Synaptic organization of the dopaminergic neurons in the retina of the cynomolgus monkey

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Dopaminergic neurons have previously been demonstrated in the retina of cynomolgus monkeys (Macaca fascicularis) by fluorescence microscopy. These neurons take up 5,6-dihydroxytryptamine, which alters their ultrastructure, and this technique has been used in the present study to identify the dopaminergic retinal neurons in the electron microscope. There are no indoleamine-accumulating neurons in the cynomolgus monkey retina to interfere with the analysis of the dopaminergic cells. The dopaminergic neurons have their cell bodies among those of the amacrine neurons in the inner nuclear layer. Their processes ramify mainly in the outermost sublayer of the inner plexiform layer. However, dopaminergic processes can be found occasionally to extend into the middle part of the inner nuclear layer and rarely into the innermost parts of the inner plexiform layer. All their output synapses are of the conventional kind. They appear to form synaptic connections only with other amacrine neurons, indicating that the dopaminergic amacrine cells in the retina of the cynomolgus monkey are interamacrine neurons. (INVEST OPHTHALMOL VIS SCI 22:8-24, 1982.)

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The distribution of the dopamine-containing cells and their processes in the retina can be readily visualized with the histochemical method of Falck and Hillarp (reviewed in refs. 1 and 2). In all species investigated so far they have their perikarya in the inner nuclear layer along the junction of the inner nuclear and inner plexiform layers. Occasionally a dopamine-containing perikaryon can be found in the ganglion cell layer or rarely in the inner plexiform layer.

In most species the processes of the dopaminergic neurons are found mainly within the inner plexiform layer, and they usually ramify near the junction of the inner nuclear and inner plexiform layers. This is the case in the retinas of the mouse, rat, cat, some monkeys such as the cynomolgus (an Old World monkey), and of man. In other animals the dopaminergic processes form two or three sublayers in the inner plexiform layer. This pattern of ramification is found in the toad, mudpuppy, pigeon, chicken, guinea pig, rabbit, and baboon among others. In many species, including most mammals, the dopamine-containing processes also extend into.
Fig. 1. A, Fluorescence micrograph of a control retina of a cynomolgus monkey, processed according to the Falck and Hillarp method. A fluorescent dopaminergic cell body (large arrow) is seen in the innermost part of the inner nuclear layer. Fluorescent processes arborize at the junction of the inner nuclear and inner plexiform layers. One fluorescent process is seen to extend between the innermost perikarya of the inner nuclear layer (small arrow). PH, Photoreceptors; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. (×265.) B, Fluorescence micrograph of a cynomolgus monkey retina processed according to the Falck and Hillarp method after intravitreal injection of 5,6-dihydroxytryptamine. There is a fluorescent perikaryon in the innermost row of amacrine cell bodies in the inner nuclear layer (large arrow). The fluorescent neuronal processes ramify at the junction of the inner nuclear and inner plexiform layers, with occasional processes extending between and around the innermost nonfluorescent cell bodies in the inner nuclear layer (small arrow). The distribution of fluorescent perikarya and processes is similar to that seen in the control retina (a). There is no evidence of uptake of 5,6-dihydroxytryptamine into any other cell population in the retina than the dopaminergic cells. (×430.).
the inner nuclear layer. Their numbers and distribution in this layer vary from species to species. Most commonly there are only a few processes between the nondopaminergic perikarya in the innermost half of the inner nuclear layer, but in several New World monkeys they form a plexus of fibers within the inner nuclear layer.\textsuperscript{4-6}

In teleost fish and several New World monkeys such as the \textit{Cebus}, the dopaminergic neurons have processes that extend widely both in the inner and outer plexiform layers. These neurons make abundant synapses in the outer plexiform layer, on horizontal and bipolar cells, and for this reason they are classified as a separate kind of retinal neuron, the interplexiform cell.\textsuperscript{4, 5, 7-10} Interplexiform cells have also been demonstrated in other animal species such as the dolphin, rat, cat, rabbit, and rhesus monkey.\textsuperscript{6, 11-15} However, the interplexiform cells of these animals are not visualized with the technique of Falck and Hillarp and consequently they are not believed to be dopamine-containing.

The dopaminergic cells and their processes are not ordinarily identifiable in the electron microscope. Therefore, to analyze the synaptic connections of these cells, the neuronal processes must be labeled. Dopaminergic cells will actively accumulate both dopamine and certain analogous amines such as a-methyl-\textit{noradrenaline}, 6-hydroxydopamine, or 5,6-dihydroxytryptamine,\textsuperscript{9, 16, 17} and certain of these analogues will alter the fine structure of the neurons. Of these analogues, 5,6-dihydroxytryptamine has the advantage of producing distinctive ultrastructural changes in the dopaminergic processes as well as increasing the intensity of their fluorescence.\textsuperscript{8-10, 16, 17}

However, 5,6-dihydroxytryptamine is taken up not only by the dopaminergic retinal neurons but also by the indoleamine-accumulating neurons present in the retinas of many animal species,\textsuperscript{10, 18, 19} which complicates the analysis of the dopaminergic cell synaptic organization. On the other hand, there are no indoleamine-accumulating retinal neurons in the cynomolgus monkey.\textsuperscript{17} Hence, to study the distribution of the dopaminergic neurons in the fluorescence microscope or their ultrastructure in the electron microscope, 5,6-dihydroxytryptamine may be used.

The cynomolgus monkey is of interest not only because it is a primate but also because its retina is particularly similar to that of man.\textsuperscript{17, 20} It is obvious that care must be taken not to draw too generalized conclusions about man from facts obtained from cynomolgus monkeys. Nevertheless, the similarities give a special significance to the elucidation of the synaptic organization of the dopaminergic retinal neurons of the cynomolgus monkey.

\textbf{Materials and methods}

Three adult cynomolgus monkeys (\textit{Macaca fascicularis}) were used. All injections were given intravitreally with a precision syringe. Four eyes were injected with 50 \(\mu\)g of 5,6-dihydroxytryptamine. The drug was dissolved in 50 \(\mu\)l of 0.9% NaCl with ascorbic acid added as antioxidant (1 mg/ml). One control eye did not receive any injection, and another was injected with the solvent only. The eyes were enucleated 4 hr after the intravitreal injection. The posterior segments of experimental and control eyes were prepared for electron microscopy by fixation in 2% OsO\textsubscript{4} in barbital (Veronal) acetate buffer (pH 7.6 to 7.8) containing 28 mM barbital sodium, 28 mM sodium acetate, 66 mM sucrose, and 1.8 mM CaCl\textsubscript{2} for 1 hr in an ice bath and for another half hour at room temperature.\textsuperscript{21} The tissue was then dehydrated, embedded in Epon 812, sectioned, and stained with uranyl acetate and lead citrate according to conventional methods. Tissue blocks from at least three different parts of the posterior segments were taken. The sections were analyzed in Philips 300 and JEOL 100 B electron microscopes.

As additional controls, pieces of retina from all eyes were processed for fluorescence microscopy according to the Falck and Hillarp method.\textsuperscript{2} 5,6-Dihydroxytryptamine creatinine sulfate was obtained from the Regis Chemical Co., Morton Grove, Ill.

\textbf{Results}

\textbf{Light microscopy.} The fluorescent dopaminergic cell bodies were situated almost entirely in the innermost row of amacrine cell bodies in the inner nuclear layer (Fig. 1). There were fluorescent cell bodies occasionally seen in the ganglion cell layer. These \textquotedblleft alloganglion\textquotedblright cells\textsuperscript{22} constituted no more than 5% to 10% of the number of dopa-
Dopaminergic retinal neurons in monkey

Fig. 2. a, Electron micrograph of a labeled dopaminergic process (asterisk) with an output synapse to an unidentified neuronal process (P) in the outermost sublayer of the inner plexiform layer. There is a presynaptic cluster of small synaptic vesicles in the labeled process. On the presynaptic and postsynaptic membranes as well as in the synaptic cleft there is electron-dense material. Many of the small synaptic vesicles in the labeled process have increased electron density of their membranes with eccentric spots of dense material. A few of them are flattened in shape. A large synaptic vesicle, completely electron dense, is seen with a thin process extending out. There are also a few vesicular structures of intermediate size. b, Small synaptic vesicles are shown in higher magnification. (OsO₄ fixation; a ×52,000, b ×92,000.)

Dopaminergic cell bodies found in the innermost part of the inner nuclear layer. Dopaminergic cell bodies were also very rarely detected in the inner plexiform layer ("eremite" cells\(^2\)).

Nearly all of the dopaminergic neuronal processes were confined to the outermost part of the inner plexiform layer. However, dopaminergic processes were sometimes observed extending between nonfluorescent cell bodies in the innermost part of the inner nuclear layer and occasionally surrounding such a cell body. Only very rarely could a dopaminergic process be seen further out in the inner nuclear layer or in the inner parts of the inner plexiform layer.

Electron microscopy

Ultrastructural alterations. The ultrastructural changes produced in the cynomolgus...
monkey by 5,6-dihydroxytryptamine were similar to those described in previous studies that used this labeling method. For example, the membranes of the small synaptic vesicles (20 to 40 nm) in the labeled processes usually showed increased density, and within the vesicles an eccentrically located dense spot of material was often present. The vesicles were also sometimes elongated or flattened in shape (Fig. 2). Large vesicles (50 to 70 nm) with enhanced electron-dense cores were frequently present in the processes. Sometimes these large vesicles were completely filled with electron-dense material, but at other times their outer membrane was distorted into a long process or was "balloned" to one side (Figs. 2 and 3, a).

The cell membranes of labeled neurons usually did not show any increased electron density except in synaptic or other junctional regions (Figs. 4 and 5). The increase in electron density of mitochondrial membranes, however, was frequently quite marked and the mitochondria were often swollen (Figs. 4 and 5).

Fig. 3. a, Electron micrograph of a synapse from an amacrine process (A) to a labeled varicosity (asterisk). The presynaptic vesicles are few (large arrow) and the presynaptic and postsynaptic membrane densities are inconspicuous. The electron-dense material in the synaptic cleft is clearly visible. A desmosome-like junction between the labeled varicosity and an adjacent neuronal process is also seen (small arrow). There are fairly symmetric electron-dense depositions on the junctional cell membranes and dense material in the intercellular cleft. No vesicles are seen in the vicinity of this junction. The labeled varicosity contains a large synaptic vesicle filled with dense material to its limiting membrane and a few labeled small vesicles. b, The same input synapse is seen in an adjacent section. There are no clearly labeled cell structures in the dopaminergic process. Outer sublayer of the inner plexiform layer. (OsO₄ fixation; a and b ×54,000.)
Fig. 4. Electron micrograph of one labeled dopaminergic process (asterisk) and one unlabeled amacrine process (A) with output synapses (arrows) to the same unlabeled varicosity (P) at the junction of the inner nuclear and inner plexiform layers. The labeled process contains a swollen mitochondrion with increased density of the outer membrane (M) and some labeled small synaptic vesicles. The synapses are of the conventional kind, with a presynaptic cluster of synaptic vesicles, dense depositions on the presynaptic and postsynaptic membranes as well as increased density of the membranes themselves, and electron-dense material in the synaptic cleft. The synaptic cleft between the dopaminergic process and the unlabeled varicosity appears to be slightly widened. In the upper left-hand corner the basal membrane of a retinal capillary is seen. (OsO4 fixation; x46,000.)

to 6). Most labeled processes seemed to have their cell structure well preserved in spite of the above-mentioned changes in the organelles (Figs. 7 and 8). Other processes had obvious signs of degeneration. Some of them were swollen, and in these processes the usual organelles, including synaptic vesicles, were scarce. In others tight clusters of small synaptic vesicles were found in one part of the process. The rest of the varicosity was then empty (Fig. 6). Other processes seemed to be shrunken. Shrunken varicosities were usually filled with small synaptic vesicles of increased electron density (Fig. 9, a).

In addition to a variation in the labeling from process to process, there was also variability in the labeling within each given neuronal process. A dopaminergic process that in one section contained both mitochondria and synaptic vesicles showing alterations could in
Fig. 5. a, Electron micrograph of two juxtaposed labeled processes (asterisks) in the outermost sublayer of the inner plexiform layer. One labeled process is filled with small synaptic vesicles and has a distorted labeled mitochondrion. The other dopaminergic process displays only a few labeled small synaptic vesicles and three large vesicles whose central cores have various degrees of electron density. The processes are connected by a desmosome-like junction (arrow). In this region there is increased density of the cell membranes, dense depositions on the cell membranes, and electron-dense material in the intercellular space. (OsO₂ fixation; ×55,000.) b, A high-magnification electron micrograph of another desmosome-like junction between two labeled processes. Symmetric depositions of electron-dense material on the cell membranes are seen as well as dense material in the intercellular space. (OsO₂ fixation; ×95,000.)

an adjacent section display only unlabeled organelles (Fig. 3). Also, in a process in one particular section, the mitochondria could be labeled and the synaptic vesicles unlabeled, whereas in an adjacent serial section the mitochondria were unlabeled and the synaptic vesicles labeled.

Distribution of labeled processes. The labeled processes consisted of the usual varicosities alternating with thin intervaricose segments. When cut in cross-section the labeled intervaricose segments were difficult to detect. The varicosities were generally of intermediate size, most with a diameter of 0.3 to 0.7 μm.

The distribution of the labeled dopamin-
Dopaminergic processes was in agreement with the fluorescence microscopic findings. The vast majority of the dopaminergic processes were found to be evenly distributed in the outermost sublayer of the inner plexiform layer, near the junction between the inner plexiform and inner nuclear layers. At times labeled varicosities were seen in the vicinity of retinal capillaries, but they were never to be found in direct contact with capillary structures. On occasion degenerating labeled processes were observed inside glial cells in the outer parts of the inner plexiform layer.

Processes were occasionally found between and on the outside of cell bodies in the innermost row of amacrine cell bodies in the inner nuclear layer (Fig. 10), but they were not seen further outward. Labeled processes were extremely rare in the middle and inner parts of the inner plexiform layer. No labeled processes were detected in the outer plexiform layer.

**Synaptic structures.** All synapses made by the dopaminergic processes were of the conventional kind, with a cluster of small synaptic vesicles on the presynaptic side of the junction. These vesicles were occasionally labeled but sometimes were not. The large synaptic vesicles with a dense core were at times seen in close proximity to a synapse, but more often they appeared to be distributed in the varicosity without any special relationship to the synaptic structures. On the presynaptic membrane there was always an aggregation of electron dense material with either a fibrillar, granular, or flocculent appearance. The
Fig. 7. Electron micrograph of a serial synapse with an output synapse (large arrow) from a dopaminergic varicosity (asterisk) to an amacrine cell body (A), which makes synaptic contact (small arrow) onto a bipolar terminal (B). The bipolar cell has a ribbon synapse onto two other neuronal processes in the dyad arrangement. (OsO₄ fixation; X36,000.)

The output synapses were found in the varicosities of the dopaminergic processes and contacts were made onto the varicosities of unlabeled neurons as well as onto their inter-varicose segments. In at least five instances output synapses from labeled varicosities were seen to contact an amacrine process adjacent to its cell body (Fig. 8). Several of the dopaminergic output synapses made contacts onto varicosities, which themselves contained conventional synapses (Fig. 6). In these cases the postsynaptic neurons were likely to be amacrine cells. Synapses from labeled dopaminergic processes contacting unlabeled amacrine perikarya in the innermost row of cell bodies in the inner nuclear layer were also frequently seen (Fig. 7).

At many synapses the postsynaptic neuron
Fig. 8. a, Electron micrograph of an amacrine cell body (A) in the inner nuclear layer with a process extending into the inner plexiform layer. Three dopaminergic varicosities are juxtaposed to this amacrine cell process (asterisks). Two of the dopaminergic varicosities have output synapses to the process (arrows). There are two more labeled processes in the figure, and to the left in the picture there is a retinal capillary. b, One of the synapses is seen in higher magnification. (OsO₄ fixation; a × 25,000, b × 36,000.)
Fig. 9. a, Electron micrograph of a labeled dopaminergic process at the junction of the inner nuclear and inner plexiform layers. The process is shrunken and filled with small synaptic vesicles of increased electron density and swollen distorted mitochondria. A junction between the labeled process and an unidentified neighboring process is seen at arrow and in b at higher magnification. (OsO4 fixation; a \( \times 29,000 \), b \( \times 110,000 \).)

was hard to identify with certainty, but most of them had characteristics considered typical of amacrine neurons, e.g., unevenly scattered synaptic vesicles and few other cytoplasmic organelles such as mitochondria and neurotubules in the processes. In no case did the postsynaptic neuron have features similar to those of bipolar terminals, such as baglike shape, numerous evenly distributed synaptic vesicles, and synaptic ribbons. Also, none of the postsynaptic processes had the appearance of ganglion cell dendrites with abundant ribosomes, vesicular endoplasmic reticulum, and absence of synaptic vesicles.

A small number of contacts between labeled processes were observed. A few of those contacts resembled the typical amacrine synapses described above. In other cases the contacts between two labeled processes more closely resembled desmosomes with very symmetric aggregations of fibrillar electron-dense material and increase in membrane electron density (Fig. 5). The intercellular cleft also contained electron-dense material. Vesicular structures were occasionally
Fig. 10. Electron micrograph of a labeled dopaminergic process (arrow) on the outside of an amacrine cell perikaryon (A) in the innermost row of cell bodies in the inner nuclear layer. (OsO₄ fixation; ×14,000.)

seen in the processes close to a junction like this, but they were not arranged in presynaptic clusters. These junctions were also seen occasionally between labeled and unlabeled processes (Figs. 3 and 9), but they tended to be more inconspicuous and asymmetric in that the deposition of material on the cell membrane of the labeled process was normally larger. Electron dense material was observed in the intercellular space at these junctions. A majority of the unlabeled elements were clearly neurons with amacrine characteristics, but one or two of them were presumed to be glial processes.

A smaller number of synapses onto labeled processes were detected. Of the synapses observed involving labeled processes only about 5% were input synapses. Furthermore, these synapses were inconspicuous and difficult to find. The presynaptic cluster of synaptic vesicles was always of the conventional kind, but usually only a few small synaptic vesicles were observed close to the presynaptic membrane. In most input synapses there was a
Fig. 11. Summary diagram of a dopaminergic neuron (DA) and its synaptic connections in the cynomolgus monkey retina. Its perikaryon is situated in the innermost row of amacrine cell bodies (A) in the inner nuclear layer (INL). Its dendrites arborize almost exclusively in the outermost sublayer of the inner plexiform layer (IPL). One process extends into the inner nuclear layer to the outside of an amacrine cell perikaryon. The dopaminergic processes make synaptic contacts onto the amacrine cell bodies and their processes as they emerge from the cell bodies at the junction of the inner nuclear and inner plexiform layers. They also have numerous output synapses onto the more distal varicosities and intervaricose segments of amacrine neurons. A dopaminergic output synapse forming part of a serial synapse with two other nondopaminergic amacrine cells is depicted. The dopaminergic neuron receives synaptic input from a nondopaminergic amacrine process. The synaptic structures of this connection are discrete. A desmosome-like junction is shown between two dopaminergic processes. No direct synaptic contacts between the dopaminergic neuron and bipolar (B) or ganglion cells are found. Thus the dopaminergic cells form a class of interamacrine neurons.

deposition of electron-dense material on both the presynaptic and postsynaptic membranes, but the depositions were often inconspicuous. Both membranes occasionally had slightly increased electron density, and the synaptic cleft contained fibrillar material (Fig. 3). Because of their synaptic structure, all presynaptic contacts were identified as likely to be coming from amacrine cells. The presynaptic process was usually a small-sized varicosity, whereas the postsynaptic dopaminergic processes were mostly middle-sized varicosities.

Synaptic organization. Serial synapses involving synapses from a dopaminergic process making contact to an amacrine cell process or perikaryon and from that element onto another unlabeled process were rather frequent (Fig. 6). There were also serial synapses involving a bipolar terminal as the third process (Fig. 7).

Single dopaminergic processes were seen in serial sections to make two separate synaptic contacts onto the same amacrine process close to its cell body, or onto the same unidentified unlabeled process. In other instances, two labeled processes with synapses to the same amacrine process were seen in one section. Conversely, labeled and unlabeled amacrine processes were observed to have synapses to the same unlabeled varicosity (Fig. 4). Finally, there were dopaminergic processes with synaptic output to two separate unlabeled varicosities as well as to one unlabeled process and an amacrine cell body. Only one or two suspected reciprocal synapses between labeled and unlabeled amacrine cells were observed.
Discussion

In this study, the technique of labeling monoaminergic retinal neurons with 5,6-dihydroxytryptamine produced enhanced fluorescence and ultrastructural changes similar to those described in earlier studies.\(^8\)\(^{-10}\), \(^{16}\)\(^{-17}\), \(^{23}\) Compared with autoradiographic techniques, the labeling with 5,6-dihydroxytryptamine has the advantage of superior resolution, but one of the greatest advantages is the opportunity to study both the distribution of labeled neurons and their processes with fluorescence microscopy and the ultrastructure and synaptic connections of the same neurons in a single retina. The distribution of labeled perikarya and processes found in this study is in agreement with the findings of earlier fluorescence microscopy studies of untreated cynomolgus monkey retinas as well as 5,6-dihydroxytryptamine-treated retinas.\(^{17}\), \(^{20}\), \(^{22}\), \(^{25}\)

Since the distribution of labeled neurons and their processes was the same with both fluorescence and electron microscopy, it is clear that the labeled neurons were dopaminergic and that no other neurons have been labeled with this experimental procedure. In addition, light microscope autoradiography after incubation of cynomolgus monkey retinas in \(^{3}\text{H}\)-5-hydroxytryptamine has shown radioactivity in only a single layer in the inner plexiform layer near the junction between the inner nuclear and plexiform layers, and radioactive cell bodies only among the amacrine cells in the innermost cell row of the inner nuclear layer (Ehinger and Holmgren, unpublished observations). Thus autoradiography confirms the earlier findings that there are no indoleamine-accumulating neurons in the retina of the cynomolgus monkey.

Nearly all the dopaminergic neurons in the cynomolgus monkey retina belong to a cell type that was originally called the "adrenergic junctional" cell.\(^5\) All available evidence indicates that the neurotransmitter of these neurons is dopamine. Their perikarya are situated among those of the amacrine cells in the inner nuclear layer, and apart from some ramifications in the inner nuclear layer, their processes are confined to the inner plexiform layer. Also like amacrine cells, the dopaminergic neurons make synapses of the conventional kind. Thus the dopaminergic neurons in the cynomolgus monkey have all the characteristics of amacrine cells, and it seems preferable to call them dopaminergic amacrine cells.

In teleost fish and several New World monkeys the interplexiform cells are dopaminergic.\(^4\), \(^5\), \(^7\), \(^8\)\(^{-10}\) The interplexiform cells have been extensively studied in the goldfish and in the *Cebus* monkey by Dowling and Ehinger\(^9\) and Dowling et al.\(^{10}\) These investigators infer that most of the dopaminergic neurons in goldfish and *Cebus* monkey retinas are interplexiform. However, since no method of labeling is available that distinguishes between different kinds of dopaminergic neurons, it is at present impossible to exclude the possibility that there are also dopaminergic amacrine cells in the retinas of these animals. To further complicate the analysis of the dopaminergic neurons and their synaptic connections in the *Cebus* mon-
key, there may be an additional third kind of dopaminergic neuron, the “pleomorph” cell, present in the retina of this monkey. This cell has not been seen in the cynomolgus monkey, however.

When comparing the light- and electron-microscopic appearance of the dopaminergic amacrine cell in the cynomolgus monkey retina with earlier descriptions of Golgi-stained amacrine cells, one finds that this neuron most closely resembles the “smaller unistratified amacrine cell” described by Boycott and Dowling. The processes of this unistratified cell ramify within only one plane of the inner plexiform layer, generally in the scleral half. In the paper of Boycott and Dowling there is a picture of a unistratified amacrine cell in the rhesus monkey retina (Fig. 62, p. 152), which has a very similar appearance to that of the dopaminergic amacrine cell in the cynomolgus monkey. The amacrine cell pictured has its perikaryon in the innermost part of the inner nuclear layer, and its processes ramify in the inner plexiform layer along the junction of the inner nuclear and inner plexiform layers. Boycott and Dowling do not describe any processes extending from this particular amacrine cell into the inner nuclear layer, but in the picture mentioned, a process can be seen extending into the inner nuclear layer close to the stained perikaryon in the same way that dopaminergic processes are seen in cynomolgus retinas processed according to the Falck and Hillarp method. However, it is not clear from the picture or the text of the paper whether this particular process is connected with the stained neuron in question. It is noteworthy that both the rhesus and cynomolgus monkeys are Old World monkeys.

Fig. 11 is a summary diagram showing a dopaminergic amacrine neuron with its perikaryon in the innermost row of amacrine cell bodies in the inner nuclear layer and the vast majority of its processes limited to the outermost sublayer of the inner plexiform layer. One process is drawn partially surrounding another amacrine cell perikaryon. The dopaminergic processes make synapses onto both cell bodies and processes of other amacrine cells. One synapse is depicted from a nondopaminergic amacrine cell process to a varicosity of a dopaminergic process. There are no direct synaptic connections between the dopaminergic neuron and bipolar or ganglion cells.

One puzzle is the difficulty with which the input synapses to dopaminergic processes are detected. The same difficulties were encountered when the synaptic connections of the dopaminergic neurons in the retina of the rabbit and of the Cebus monkey were analyzed. In the rabbit the synaptic input from other amacrine neurons was found to be always onto the intervaricose segments of the dopaminergic cells. The chance of finding labeled organelles such as synaptic vesicles or mitochondria in the thin intervaricose segments is small, and it is also hard to trace these intervaricose segments in serial sections. This may explain some of the difficulties in finding the input synapses from nondopaminergic neurons in that animal. In the present study, the number of input synapses onto dopaminergic processes observed was also very small, and generalized conclusions about the parts of neuronal processes involved cannot be made. Another reason why input synapses are so infrequently observed is that they tend to be inconspicuous, with only three or four synaptic vesicles seen in the presynaptic process in any one section and very little in the way of membrane specialization at the presumed presynaptic site. However, the difference in the number of output and input synapses is substantial enough to warrant the conclusion that output synapses are far more common than input synapses.

The synaptic arrangement of the dopaminergic amacrine cells is similar to that of the dopaminergic neurons in the retina of the rabbit and that seen in the inner plexiform layer of goldfish and Cebus monkey. Thus, where investigated, the dopaminergic neurons in the inner plexiform layer have been found to contact only amacrine cells. Other kinds of amacrine cells, on the other
hand, are known to have contacts with bipolar neurons and ganglion cells, as well as with other amacrine cells. From the time of Ramón y Cajal it has been recognized that the amacrine cells of the retina can be divided into subclasses on a morphologic basis. In recent years a multitude of neurotransmitters have been indicated for the amacrine neurons (acetylcholine, dopamine, an indole, amino acids, and possibly peptides). Thus a number of pharmacologically distinct subclasses of amacrine cells have emerged. Ultrastructural analysis has demonstrated that within one animal, different pharmacologic subclasses of amacrine neurons may have different morphologic traits and different synaptic organization. In the rabbit retina, for instance, the dopaminergic neurons have their dendritic arborizations within three sublayers in the inner plexiform layer but mainly within the outermost sublayer. They exchange synaptic information only with other amacrine cells. The indoleamine-accumulating neuronal processes, on the other hand, ramify chiefly in the innermost sublayer of the inner plexiform layer. The vast majority of their synaptic connections are formed with bipolar cells, generally in a reciprocal arrangement.

It is also known from electrophysiologic studies that neurotransmitter substances such as acetylcholine, \( \gamma \)-aminobutyric acid, and glycine and their agonists and antagonists have different effects on the ganglion cells of the retina and that these effects often vary considerably from species to species. Dopamine has been shown to depress light-evoked excitation and to enhance light-evoked inhibition of ganglion cells in the cat retina after iontophoretic application or close intraarterial injection. These responses are the same for on, off, and on-off ganglion cells. In the isolated and perfused rabbit retina, dopamine increases the activity of off ganglion cells and decreases the spontaneous and evoked activity of on and on-off ganglion cells. An extensive study of the effects of dopamine on the retina of the goldfish suggests that the dopaminergic neurons (which are interplexiform in the goldfish) act on the luminosity-type cone horizontal cells and bipolar cells in the outer plexiform layer and on the transient amacrine cells in the inner plexiform layer. Dopamine decreases the light responsiveness of the horizontal cells, suggesting that the dopaminergic cells regulate lateral inhibitory effects and center-surround antagonism in the outer plexiform layer. The above results indicate several functions for dopaminergic neurons in the retina. However, much more detailed investigations are needed before the function of the various types of dopaminergic neurons throughout the animal series is clear.

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