Histamine Reduces Flash Sensitivity of ON Ganglion Cells in the Primate Retina

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Purpose. In Old World primates, the retina receives input from histaminergic neurons in the posterior hypothalamus. They are a subset of the neurons that project throughout the central nervous system and fire maximally during the day. The contribution of these neurons to vision, was examined by applying histamine to a dark-adapted, superfused baboon eye cup preparation while making extracellular recordings from peripheral retinal ganglion cells.

Methods. The stimuli were 5-ms, 560-nm, weak, full-field flashes in the low scotopic range. Ganglion cells with sustained and transient ON responses and two cell types with OFF responses were distinguished; their responses were recorded with a 16-channel microelectrode array.

Results. Low micromolar doses of histamine decreased the rate of maintained firing and the light sensitivity of ON ganglion cells. Both sustained and transient ON cells responded similarly to histamine. There were no statistically significant effects of histamine in a more limited study of OFF ganglion cells. The response latencies of ON cells were approximately 5 ms slower, on average, when histamine was present. Histamine also reduced the signal-to-noise ratio of ON cells, particularly in those cells with a histamine-induced increase in maintained activity.

Conclusions. A major action of histamine released from retinopetal axons under dark-adapted conditions, when rod signals dominate the response, is to reduce the sensitivity of ON ganglion cells to light flashes. These findings may relate to reports that humans are less sensitive to light stimuli in the scotopic range during the day, when histamine release in the retina is expected to be at its maximum. (*Invest Ophthalmol Vis Sci.* 2010;51:3825–3834) DOI:10.1167/iovs.09-4806

As in other vertebrates, the retinas of primates, including humans, receive inputs from the brain, but it is still uncertain how these retinopetal axons contribute to vision.[1] There is good evidence that histamine is one of the neurotransmitters in this pathway. In human retinas, the levels of histamine are comparable to those of other regions of the central nervous system, and the synthetic enzyme, histidine decarboxylase, is present.[2] Histamine has been localized to retinopetal axons that terminate in the inner plexiform layer (IPL) of monkey retinas.[3] These axons originate from the tuberomamillary nucleus of the posterior hypothalamus,[4] the only site where histaminergic neurons occur in the central nervous system.[5] In other mammals, histaminergic neurons play an important role in maintaining the waking state, firing at maximum during the animal's active period.[6] In macaques, levels of histamine metabolites in the third ventricular cerebrospinal fluid are higher during the day than at night, a finding suggesting that the retinopetal axons release histamine during the day.[7]

In the central nervous system, histamine acts on three types of G-protein-coupled receptors: HR1, HR2, and HR3.[8] There is evidence suggesting that all three are present in primate retinas. HR1 has been characterized in human retinal membranes by [^{3}H]-mepyramine binding.[9] Anatomic techniques have localized immunoreactive HR2 to cone pedicles in macaque retina.[10] HR3 has been localized to the dendrites of ON bipolar cells in macaque retinas by light and electron microscopic immunohistochemical techniques,[11] and an agonist of HR3, (*R*) α-methylhistamine (RAMH), reproduces many, although not all, of the effects of exogenous histamine on monkey retinal ganglion cells.[12] Both histamine and RAMH also increase the delayed rectifier component of the voltage-gated potassium conductance in macaque ON bipolar cells.[13]

Previously, we studied the effects of histamine on the responses of monkey retinal ganglion cells to a white light of single-stimulus strength in the photopic range in an eye cup preparation in vitro with conventional extracellular electrodes. Under these conditions, histamine either decreased the amplitude of the light responses or had no effect; ON and OFF cells had very similar responses to histamine.[12] We now have repeated those experiments, using a multielectrode array, a thoroughly dark-adapted baboon eye cup preparation, and a range of monochromatic, scotopic stimuli. On average, histamine decreased the rate of maintained firing in darkness and reduced the sensitivity of all types of ON cells. In contrast, the histamine effects on the two types of OFF ganglion cells found in our sample were not statistically significant; however, conclusions about OFF cells must be tempered due to the limitations of the stimuli used, which did not support full characterization of the OFF cells.

Methods

Superfused Retina Preparation

Adult baboon (*Papio cynocephalus*) eyes (n = 17) were obtained through the Biological Materials Distribution Program at the Southwest Foundation for Biomedical Research (SFBR; San Antonio, TX). All veterinary procedures were performed by SFBR personnel. The ani-

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mals were sedated with an intramuscular injection of ketamine (15 mg/kg, IM) and then euthanized with an intravenous injection of pentobarbital (100 mg/kg, IV). These agents and procedures were approved by the Institutional Animal Care and Use Committee of the SFBR, and were in conformance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The eyes were enucleated at the beginning of the necropsy and hemisected. The vitreous humor was removed with fine forceps, and the eye cups were transported to the laboratory in the dark in Ames medium (Sigma-Aldrich, St. Louis, MO) equilibrated with 95% O_2/5%CO_2. This medium was also used in all the following procedures. The eye cups were cut into pieces approximately 7 mm^2, and those taken from the midperipheral region of the retina (20–40° eccentricity) were used for the recordings. One piece was placed in a superfusion chamber maintained at 37°C with an in-line heater (TC-324B; Warner Instruments Corp., Hamden, CT). The remaining pieces were stored at 20°C in the dark. The tissue remained responsive to light for 6 to 8 hours under these conditions.

Multielectrode Array Recording of Retinal Ganglion Cell Activity

Recordings were made with 16-channel silicon probe electrodes (Neuronexus Technologies, Ann Arbor, MI). The silicon probes consisted of four shanks 3 mm long and 15 μm thick, each with four active sites. The distance between shanks was 150 μm, and the separation between recording sites on each shank was 25 μm. Two types of electrodes were used; they differed in recording areas, but otherwise were identical. One type had a recording area of 312 μm^2 and the other type an area of 177 μm^2. Because the results with the two electrode types were very similar, they were analyzed together. Under dim red light, the array was positioned in the ganglion cell layer of the retinal midperiphery or periphery with a hydraulic micromanipulator (MX630R; Newport, Irvine, CA). After 30 minutes of dark adaptation, ganglion cell recording commenced. Typically, at least two cells, and no more than four, were recorded per electrode channel.

Action potential recordings were amplified and continuously acquired with a 16-channel preamplifier (RA16PA Medusa; Tucker-Davis Technologies [TDT], Alachua, FL) and a multiprocessor (RX-5 Pentusa Base Station; TDT) with a sampling rate of 25 kHz. The accompanying system software (OpenEx Suite; TDT) was used to control acquisition. Continuous activity, spike waveforms (recorded from 0.2 ms before the point at which the spike was triggered to 0.64 ms after this point), spike timestamps, stimulus timestamps, and light intensities were recorded simultaneously for each channel. The software was used to sort the spikes in several ways: Bayesian expectation-maximization, k-means, and closest centers algorithms. The various statistical methods were used to confirm consistent cell sorting. Manual cluster cutting and waveform selection were also used. Sorted records were extracted from the files with custom software, which also made a preliminary calculation of mean firing rate for the light responses and maintained neural activity. Two commercial software programs were used for the final statistical analysis (Statistica 6; StatSoft, Tulsa, OK, and SAS, Cary, NC). Unless otherwise noted, groups of cells were compared by *t*-test for independent samples.

Light Stimulation and Calibration

Light responses were elicited in complete darkness with an LED photostimulator with a peak wavelength of 560 nm (50 nm bandwidth), producing full-field flashes in the scotopic range that were 5 ms in duration.[14] The stimulator, with an opal glass diffusing filter at its output, was mounted onto the camera port of a dissecting microscope (SZH-ILLK; Olympus, Center Valley, PA). The stimuli were calibrated by using a radiometer/photometer (UDT350; Graseby Optronics, Orlando, FL) with a silicon detector (M268R; Graseby Optronics). Output irradiance at the plane of the retina varied from 3×10^{-6} to 3.9×10^{-2} $\mu W/cm^2$. The output was converted (using the equivalence that the photon energy [e] at 560 nm is 3.55×10^{-19} J) to a quantal irradiance range for the 5-ms flash of 4.25×10^{-4} to 5.6 quanta/μm^2 per flash delivered to the retina. For the 500-ms stimuli used to classify cells, the five weakest stimuli were used, ranging from 4.25×10^{-4} to 68×10^{-4} quanta/μm^2 per flash. To convert to photoisomerizations per rod (Rh*) per flash, we used a modified version of the equation of Lyubarsky and Pugh,[15] that included a term, $E(\lambda)$, for the efficiency of the 560-nm light at 507 nm for the rods. This term represented the efficiency of absorption of the 560-nm photon in a rod photoreceptor and was based on the relative overlap of the spectral output of the visual stimulator, centered at 560 nm, with the V_λ' function (the scotopic relative luminous efficiency function), which is centered at 507 nm.

The equation used for the conversion was

$$\text{Rh}^* \text{ per rod per flash} = Q(\lambda) \times \tau(\lambda) \times A_c(\lambda) \times E(\lambda) \qquad (1)$$

where $Q(\lambda)$ is the photon density per flash; $\tau(\lambda)$ is the transmission coefficient of the neural retina; and A_c is the effective collecting area of a single rod, a value that includes the geometric factor (2-μm diameter rod aperture) as well as common values for optical absorbance factor (0.7) and quantum efficiency (0.67). A value of 1.8 μm^2 was selected for A_c, which lay between values estimated for mouse (1.3 μm^2) and human retinas (2.3 μm^2).[15] The value of $\tau(\lambda)$ established for rat retina at 510 nm is 0.7,[16] 0.79 at 514 nm for bovine retina,[17] and between 0.74 and 0.8 (including both direct and forward scattered light) for human retina at 510 to 514 nm.[18,19] Therefore, for the baboon retina, an intermediate value of 0.75 was used. The effectiveness factor, $E(\lambda)$, to represent the effective light at 507 nm, was estimated to be 0.3, yielding a final stimulus range of 1.72×10^{-4} to 2.27 Rh*/rod /flash.

The specificity of this LED stimulus for rod photoreceptors may be evaluated by noting that a previous study of scotopic responses in macaque retina reported that "the photopic luminance provided by the green LEDs [used in this particular stimulator] was 2.4 times the scotopic luminance (assuming CIE functions)"[20]; however, the weak stimuli used in the present study of ganglion cells in fully dark-adapted baboon retina were still much more effective for rods than for cones. Suction electrode recordings from single photoreceptors of primates have shown L- and M-type cones to be about two orders of magnitude less sensitive than rods.[21] Furthermore, the sparseness of cones relative to rods in the midperipheral retina,[22] where the present recordings were made, further limited the possibility of finding cone-driven responses for stimuli that were near the lowest limit of sensitivity for fully dark-adapted, rod-driven responses.

Characterization of Retinal Ganglion Cell Responses to Light and Histamine

With an audio monitor and an oscilloscope, a series of weak stimuli were used to find the threshold (i.e., a just detectable response) for the best isolated cell recorded by the array. The mean threshold stimulus strength was 0.034 ± 0.057 Rh*/rod. After the characterization of the cell's threshold, the cell's responses were obtained to test flashes of 5-ms duration, presented in steps of increasing strength. Sets of five stimuli, beginning with the threshold stimulus, and with each successive stimulus double the strength of the preceding one, were presented so that a 1.2-log unit flash energy range was tested. The interval between all stimuli in a set of five stimuli was 2 seconds, and there were 2 seconds between stimulus sets. The maintained activity during the 500-ms interval before each flash was averaged and combined to create pseudocontinuous plots that reflected maintained activity between flashes. The light responses were measured by counting action potentials (spikes) in an interval selected to include the longest response, typically to the strongest stimulus strength. This interval was used for all the light responses of that cell. The intervals ranged from 150 to 300 ms and were typically 200 ms for ON cells and 250 ms for OFF cells. For ON cells, the maintained activity for a 500-ms interval before each stimulus was subtracted from the corresponding response to yield a measure of the light-activated component of the response.

Histamine dihydrochloride (Sigma-Aldrich) was applied to the retina via the superfusate; the dose was typically 5 μM (mean concentration was 4.52 ± 1.13 μM). The data acquisition period was 10 to 12

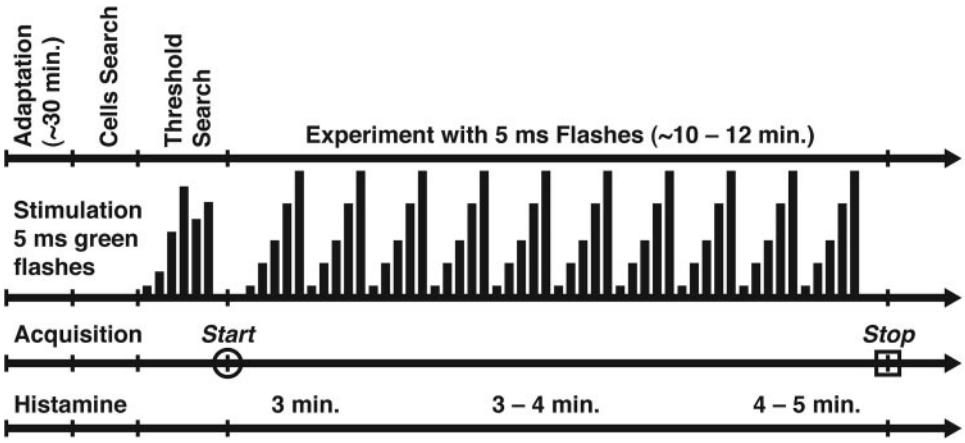

FIGURE 1. Experimental protocol. A piece of baboon eye cup was placed in the chamber and dark adapted for 30 minutes. Then, in complete darkness, the electrode array was positioned to obtain strong signals on at least three electrodes. The retina was stimulated with LED flashes of 5-ms duration. At each recording site, the threshold for one of the best-isolated cells was found and used as the first stimulus. The flashes were grouped into trials, each containing five flashes with strength increased by a factor of two on successive presentations. There were 2 seconds between flashes and 2 seconds between trials. The main experiment consisted of three intervals—control, histamine application, and washout—each with 18 to 24 trials. The intervals are not drawn to scale.

minutes, divided into three intervals: 3 minutes before histamine application, 3 to 4 minutes with histamine present, and 4 to 6 minutes of washout, which was sufficient to reverse the effects of histamine on both maintained activity and on light-evoked responses. In each period, there were 18 to 24 trials, each consisting of the five steps of stimulus strength. Typically, after the experiments with histamine had been completed, flashes of the same five stimulus strengths, but each with a duration of 500 ms, were presented to aid in classifying the light response type of the ganglion cells, as well as to confirm the recovery of the baseline light response after washout of histamine. The experimental protocol is shown in Figure 1.

The intensity-response functions before and during histamine application were compared by using a Naka-Rushton analysis.[23] More specifically, each cell's light response (isolated by subtraction of the maintained activity) was averaged and fitted with a simple hyperbolic function that describes an increase of response in proportion to the stimulus, followed by a characteristic saturation (i.e., the generalized Naka-Rushton equation):

$$R = \frac{aI^n}{I^n + b^n} \quad (2)$$

where R is the mean firing rate (impulses per second) in response to a particular value of I, a is the maximum response (impulses per second), I is the stimulus strength (Rh*/rod/flash), b is the stimulus strength producing a half-maximum response (Rh*/rod/flash), and the exponent n is equal to 1, as it was in the initial analysis.[23] The assumption of an initially linear stimulus response relation is based on observations in numerous studies of dark-adapted mammalian retina, as reviewed elsewhere.[14] A derived measure, the slope in the linear response range (before saturation), a/b, was used to characterize the sensitivity to the light flash, S_f, expressed as impulses per second per Rh*/rod/flash: hereafter as imp/s per Rh*. To determine the effect of histamine on flash sensitivity, the ratio (S_f during histamine)/(S_f before histamine) was calculated for each cell.

Latency

The latencies were defined as the time to reach the 95% confidence level that the cell was firing in response to the light stimulus, in averaged peristimulus time histograms constructed from the cell's response to the fourth stimulus in the sets of five increasing stimulus strengths. This stimulus strength was chosen for the latency calculations because it elicited a reliable light response.

Signal to Noise

The signal-to-noise ratio was defined as the light response minus maintained activity, divided by the light response with maintained activity included. It was calculated by using the response to the third stimulus intensity instead of the fourth. This intensity level was chosen to minimize the effects of response saturation.

Peristimulus Time Histograms

Standardized peristimulus time histograms (PSTHs) were constructed to represent the light response of the cell—specifically, its characteristic waveform. Eighteen to 24 responses to stimulus level four were recorded from a given retinal ganglion cell, averaged, and used as the input data for the PSTH. According to Tolhurst et al.,[24] the variance of a neuron's firing rate is directly proportional to its mean firing rate. To reduce the variability inherent in a cell's response, a standardization procedure was followed. The input data for the PSTH was a cell's response to the standard stimulus level four, which was chosen to ensure a high probability of eliciting a light response. The mean value of the PSTH data was calculated and subtracted from the amplitude value at each time point in the PSTH. Then, the remainder was divided by the overall standard deviation of the response amplitude. This standardization procedure minimized the variability in the cell's firing rate due to maintained activity and also compressed the response, so that the PSTH had a 0 mean with an SD of 1. This standardization procedure was more efficient than simple normalization, because it facilitated the comparison of the time course of the cells' light responses by making all the response waveforms superimposable. Response kinetics were unaffected by the standardization procedure.

RESULTS

The dataset consisted of 154 ganglion cells, including 117 ON cells and 37 OFF cells.

ON Ganglion Cells

It was possible to fit the responses of 100 of the ON cells with the Naka-Rushton equation and determine their flash sensitivity

TABLE 1. Characteristics of the ON and OFF Ganglion Cells under Control Conditions

Light Response Type	*n*	Maintained Rate (Hz)	*n*	Flash Sensitivity (imp/s per Rh*)	*n*	Latency (s)
ON fast sustained	17	1.84 ± 2.59	15	156.9 ± 261.5	14	0.071 ± 0.019
ON slow sustained	16	7.96 ± 11.24	9	155.4 ± 171.3	10	0.108 ± 0.034
ON fast transient	13	14.58 ± 8.65	13	1353.5 ± 983.7	14	0.058 ± 0.011
ON slow transient	68	21.03 ± 16.67	63	960.6 ± 1041.0	67	0.060 ± 0.015
OFF (sust. and trans.)	37	10.4 ± 11.8	—	NA	—	NA
Total or average	151	14.34 ± 14.93	100	818.6 ± 982.1	107	0.066 ± 0.023

The maintained firing rate in darkness was calculated for 500 ms before each flash and averaged. Light responses were fitted with the Naka-Rushton equation, equation 2, and flash sensitivity, S_f, was determined as described in Methods. Latencies were calculated from the averaged responses to the fourth stimulus strength in each trial. The data from OFF sustained and transient cells are combined in the table. Only maintained activity is reported for the OFF cells, because flash sensitivity and latency were not reliably measured for them, sust., sustained; trans., transient; NA, not available.

(S_f) by using the equation parameters described herein. The group data for those cells are summarized in Table 1. The response of the remaining cells was so variable that a reliable fit to the equation could not be made. Two representative examples of analyses of the 100 cells, one from a cell with high sensitivity and the other from a cell with low sensitivity, are illustrated in Figure 2. Table 1 also summarizes the rates of maintained activity for 114 ON cells that had detectable maintained activity and the response latencies to the fourth in the series of flash strengths. The latencies were determined from the standardized PSTHs generated from the ganglion cells' responses to the fourth stimulus strength. Of the sample of ON cells, 115 could be subdivided into the types illustrated in Figure 3, on the basis of the kinetics of their responses to the brief flashes.

The sustained cells ($n = 33$) had similar, low flash sensitivities relative to the transient types to be described next (Table 1). Sustained cells could be further subdivided into two groups on the basis of maintained firing rate. One subgroup (fast sustained, $n = 17$, Fig. 3A) had a low rate of maintained firing in the dark of 1.84 ± 2.59 (SD) Hz and responded with an average latency of 71 ± 19 ms. Their response amplitude declined gradually, returning to the baseline by 250 ms after the flash. The other subgroup (slow sustained, $n = 16$, Fig. 3B) had a higher maintained rate 7.96 ± 11.2 Hz ($P < 0.05$) and a much longer latency 108 ± 34 ms ($P < 0.01$). The responses of the slow sustained cells also decreased more gradually.

ON transient cells ($n = 81$) had higher flash sensitivities than those of the sustained cells ($P < 0.001$). The maintained firing rates of the transient cells were also similar, 20 Hz on average, and were significantly higher than those of the sustained cells ($P < 0.0001$). The transient responding cells could also be subdivided into two subgroups: fast and slow. The properties of these subgroups are summarized in Table 1. The response latencies of both fast and slow transient cells to the fourth stimulus were similar, 60.2 ± 1.44 ms on average, and therefore much shorter than the average for all sustained cells, which was 86.1 ± 32.1 ms ($P < 0.001$). However, the return of the firing rate to baseline in the late phase of the response differed for the fast and slow transient types, which was the basis for subdividing the response types. The firing rates of fast transient cells ($n = 13$) returned to the baseline rate within 150 ms after the flash onset, oscillating slightly afterward (Fig. 3C). In contrast, the firing rates of slow transient cells ($n = 68$) declined more slowly, reaching a plateau between 175 and 225 ms after the flash onset, without oscillations (Fig. 3D).

OFF Cells

With the 500-ms stimuli used to confirm the response type, 37 cells were classified as OFF cells (Fig. 4), and 25 of these also responded to brief flashes (5 ms). OFF cells fired, on average, at 10.4 ± 11.8 Hz in darkness and thus had lower maintained firing rates than did ON cells, which fired at 15.6 ± 15.7 Hz, on average, under these conditions (Table 1). OFF cells were further subdivided into two types on the basis of the kinetics of their responses to 500-ms steps of light. Cells with transient responses to the offset of 500-ms steps of light had low rates of maintained firing in darkness: 4.0 ± 3.7 Hz, on average. In response to brief flashes, transient OFF cells ($n = 4$) increased their firing rates, beginning approximately 200 ms afterward (Fig. 4A). Cells with sustained OFF responses to 500-ms steps of light had higher rates of maintained firing in darkness than did the transient OFF cells: 12.4 ± 12.9 Hz, on average. In

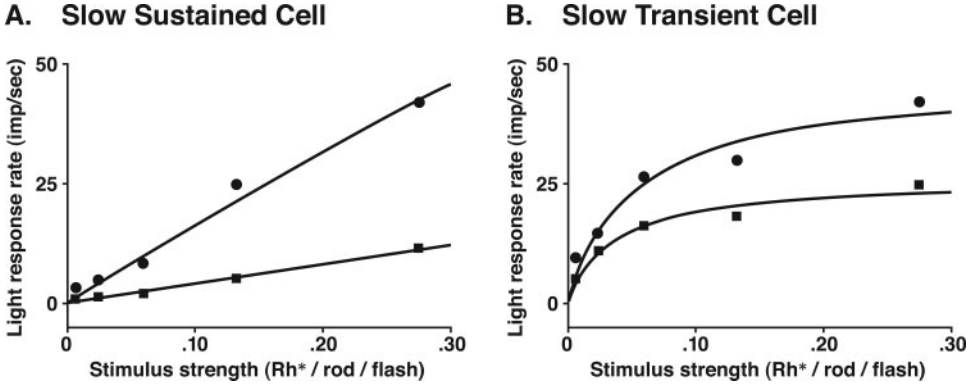

FIGURE 2. Intensity–response curves. Each point is the averaged firing rate for 200 ms after beginning the 5-ms flashes. The light response of the cell was isolated by subtracting the average maintained firing rate recorded in the 500 ms before the start of the flashes. Intensity response data were fitted with the original Naka-Rushton[23] equation (i.e., equation 2). The initial slope of the fitted function was used to characterize light flash sensitivity (S_f) under control conditions (*circles*) and during histamine application (*squares*). (**A**) A representative slow sustained cell. The control S_f was 167 imp/s per Rh*, and the S_f during histamine was 40 imp/s per Rh*. (**B**) A representative slow transient cell. The S_f under control conditions was 906 imp/s per Rh*, and the S_f during histamine application was 713 imp/s per Rh*.

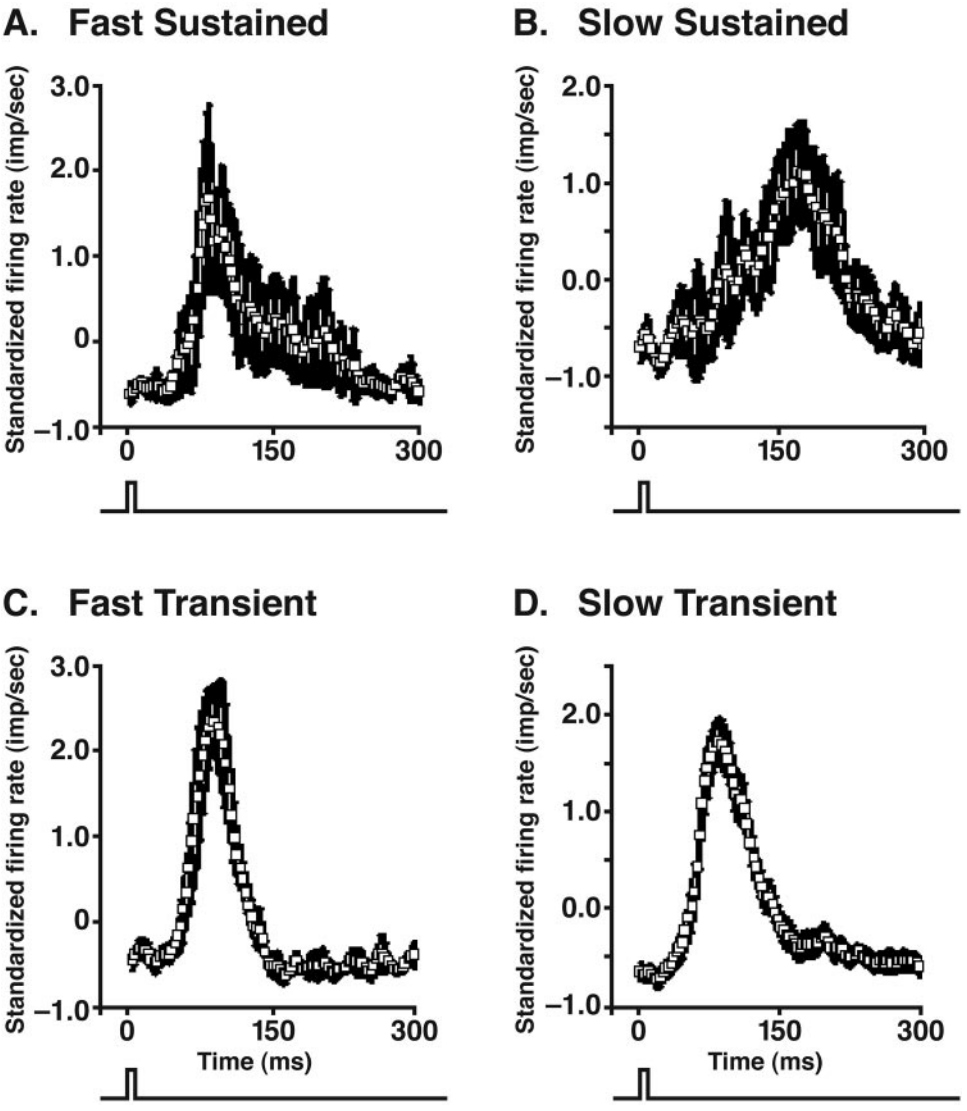

FIGURE 3. Four types of ON light responses. Standardized, averaged peristimulus time histograms for four types of light responses. The flash stimulus (5-ms duration) began at time 0 and was the fourth in the intensity series. Error bars, standard deviations. (**A**) Fast sustained cells (n = 17) had relatively long response latencies and gradually returned to baseline levels. (**B**) Slow sustained cells had even longer response latencies and times to peak response (n = 16). (**C**) Fast transient cells (n = 13) had responses that returned to baseline levels after 150 ms, followed by oscillations in the firing rate. (**D**) Slow transient cells showed small plateaus in the firing rate after 150 ms and returned to baseline 50 ms later (n = 68). The transient cell types had shorter response latencies than did the sustained types. Some of the amplitudes in the PSTHs have negative values because of the standardization procedure, in which the mean amplitude of the entire histogram was subtracted from the amplitude of each time point in the histogram. Repeated ANOVA tests, with the cell type as the factor, were used to compare the fast and slow transient types, to ensure that they were significantly different. (**C**, **D**) Responses compared at times ranging from 123 to 225 ms after the flash, when the responses of the slow transient cells remained elevated. The responses of the two types of ganglion cell were found to be significantly different in this range (F = 5.662, P = 0.02), justifying the subdivision into two separate groups.

response to brief flashes, they decreased their firing rates, with a maximum decrease of approximately 100 ms after the flash (Fig. 4B). Because of the difficulty in characterizing the OFF cell types further, the sustained and transient OFF-responding ganglion cells were considered as a single group.

Effects of Histamine on Maintained Activity

In 95% of ON ganglion cells that exhibited maintained firing (146/154 cells, Table 2), histamine had a detectable effect on the rate of maintained firing, either decreasing (75%) or increasing (23%) the firing rate (Table 2, Fig. 5). In the remainder of the cells (n = 2), histamine either had no effect or else its effect could not be quantified because of very low or unstable rates of maintained activity (Table 2). In the ON cells, histamine produced a statistically significant ($P < 0.0001$, paired t-test) decrease in the maintained firing rate in darkness to 81% of the control value; however, the high coefficient of variation (62%) indicates that the effects were highly variable. Although maintained firing rates of OFF cells tended to decrease in response to histamine (24/37 cells, Table 2), there were no statistically significant changes in the maintained rates of such cells.

Effects of Histamine on Light Responses

Histamine had a variety of effects on the flash sensitivity of ON ganglion cells, as measured by the parameters of the best fit Naka-Rushton equations. Some ON cells showed increases in sensitivity or no change after histamine, but in most, sensitivity was decreased (Fig. 6). On average, the flash sensitivity during histamine application was 73.4% ± 45.2% of the control value, a significant difference ($P < 0.0001$). There were no statistically significant differences among the ON ganglion cell types in the effect of histamine on flash sensitivity, nor were the magnitude and sign of the histamine effect related in a significant way to the prehistamine flash sensitivity. Although it was not possible to derive sensitivity for a sufficient number of OFF cells to make these calculations, histamine qualitatively had little effect on these cells, which is notable in view of findings that the human psychophysical responses to stimulus decrements are more sensitive and faster than to stimulus increments.[25–27] In a separate set of recordings, nine OFF cells were studied in the presence of an adapting background with a strength of 1.25 Rh*/rod/s; these stimulus conditions gave better-defined OFF responses. Similar to the findings in dark-adapted cells, however, histamine had only weak and inconsistent effects on the OFF cells.

The light responses of ON ganglion cells were slower, on average, in the presence of histamine. The changes in response kinetics were small, but significant differences emerged, when the dataset was analyzed with paired t-tests. Kinetics were measured before and after histamine treatment in 14 of 33 ON

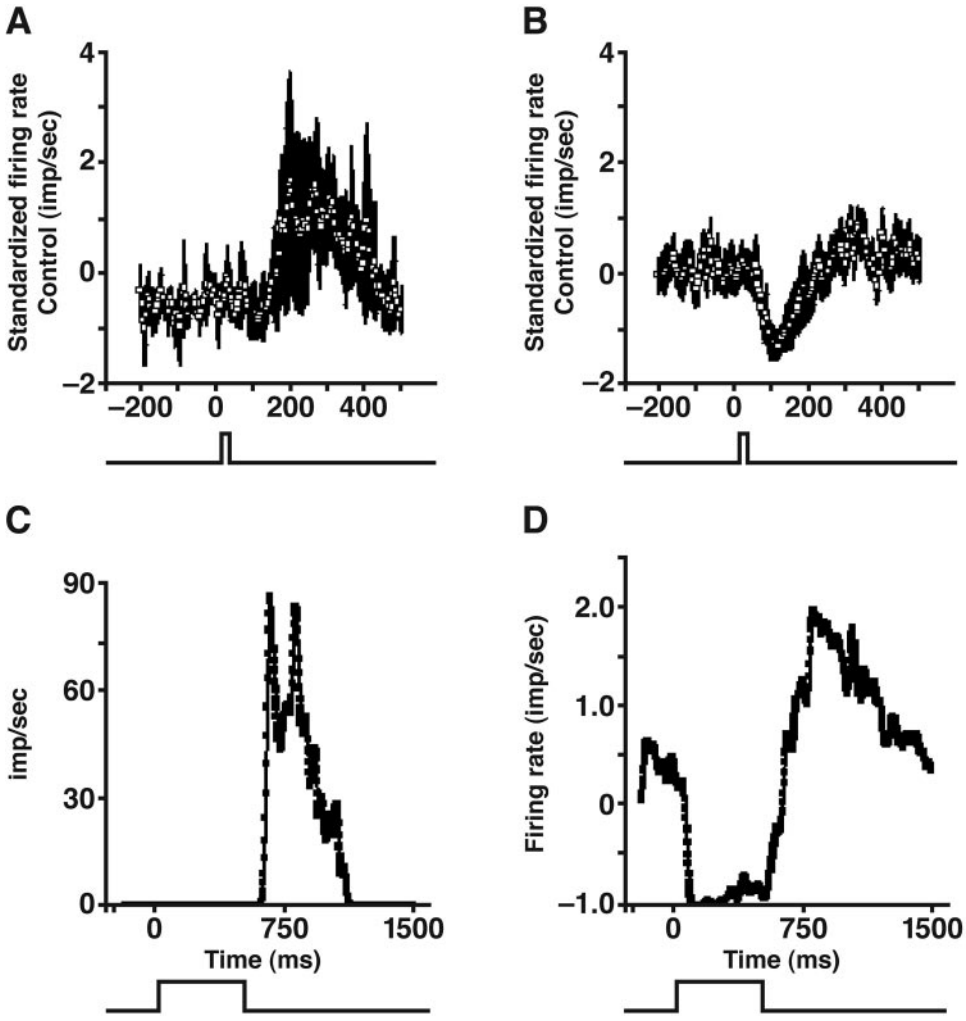

Figure 4. Two types of OFF light responses. OFF cells were subdivided into two groups, sustained and transient, on the basis of the kinetics of their responses to 500-ms steps of light presented after completion of the tests with 5-ms flashes. (**A**) Peristimulus time histogram of the response of a representative OFF transient cell to a 5-ms flash. (**B**) Peristimulus time histogram of the response of a representative OFF sustained cell to a 5-ms flash. The 5-ms flash stimulus began at time 0 and was the fourth in the series of increasing stimulus strengths. Error bars, standard deviations. (**C**, **D**) Standardized, averaged peristimulus time histograms for both types of light responses to 500-ms steps of light. (**C**) Transient OFF cells ($n = 4$) had no maintained activity in the dark but increased their firing rates in response to the offset of the light stimulus. (**D**) Sustained OFF cells ($n = 21$) decreased their rate of maintained firing in response to the light.

sustained cells, and in 64 of 81 ON transient cells. Under control conditions, the average response latency of ON transient cells was 60.5 ± 14.7 ms, whereas in the presence of histamine, the latency was 64.2 ± 13.6 ms ($P \approx 0.008$). For the ON sustained cells, the latency in control conditions was 102 ± 31.7 ms, whereas in the presence of histamine, the latency was 118 ± 41.4 ms ($P \approx 0.02$). For the time to peak response, ON transient cells had a value of 90.5 ± 21.7 ms in control conditions and 95.6 ± 20.5 ms in the presence of histamine ($P \approx 0.002$), whereas ON sustained cells had a value of 150.0 ± 41.8 ms in control conditions and 141.9 ± 46.7 ms in the presence of histamine (difference not significant). There were no statistically significant histamine-induced changes in the response width of the peristimulus time histogram at half maximum for either ON sustained or ON transient cells.

It was possible to calculate the signal-to-noise ratio (SNR) for 102 ON ganglion cells on the basis of their response to the third stimulus intensity. Histamine slightly decreased the average SNR of ON ganglion cells from 0.62 ± 0.20 to 0.57 ± 0.22. This change was statistically significant when analyzed with a paired *t*-test ($P < 0.001$). For cells with maintained firing rates that were decreased by histamine, the SNR did not change, but for cells with increased maintained firing rates with histamine, the SNR was decreased to 76.4% ± 20.5% of the control value. The difference between the two groups was statistically significant ($P < 0.0001$).

Discussion

Visual Stimulation and Retinal Ganglion Cell Responses

Stimuli. The briefly flashed stimuli in the present study were sufficiently weak that all responses in ganglion cells were rod driven and most were mediated by the sensitive primary rod pathway. That is, the quantal signals from the rods were conveyed via rod bipolar cells to AII amacrine cells and then, via cone bipolar cells, to retinal ganglion cells.[28] Responses to single photoisomerizations in the rods are known to decrease synaptic transmission from rod bipolar cells to AII amacrine cells in mouse retina,[29] and the same is assumed to be true in primates. This effect, however, lasts only a few hundreds of milliseconds, and the sensitivity of these synapses should have recovered completely during the 2 seconds of darkness between stimuli in the present study. The weakest stimulus that was used, 0.002 Rh*/rod, elicits a robust scotopic threshold response (STR) in the full-field, dark-adapted electroretinogram (ERG), recorded noninvasively in vivo, from macaque monkeys,[20] with the same stimulator. The stimulator used in the present study was found to elicit a just distinguishable STR in monkeys when the stimulus was only 10 times weaker, near the limit of vision in humans determined psychophysically.[30] In monkeys, the STR is thought to be generated by retinal ganglion cells that have received signals via the primary rod pathway,[20] but in other species, AII amacrine cells may be directly involved in generating the response.[31]

TABLE 2. Effects of Histamine on Maintained Activity and Light Responses

		Maintained Spike Rate (Hz)			Light Response				Flash Sensitivity		
Light Response Type	***n***	**Incr.**	**Decr.**	**Unclass.**	**Incr.**	**Decr.**	**No Eff.**	**Abol.**	**Incr.**	**Decr.**	**Unclass.**
ON fast sustained	17	6	11	0	1	13	2	1	2	12	3
ON slow sustained	16	6	9	1	3	7	6	0	2	6	8
ON fast transient	14	1	12	1	0	12	2	0	1	12	1
ON slow transient	68	14	54	0	3	47	14	4	17	44	7
OFF (sust. and trans.)	37	8	24	5	—	—	—	—	—	—	—
Unclassified	2	0	1	1	0	1	0	1	0	0	2
Total	154	35	111	8	7	80	24	6	22	74	21

The maintained firing rate and flash sensitivity were calculated as for Table 1. The effects on the light responses described in the table are summaries of the changes in S_r (the derived flash sensitivity) of the cells, before and during histamine application. For the OFF cells (sustained and transient types combined), histamine effects on their light responses could not be reliably determined; therefore, only histamine effects-on-maintained firing rate are reported. sust., sustained; trans., transient.

The strongest stimulus used in the present study, 2.3 Rh*/rod, is close to the level, ~1 Rh*/rod, that just saturates the ERG b-wave in macaques.[20] The dominant component (PII) of the rod-driven b-wave is thought to reflect mainly the activity of rod bipolar cells.[14,32] This notion is consistent with the finding that a stimulus of ~2 Rh*/rod produces half-maximum responses from rod bipolar cells recorded in mouse retinal slices.[33] The strongest stimulus used in the present experiments was likely to be just above the activation of the secondary rod pathway, in which signals pass from rods to cones via gap junctions.[28] In isolated, dark-adapted monkey retina, the threshold for rod responses to brief flashes that produce detectable hyperpolarizations in cones is ~1 Rh* rod,[34] which is higher than the strength of the typical stimuli used in the present study.

Light Responses. The ganglion cells from peripheral baboon retina were classified into four types (two ON and two OFF) on the basis of the kinetics of their light responses, their maintained activity in darkness, and their scotopic sensitivity. The flashes were full-field, and, because the retinas were thoroughly dark adapted, receptive field surrounds would not be expected to contribute to the responses.[35] Although the stimuli used in the present study were appropriate for studying the effects of histamine on the flash sensitivity of dark-adapted ganglion cells, they were not optimal for classifying the cells. There are 20 or more morphologic types of retinal ganglion cells in primates, and in some cases, their light responses have not been described.[36,37]

ON ganglion cells were distinguished on the basis of their light response kinetics, as described previously.[38] ON cells with relatively sustained responses to brief flashes had low rates of maintained firing in the dark and relatively low flash sensitivity. In these respects, sustained cells resembled ganglion cells projecting to the parvocellular layers of the lateral geniculate nucleus (LGN).[39–42] Transient ON ganglion cells were different from sustained ON cells in several respects. They had shorter response latencies and times to peak and higher rates of maintained activity in darkness than sustained ON cells, a finding opposite that was reported in another study of light-adapted macaque ganglion cells.[43] Transient ON cells also had higher flash sensitivities than did sustained cells, like ganglion cells that project to the magnocellular layers of the LGN,[39–42] and to the superior colliculus (SC).[44] The difference in flash sensitivities (gain) of the two cell systems resembles gain differences reported for P and M cells.[45] The effects of histamine on both types of ON ganglion cells were similar, a finding consistent with the localization of HR3 to the dendritic

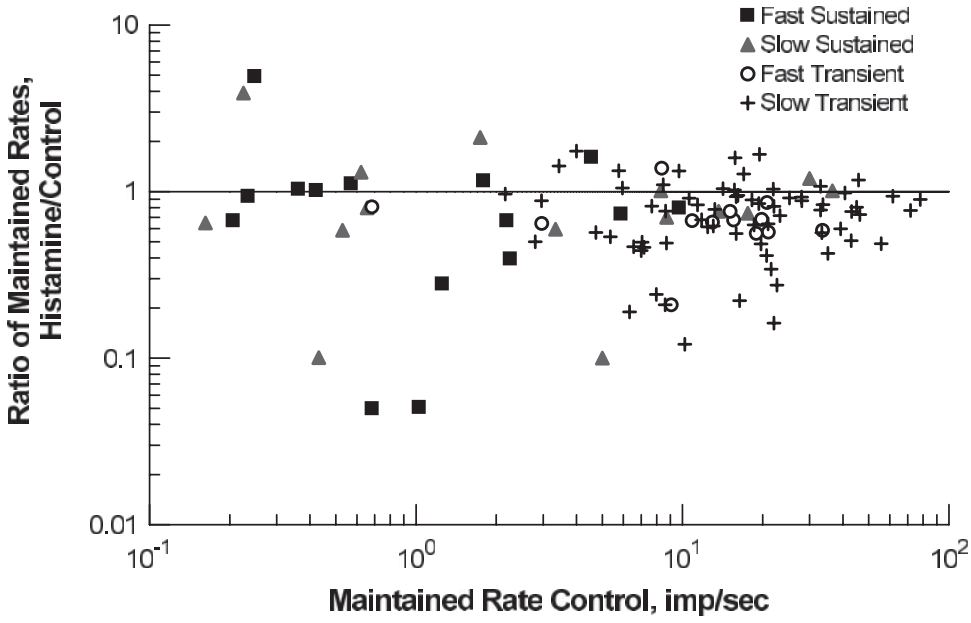

FIGURE 5. Effects of histamine on maintained firing of ON ganglion cells in darkness. The maintained firing rate was averaged from 500-ms intervals recorded before each flash. For each ON cell with detectable maintained activity, the ratio of the maintained rate during histamine application, to the control rate before, is plotted versus the control rate. Note that both scales are logarithmic. In 75% of ON cells, histamine decreased the maintained rate. In the entire sample, histamine decreased the maintained rate to 81% of the baseline value ($P < 0$ 0.0001 paired *t*-test). Although the four subtypes of ON cells differed in their rates of maintained firing under control conditions, there were no statistically significant differences in the effect of histamine among the subtypes.

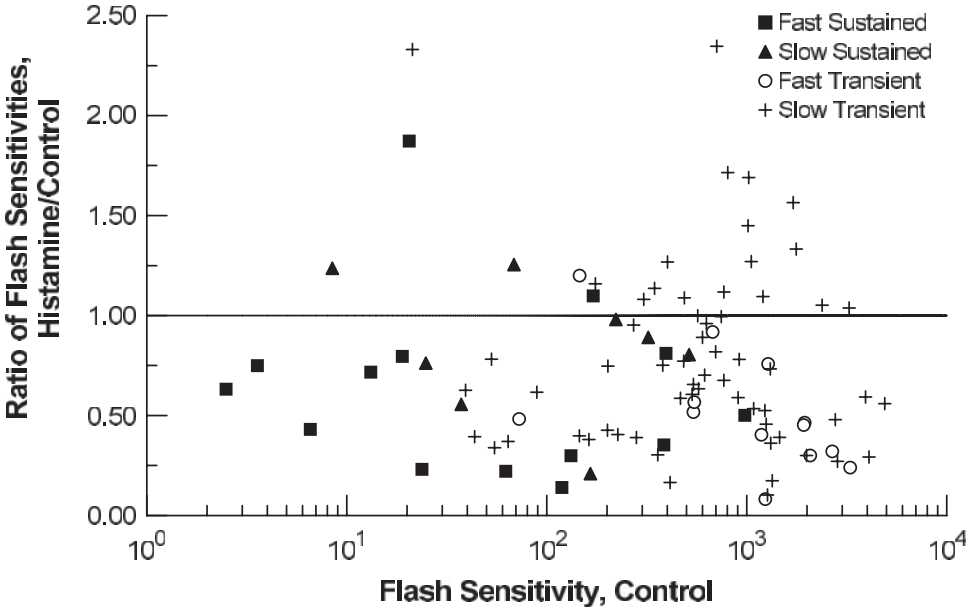

FIGURE 6. Effects of histamine on the flash sensitivity of ON ganglion cells. The intensity response curves were fitted with the Naka-Rushton equation (equation 2), and the flash sensitivity (imp/s per Rh*) was calculated from the initial slopes (Fig. 2). Under control conditions, the flash sensitivity of ON transient cells was higher than that of ON sustained cells. For all ON cells, the flash sensitivity was decreased to 73.4% of the control value with histamine, although there were no statistically significant differences in the effects of histamine among the four subtypes.

tips of all types of ON bipolar cells, including the rod bipolar cells of the primary rod circuit, in the macaque retina.[11]

Two subtypes of OFF ganglion cells, sustained and transient, were also distinguished based on their response kinetics.[38] It is likely that the transient cells correspond to the OFF parasol cells, and the sustained cells correspond to the OFF midget cells, because they are the two most common types of OFF ganglion cells in macaque retina.[46] It is possible that midget ganglion cells were included in the dataset. Although midget ganglion cells in central retina get very little, if any, rod input,[47] they do receive significant rod input in the more peripheral retina,[48,49] from which the retinal tissue used in the present investigation was obtained.

Histamine did not have any statistically significant effects on the OFF ganglion cells, but it remains to be shown whether histamine selectively affects the ON pathway. Effects of histamine on scotopic responses of OFF ganglion cells would be expected, because the OFF ganglion cells also receive input from rod bipolar cells via AII amacrine cells, and rod bipolar cells are among those that express HR3[11] and possess voltage-gated potassium currents that are enhanced by histamine.[13] A possible explanation is that the effect of histamine is more prominent in the ON pathway because ON cone bipolar cells are also sensitive to histamine, providing a second site for histamine action that is not present in the OFF pathway. Another possibility is that OFF ganglion cells were underrepresented in these experiments. They comprised only 24.5% of the sample, even though they have higher spatial densities than ON ganglion cells.[46] OFF cells may have been underrepresented because, on average, they had lower rates of maintained activity in darkness than did ON cells, as reported previously in light-adapted macaque retinas.[43,50,51]

OFF parasol and midget ganglion cells are known to have smaller dendritic fields and receptive field centers than do the corresponding types of ON cells, according to findings in anatomic and physiological studies.[37,51–53] As a result, OFF ganglion cells would be expected to have lower flash sensitivities. The brief flashes from darkness near absolute threshold, used in the present study, by design, to maintain dark adaptation, were clearly not optimal stimuli for OFF ganglion cells, for which decrements from steady light, or rapid-off sawtooth waveforms would be more adequate.[50] One-third of the OFF cells, identified in the present study using 500-ms steps of light, failed to respond to brief (5 ms) flashes. Nevertheless, it is noteworthy that, in a small group of nine additional OFF cells studied in the presence of a background light (a better stimulus for eliciting an OFF response), histamine had only a weak or no effect on the OFF response.

Effects of Histamine on Flash Sensitivity

Histamine, on average, reduced the sensitivity of both transient and sustained ON retinal ganglion cells to scotopic, full-field, flash stimuli. There is evidence of a similar reduction in sensitivity of the human ERG at the time when histamine release in the retina is expected to be at maximum. Threshold responses to scotopic stimuli, as determined by measuring the amplitude of the b-wave of the ERG, are higher 1.5 hours after the onset of light. This effect is larger than would be predicted by the shedding of outer segment discs, and it is only observed in eyes entrained to a regular light–dark cycle.[54] There have not been any recordings of the activity of histaminergic neurons in primates, but a diurnal rhythm of the primary metabolite of histamine, *N*-methylhistamine (*tele*-methylhistamine), in macaque cerebrospinal fluid (CSF) has been observed.[7] There is a sharp increase at the onset of light with a peak at 3 hours later—a finding consistent with the ERG results, assuming a lag in the release of histamine and the detection of its metabolites in the CSF. Another study of the diurnal variation in the human scotopic b-wave reported a decrease in sensitivity in the early morning in most of the subjects[55]; however, because observations were made at 6-hour intervals and the subjects were not entrained to a light–dark cycle, it is difficult to compare directly the two sets of results.

The sensitivity of human observers to scotopic stimuli also shows diurnal variation. In a study comparing the absolute thresholds of human observers at noon and midnight, five of seven subjects showed a decrease in sensitivity during the day.[56] Similar results were obtained using brighter stimuli in the scotopic range.[57] The sensitivity of human observers to stimuli in the mesopic range is also lowest in the morning[58,59]; however, there have been other, contradictory reports in psychophysical studies. For subjects maintained in constant darkness and tested hourly, only one subject in three showed a decrease in absolute sensitivity during the day.[56] Another group using somewhat different methods found higher scotopic thresholds at night.[60] The most recent study of diurnal variation in visual sensitivity used subjects who had been

dark adapted for 30 minutes before testing, as were the retinal preparations in our study, and this study design also provided a rest period, so that sleep deprivation was not a factor. The stimuli were full-field flashes in the low scotopic range, with 2-second intervals between stimuli, also as in our study. The thresholds for detection were highest in the morning, peaking at 7:30 to 8:00 AM.[61]

Other Effects of Histamine

Histamine also had other, more subtle effects on light responses of ON ganglion cells. On average, histamine reduced the maintained rate of firing by approximately 20%. Like the effect on flash sensitivity, this is consistent with the finding that histamine hyperpolarizes ON bipolar cells and increases the voltage-sensitive potassium conductance of ON bipolar cells in slice preparations from macaque retina.[13] These findings, however, do not account for the 5-ms increases in the latency and time to peak of the light responses produced by histamine. The increases in potassium conductance produced by histamine would be expected to make the light responses of ON bipolar cells faster, not slower.[62] Nor were there were any obvious explanations for the increases in maintained firing rates or the more complex patterns of changes in maintained activity observed during histamine application. Histamine also decreased the signal-to-noise ratio in the light responses of ON ganglion cells by approximately 10% on average. This effect was particularly prominent in cells with maintained firing rates that were increased throughout the application of histamine. Taken together, these findings are consistent with those in earlier studies showing that HR1 and HR2 are also present in primate retinas,[9,63] and they suggest that these other types of histamine receptors are functionally important. Considered with our results, the findings indicate that endogenous histamine released from retinopetal axons contributes to the reduction in absolute visual sensitivity in humans during the day.

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