Histamine Immunoreactive Axons in the Macaque Retina

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Purpose. The goal of these experiments was to identify the neurotransmitter in centrifugal axons of the macaque retina.

Methods. *Macaca mulatta* retinas and optic nerves were fixed overnight in carbodiimide and labeled with an antiserum to histamine with the use of an immunofluorescence technique.

Results. Several large histamine-immunoreactive axons ran from the optic nerve head to the peripheral retina, where they branched extensively and terminated in the inner plexiform layer, occasionally alongside retinal blood vessels. Other axons that emerged from the optic nerve head ran in the optic fiber layer to the central retina, circled the fovea, and then returned to the optic disc. These may be the source of histamine-immunoreactive axons that have been observed in central visual areas. No labeled cell bodies were present in the retina. Because perikarya in the posterior hypothalamus are the only known source of histamine in the primate central nervous system and because neurons there can be retrogradely labeled from the cut optic nerve, the histamine-immunoreactive axons must have originated there.

Conclusions. Centrifugal axons in the macaque retina are part of the system of axons containing histamine that originate in the hypothalamus and project throughout the brain. Because the activity of these neurons is highest during the morning, histamine might play a role in preparing the retina to operate in daylight. The contacts of histamine-immunoreactive axons with blood vessels suggest that histamine may also play a role in regulating the retinal microvasculature. (*Invest Ophthalmol Vis Sci.* 1999;40:487-495)

Anatomic studies have shown that there is a centrifugal projection from the brain to the retina in primates. Large-diameter axons in the optic fiber layer (OFL) that emerged from the optic disc and produced branches in the inner plexiform layer (IPL) have been labeled in the primate retina with the Golgi method[1] and with silver stains.[2-7] In macaques, cell bodies in the posterior hypothalamus were retrogradely labeled when horseradish peroxidase was applied to the cut optic nerve.[8] Degenerating terminals in the IPL were observed after optic nerve lesions,[9] and axons in the retina were anterogradely labeled when horseradish peroxidase was applied to the cut optic nerve.[10]

The role of this pathway in vision is uncertain, however, because the early physiological studies of centrifugal axons relied on optic nerve stimulation, which produced antidromic and orthodromic effects.[11] To study the functions of centrifugal axons more rigorously, we undertook these experiments to identify their neurotransmitter. Histamine-immunoreactive (IR) axons were briefly described in the retina and optic nerves of guinea pigs[12] and rats,[13] but labeled cell bodies were found only in the posterior hypothalamus. Using the same technique on whole-mount preparations of macaque retinas, we labeled axons that resembled centrifugal axons described previously but that were clearly different from other types of axons observed in the retina.[7,14] Taken along with evidence from previous studies that histamine is a retinal neurotransmitter,[15] these findings indicate that histamine and its agonists would mimic the effects of activity in the centrifugal axons and that antagonists would be useful to distinguish the two effects of optic nerve stimulation. Another unexpected finding was that some histamine-IR axons made numerous collaterals that circled the fovea and returned to the optic disc.

Materials and Methods

Tissue Preparation and Fixation

Right eyes of adult *Macaca mulatta* were enucleated after an overdose of pentobarbital and were transported to the laboratory in glass jars packed with ice. The eyes were hemisected, and the posterior halves with the optic nerve attached were fixed overnight in 1% to 4% 1-ethyl-3(3-diethlyaminopropyl)-carbodiimide (Sigma, St Louis, MO) at 4°C in 0.1 M phosphate buffer (pH 7.4) made fresh no more than 15 minutes before the dissection.[16] After washing in phosphate-buffered saline (PBS), the retinas were carefully dissected away from the pigment epithelium, and the remaining vitreous humor was removed.

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Immunocytochemistry

The isolated macaque retinas were first labeled with rabbit antiserum to histamine (1:200 or 1:500; Chemicon International AB134) containing 0.3% Triton X-100 and 0.25% bovine serum albumin (Sigma) in 0.3% sodium azide PBS (PBSa) for 7 to 8 days followed by affinity-purified secondary antibodies and avidin-conjugated fluorophores (Jackson Immunoresearch) diluted in PBS. For the double-labeling experiments, donkey anti-rabbit Cy3™ (1:100) was applied overnight; after washing in PBS, a mouse monoclonal antibody to tyrosine hydroxylase (TH, 1:10,000; Sigma #T-2928) containing 0.3% Triton X-100 in PBSa was applied for 7 to 8 days, followed by a biotinylated donkey anti-mouse IgG (1:100) for 2 days and streptavidin Cy5™ (1:100) overnight. Some tissue incubated with only the histamine antiserum was labeled with biotinylated goat anti-rabbit IgG (1:100) for 2 days and then streptavidin Cy3™ (1:100) overnight. The retinas were mounted photoreceptor-side down on glass slides in a 1:3 glycerol/PBSa solution containing *p*-phenylenediamine.

To label histamine-immunoreactive axons in the optic nerve, pieces of fixed nerve were cryoprotected in 30% sucrose in PBS at 4°C overnight and sectioned in a cryostat. Sixty-micrometer-thick longitudinal sections were processed free-floating, or else 30-μm-thick cross sections were mounted on slides and dried. All sections were treated with goat serum (1:100 in PBS) containing 0.3% Triton X-100 and 0.25% bovine serum albumin for 3 hours. They were then rinsed and incubated in the same solutions described above for the single-labeled tissue, except with shorter incubation times: 3 to 4 days at 4°C for the primary antibody, 2 hours for the biotinylated secondary antibody, and 1 hour for the streptavidin-conjugated Cy3™. Control experiments were performed on the 30-μm cross sections of the optic nerve. The histamine antiserum was preabsorbed overnight at 4°C with 1 mg histamine per milliliter of diluted antiserum. Control sections showed no evidence of labeled axons. The specificity of this histamine antiserum has also been demonstrated previously.[17]

Image Acquisition

Images were acquired using a Zeiss confocal laser-scanning microscope (Carl Zeiss, Oberkochen, Germany) with a krypton-argon laser at a 512 × 512 pixel image size. Length and diameter of axons and collaterals were determined using Zeiss laser-scanning microscopy software from single optical sections and from reconstructed stacks of optical sections. To make photomontages, each stack of images was aligned in Adobe Photoshop 3.0 (Adobe Systems, Mountain View, CA). The dimensions of the retinal layers and the dopamine plexus were measured from a reconstructed stack of dual-scanned optical sections of histamine and tyrosine hydroxylase-immunoreactivity through the whole-mounted retina and projected onto the Z axis. The histamine-IR axons in the macaque retina were drawn using a 40× water-immersion lens on a Zeiss Axiophot microscope with a motorized stage, and the areas of the terminal branches were calculated using with Neurolucida 3.0 (Microbrightfield).

Results

A subpopulation of axons in the macaque retina was labeled with antiserum to histamine, but cell bodies were not labeled. The axons ran from the optic nerve head to the temporal side of the parafovea, where they made numerous collateral branches. Several of the histamine-IR axons branched extensively in the IPL of the peripheral retina and resembled the centrifugal axons that have been described previously in primate retinas.[2,4-7,10] These axons had a much larger diameter and branched far more extensively in the IPL than those of the ganglion cells with intraretinal collaterals.[7] Another finding suggesting that the histamine-IR axons were centrifugal was that they had large diameters and broadly striated terminals in the IPL, like the degenerating axons observed in the macaque retina after lesions of the optic nerve.[9] In addition, the only cells in the primate central nervous system known to contain histamine are found in the posterior hypothalamus,[18-20] and neurons there have been retrogradely labeled after horseradish peroxidase was applied to the proximal stump of the macaque optic nerve.[8]

Histamine-Immunoreactive Centrifugal Axons in Macaque Retinas and Optic Nerves

Five to 10 large histamine-IR axons, approximately 2.5 μm in diameter, ran through the OFL from the optic disc to the parafovea. The majority ran at approximately a 30° angle above or below the horizontal meridian, but these axons occasionally followed the horizontal meridian, passing within 250 μm of the fovea (Fig. 1). They did not branch until they reached the temporal half of the retina, where they had numerous collateral branches running in either direction around the fovea (Fig. 2), a few of which terminated with small swellings in the temporal OFL. The majority of these collaterals ran back into the nasal half of the retina and returned to the optic disc. Some collateral branches returned via the superior retina and others via the inferior retina (Fig. 3). Smaller histamine-IR axons, less than 2.0 μm in diameter, emerged from the optic disc at larger angles, up to 60°, and also circled the fovea in both directions. No axons emerged from the nasal side of the optic disc.

In each retina, four to five histamine-IR axons, of both large and small types, also had branches that descended through the ganglion cell layer orthogonally into the IPL (Fig. 4). These orthogonal branches then ran toward the temporal retina until they were at least 2 mm past the fovea, and then they branched into the IPL. Some terminal branches were also seen in the IPL in the parafovea (Fig. 3). The axons were found in sublaminas a and b and most commonly found in a broad band around the center of the IPL. In a few instances, the terminal branches in the IPL had orthogonal branches that ran back to the OFL, but none returned to the optic disc (Fig. 5A).

The histamine-IR terminals in the superior retina could be traced back to a single axon emerging from the optic disc at approximately a 30° angle from the horizontal meridian (Fig. 5B). It circled the fovea in the OFL and made an orthogonal projection to the IPL just nasal to the fovea. Unlike the axons supplying the temporal retina, this axon made an extremely long arc with a radius of approximately 6 mm. It made only a few collateral branches as it ran through the inferior retina until it reached the superior retina, where the axons branched repeatedly into the IPL.

The smaller axons had swellings of approximately 1 μm in diameter. Larger irregularly spaced varicosities of as much as 2.8 μm in diameter were found along the larger axons in the IPL (Fig. 6A). The axons did not form pericellular baskets or other specialized endings; instead, they ended in small swell-

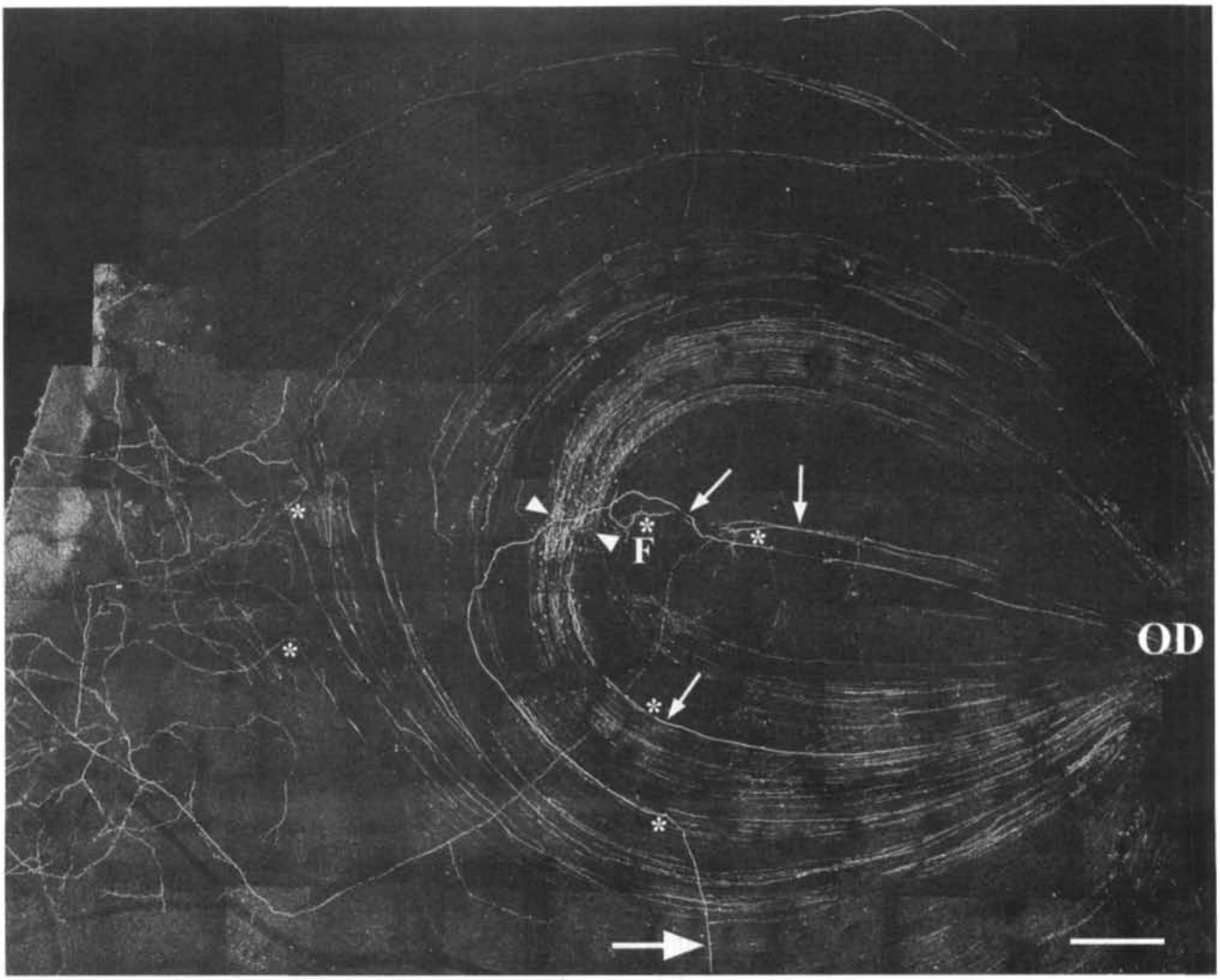

FIGURE 1. Histamine-immunoreactivity in the central macaque retina. A low-power magnification photomontage of the histamine-immunoreactive (IR) centrifugal axons, including the optic disc (OD), fovea (F), and temporal retina. The histamine-IR axons emerged from the optic disc and ran into the optic fiber layer toward the fovea. Several axons (*small arrows*) had branches projecting orthogonally (*) to the inner plexiform layer, where they ran to the temporal retina, branched repeatedly, and terminated. A single axon (*large arrow*) ran through the inferior retina and branched in the superior retina. Another set of collateral branches arose just temporal to the fovea (*arrowheads*) within 1 mm of the horizontal meridian, ran around the fovea, and returned to the optic disc. The superior retina is up, and the temporal retina is on the left. Scale bar, 0.5 mm.

ings. Histamine-IR axons also interacted with some of the larger blood vessels in the OFL and IPL. The axons ran alongside the blood vessels, forming varicosities (Fig. 6B). Varicose histamine-IR axons were also found among the ganglion cell axons in the optic nerve (Fig. 6C). The labeled axons were not confined to any part of the optic nerve, but some were found adjacent to the central retinal artery. None of the axons showed any evidence of branching within the optic nerve.

On the basis of results found in the fish retina,[21] centrifugal axons were expected to contact dopaminergic amacrine cells that ramify in stratum 1 of the IPL. In the macaque retina, however, several double-label experiments with rabbit antiserum to histamine and mouse monoclonal antibody to tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, indicated no such interaction. Most of the histamine-IR terminals ramified vitreal to the dopamine plexus, and the axons that ran in the same stratum as the dopaminergic processes were not associated with them (Fig. 4).

DISCUSSION

A subpopulation of axons was labeled with antiserum to histamine in the macaque retina. The extensive arbors of these axons were characteristic of retinopetal axons that originate from areas other than the isthmo-optic nucleus.[22] These axons ran from the optic disc to the temporal side of the parafovea, where they made numerous collateral branches. The collaterals ran back around the fovea to the optic disc and left the retina. It is uncertain where these axons terminate, but histamine-IR axons are known to be present in the lateral geniculate nucleus and superior colliculus of macaques.[20] Axons with this unusual course have never been described previously in mammals, but in turtle retinas centrifugal axons containing immunoreactive nitric oxide synthase and NADPH diaphorase activity ran from the optic nerve head, into the central retina, and then back.[23]

The histamine-IR varicosities in the IPL might be sites of synaptic contact or sites of histamine release without postsyn-

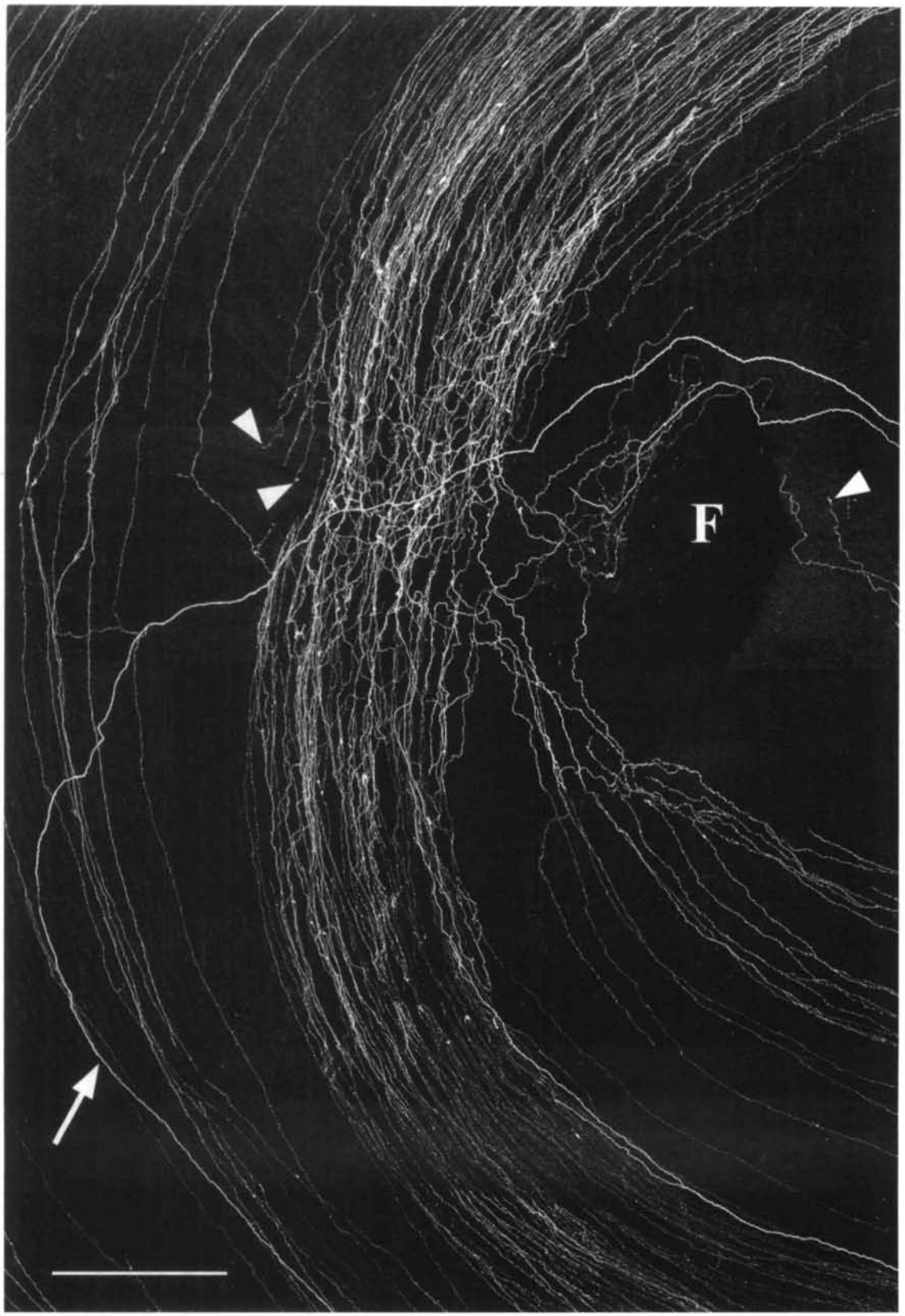

FIGURE 2. A high-power magnification photomontage of histamine-immunoreactive (IR) axons from the area temporal to the fovea (F) shown in Figure 1. In this region, most histamine-IR axons made collateral branches in the optic fiber layer that continued around the fovea and returned to the optic disc. One large axon (*arrow*) passed through this dense network of collaterals and ultimately made an orthogonally projecting branch to the inner plexiform layer. In the parafovea, a few of the smaller collaterals terminated in the inner plexiform layer, and the remainder ended in small swellings in the optic fiber layer (*arrowheads*). The superior retina is up, and the temporal retina is on the left. Scale bar, 200 μm.

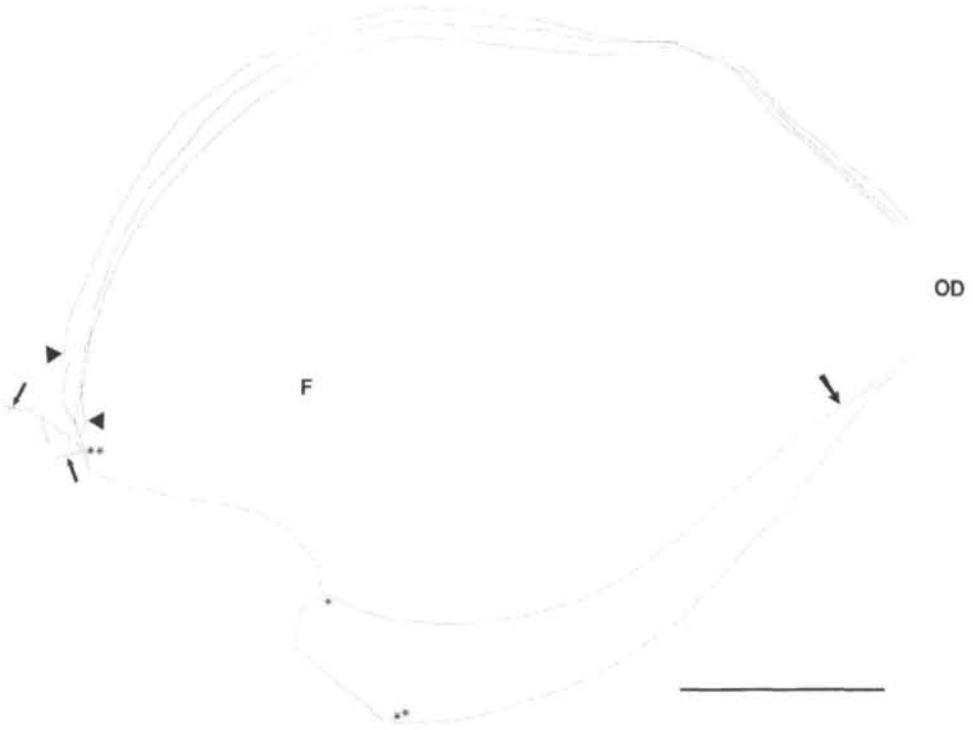

FIGURE 3. A Neurolucida drawing of one large histamine-immunoreactive axon (*large arrow*) and its collaterals that returned to the optic disc. After making an orthogonal projection into the inner plexiform layer (*), several branches terminated there (*small arrows*). Other branches ran back to the optic fiber layer (**) and produced at least 16 collaterals that ran back to the optic disc, 3 of which are illustrated here (*arrowheads*). The superior retina is up, and the temporal retina is on the left. Scale bar, 1.0 mm. F, fovea; OD, optic disc.

aptic specializations. After lesions of the optic nerve, degenerating terminals making synapses were observed in the IPL of macaques and cats.[9,24] However, four previous studies have examined the ultrastructure of axons containing histamine or the synthetic enzyme histidine decarboxylase (HDC) in mammalian brains, and they found that these axons made very few, if any, synapses. In the lateral geniculate nucleus of the cat, appositions between histamine-IR axons and other processes were observed, but there were no obvious synaptic densities.[25] Another group used an antiserum to HDC in the rat brain and found some varicosities that made small asymmetrical synapses, but most varicosities made no synapses at all.[26,27] Synapses from HDC-IR axons onto neuronal perikarya have also been observed in the mesencephalic nucleus of the trigeminal nerve in the rat brain.[28] In this respect, the histamine-IR axons are similar to central catecholaminergic neurons, which often make unspecialized contacts.[29] In a recent study of dopaminergic terminals in the rat neostriatum, for example, only 30% to 40% of the varicosities made synapses.[30]

A number of studies of histamine synthesis, its physiological effects, and its catabolism suggest that histamine acts as a neurotransmitter or neuromodulator in the mammalian retina. Histamine has been detected in the retinas of macaques and humans at levels comparable to that detected in the brain.[31] Histamine does not cross the blood-retinal barrier, and HDC activity has been detected in macaque retinas.[32] Mast cells, another possible source of histamine, are not present in the retina.[33] The histamine in the retina is, therefore, likely to originate from neurons. Horizontal cells have also been proposed as the source of histamine in the guinea pig retina.[34] However, it is possible that the HDC antiserum used in that study recognized an unknown decarboxylase in horizontal cells because it also labeled dopaminergic cells containing dopa decarboxylase. When the guinea pig retina was studied

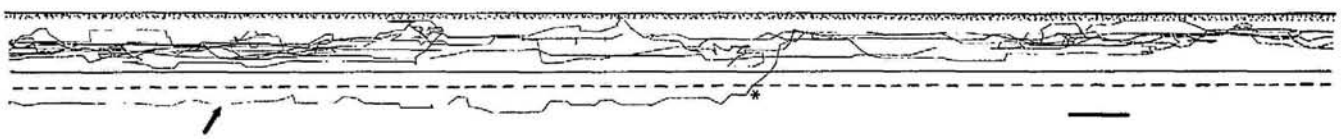

FIGURE 4. A Neurolucida drawing of a single centrifugal axon (*arrow*) from a whole-mount preparation rotated 90° about the *x* axis. A typical orthogonal projection from the optic fiber layer to the inner plexiform layer (*) is shown as it would appear in a vertical section. The terminal branches from this axon covered 2.67 mm^2 in the temporal retina and formed a broad band 10-μm to 15-μm wide near the center of the inner plexiform layer. The dopaminergic amacrine cell plexus (*stippled area*) sometimes overlapped with the terminal branches, but dopaminergic processes did not interact with the histamine-immunoreactive varicosities. Scale bar, 20 μm.

FIGURE 5. Neurolucida drawings of histamine-immunoreactive axons in the inner plexiform layer. (**A**) This axon emerged from the optic disc (OD), ran along the horizontal meridian, and made an orthogonally projecting branch (*) into the inner plexiform layer. A few branches were found nasal to the fovea (F, *arrows*), but the main axon ran to the temporal peripheral retina, where it terminated and supplied an area measuring 5.68 mm^2. A second orthogonally projecting branch (**) returned to the optic fiber layer but ended before reaching the optic disc. Scale bar, 1.0 mm. (**B**) This axon emerged from the optic disc (OD), circled the fovea (F), and made an orthogonally projecting branch (*) into the inner plexiform layer. The axon ran through the inferior retina, making only a few short branches (*arrows*), and then into the nasal superior retina. It branched extensively there, supplying an area approximately 25 mm^2. The superior retina is up, and the temporal retina is on the left. Scale bar, 3.0 mm.

using a histamine antiserum, only axons were labeled,[12] as we found in the macaque retina.

Histamine release from the retina has not been studied directly, but there is indirect evidence that histaminergic neurons in the macaque hypothalamus are active during the day. The levels of histamine metabolites in the ventricular cerebral spinal fluid increase more than threefold after the onset of light and remain significantly higher than during darkness.[35] In rabbits, retinal histamine content is decreased by light stimulation,[36] a finding consistent with histamine release in the light.

The retina can remove histamine from the extracellular space by two mechanisms. Mammalian retinas contain histamine methyltransferase, which inactivates histamine via methylation.[36] Rabbit retinas also take up ^{3}H-histamine, and this uptake decreases in a dose-dependent manner by ouabain.[37] An autoradiographic study of histamine uptake in rabbit retinas found only diffuse radioactivity throughout the retina, howev-

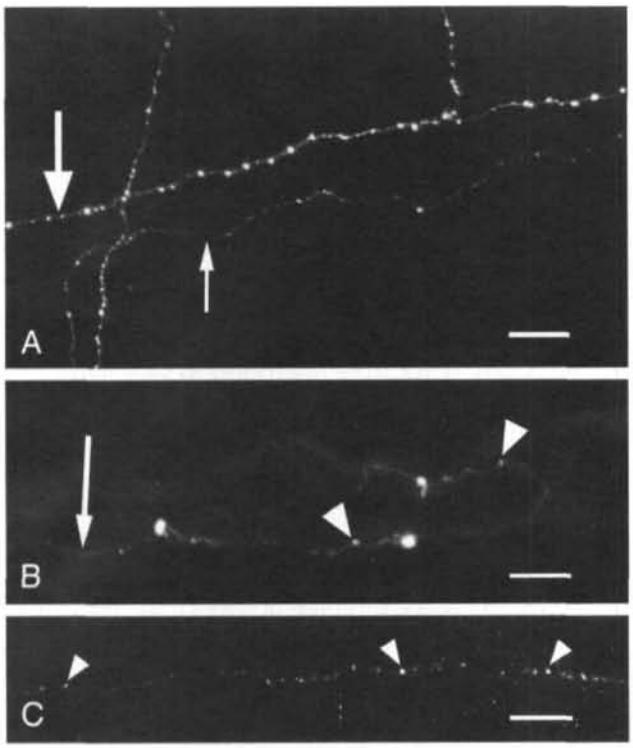

FIGURE 6. Histamine-immunoreactive varicosities in the macaque retina and optic nerve. (**A**) These labeled axons were located approximately 1-mm inferior to the horizontal meridian in the inner plexiform layer of the temporal retina. The larger axons (*large arrow*) had larger and more frequent varicosities than the smaller axons (*small arrow*). Both types of terminal branches were found throughout the inner plexiform layer. (**B**) Two histamine-immunoreactive axons running alongside a retinal blood vessel (*arrow*). This blood vessel is located in the inner plexiform layer of the superior peripheral retina. Varicosities (*arrowheads*) were common along the axons contacting blood vessels. (**C**) A labeled axon in the macaque optic nerve running longitudinally. Varicosities (*arrowheads*) were present on these axons throughout the optic nerve, but they did not branch. Scale bars, (**A**, **C**) 20 μm; (**B**) 100 μm.

er.[38] This discrepancy may be attributable to differences between the two studies in the lighting and other physiological conditions. It is also possible that uptake into the sparsely distributed centrifugal axons in the rabbit retina[39] was difficult to detect in the vertical sections of the retina used in the autoradiographic study.

H_1 receptors with a high affinity for histamine have been detected in the human retina using ^{3}H-mepyramine,[40] and some H_1 antagonists decrease the critical flicker fusion frequency in humans.[41-44] Because the responses of magnocellular-projecting retinal ganglion cells in macaques to luminance flicker are identical to those of human subjects under the same conditions,[45] it is possible that these ganglion cells are affected by the H_1 antagonists. These drugs might be acting at any level in the visual system, however, and their effects on retinal ganglion cells have not been reported. Several effects of histamine and its antagonists have been described in other mammalian retinas. One histamine effect is similar to that of dopamine, which is released by light stimulation from macaque retinas[46]; histamine increased the current through γ-aminobutyric acid ($GABA_A$) receptor-activated chloride channels in isolated rat amacrine cells.[47] One possible interpretation of these findings is that dopamine and histamine act synergistically to enhance the responses of the retina to rapidly changing stimuli at high ambient light levels.

Other reported effects of histamine in mammalian retinas are the opposite of those produced by dopamine, however. Histamine inhibited the forskolin-induced increase in cAMP in the rabbit retina,[48] and the H_1 antagonist mepyramine reduced the amplitude of the b-wave of the electroretinogram in sheep.[49] In addition, histamine decreased norepinephrine release from the pig retina[50] and stimulated the influx of calcium into dissociated rabbit retinal cells.[51] Histamine might exert some of its effects in the retina via receptors for GABA. In the brain, endogenous histamine can be oxidized to form imidazole acetic acid,[52] a potent antagonist of $GABA_C$ receptors in the retina.[53,54] In the macaque retina, this histamine metabolite would be expected to act on axon terminals of bipolar cells because $GABA_C$ receptors have been localized there.[55]

Another function of the centrifugal axons may be regulation of retinal blood flow and capillary permeability. We observed close appositions between histamine-IR axons and blood vessels in the macaque retina similar to the ones described previously in the hypothalamus,[56] but we cannot rule out the possibility that glial processes intervened between the axons and the vessel walls. In the striatum, HDC-IR axons have also been seen in close proximity to cerebral blood vessels, but the thin processes of glial cells always separated the axons from the vessel walls.[27] Varicosities and terminals in the OFL of the central retina may also be sites of histamine release, and, if so, they might influence blood vessels in that layer. Thus, it is possible that the axons passing through the retina have effects on retinal blood flow even though they do not innervate the peripheral retina.

The histamine-IR axons that contact retinal blood vessels might play a role in the etiology of diabetic retinopathy. Centrifugal axons are reported to be abnormal in retinas from diabetic donors, resembling axons undergoing pathologic regeneration. The centrifugal axons that supplied retinal blood vessels appeared to proliferate and surround microaneurysms.[57] In rats, exogenous histamine increases the permeability of retinal blood vessels when it is applied intravitreally[58]; therefore, it is possible that the histamine released from centrifugal axons has the same effect on blood vessels in primate retinas. If so, this may account for the therapeutic effects of antihistamines on leakage from retinal blood vessels in diabetic patients.[59]

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References

1. Polyak SL. *The Retina*. Chicago: University of Chicago Press; 1941: 338-342.
2. Honrubia FM, Elliott JH. Efferent innervation of the retina, I: morphologic study of the human retina. *Arch Ophthalmol.* 1968; 80:98-103.
3. Honrubia FM, Elliott JH. Efferent innervation of the retina, II: morphologic study of the monkey retina. *Invest Ophthalmol.* 1970;9:971-976.
4. Reperant J, Miceli D, Vesselkin NP, Molotchnikoff S. The centrifugal visual system of vertebrates: a century-old search reviewed. *Int Rev Cytol.* 1989;118:115-171.

5. Silveira LC, Perry VH. A neurofibrillar staining method for retina and skin: a simple modification for improved staining and reliability. *J Neurosci. Methods.* 1990;33:11-21.
6. Silveira LC, Perry VH. The topography of magnocellular projecting ganglion cells (M-ganglion cells) in the primate retina. *Neuroscience.* 1991;40:217-237.
7. Usai C, Ratto GM, Bisti S. Two systems of branching axons in monkey's retina. *J Comp Neurol.* 1991;308:149-161.
8. Labandeira-Garcia JL, Guerra-Seijas MJ, Gonzalez F, Perez R, Acuna C. Location of neurons projecting to the retina in mammals. *Neurosci Res.* 1990;8:291-302.
9. Brooke RN, Downer JDC, Powell TP. Centrifugal fibres to the retina in the monkey and cat. *Nature.* 1965;207:1365-1367.
10. Perry VH, Oehler R, Cowey A. Retinal ganglion cells that project to the dorsal lateral geniculate nucleus in the macaque monkey. *Neuroscience.* 1984;12:1101-1123.
11. Ogden T. On the function of efferent retinal fibers. In: CV Euler, S Skogland, U Soderberg, eds. *Structure and Function of Inhibitory Neuronal Mechanisms.* Oxford: Pergamon Press; 1968:89-109.
12. Airaksinen MS, Panula P. The histaminergic system in the guinea pig central nervous system: an immunocytochemical mapping study using an antiserum against histamine. *J Comp Neurol.* 1988; 273:163-186.
13. Panula P, Pirvola U, Auvinen S, Airaksinen MS. Histamine-immunoreactive nerve fibers in the rat brain. *Neuroscience.* 1989;28:585-610.
14. Peterson BB, Dacey DM. Morphology of human retinal ganglion cells with intraretinal axon collaterals. *Vis Neurosci.* 1998;15:377-387.
15. Nowak JZ. In: T Watanabe, H Wada, eds. *Histaminergic Neurons: Morphology and Function.* Boca Raton, FL: CRC Press; 1991:365-382.
16. Panula P, Happola O, Airaksinen MS, Auvinen S, Virkamaki A. Carbodiimide as a tissue fixative in histamine immunohistochemistry and its application in developmental neurobiology. *J Histochem Cytochem.* 1988;36:259-269.
17. Orona E, Battelle BA, Ache BW. Immunohistochemical and biochemical evidence for the putative inhibitory neurotransmitters histamine and GABA in lobster olfactory lobes. *J Comp Neurol.* 1990;294:633-646.
18. Panula P, Airaksinen MS, Pirvola U, Kotilainen E. A histamine-containing neuronal system in human brain. *Neuroscience.* 1990; 34:127-132.
19. Airaksinen MS, Paetau A, Paljarvi L, et al. Histamine neurons in human hypothalamus: anatomy in normal and Alzheimer diseased brains. *Neuroscience.* 1991;44:465-481.
20. Manning KA, Wilson JR, Uhlrich DJ. Histamine-immunoreactive neurons and their innervation of visual regions in the cortex, tectum, and thalamus in the primate Macaca mulatta. *J Comp Neurol.* 1996;373:271-282.
21. Zucker CL, Dowling JE. Centrifugal fibres synapse on dopaminergic interplexiform cells in the teleost retina. *Nature.* 1987;330: 166-168.
22. Uchiyama H. Centrifugal pathways to the retina: influence of the optic tectum. *Vis Neurosci.* 1989;3:183-206.
23. Blute TA, Mayer B, Eldred WD. Immunocytochemical and histochemical localization of nitric oxide synthase in the turtle retina. *Vis Neurosci.* 1997;14:717-729.
24. Wakakura M, Ishikawa S. Ultrastructural study on centrifugal fibers in the feline retina. *Jpn J Ophthalmol.* 1982;26:282-291.
25. Uhlrich DJ, Manning KA, Pienkowski TP. The histaminergic innervation of the lateral geniculate complex in the cat. *Vis Neurosci.* 1993;10:225-235.
26. Hayashi H, Takagi H, Takeda N, et al. Fine structure of histaminergic neurons in the caudal magnocellular nucleus of the rat as demonstrated by immunocytochemistry using histidine decarboxylase as a marker. *J Comp Neurol.* 1984;229:233-241.
27. Takagi H, Morishima Y, Matsuyama T, et al. Histaminergic axons in the neostriatum and cerebral cortex of the rat: a correlated light and electron microscopic immunocytochemical study using histidine decarboxylase as a marker. *Brain Res.* 1986;364: 114-123.
28. Inagaki N, Yamatodani A, Shinoda K, et al. The histaminergic innervation of the mesencephalic nucleus of the trigeminal nerve in rat brain: a light and electron microscopical study. *Brain Res.* 1987;418:388-391.
29. Pickel VM. Ultrastructure of central catecholaminergic neurons. In: P Panula, H Paivarinta, S Somila, eds. *Neurohistochemistry: Modern Methods and Applications.* New York: Academic Press; 1986:397.
30. Descarries L, Kenneth WC, Garcia S, Bosler O, Doucet G. Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: a quantitative autoradiographic and immunocytochemical analysis. *J Comp Neurol.* 1996;375:167-186.
31. Nowak JZ, Nawrocki J. Histamine in the human eye. *Ophthalmic Res.* 1987;19:72-75.
32. Sawai S, Fukui H, Fukuda M, et al. [3H]mepyramine binding sites, histamine H1-receptors, in bovine retinal blood vessels. *Curr Eye Res.* 1991;10:713-718.
33. Smelser GK, Silver S. The distribution of mast cells in the normal eye. *Exp Eye Res.* 1963;2:134-141.
34. Ando-Yamamoto M, Kiyama H, Hayashi H, et al. Demonstration of histaminergic neurons in horizontal cells of guinea pig retina. *Brain Res.* 1987;410:269-274.
35. Prell GD, Khandelwal JK, Burns RS, Green JP. Diurnal fluctuation in levels of histamine metabolites in cerebrospinal fluid of rhesus monkey. *Agents Actions.* 1989;26:279-286.
36. Nowak JZ, Nawrocki J, Malinski C. Histamine in the rabbit eye: distribution, synthesis, catabolism, and changes by light stimulation. *Agents Actions.* 1985;16:80-83.
37. Nowak JZ, Kulinski JZ. The origin and fate of histamine in the rabbit retina. *Neurochem Int.* 1986;8:53-58.
38. Ehinger B, Falck B. Autoradiography of some suspected neurotransmitter substances: GABA glycine, glutamic acid, histamine, dopamine, and L-dopa. *Brain Res.* 1971;33:157-172.
39. Luciano L, Spitznas M, Reale E. Morphologic evidence of centrifugal fibers in the retina. *Ophthalmologica.* 1971;163:281-287.
40. Sawai S, Wang NP, Fukui H, et al. Histamine H1-receptor in the retina: species differences. *Biochem Biophys Res Comm.* 1988; 150:316-322.
41. Turner P. Critical flicker frequency and centrally-acting drugs. *Br J Ophthalmol.* 1968;52:245-250.
42. Large AT, Wayte G, Turner P. Promethazine on hand-eye coordination and visual function. *J Pharm Pharmacol.* 1971;23:134-135.
43. Nicholson AN, Smith PA, Spencer MB. Antihistamines and visual function: studies on dynamic acuity and the pupillary response to light. *Br J Clin Pharmacol.* 1982;14:683-690.
44. Nicholson AN, Stone BM. The H1-antagonist mequitazine: studies on performance and visual function. *Eur J Clin Pharmacol.* 1983; 25:563-566.
45. Lee BB, Martin PR, Valberg AV. Sensitivity of macaque retinal ganglion cells to chromatic and luminance flicker. *J Physiol (Lond).* 1989;414:223-243.
46. Boelen MK, Boelen MG, Marshak DW. Light-stimulated release of dopamine from the primate retina is blocked by L-2-amino-4-phosphonobutyric acid (Apb). *Vis Neurosci.* 1998;15:97-103.
47. Feigenspan A, Bormann J. Facilitation of GABAergic signaling in the retina by receptors stimulating adenylate cyclase. *Neurobiology.* 1994;91:10893-10897.
48. Nowak JZ, Sek B, Szkiel B. Histamine-mediated regulation of cAMP levels and inositol phosphate metabolism in isolated rabbit retina. *Agents Actions.* 1989;27:131-134.
49. Caslissendorff B. Melanotropic drugs and retinal functions. *Acta Ophthalmologica.* 1976;54:118-128.
50. Schlicker E, Schunack W, Gothert M. Histamine H3 receptor-mediated inhibition of noradrenaline release in pig retina discs. *Naunyn Schmiedebergs Arch Pharmacol.* 1990;342:497-501.
51. Xuan B, Liu F, Zhang MY, Xiao JG. Inhibitory effects of tetrandrine on intracellular free Ca2+ increase induced by glutamate, serotonin and histamine in dissociated retina cells. *J Ocul Pharmacol Ther.* 1996;12:331-336.

52. Prell GD, Morrishow AM, Duoyon E, Lee WS. Inhibitors of histamine methylation in brain promote formation of imidazoleacetic acid, which interacts with GABA receptors. *J Neurochem.* 1997; 68:142-151.
53. Qian H, Dowling JE. Pharmacology of novel GABA receptors found on rod horizontal cells of the white perch retina. *J Neurosci.* 1994;14:4299-4307.
54. Qian H, Dowling JE. GABAa and GABAc receptors on hybrid bass retinal bipolar cells. *J Neurophysiol.* 1995;74:1920-1928.
55. Enz R, Brandstatter JH, Wassle H, Bormann J. Immunocytochemical localization of the GABAc receptor p subunits in the mammalian retina. *J Neurosci.* 1996;16:4479-4490.
56. Steinbusch HWM, Verhofstad AAJ. Immunocytochemical demonstration of noradrenaline, serotonin and histamine and some observations on the innervation of the intracerebral blood vessels. In: Owman C, Hardebo JE, eds. *Neural Regulation of Brain Circulation.* New York: Elsevier Science; 1986:181-194.
57. Wolter JR. Diabetic retinopathy. *Am J Ophthalmol.* 1961;51: 1124-1141.
58. Bamforth SD, Lightman SL, Greenwood J. Interleukin-1 beta-induced disruption of the retinal vascular barrier of the central nervous system is mediated through leukocyte recruitment and histamine. *Am J Pathol.* 1997;150:329-340.
59. Gardner TW, Eller AW, Friberg TR, D'Antonio JA, Hollis TM. Antihistamines reduce blood-retinal barrier permeability in type I (insulin-dependent) diabetic patients with nonproliferative retinopathy: a pilot study. *Retina.* 1995;15:134-140.