RETINAL CIRCUITRY AND CLINICAL OPHTHALMOLOGY*

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ABSTRACT

Six different neurotransmitters are now comparatively well established in the retina, and for several we know both which cells they are in and what contacts these cells make with other neurons. We also know there must be many more neurotransmitters, although they have not yet been clearly identified.

Psychoactive drugs can be expected to affect not only neurons in the brain but also in the retina. Dopaminergic neurons, GABA neurons, and indoleamine neurons are among the ones likely to be affected. Drugs which influence these systems are already in widespread use, and new and more powerful ones are constantly being developed. However, comparatively little is known about what the drugs do to vision. Very likely, this is so because clinical ophthalmological methods are usually not sophisticated enough to detect the effects of the drugs on vision. There is a great need for better clinical diagnostic methods, based on experimental knowledge of what different types of neurons may do in the retina and what different signal systems there are.

There may be as many different specialized tasks for the retina as there are neurotransmitters. It is important that these tasks are duly identified in the laboratory and that the results are brought to the ophthalmologists so that sensitive and selective clinical testing procedures can be developed.

INTRODUCTION

The eye is, in many respects, similar to a camera, and light induces some special chemical reactions both in the photographic film and in the retina at the back of the eye. However, unlike the film, the retina cannot be taken out to be developed and therefore it has to transform the chemical reactions into nerve signals that can be sent into the brain so we can see. This is done by means of various interactions between the many and different nerve cells that it contains, producing a coded signal that can be efficiently transmitted to the brain.

Ordinary microscope sections of the retina show a number of nerve cells, but not much of the different connections they make. With the more than a century-old silver staining methods, the form of individual nerve cells can be studied in great detail, and with the procedure, very complex network diagrams of different cell types have been produced (Polyak, 1941). However, as intricate and detailed as the diagrams are, they have so far been of only limited value. The reason for this is that some very important information is missing. The diagrams give no details about what the different nerve cells actually do, or how they do it.

Modern neurobiology is changing this. We are now able to say a little about the actions of the different components in the retina and some examples will be
presented here on how the cells in the retina send signals to each other. Some special attention will be given to systems which seem particularly relevant to clinical ophthalmology.

It was once maintained by some that the signal transfer between different neurons was rather trivial. All that was needed, it was said, was something similar to the binary system of a computer: an excitatory signal (or plus signal if you wish) and an inhibitory (or minus) signal. As will be seen, this is far from what the situation is like in the retina or, for that matter, in any other part of the nervous system.

**THE SIMPLIFIED RETINA**

It is useful to simplify the structure of the retina as in Figure 1, where one cell of each of the main types is shown.

The pigment epithelium cells serve to reduce reflections in the eye and thus to prevent glare. However, more importantly, they are positioned in between the blood vessels of the choroid and the photoreceptor cells, and, as will be further discussed, they therefore interact with them as a kind of helper cell.

The photoreceptor cells are highly specialized for capturing light. As indicated in Figure 1, light passes through most of the retina before it reaches these cells and excites them. They will in turn affect the next neuron, the bipolar cell. From the structure of the junction between the two types of cells we know that it is a chemical synapse, that is, a neurotransmitter is released from the photoreceptor cells to influence the next cell in line. There is also a different kind of synapse in the retina, gap junctions, which couple cells together electrically (Yamada and Ishikawa, 1965; Witkovsky and Dowling, 1969; Kolb and Nelson, 1983, 1984; Witkovsky et al., 1983). But our knowledge about them is still not sufficient to make it possible to discuss how they might affect vision, however interesting they may be.

The bipolar cell passes the signal received from the photoreceptor cell on to the ganglion cell in a second chemical synapse. The ganglion cell has a long axon with which it sends the extensively coded information to the brain.

There are also cells that modify the transmission of information through the retina. Horizontal cells are present at the first synapse between the photoreceptor cells and the bipolar cells. Amacrine cells affect the signal transfer at the second synapse, e.g., the synapse between the bipolar cells and the ganglion cells. They also form important links in multineuron signal chains. For instance, in the cat retina, certain amacrine cells are intercalated between rod bipolar cells and ganglion cells so that, in effect, a four-neuron path is produced from the photoreceptor cells to the brain (Kolb and Nelson, 1983). In the ground squirrel, some ganglion cells get their information exclusively from amacrine cells, and also in these cases a four-neuron path must be assumed (West and Dowling, 1972).

A recently discovered, slightly different cell type, the interplexiform cell, appears to send information backwards from the second to the first synapse, in what electronic engineers would call a feedback loop. This arrangement was originally recognized as the result of studies on a special neurotransmitter in fish retinas, dopamine (Dowling and Ehinger, 1975; Dowling et al., 1976), but the cells have more recently also been observed with other methods (Ehinger, 1982, 1983a).

A special type of ganglion cell has recently been described (Mariani, 1983; Zrenner et al., 1983). It contacts the photoreceptor cells directly without any intermediary cells, but has so far been seen only in cynomolgus monkeys.

The image on the retina is thus in most cases sent from the photoreceptor cells
FIGURE 1. Simplified diagram of the retinal neurons. Light enters from below and passes through the various cells until it reaches the photoreceptors (Ph), where it is converted to an electrical signal. PE is the pigment epithelium. The signal is transmitted by means of a network of cells to the ganglion cell (G) which sends it to the brain. The network consists of horizontal cells (H), bipolar cells (B), amacrine cells (A), and interplexiform cells (I). The arrows indicate the possible flow of information in the different parts.

to the brain through a chain of at least three neurons, the photoreceptor cell, the bipolar cell, and the ganglion cell. The signals will be modified when they pass from one cell to the next at the synapses and therefore it is important to understand how they are being transformed when passing through this chain of neurons.
THE NEUROTRANSMITTERS

Since most of the neurons operate with the aid of neurotransmitters, it is of interest to know what these chemicals are and how they act. This is a field where substantial progress has been made the last few years. Six substances are relatively well documented as retinal neurotransmitters. They are, in approximate descending order of documentation: dopamine, acetylcholine, glycine, GABA, 5-hydroxytryptamine (in non-mammalian vertebrates), and glutamic acid. Also more than a dozen other substances are suspected to be neurotransmitters for different reasons. These include many neuropeptides, but the evidence is in these cases less complete. Most likely, there are also many neurotransmitters in the retina which we have not recognized.

The three main criteria for classifying a substance as a neurotransmitter are: (1) that the substance is present or can be rapidly synthesized in neurons, (2) that it can be released by nerve activity, and (3) that selective receptors are present. In addition, some other criteria are often used: (4) there should be some inactivation mechanism for the transmitter. (5) Synthesizing enzymes should be demonstrable. (6) Some protected storage mechanism should be present. These criteria open up many ways to identify a neurotransmitter: by chemical analysis, by physiological or pharmacological experiments, or by morphological work. All approaches have been used in different studies, and various examples will follow, although some emphasis will be given to morphological results.

DOPAMINERGIC NEURONS

Many of the drugs used today by psychiatrists affect the function of, among others, dopaminergic neurons, and such cells have been demonstrated in the retina. Figure 2 is a fluorescence micrograph of the dopaminergic neurons in the cynomolgus monkey retina. Human retinas are very similar. Dopamine has been turned into a fluorescent compound in the tissue section by reacting it with formaldehyde with the method of Falck and Hillarp (see Björklund et al., 1972). Fluorescence is seen in amacrine cells, indicating they contain dopamine.

Dopaminergic neurons vary in intriguing ways between different species, but nevertheless occur in all vertebrates investigated so far (Fig. 3). Evidently, then, they must be important for vision. However, simple clinical observations tell us that powerful as they are, the neuroleptics used by the psychiatrists do not seem to affect vision in any readily discernible way even though they are well known to interfere

Figure 2. Formaldehyde induced fluorescence in a baboon retina, demonstrating a dopaminergic cell body (arrow) and dopaminergic processes forming a narrow sublayer in sublamina 1 at the border between the inner plexiform layer and the inner nuclear layer (arrowheads). Fluorescence micrograph, 110X.
with dopaminergic systems similar to the one found in the retina. This is of course good and well for the psychiatric patient but may seem somewhat surprising, and perhaps also a little disappointing from the neurobiologist's viewpoint. However, something of an explanation can be found if one goes a little more into the details of how these cells connect to other cells.

Dopaminergic neurons very actively accumulate their transmitter and certain similar substances. 5,6-dihydroxytryptamine is such a dopamine-like substance, and it makes the membranes and the cytoplasm appear darker in the electron microscope, and also induces some swelling of the processes and organelles of the dopaminergic neurons. Therefore these neurons can be identified at the ultrastructural level (Fig. 4; Dowling and Ehinger, 1975, 1978). Indoleamine accumulating neurons also are labeled by the treatment, but they can be removed so that they will not interfere with the analysis (Ehinger and Florén, 1978).

In favorable sections, one can identify the pre- and postsynaptic members of the synapse and thus determine which contacts the dopaminergic cell makes. The analysis shows that they contact only other amacrine cells (Fig. 5; Dowling and Ehinger, 1976, 1978; review: Ehinger, 1983b).

The main and most direct signal pathway from the photoreceptor cells to the brain is through the bipolar and ganglion cells. Dopaminergic neurons are not coupled directly to either of these cells (Ehinger, 1983a). Consequently, it seems reasonable to guess that the dopaminergic neurons have some general but specialized governing function rather than an influence on details in the visual field. Actually, the overall morphology of the dopaminergic neurons suggests the same: the cells are relatively sparse, but branch widely so that a single cell can affect many others. Such a general but special function may be difficult to observe clinically because the methods routinely available do not test any special function of vision. This might be the explanation why psychoactive drugs have not been observed to influence human vision.

If dopaminergic drugs do not affect vision in a readily detectable way because dopaminergic neurons are not directly connected to the neurons which form the
most direct link between photoreceptors and the brain, then other cells which do contact this link should affect vision significantly. As the following examples will show, this is perhaps the case, although only too few studies directly address this point.

**Cholinergic Neurons**

There is now very good evidence that cholinergic amacrine neurons occur in the retina. Acetylcholine is present in the tissue, as is its synthesizing and degrading enzymes, and specific receptors have also been demonstrated with several different methods (reviews: Neal, 1976, 1983). Moreover, there is a calcium-dependent and light-driven release system for acetylcholine (Masland and Livingstone, 1976; Baughman and Bader, 1977; Baughman, 1980; Neal and Massey, 1980; Neal, 1982, 1983). By autoradiography and other morphological methods, the cholinergic cells have been identified as amacrine cells, most likely of the special, so-called starburst type (Masland and Mills, 1979; Masland, 1980; Famiglietti, 1983a, b). Both physiological and pharmacological experiments suggest that these neurons contact the bipolar cells and presumably also the ganglion cells (Fig. 6). In birds, autoradiography suggests that certain bipolar cells perhaps are cholinergic (Baughman and
Bader, 1977) and in toads and goldfish there is evidence that a small percentage of the ganglion cells may be cholinergic (Oswald and Freeman, 1980).

In clinical ophthalmology, cholinomimetic drugs such as pilocarpine or various cholinesterase inhibitors have long been used as topical eye drops for the treatment of glaucoma. These drugs are likely to reach the retina in significant concentrations, especially in patients which have had their lenses removed because of cataract. However, neither textbooks nor the scientific literature usually suggest that the treatment has any effect on the retina. Nevertheless, all patients on pilocarpine or similar eye drops complain about blurred and obscured vision, at least initially. However, they still usually perform well on ordinary visual testing, so doctors tend not to pay very much attention to these complaints. The standard explanation the doctor gives to the patient is that his or her vision gets obscured because the pupil...
FIGURE 6. Cholinergic neurons (ACh) in the mouse and rabbit retina seem to contact both amacrine cells (A), bipolar cells (B) and, perhaps, ganglion cells (G). The diagram is somewhat tentative and based on receptor binding studies with alpha-bungarotoxin, autoradiography, electron microscopy, and certain electrophysiological experiments. Ph, photoreceptor; H, horizontal cell.

has been made so small by the drug. This effect is easily seen by the doctor, and the patient (or at least some relative) can readily confirm the observation, so the statement is usually accepted without any further discussion. The doctor would ask the patient to put up with the problem or lose vision. The choice seems easy.

However, the retina is able to change its sensitivity about a hundred thousand fold by adjusting the gain in its amplifier system. Constriction of the pupil by a
drug can diminish the light falling on the retina only about fifteen-fold, provided that no complicating factor is present, such as opacification of the lens by a cataract. Clearly, the doctor’s simple standard explanation must be in doubt in many cases. One can reasonably suspect a pharmacological action of the cholinergic drugs on the retina in addition to the change in pupil size. However, currently our standard methods for clinical investigations are too crude to tell easily what is actually happening. Clinicians will be able to design effective clinical testing methods only when they can be guided by results from experimental neurobiology, showing what functions the cholinergic neurons may have.

**GABA NEURONS**

There is a similar story for another neurotransmitter, *gamma*-amino butyric acid, usually abbreviated GABA.

There are good reasons to believe that GABA is a neurotransmitter in certain amacrine cells in the mammalian retina. It is present in high concentration in the same region of the retina as the amacrine cells, where the synthesizing enzyme can also be found (Kuriyama et al., 1968; Graham, 1972; Voaden, 1978; Brandon et al., 1979; Lam et al., 1979; Brandon et al., 1980; Famiglietti and Vaughn, 1981; Vaughn et al., 1981; Wu et al., 1981; Zucker et al., 1984). There is also a highly selective and very active uptake mechanism for GABA in certain amacrine cells which can be illustrated by autoradiography (Fig. 7). Moreover, tritiated GABA can be released from the retina by light flashes. Finally, different types of experiments in several laboratories have shown GABA receptors to be present in the retina (reviews: Ehinger, 1982, 1983b).

![Figure 7. Autoradiogram of a rabbit retina 4 hours after the intravitreal injection of (H)-GABA. Amacrine cells, some ganglion cells (G), and the entire inner plexiform layer (IPL) have become labeled. Ph, photoreceptors; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; G, ganglion cell layer. Phase contrast micrograph, 400×.](image-url)
Immunohistochemical analyses of the localization of the enzyme that synthesizes GABA have suggested that the GABA neurons connect directly to bipolar cells and, possibly, ganglion cells (Brandon et al., 1980; Famiglietti and Vaughn, 1981; Vaughn et al., 1981; Zucker et al., 1984). The postulated organization is shown in Figure 8.

When driving an automobile, it is extremely important that you are able to detect moving objects in the periphery of your field of vision, like a running child emerging from the sidewalk, for instance. It has been shown that GABA receptor blockers degrade the function of cells in the rabbit retina which detect movements (Wyatt and Daw, 1976; Caldwell et al., 1978). Similar results have been seen more recently in frogs (Bonaventure et al., 1983). Certain benzodiazepine tranquilizers like Valium (R) and Librium (R) are known to affect the GABA neurons and it would seem that one of the reasons why benzodiazepine drugs are unfit for drivers is that they make it difficult to detect the child running in from the side. Regrettably,
This has not been properly tested, presumably because there are no suitable and well-developed clinical test systems. Therefore we do not know if some benzodiazepines are more detrimental than others. However, one report suggests that this should be testable. The ability of humans to track moving stripes after having been dazzled by a strong light decreased with increasing concentrations of a benzodiazepine in their blood (Bergman et al., 1979). The authors' interpretation of their results did not include any discussion of the GABA receptors in the retina, and any inference about an effect on the retinal GABA neurons must await appropriate control experiments. However, the results do show that with properly refined clinical methods one is likely to be able to make significant observations on the function of neurotransmitters, but the tests will need a theoretical background that only animal experimentation can provide.

**Indoleamine Accumulating Neurons**

There are other neurotransmitters in the retina which should be of some future concern to both ophthalmologists and neurobiologists. In cold-blooded vertebrates, 5-hydroxytryptamine has been shown to be present in amacrine cells (Fig. 9) and it seems likely that it is a neurotransmitter. The substance is present in presynaptic terminals, it can be inactivated by re-uptake into neurons, receptors have been demonstrated for it, and there appears to be stores for it (reviews: Ehinger, 1982; Osborne, 1984; Redburn, 1984). Furthermore, tritiated hydroxytryptamine can be released by depolarizing stimuli and by light (Thomas and Redburn, 1979; Osborne, 1980, 1982a, b; Redburn, 1984; Ehinger, Tornqvist, and Waga, unpubl.). There are similar neurons in mammals, but it is not certain that 5-hydroxytryptamine is their transmitter because their content of the substance is at least 50 times less than in neurons known to be 5-hydroxytryptaminergic (Ehinger, 1982; Redburn, 1984). However, collectively these neurons can be called indoleamine accumulating neurons, and their similarities warrant that they be discussed together.

The indoleamine accumulating neurons can be identified using the electron microscope because they can take up 5,6-dihydroxytryptamine. This drug induces morphological changes similar to the ones seen in Figure 4 (Dowling et al., 1980; Ehinger, 1982; Holmgren-Taylor, 1982a–c) and makes it possible to trace their connections with the electron microscope. Since the dopaminergic neurons also accumulate the substance, these neurons must first be eliminated so that they do not interfere with the analysis. This can be done with the aid of the selective neurotoxin, 6-hydroxydopamine (Ehinger and Nordenfelt, 1977).

When the indoleamine-accumulating neurons are studied in the electron microscope, they are seen to contact bipolar cells (Fig. 10). Bipolar cells form part of the main signal pathway from the photoreceptor cells to the brain, and the indoleamine-accumulating neurons can thus be suspected to influence vision directly; but almost nothing is known about their function. Therefore it is not possible to predict what interfering with the indoleamine accumulating neurons will do to vision. Currently, however, many pharmaceutical companies are very actively testing new psychotropic drugs which act on the 5-hydroxytryptamine neurons, and it is likely that they will affect the function of the retina. Since there is at present no way of knowing what type of side effects to expect, it is not very likely that they will be discovered in the initial drug testing phases, but only when the drugs have come into extensive use. Again, we do not have good enough clinical methods available to analyze routinely the different components of vision.

As mentioned above, indoleamine accumulating neurons are somewhat complicated. It is now quite clear that there are two types, one that contains significant
FIGURE 9. Left, immunohistochemical demonstration of 5-hydroxytryptamine in the skate retina. There is an immunoreactive amacrine cell body (arrow) and there are numerous immunoreactive processes in the outer half of the inner plexiform layer. Right, phase contrast micrograph of the same region. Ph, photoreceptors; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; 280×.

amounts of 5-hydroxytryptamine and one that does not (Bruun et al., 1984). Mammalian retinas contain the type that does not contain very much 5-hydroxytryptamine. However, the pharmaceutical companies direct their work towards the ones which do contain 5-hydroxytryptamine and which are found in the mammalian brain. Some drugs that will be developed are likely to affect only the type containing much 5-hydroxytryptamine, others will presumably affect both types, and yet others may perhaps even affect only the type with little 5-hydroxytryptamine. This will cause confusion, and will make it even more difficult to detect whatever side effects on vision drugs acting on the indoleamine systems may have.

NEUROPEPTIDES

Neuropeptides presumably act as neurotransmitters, and many have now been found in the retina, most of them in amacrine cells (Table I). Much of the work has been done with immunohistochemistry (Tornqvist, 1983; Brecha et al., 1984),
and exact identification is available only for a few of the substances and in a limited number of animal species. Also, no retina has been shown to have all of them. The number of known neuropeptides is growing rapidly, and many will no doubt be added to the list.

Little is known about the function of the neuropeptides in the retina, so it is more than premature to speculate about their possible clinical implications. However, it is important to note that they are present, because drugs acting as either agonists or antagonists are now very actively being developed in many laboratories. Substance P may be taken as an example. A substance P antagonists [Spantid (R)] has been shown to have anti-inflammatory effects in rabbits (Holmdahl et al., 1981; Bynke,
TABLE I
An alphabetical list of some neuropeptides shown or presumed to be present in the retina of different animals

<table>
<thead>
<tr>
<th>Neuropeptide</th>
<th></th>
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<tbody>
<tr>
<td>Bombesin</td>
<td>NPY</td>
</tr>
<tr>
<td>Cholecystokinin (CCK)</td>
<td>PHI</td>
</tr>
<tr>
<td>Endorphins</td>
<td>Somatostatin</td>
</tr>
<tr>
<td>Enkephalins</td>
<td>Substance P</td>
</tr>
<tr>
<td>Glucagon</td>
<td>TRH (no immunohistochemistry)</td>
</tr>
<tr>
<td>LHRH</td>
<td>VIP</td>
</tr>
<tr>
<td>Neurotensin</td>
<td></td>
</tr>
</tbody>
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1984) and it seems possible that it may find use in ophthalmology as an anti-inflammatory drug. Others are likely to follow. Therefore there are good reasons to investigate what the drugs affecting the function of neuropeptides might do to vision, first by finding out where the neuropeptides are in the retina and then to continue with physiological and pharmacological work.

PIGMENT EPITHELIUM

The examples above all show how signals are being sent between nerve cells in the retina. There are several other very important signal systems in it, and one is of particular significance to ophthalmologists.

It has been known for some time that normal functioning of the photoreceptor cells is dependent on the pigmented epithelium. Figure 1 shows how the pigment epithelium sits just next to the photoreceptor cells. In some very elegant studies only little more than a decade ago Young (1967) showed that photoreceptor cells grow continuously and shed their tips in order to keep their length constant. The pigment epithelium phagocytizes the discarded photoreceptor tips (Hollyfield and Basinger, 1978).

Since the phagocytosis of photoreceptor tips has been found to take place only during a few hours and only once daily for a given class of cells, there must be a very precise system to tell the pigment epithelium when it is time to consume a little of the photoreceptor cells, and which ones. We are just beginning to suspect that certain chemicals, present in the retina, may turn this system on or off, although the picture is still very far from clear (Ogino et al., 1983; Besharse et al., 1984). Different disturbances in the turnover system of the photoreceptor cells are well known in various laboratory animals. In humans, there may be similar situations in a special group of diseases, collectively called retinitis pigmentosa. These disorders are relatively common, stealing the eyesight from some 50,000 or perhaps more Americans. In most cases the patients are young, and otherwise the disease does not effect them so that they live an ordinary life except that they are blind. It is tantalizing that it is realistic to hope for a treatment in these cases. However, there is much work to be done before any treatment will be available. Therefore, it seems extremely important to point out that studies on the interactions between the retinal pigment epithelium and the photoreceptor cells carry the potential of helping to retain the eyesight of tens of thousands of people in the U. S. alone and many many more in other countries. High priority should be given to such work.

CONCLUSIONS

We now know a number of neurotransmitters in the retina (Fig. 11) and for several, the circuits of the cells they are in have been traced. This knowledge is
potentially useful in two ways: (1) it can help us pinpoint the cause of certain diseases so that remedies can constructively be sought for and (2) it can help us predict the effects of new drugs, favorable or unfavorable.

At the same time it seems evident that with this multitude of different transmitters, there are perhaps as many different tasks that the retina performs. There is a shortage of adequate methods for testing possible functions in the clinical setting. This is the task for the neurobiologists: find the functions of the different neurotransmitters and their circuits and tell the clinicians how to search for the function also in patients. No doubt such work will have to start on cold blooded vertebrates which are comparatively easy to work with and therefore suitable as models for more complicated systems.

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