates. In: Comparative Neurochemistry, Proceedings of the 5th International Neurochemistry Symposium. Oxord: Pergamon Press, pp. 355–366.

Witkovsky, P., and W.K. Stell (1973) Retinal structure in the smooth dogfish, *Mustelus canis*: Light microscopy of bipolar cells. J. Comp. Neurol. *248*:147–160.