Dopamine-Accumulating Retinal Neurons Revealed by in Vitro Fluorescence Display a Unique Morphology

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Dopamine is the principal catecholamine neurotransmitter in the vertebrate retina. The shape of retinal neurons that accumulate dopamine has been demonstrated in an in vitro preparation of cat retina. This was achieved by the discovery that the combined uptake of dopamine and the indoleaminergic transmitter analog 5,7-dihydroxytryptamine leads to an intense, catecholamine-like fluorescence in the cell bodies and processes of presumed dopaminergic amacrine cells in the living retina. This fluorescence served as an in vitro marker for these cells, and their detailed morphology was analyzed after intracellular injection of horseradish peroxidase under direct microscopic control. The horseradish peroxidase–filled cells show an unprecedented neuronal morphology: each cell gives rise to multiple, axon-like processes that arise from, and extend for millimeters beyond, the dendritic tree. The unique structure of this type of amacrine cell suggests a function for dopamine in long-range lateral interactions in the inner plexiform layer.

DOPAMINE IS PRESENT IN DISTINCT subpopulations of neurons in the vertebrate retina. Dopaminergic interplexiform cells in teleost fish retinas make synaptic contact with horizontal cells in the outer plexiform layer (OPL) where the release of dopamine during dark adaptation plays a neuromodulatory role, acting via adenosine 3′,5′-monophosphate (cyclic AMP), to effect long-lasting changes in the strength of lateral inhibition mediated by the horizontal cells (*1*). Dopaminergic amacrine cells make synaptic contact in the inner plexiform layer (IPL) and appear to be the principal type of catecholaminergic neuron in the mammalian retina (*2–4*), but, by contrast with the evidence in the OPL of teleost retina, the significance of dopamine release here is not understood. This report provides a detailed view of the shape of the putative dopaminergic amacrine cell by making use of a fluorescent marker for cells that accumulate dopamine in the living retina.

In vitro fluorescence of dopamine-accumulating (DA) neurons was demonstrated in cats that received intravitreal injections of dopamine (50 to 500 μg) and 5,7-dihydroxytryptamine (5,7-DHT) (100 to 500 μg) while under deep halothane anesthesia. The eye was removed 3 to 8 hours after the injection and hemisected, and the retina was dissected in oxygenated Ames medium (*5*). The retina was then laid flat, vitreal side up, in a tissue chamber mounted on the stage of a light microscope and continuously superfused with oxygenated Ames medium at room temperature. When the retina was observed under blue episcopic illumination, an intense green fluorescence was present in a distinct population of multipolar cells located at the inner margin of the inner nuclear layer (INL) (Fig. 1). The chemical basis for this fluorescence is unknown, but it appears to involve uptake of both 5,7-DHT and dopamine because the intravitreal injection of either alone does not produce the effect. Uptake of 5,7-DHT alone in rabbit retina leads to a weak in vitro fluorescence in the cell bodies of serotonin-accumulating neurons (*6*), but this fluorescence appears in a population of cells morphologically distinct from those demonstrated here. It is known that 5,7-DHT is highly reactive (*7*) and may be converted to an aldehyde by monoamine oxidase (*8*). The fact that formaldehyde reacts with dopamine in fixed tissue to yield the catecholamine fluorophore suggests that 5,7-DHT–aldehyde may react with dopamine to yield a similar fluorophore in vitro.

The fluorescing cells gave rise to thick, sparsely branching, primary dendrites embedded in a plexus of intensely fluorescing varicosities and fine processes. This plexus was narrowly stratified at the outer border of the IPL and was interrupted by round holes (7 to 10 μm in diameter), which gave it a honeycomb appearance (Fig. 1). Counts of fluorescing cells from retinas maintained in vitro show densities ranging from 12 to 16 cell/mm^2 in the peripheral retina up to 120 cell/mm^2 near the area centralis (Fig. 2).

The appearance, stratification, and density of these cells correspond to a subpopulation of putative dopaminergic amacrine cells identified in the retina of several mammalian species (*9–15*). In cats, large multipolar cells (11 to 19 μm in diameter) located at the inner border of the INL at relatively low densities (6 to 50 cell/mm^2) have been observed with histochemical fluorescence (*16*), tyrosine hydroxylase immunoreactivity (*17*), and autoradiographic localization of [^{3}H]dopamine uptake (*4*). These methods also reveal the varicose plexus in the IPL and its characteristic honeycomb appearance (*13, 16, 17*).

To determine their detailed morphology, fluorescing cells were impaled under direct microscopic control with high-resistance microcapillary electrodes and iontophoretically injected with Lucifer yellow (LY) and rhodamine-conjugated horseradish peroxidase (HRP). When viewed for LY fluores-

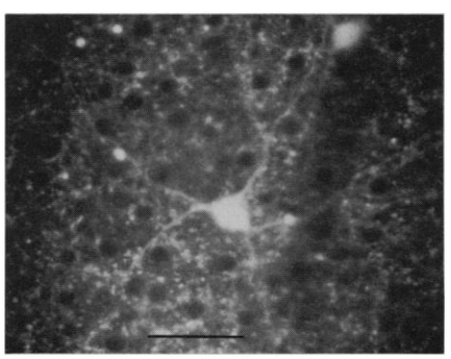

Fig. 1. Intense green fluorescence in a distinct subpopulation of large amacrine cells in an in vitro wholemount preparation of cat retina. The fluorescence, shown under the blue episcopic illumination, resulted from intravitreal injections of both dopamine (400 μg) and 5,7-DHT (300 μg), 6 hours before eye removal. Fluorescing somata are large and fusiform (12 to 20 μm along their long axes), and their primary dendrites are thick, straight, and sparsely branching. Fluorescence is also present in a plexus of fine varicose processes restricted to the outer border of the IPL. The somata of other, nonfluorescing cells in the INL protrude into the fluorescing meshwork to give it a honeycomb appearance. Excitation filter, 410 to 490 nm; barrier filter, 515 nm long pass; scale bar, 50 μm.

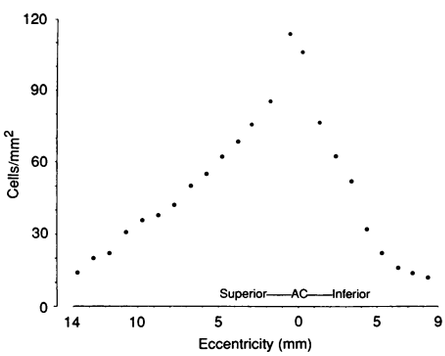

Fig. 2. The density of DA amacrine cells in vitro. Counts of fluorescing cell bodies were made in the in vitro retina. Sample areas measured 1 mm by 0.5 mm, and the counts were multiplied by 2 to give the number of cells per square millimeter. In a transect along the dorsoventral axis, cell densities ranged from 12 in the far periphery to 120 within 0.5 mm of the area centralis (AC).

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cence, all cells showed four to eight radiate dendrites with a mean length of 175 μm (n = 143; SD = 36 μm) and a moderate covering of thin branchlets. After HRP histochemistry, an additional and major component of the cell's morphology was observed; each of the main dendrites abruptly tapered and gave rise to a thin process that projected beyond the cell's apparent dendritic field (Fig. 3). These axon-like processes were smooth and spine-free but bore distinct boutons en passant at a density of about 100 per millimeter of axon. They were 2 to 4 mm (n = 41; mean = 3.2 mm; SD = 0.5 mm) long and thus comprised about 90% of the total length of the cell's processes (*18*). Given an approximate length of 18 mm of axon-like process per cell (six dendrites per cell and 3-mm axon per dendrite), a single DA amacrine cell would bear about 1800 boutons, and the bouton density that derives from this cell population (12 to 120 cell/mm^2) should range from about 20,000 to 200,000 per square millimeter of retina. The varicose plexus in Fig. 1 thus results from the overlap of the long, axon-like processes that project for millimeters beyond the classical dendritic field (Fig. 3, D and E).

Many, if not all, of the holes in the DA plexus are filled by the somata of a single type of amacrine cell, the AII amacrine cell (*4, 19*). This plexus provides a significant source of synaptic input to the AII cell's somata and proximal dendrites (*4*). The AII amacrine cells also receive input from rod bipolar cells and are coupled to each other and to cone bipolar cells by gap junctions

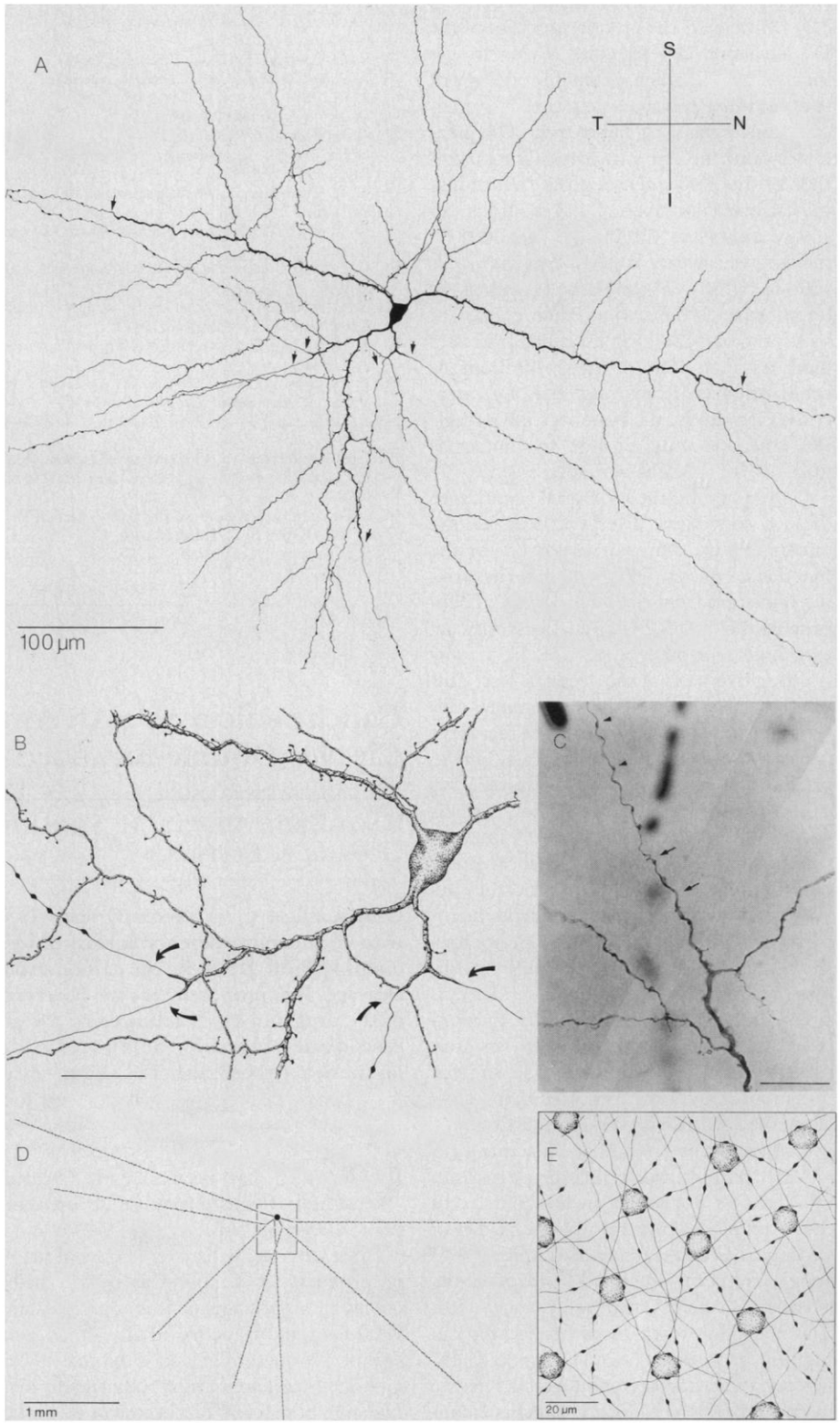

Fig. 3. (**A**) Camera lucida tracing of an amacrine cell identified by in vitro fluorescence and recovered after intracellular HRP injection. Each primary dendrite gives rise to one or two thin processes (arrows) that extend beyond the field shown [see (B)]. Injections were made with high-resistance microcapillary electrodes (80 to 100 megohms) that contained both LY and rhodamine-conjugated HRP (Sigma, type VI) in tris buffer (*p*H 7.6). Cell penetration was confirmed under blue excitation by passing LY into the cell with negative current (1 to 2 nA for 10 s). Rhodamine-conjugated HRP was then passed into the cell with the same electrode by positive current (8 to 12 nA for 1 to 3 min). The HRP was visualized with diaminobenzidine as the chromagen. S, superior; I, inferior; N, nasal; T, temporal. (**B**) Closer view of part of the dendritic field of the cell in (A) showing the morphology of the transition from dendrite to axon-like process. The thick, spiny dendrites taper over a distance of 5 to 10 μm and project beyond the dendritic field as thin processes that are smooth and spine-free but bear distinct varicosities. Scale bar, 20 μm. (**C**) Another axon-like process arising from the tip of a major dendrite 175 μm from the soma. The arrows indicate the region of transition, and the arrowheads indicate varicosities along the length of the axon-like process. The transition from a thick, spiny process to a thin, axon-like process was observed on each of the major dendrites of over 60 cells recovered after intracellular HRP injections. Scale bar, 20 μm. (**D**) Semischematic illustration of the morphological basis for the DA plexus shown in Fig. 1. The axon-like processes of the DA amacrine project for 2 to 4 mm along a straight course without branching. Boutons en passant are irregularly spaced at a density of about 100 per millimeter. The cell densities of the DA amacrines (Fig. 2) result in an overlap of axon-like processes that form a dense plexus of fine processes and varicosities. The proximal dendritic morphology of the cell in the inset is shown in (A). (**E**) The somata of AII amacrine cells (shown as stippled) protrude into the dopaminergic plexus and are ringed by boutons. The rings are formed by boutons en passant that derive from a number of axon-like processes. Each axon-like process originates from a different DA amacrine cell.

(*20*). By analogy with dopamine's effect on teleost horizontal cells (*21, 22*), dopamine release in the IPL may also alter the electrical coupling among AIIs and between AIIs and cone bipolar cells, thereby regulating the passage of rod signals to ganglion cells (*14*).

The present results suggest that the boutons along the axon-like component provide the synaptic connection between the DA amacrine and the AII amacrine cells (Fig. 3D). Moreover, the unique structure of the DA amacrine cell provides a clue to the function of this synapse; the morphology of the axon-like processes indicates a significant convergence of input from DA amacrines onto the AII amacrines. The magnitude of this convergence, given by dividing the estimated number of DA boutons per square millimeter (20,000 to 200,000) by the known density of AII amacrine cells (500 to 5,000) (*23*) could be as high as 40 boutons per AII amacrine. Their en passant location along the long axon-like processes indicates that each of these 40 boutons could originate from a different DA amacrine. Conversely, the boutons from a single DA amacrine must diverge to contact as many as 1800 AII amacrine cells.

Understanding the biological significance of such convergent-divergent neuronal geometry requires, in part, discovery of the function of the axon-like process. The striking transition from the thick, proximal dendrite to the thin, axon-like process may act as a high-resistance bottleneck, electrically isolating two parts of the dendritic tree. This would be analogous to those horizontal cells that have thin, axon-like processes that serve not to conduct action potentials but to prevent the passive spread of current between two independent dendritic systems (*24*). If this were the case, the DA boutons could operate independently, as local input-output units (*25, 26*). However, this hypothesis is not supported by electron microscopic studies that have found DA boutons in the IPL to be presynaptic only, resembling typical axonal synapses (*2–4*). Alternatively, the transition from dendritic to axon-like process may be equivalent to an axon hillock and may function as the site for initiation of an action potential that travels along the axon-like process. Dendritic action potentials occur in retinal amacrine cells (*27–29*), and in turtle retina the physiological effects of dopamine on horizontal cells can be mimicked by veratridine, a releaser of transmitter from the terminals of spike-generating neurons and prevented by tetrodotoxin, a blocker of voltage-gated Na^+ channels (*30*). The possibility that DA amacrine cells can transmit information laterally over long distances via an excitable axon suggests a synaptic mechanism by which global changes in illumination during light and dark adaptation could influence local changes in ganglion-cell receptive-field sensitivity.

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Calicheamicin γ_1^I: An Antitumor Antibiotic That Cleaves Double-Stranded DNA Site Specifically

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Calicheamicin γ_1^I is a recently discovered diyne-ene–containing antitumor antibiotic with considerable potency against murine tumors. In vitro, this drug interacts with double-helical DNA in the minor groove and causes site-specific double-stranded cleavage. It is proposed that the observed cleavage specificity is a result of a unique fit of the drug and DNA followed by the generation of a nondiffusible 1,4-dehydrobenzene–diradical species that initiates oxidative strand scission by hydrogen abstraction on the deoxyribose ring. The ability of calicheamicin γ_1^I to cause double-strand cuts at very low concentrations may account for its potent antitumor activity.

CALICHEAMICIN γ_1^I (STRUCTURE 1) (*1, 2*) has been recently isolated from fermentations of *Micromonospora echinospora* ssp. calichensis and is a member of a newly discovered class of natural products (*3, 4*) found to be unusually potent antitumor agents. It is approximately 1000-fold more active than adriamycin against murine tumors and is optimal at 0.5 to 1.5 μg per kilogram of body weight (*5*). The high potency of calicheamicin γ_1^I in the biochemical assay of prophage induction (*6*) (active at picogram per milliliter concentrations) suggested that its activity was due to its ability to damage DNA. In addition, the drug can also cause chromosome aberrations

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