Histamine interferes with the early visual processing in mice

Authors: Matteo Tripodi[1], Hiroki Asari*[1]

Affiliations:

1. Epigenetics and Neurobiology Unit, EMBL Rome, European Molecular Biology Laboratory, Monterotondo, 00015, Italy

***Corresponding author:** Hiroki Asari, E-mail: asari@embl.it

Author contributions: M.T. and H.A. designed the study; M.T. performed experiments; M.T. and H.A. analyzed the results; and M.T. and H.A. wrote the manuscript.

Competing interests: The authors declare no competing financial interests.

Key words: retinal ganglion cells; lateral geniculate nucleus; histaminergic modulation; tuberomammillary nucleus.

Acronyms: AAV, adeno-associated virus; ANOVA, analysis of variance; DLP, digital light processing; DRN, dorsal raphe nucleus; HDC, histidine decarboxylase; GABA, γ-aminobutyric acid; IQR, interquartile range; IR, infrared; LED, light emitting diode; LGN, lateral geniculate nucleus; OT, optic tract; PFA, paraformaldehyde; PSAM/PSEM, pharmacologically selective actuator/effector module; RF, receptive field; RGC, retinal ganglion cell; STA, spike-triggered average; tSNE, t-distributed Stochastic Neighbor Embedding; UV, ultraviolet.

Abstract

Sensory processing is dynamically modulated by an animal's behavior and internal states. Growing evidence suggests that such modulation starts from early stages, even at the retina, but the underlying mechanisms remain elusive. Combining pharmacological and chemogenetic tools with single-unit extracellular recordings in awake head-fixed mice, here we identified that the visual responses of retinal ganglion cells and the lateral geniculate nucleus were both made weaker and slower by histaminergic projections from the tuberomammillary nucleus of the posterior hypothalamus. The observed changes in the visual responses were, however, not directly linked with histaminergic modulation of pupil dynamics or locomotion behavior. Our computational modelling analysis instead suggests neuronal circuit effects, such as gain modulation via the histamine H1 receptors in the retina. As nocturnal animals, facilitation of visual processing at low histamine level is ethologically beneficial for mice to respond faster to visual threats when animals are less active during daytime.

Introduction

In the mammalian brain, histaminergic neurons are exclusively located in the tuberomammillary nucleus (TMN) and surrounding areas of the posterior hypothalamus (Haas and Panula, 2003). They express histidine decarboxylase (HDC) and innervate widely across the brain (Lin et al., 2023), including the retina (Gastinger et al., 1999; Repérant et al., 2007; Warwick et al., 2024). These neurons remain silent during sleep, but increase their firing during wakefulness and reach their maximal activity at the state of high vigilance, much as other monoaminergic systems do, such as the serotonergic neurons in the dorsal raphe nucleus (DRN) of the brain stem (Lin et al., 2023; Takahashi et al., 2006; Thakker, 2011). Brain histamine exerts its effects via three types of receptors: the H1 and H2 receptors mediate excitation of postsynaptic cells, while the H3 receptors serve as an auto-receptor to presynaptically inhibit histamine release (Haas and Panula, 2003). The central histaminergic

system has been implicated in regulating an animal's state and a broad range of behavior, including the sleep-wake cycle (John et al., 2004; Mochizuki et al., 1992), arousal (Parmentier et al., 2002; Scammell et al., 2018), suppression of food intake (Khouma et al., 2023; Valdés et al., 2010; Wang et al., 2015) and learning (Kamei et al., 1993; Klapdor et al., 1994; Orsetti et al., 2001; Rubio et al., 2002). Histaminergic neurons, however, corelease γ-aminobutyric acid (GABA) and interact with other neuromodulatory systems, such as the cholinergic and serotonergic systems (Brown et al., 2002; Khateb et al., 1995; Lin et al., 2023). This makes it difficult to dissect the effects of histamine by itself on neural circuit functions. In particular, histaminergic modulation of sensory processing remains largely elusive (Lee and Dan, 2012; McCormick et al., 2020).

The retina performs the first stage of visual processing, and conveys visual information to the brain via spike trains of retinal ganglion cells (RGCs). Growing evidence supports that the activity of RGCs is correlated with various behavioral measures, such as locomotion and pupil size (Schröder et al., 2020; Liang et al., 2020; Reggiani et al., 2023; Boissonnet et al., 2023), pointing to a more dynamic role of the retina than is assumed for a deterministic processor. The vertebrate retina is part of the central nervous system equipped with both intrinsic and extrinsic neuromodulation mechanisms, much as other brain regions are (Lee and Dan, 2012; McCormick et al., 2020). For instance, dopamine is synthesized and released by a group of retinal amacrine cells under the control of circadian rhythm, and contributes to adjusting the retinal circuitry for rod or cone vision by modulating gap-junction couplings (Bloomfield and Völgyi, 2009). Such retinal intrinsic mechanisms of neuromodulation are, however, much slower than needed to modulate retinal function to follow rapid behavioral changes. To this end, the retina seems to exploit the centrifugal system to receive inputs from a variety of brain regions in a species-specific manner (Repérant et al., 2007). For example, histaminergic and serotonergic projections from TMN and DRN, respectively, have been anatomically described in the mammalian retina (Gastinger et al., 1999; Greferath et al., 2009; Labandeira-Garcia et al., 1990; Warwick et al., 2024). However, compared to the isthmo-optic

pathway in birds (Wilson and Lindstrom, 2011) or the olfacto-retinal circuit in fish (Esposti, et al., 2013), little is known about the function of the mammalian centrifugal system.

Combining in vivo single-unit extracellular recordings from either the lateral geniculate nucleus (LGN) or the optic tract (OT) with pharmacological and chemogenetic tools, here we explored how histamine affects the early visual processing in awake head-fixed mice. During the recording sessions, we simultaneously monitored the subject animal's behavior, such as pupil dynamics and locomotion, to better correlate the effects of histamine on the visual response properties with those on the behavioral measures. We also took a computational approach to clarify the net effect of histamine on the visual responses from functional viewpoints. Because visual processing in the brain fully relies on the signals from the retina, characterization of the retinal operation in vivo is indispensable to better understand how the visual system works.

Results

Histamine leads to weaker and slower visual responses in the mouse lateral geniculate nucleus

To investigate how histamine influences the mouse visual system, we first performed in vivo single-unit extracellular recordings from the lateral geniculate nucleus (LGN) in awake head-fixed mice, and compared the visual response properties of the recorded cells before and after perturbing the central histaminergic system (Figure 1A; see Methods for details). Stimulus ensemble statistical techniques ("reverse correlation"; Chichilnisky, 2001) were employed to systematically characterize the visual response properties of the recorded cells in the framework of a linear-nonlinear cascade model (Boissonnet et al., 2023). In particular, using full-field white-noise stimuli, we analyzed histaminergic effects on 1) the linear temporal filter, estimated by the spike-triggered average (STA) stimulus, i.e., the mean stimulus that triggered spiking responses; and 2) the static nonlinear gain function, i.e., an instantaneous mapping of

79 the STA output to the neural responses (Figure 1B). We also functionally mapped the cells'
80 receptive field (RF) by calculating the linear spatial filter from the responses to random binary
81 "checkerboard" stimuli, where the contrast of each spatial location was randomly and
82 independently inverted over time. To manipulate the histaminergic system, we used two
83 different approaches (Supplementary Figure 1). The first was the chemogenetic activation of
84 the central histaminergic system (Magnus et al., 2011). We injected cre-dependent adeno-
85 associated viruses (AAVs) carrying pharmacologically selective actuator module (PSAM)-
86 5HT3HC channels into the tuberomammillary nucleus (TMN) of the posterior hypothalamus in
87 histidine decarboxylase (HDC)-cre transgenic mice, and then achieved selective activation of
88 the target neurons by systemic administration of the pharmacologically selective effector
89 molecule (PSEM). The second approach was the use of pharmacological tools in wild-type
90 mice (Thakkar, 2011). Specifically, we used chlorphenamine (5 mg/kg), an antagonist of a
91 postsynaptic histamine receptor H1; and ciproxifan (12 mg/kg), an antagonist of the H3 auto-
92 receptor that presynaptically inhibits histamine release. These drugs effectively down- and up-
93 regulate the action of histamine in the nervous system, respectively. Thus, resulting changes
94 in visual processing, if any, were expected to be in opposite directions.

95 Upon chemogenetically activating HDC+ neurons in TMN, we found that LGN neurons
96 generally showed weaker and slower visual responses (Figure 1C,F; N=58 from 3 animals).
97 The mean firing rate during the visual stimulation significantly reduced (median, -5.0 Hz;
98 interquartile range (IQR), 9.9 Hz; p(U-test)<0.001); and the response gain also significantly
99 decreased (median, -41%; IQR, 54%; p(U-test)<0.001) that we computed as the ratio of the
100 estimated static nonlinearity functions before and after PSEM injection. The temporal filter
101 became elongated regardless of their functional cell types (Supplementary Figure 2), leading
102 to significantly longer peak latencies (median, +32 ms; IQR, 27 ms; p(U-test)<0.001) and lower
103 peak frequencies (median, -16%; IQR, 5 %; p(U-test)=0.01).

104 Consistent results were obtained when histamine release was pharmacologically up-
105 regulated. Specifically, after the application of ciproxifan (H3 antagonist; Figure 1D,G; N=48

106 from 2 animals), we found significantly reduced mean firing rates (median, -3.7 Hz; IQR, 5.0
107 Hz; p(U-test)<0.001) and response gain (median, -30%; IQR, 52%; p(U-test)=0.003), and
108 significantly longer peak latency (median, +17 ms; IQR, 13 ms; p(U-test)<0.001) and lower
109 peak frequency (median, -9%; IQR, 14%; p(U-test)<0.001) of the linear temporal filters. These
110 effects of the pharmacological treatment were less prominent than those of the chemogenetic
111 manipulation (Figure 1I-L), except for the changes in the receptive field size where the
112 reduction reached the statistically significant level with ciproxifan (median, -26%; IQR, 38 %;
113 p(U-test)=0.004, N=28 from 2 animals) but not with the chemogenetic manipulation (median,
114 -11%; IQR, 32 %; p(U-test)=0.11, N=19 from 2 animals; Supplementary Figure 3A-F).

115 In contrast, chlorphenamine (H1 antagonist) led to marginal changes in the visual
116 response properties of LGN neurons in the opposite direction (Figure 1E,H; N=116 from 3
117 animals). We observed a tendency towards an increased mean evoked firing rate (median,
118 +1.0 Hz; IQR, 7.2 Hz; p(U-test)=0.09) and response gain (median, +13%; IQR, 87%; p(U-
119 test)=0.3), and facilitated response kinetics with significantly higher peak frequency of the
120 linear temporal filter (median, +4%; IQR, 15%; p(U-test)=0.03) but no significant changes in
121 its peak latency (median, 0 ms; IQR, 5 ms; p(U-test)=0.7) or the receptive field size (median,
122 +5%; IQR, 34 %; p(U-test)=0.08, N=38 from 3 animals; Figure 1I-L and Supplementary Figure
123 3A-F). Taken together, these results indicate that histamine reduces the response gain and
124 slows down the response kinetics in the mouse LGN.

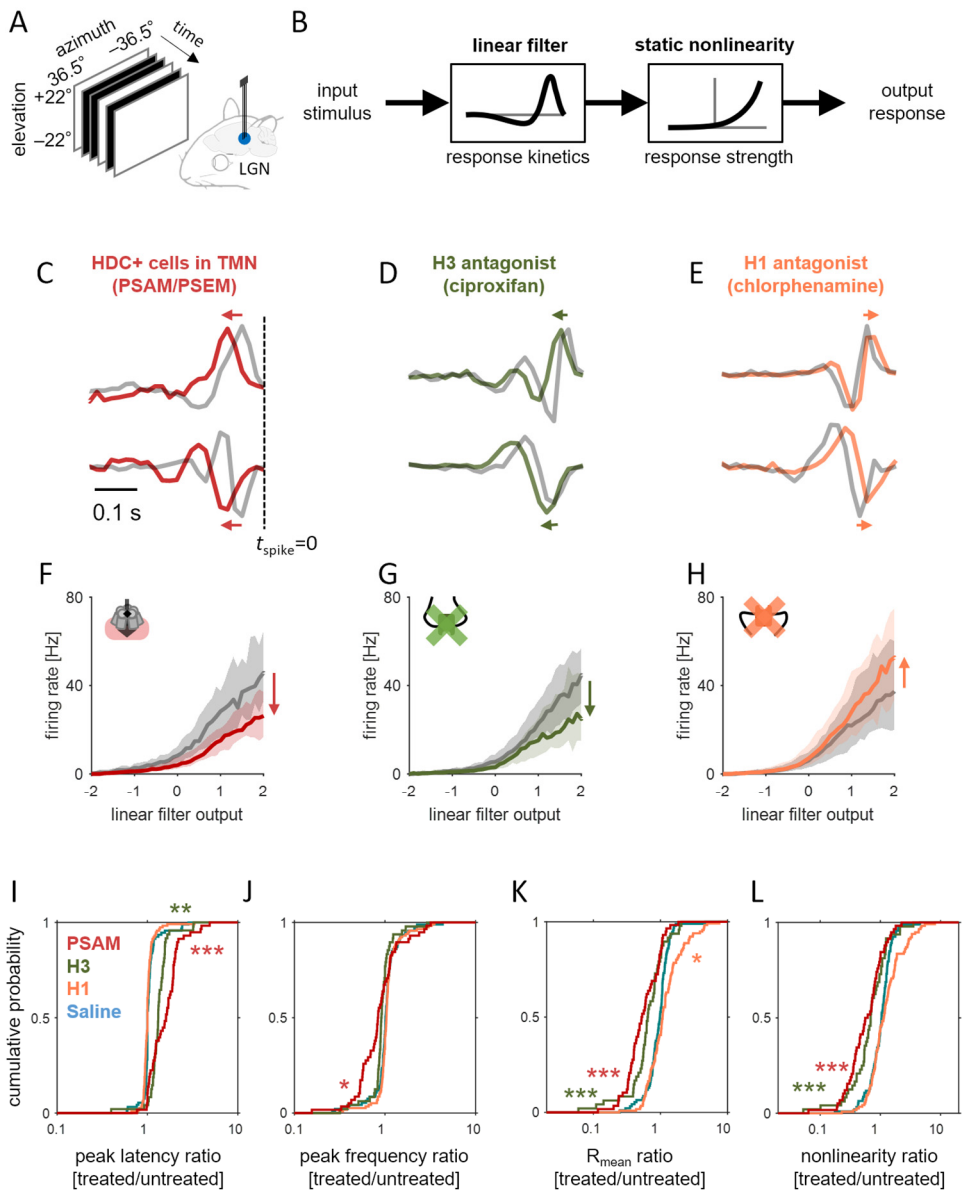
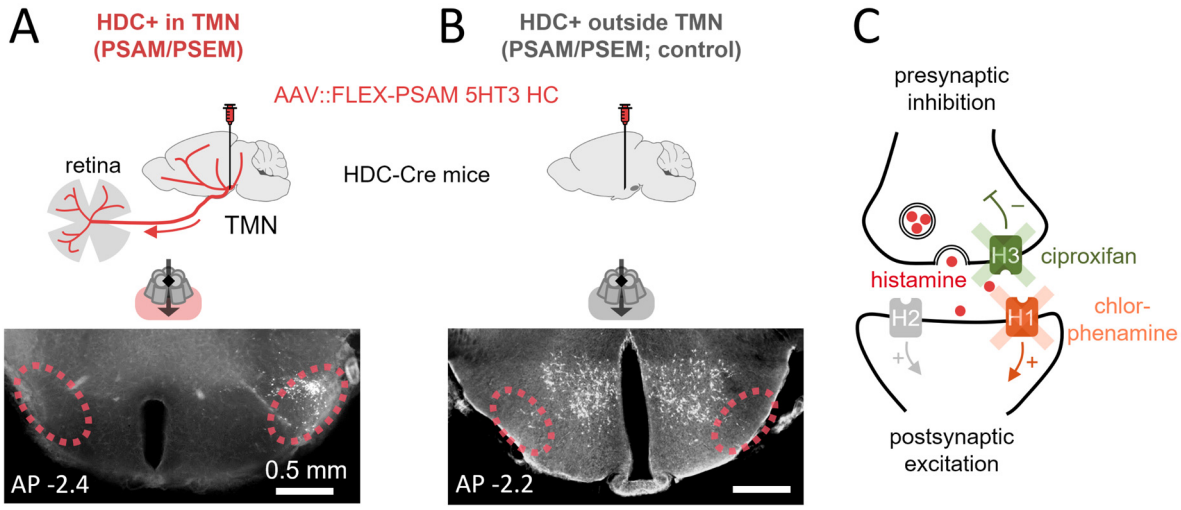
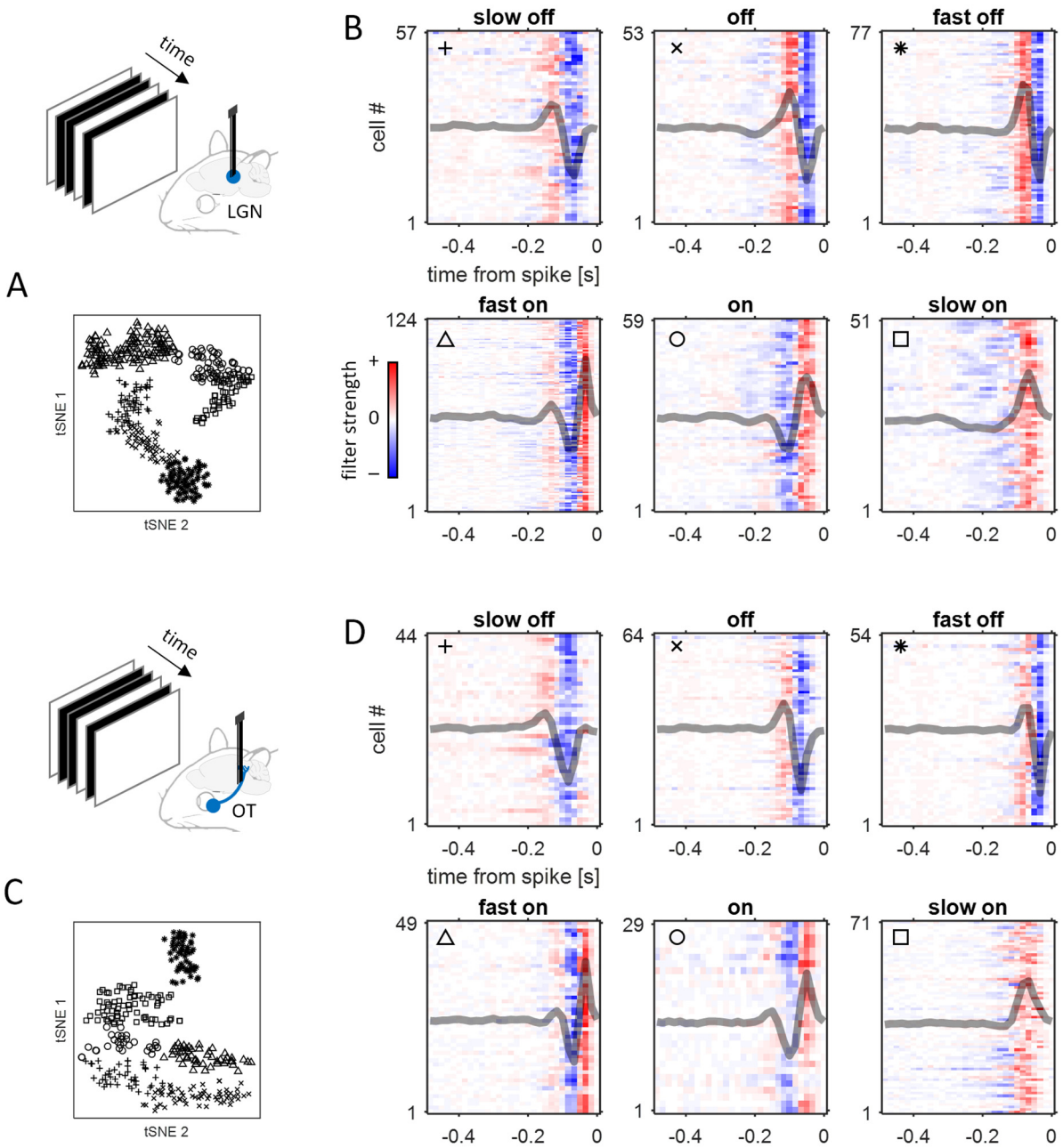


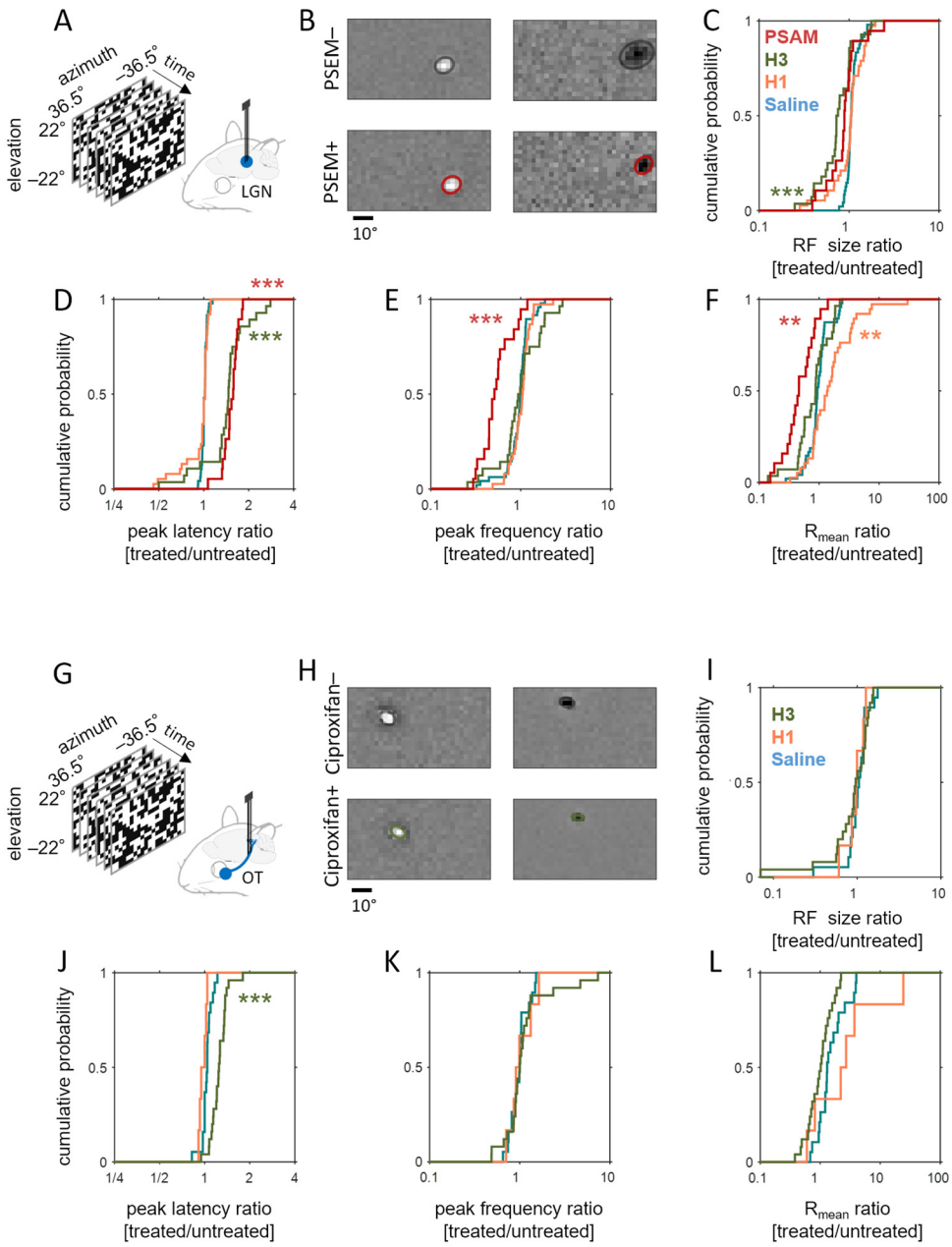
Figure 1: Histamine made the visual responses of LGN weaker and slower in awake head-fixed mice. *A,B*: Schematic diagram of LGN recordings (A) and linear-nonlinear cascade model (B). Reverse-correlation was used to estimate the linear filter and static nonlinearity that represent response kinetics and strength, respectively. *C*: Linear filters of two representative LGN cells before (gray) and after chemogenetic activation of HDC+ cells in TMN (red). *D,E*: Representative LGN linear filters before (gray) and after pharmacological treatment (D, 12 mg/kg ciproxifan, green; E, 5 mg/kg chlorphenamine, orange). *F-H*: Static nonlinearities averaged across LGN population before and after treatment in corresponding colors (F, chemogenetics, N=58 from 3 animals; G, ciproxifan, N=48 from 2 animals; H, chlorphenamine, N=116 from 3 animals): line, median; gray shade, 25 and 75 percentiles. *I-L*: Cumulative distributions of response feature ratio before and after each treatment (in corresponding color): peak latency (I) and frequency (J) of the linear temporal filters, mean evoked firing rate during stimulation (K), and static nonlinearity (i.e., response gain; L). One-way analysis of variance (ANOVA) with Tukey-Kramer correction was used to compute the p-value against saline control (blue, N=93 from 3 animals): *, p<0.05; **, p<0.01; ***, p<0.001.



Supplementary Figure 1: Pharmacological and chemogenetic approaches to manipulate the central histaminergic system in the mouse brain. *A,B*: Viral delivery of PSAM 5HT3HC channel to HDC+ cells in the TMN (marked with dotted lines) of the posterior hypothalamus (A) or those in the anterior hypothalamus (B; control). *C*: Schematic diagram of histamine receptor localizations and their cellular function. Chlorphenamine blocks the postsynaptic receptor H1 (orange) that mediates the action of histamine postsynaptically, while ciproxifan blocks the auto-receptor H3 (green) that inhibits presynaptic histamine release.



Supplementary Figure 2: Functional classification of LGN/RGC visual response types. *A*: t-SNE embedding of the STAs of LGN cells. Different markers are used for distinct response categories (shown in B). *B*: Each panel represents one of the six response categories: slow off, off, fast off, fast on, on, and slow on. In each panel, each row represents a cell's STA (color-coded with red and blue indicating positive and negative filter values, respectively); and the overlaid gray line shows the average STAs in each response type. *C,D*: Corresponding data for RGCs obtained by in vivo optic tract (OT) recordings methods.



Supplementary Figure 3: Histamine reduced the LGN but not RGC RF size. *A*: Schematic diagram of LGN recordings with white-noise "checkerboard" stimuli. *B*: Estimated spatial filter (receptive field) of two example LGN cells before (top) and after (bottom) chemogenetic activation of HDC+ cells in TMN. Note a decrease of the RF size of this OFF LGN cell (right), while no change was observed for this ON cell (left). *C-F*: Cumulative distribution of the RF size ratio of LGN cells before and after treatment (C; orange, chlorphenamine, N=38 from 3 animals; green, ciproxifan, N=28 from 2 animals; red, PSAM/PSEM for HDC+ cells in TMN, N=19 cells from 2 animals) and corresponding figure panels for the peak latency (D) and frequency (E) of the RF center dynamics as well as for the mean firing rate (F). One-way ANOVA with Tukey-Kramer correction against saline control (N=48 from 3 animals): *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. *G-L*: Corresponding figure panels for RGCs: chlorphenamine (H1 antagonist), N=6 from 3 animals; ciproxifan (H3 antagonist), N=25 from 4 animals; saline, N=18 from 3 animals.

Histaminergic modulation starts from the mouse retina

Histaminergic neurons are localized solely in the hypothalamus, most densely in TMN, and project their axons widely across the brain (Lin et al., 2023), including the mouse retina (Gastinger et al., 1999; Repérant et al., 2007; Warwick et al., 2024). To test if the observed histaminergic modulation of the visual processing in LGN originates in the retina, we next performed in vivo optic tract (OT) recordings to monitor the retinal output responses in awake head-fixed mice (Boissonnet et al., 2023) before and after manipulating the central histaminergic system (Figure 2).

Consistent with the results in LGN, chemogenetic activation of HDC+ neurons in TMN led to reduced firing (median, -13.0 Hz; IQR, 25.5 Hz; p(U-test)=0.11) with lower response gain (median, -51%; IQR, 50 %; p(U-test)=0.04); and slower response kinetics with elongated linear temporal filters in retinal ganglion cells (RGCs; peak latency change, median, 46 ms; IQR, 22 ms; p(U-test)=0.008; peak frequency change, median, -36%; IQR, 22%, p(U-test)=0.04; N=10 from 2 animals; Figure 2A,D). In contrast, no change in these response parameters was observed when targeting HDC+ populations in the anterior hypothalamus outside TMN for the PSAM/PSEM activation (firing rate change, median, -0.7 Hz, IQR, 16 Hz, p(U-test)=1; gain change, median, 0.5%, IQR, 79%, p(U-test)=1; latency change, median +2 Hz, IQR, 11 Hz, p(U-test)=0.8; frequency change, median -2.9%, IQR, 11%, p(U-test)=0.12; N=23 from 3 animals). This supports that the histaminergic modulation of the early visual system is mediated by the centrifugal projections specifically from TMN (Figure 2G-J and Supplementary Figure 1).

The effects of the pharmacological treatments were overall similar between LGN and RGCs, though certain differences existed. First, pharmacologically blocking the H3 receptors with ciproxifan led to slower response kinetics in both LGN and RGCs (latency change, median, 8 ms; IQR, 14 ms; p(Utest)<0.001; frequency change, median, -7%; IQR, 19%; p(U-test)=0.04; N=40 from 4 animals), but the firing rates and the response gain remained largely unaffected in RGCs (Figure 2B,E; firing rate change, median -0.3 Hz; IQR, 14.2 Hz; p(U-text)=0.8; gain

198 change, median, -1.3%; IQR, 68%, p(Utest)=0.8). Second, while chlorphenamine (H1
199 antagonist) drove faster kinetics with marginally higher firing in both LGN and RGCs, the
200 effects on the temporal filters were primarily on the peak frequencies in LGN (Figure 1E,H)
201 but on the peak latencies in RGCs (Figure 2C,F; latency change, median, -5 ms; IQR, 10 ms;
202 p(U-test)=0.004; frequency change, median, +4 Hz; IQR, 19 Hz; p(U-test)=0.7; firing rate
203 change, median, +2.1 Hz, IQR, 10.4 Hz; p(U-test)=0.14; gain change, median, 5%, IQR, 88%,
204 p(U-test)=0.2; N=34 RGCs from 3 animals). Finally, RGC receptive fields did not show
205 significant changes in size (Supplementary Figure 3G-L; ciproxifan, median, -4%, IQR, 54%,
206 p(U-test)=1, N=25 from 4 animals; chlorphenamine, median, ±0%, IQR, 36%, p(U-test)=1,
207 N=6 from 3 animals; saline, median, 5%, IQR, 30%, p(U-test)=0.36, N=18 from 3 animals).
208 Taken together, our electrophysiology data indicate that 1) the mouse early visual processing
209 is subject to histaminergic modulation controlled by TMN, starting from the retina; 2) histamine
210 generally suppresses the firing and slows the response kinetics; and 3) response latency and
211 frequency tuning are likely modulated by different mechanisms.

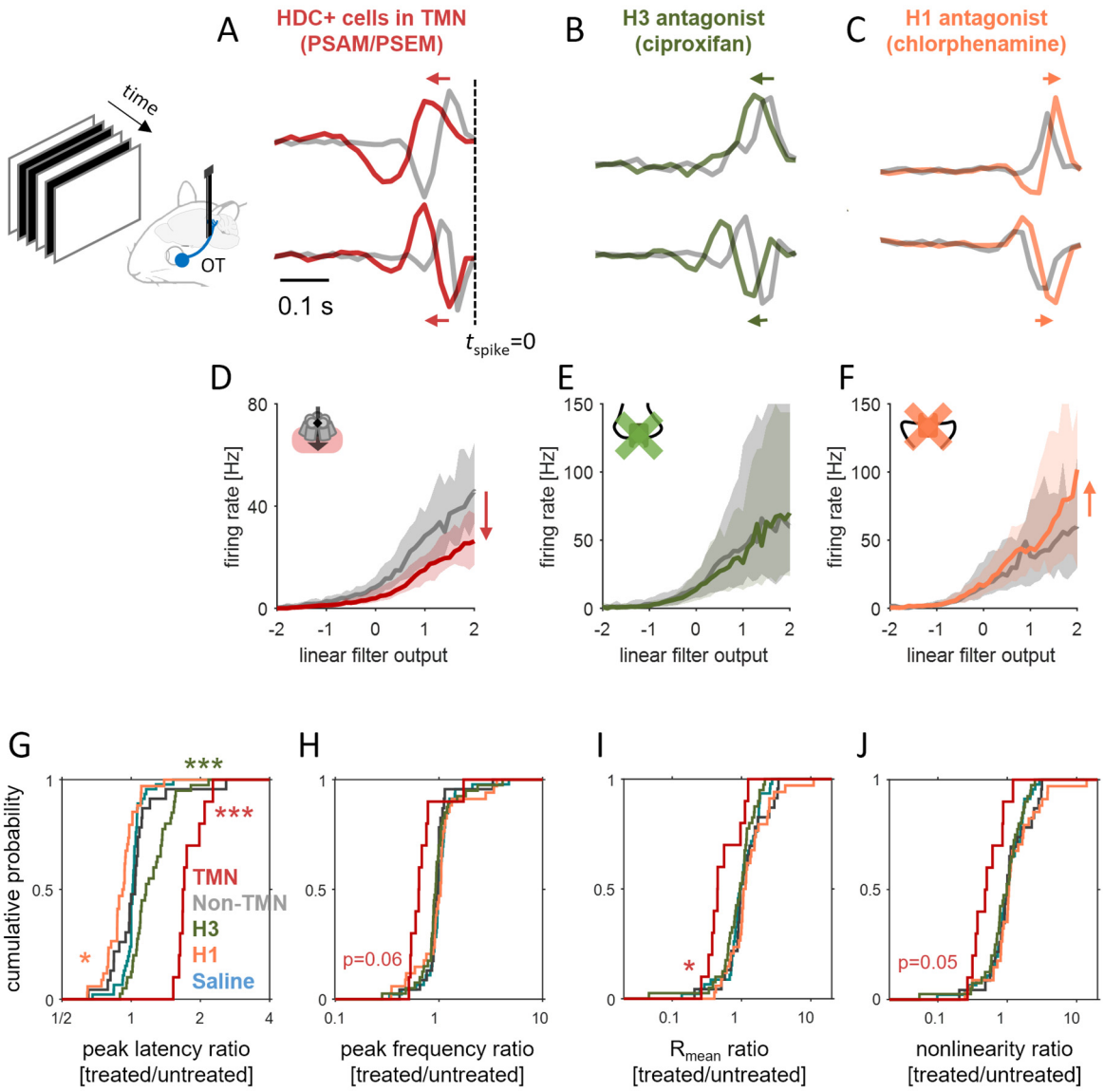


Figure 2: histamine primarily affected the visual response kinetics of RGCs. *A-C*: Linear filters of two representative RGCs before (gray) and after chemogenetic (A) or pharmacological treatment (B, ciproxifan, green; C, chlorphenamine, orange). Schematic diagram of in vivo optic tract (OT) recordings is displayed on the left. *D-F*: Static nonlinearities averaged across RGC population before and after treatment in corresponding colors (D, chemogenetics, N=10 from 2 animals; E, ciproxifan, N=40 from 4 animals; F, chlorphenamine, N=34 from 3 animals): line, median; gray shade, 25 and 75 percentiles. *G-J*: cumulative distributions of the response feature ratio before and after each treatment (in corresponding colors): peak latency (G) and frequency (H) of the linear temporal filters, mean evoked firing rate during stimulation (I), and static nonlinearity gain function (J). One-way ANOVA with Tukey-Kramer correction (chemogenetics against non-TMN targets, N=23 from 3 animals, dark gray; pharmacology against saline control, N=46 from 4 animals, blue): *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Pupil dynamics and locomotion are irrelevant to histaminergic modulation of early visual processing

Histamine is a potent neuromodulator implicated in various brain functions and behaviors, such as sleep-wake cycles and arousal (Haas and Panula, 2003; Thakkar, 2011). We then cannot deny the possibility that the observed histaminergic effects on the visual response properties in the mouse early visual system are by-products of behavioral modulation. For example, a larger pupil size leads to stronger responses because more photons reach the retina (Boissonnet et al., 2023). To address this issue, we examined the relationship between the histaminergic effects on the RGC/LGN visual responses and simultaneously monitored behavioral measures (Figure 3).

We first analyzed the histaminergic modulation of pupil dynamics (Figure 3A). We found pupil dilation in all 6 mice after chlorphenamine (H1 antagonist) application, but pupil constriction in 7 out of 8 animals when histamine release was enhanced by systemic injection of ciproxifan (H3 antagonist). Chemogenetic activation of the central histaminergic system, however, led to pupil dilation in most animals (4 out of 5 mice). Thus, the polarity of the changes in pupil size was not consistent with that of RGC/LGN visual responses. This suggests that the histamine-driven changes of pupil size cannot explain the modulation of the early visual responses.

We next examined how histamine affects locomotion of the head-fixed animals on the treadmill during recordings (Figure 3B,C). These mice often stayed still and moved fast ("running" at >2 cm/s) only about 20% of the time on average. In general, none of the perturbations of the histaminergic system strongly affected the time spent running in most mice (Figure 3B). When they did, however, we found that the chemogenetic activation of HDC+ neurons in TMN resulted in a substantial increase of no-running periods (2 out of 6 mice), whereas both chlorphenamine and ciproxifan led to a substantial decrease of no-running periods (2 out of 6 and 1 out of 7 mice, respectively). Furthermore, the mean running

speed during the recordings decreased upon the chemogenetic activation, whereas it varied in both directions after the pharmacological treatments (Figure 3C). Thus, changes in locomotion driven by histamine were not in agreement with the histaminergic effects on the RGC/LGN visual responses (Figures 1 and 2).

Taken together, neither pupil dynamics nor locomotion behavior was causally linked with the histaminergic modulation of the mouse early visual system. We conclude that the central histaminergic system modulates the mouse vision, effectively leading to slower and weaker responses.

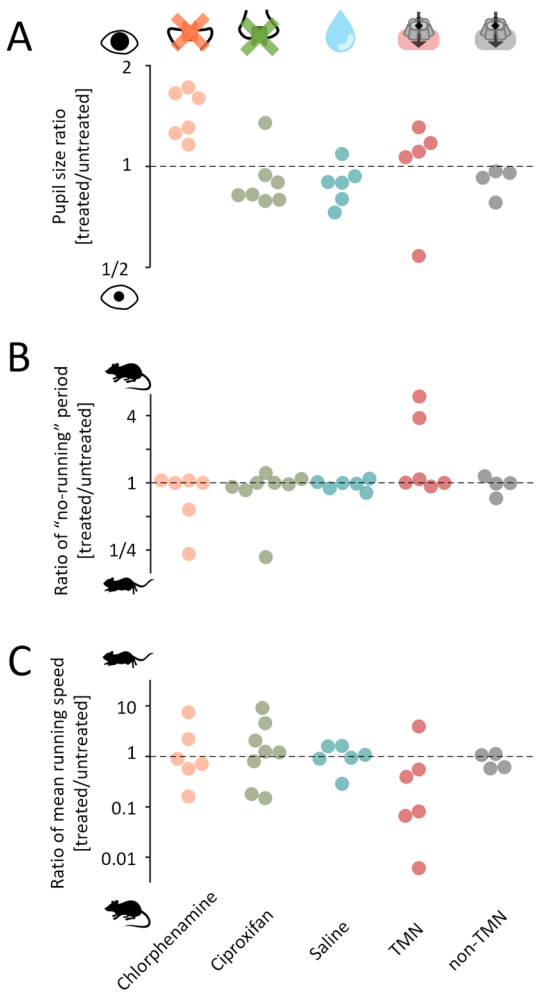


Figure 3: histaminergic modulation of pupil dynamics and locomotion behavior was not causally linked with that of RGC/LGN visual responses. *A*: the ratio of the average pupil size before and after treatment (in corresponding colors). *B*: the ratio of the "no-running" periods (threshold, 2 cm/s) before and after treatment. *C*: the ratio of the average locomotion speed during recording sessions before and after treatment.

Response gain modulation is more strongly correlated with peak latency modulation than with peak frequency modulation

What are the mechanisms underlying the histaminergic modulation of early visual processing? To address this question, we next took a computational approach to examine the relationship between different aspects of the visual response properties that we identified to be affected by histamine (Figure 4). In particular, we simulated the activity of an integrate-and-fire neuron with Poisson spiking and refractoriness in response to white-noise input stimuli (Figure 4A), and processed the output spike trains in the framework of the linear-nonlinear cascade model to characterize the simulated response properties in the same way as the experimental data (Figure 1A). Here we varied two model parameters, gain and resting baseline, to examine the effects of their changes on the following four aspects of the simulated responses: spike counts, the peak latency and frequency of the estimated linear temporal filter, and the slope of the estimated static nonlinearity expressed as half-wave rectification. The rest model parameters were fixed in all simulations, including the underlying linear filter given by a trigonometric function with logarithmic scale in time, and the refractory period filter given by an exponential function. The spike threshold was drawn from a random uniform distribution ranging from 0 to 1 for each time point to approximate the Poisson spiking properties.

When the model neuron had a higher gain or higher resting baseline, subthreshold signal reached the spike threshold faster and more frequently; hence, total spike counts became higher (Figure 4B) and the peak latency of the estimated linear temporal filter appeared shorter (Figure 4C). In contrast, the apparent peak frequency of the simulated responses was dependent more strongly on the resting baseline than on the gain (Figure 4D). These changes in the estimated linear temporal filter properties were consistent with the experimental observation, where the magnitude of the response gain modulation by histaminergic perturbation was significantly correlated with that of the peak latency modulation

292 (Figure 4F,H), but much less with that of the peak frequency modulation (Figure 4G,I). This
293 agreement between the observed and simulated data indicates that, despite the simplicity, our
294 model captures aspects of neuronal response modulation to a first approximation.

295 The model analysis supports that the histaminergic effects on RGCs should involve at
296 least gain and baseline modulations. First, the H1 receptors likely mediate the gain modulation
297 in RGCs, given that chlorphenamine (H1 antagonist) led to changes in the peak latency but
298 not the peak frequency, along with changes in the estimated static nonlinearity largely by
299 scaling (Figure 2). Second, the peak frequency was modulated as well as the peak latency of
300 RGCs upon chemogenetic activation of HDC+ neurons in TMN, accompanied by a shift of the
301 estimated static nonlinearity. This implies changes in the baseline resting potential of the cells.
302 Blocking the H3 receptors, however, led to changes in the RGC response kinetics without
303 much affecting the mean firing rates. Furthermore, the net effects of histamine perturbations
304 on LGN responses were more diverse (Figure 1) and not fully explainable by the simple model
305 (Figure 4). Thus, we expect that mechanisms besides gain and baseline modulations are also
306 involved, such as neural circuit effects through retinal amacrine cells for example (Horio et al.,
307 2018).

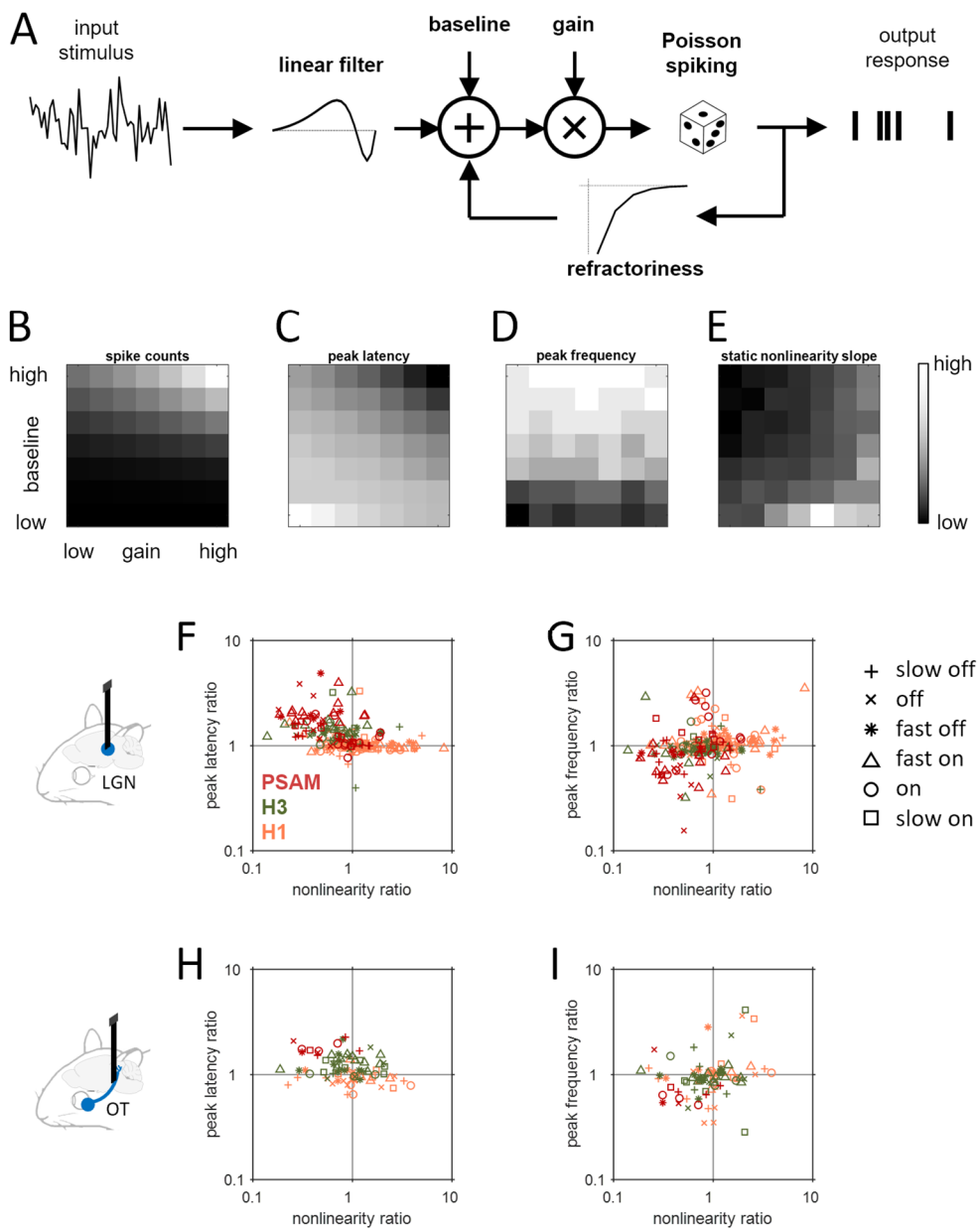


Figure 4: Gain modulation accompanies the peak latency modulation but not the peak frequency modulation via histaminergic effects. *A-E*: Using a computational model outlined in A, here we examined the consequences of varying the baseline and gain levels on the response features, such as spike counts (B), peak latency (C) and frequency (D) of the estimated linear filter from the simulated responses, and slope of static nonlinear gain function of the model neuron (E). See Methods for details. *F,G*: Relationship between the magnitudes of the gain modulation and response kinetics modulation by perturbing the histaminergic system for LGN neurons (chemogenetics, red; ciproxifan, green; chlorphenamine, orange; N=222 from 8 animals). Cell types are indicated by different markers (Supplementary Figure 2). A stronger correlation was found for the peak latencies (F, R = –0.42, p<0.001) than for the peak frequencies (G, R = 0.17, p=0.01). *H,I*: corresponding figure panels for RGC responses (N=84 from 9 animals). Significant correlation to the gain modulation was found for the peak latency modulation (R = –0.25, p = 0.02), but not for the peak frequency modulation (R= 0.20, p = 0.07).

Discussion

Here we provide in vivo electrophysiological evidence for histaminergic modulation of the mouse early visual system: the more the histamine released, the slower and weaker the LGN and RGC visual responses in awake head-fixed mice (Figures 1 and 2). This is opposite from what one would expect for the effect of histamine, given that the level of brain histamine typically increases as animals become more aroused (Thakker, 2011). At the behavioral level, antihistamine drugs lead to drowsiness in humans, impairing sensory processing (van Ruitenbeek et al., 2009). At the neurophysiological level, it has been shown that histamine leads to slow depolarization of LGN relay cells in cats and guinea pigs (McCormick and Williamson, 1991; Uhlrich et al., 2002). Histamine was thus expected to facilitate visual processing, unlike what our observations indicated. These discrepancies may arise from two reasons. First, the effects of histamine can be species-specific. In particular, as nocturnal animals, mice are less active during daytime. Thus, facilitating visual responses at low histamine level may be ethologically beneficial for mice to respond faster to visual threats during daytime, especially when mice are dormant. Future studies in the context of sleep-wake cycles as well as in diurnal animals will give further insights into this question.

Second, our unexpected results may be due to differences in experimental conditions. Electrophysiological studies on neuromodulation were conventionally conducted in vitro or in anesthetized animals (McCormick and Williamson, 1991; Uhlrich et al., 2002; Warwick et al., 2024). It has been shown, though, that substantial differences exist in the early visual processing between awake and anesthetized conditions (Durand et al., 2016), including the retinal output (Boissonnet et al., 2023). Here we took a computational modelling approach to better understand the net effect of histamine from functional viewpoints, rather than pursuing the underlying biophysical mechanisms. We suggest that the H1 receptors mediate gain modulation in RGCs: chlorphenamine (H1 antagonist) affected the response latency but not the frequency tuning (Figure 2), a distinctive feature of gain modulation identified by our computational modelling analysis (Figure 4). This action of histamine does not have to be

directly on RGCs expressing the H1 receptors, but can be mediated indirectly by circuit mechanisms. Indeed, histamine affects both inward and outward currents of amacrine cells in the mouse retina (Horio et al., 2018). The effects of histamine can then be more profound than gain modulation, much as we observed diverse outcomes in RGCs and LGN when different pharmacological and chemogenetic perturbation tools were employed (see also Warwick et al., 2024). Furthermore, in awake conditions, the histaminergic system acts in concert with other neuromodulatory systems (McCormick et al., 2020). For example, serotonergic neurons in the dorsal raphe nucleus have been suggested to project to the retina (Schütte, 1995; Repérant et al., 2000; Gastinger et al., 2006). As the brain state is dynamically controlled by multiple neuromodulators, it will be critical to further characterize neuromodulation of the retina and downstream visual areas in various behavioral contexts.

What is the biological relevance of histaminergic modulation of the early visual system, starting at the retina? Growing evidence supports significant correlations between awake retinal responses and various behavioral measures, such as pupil dynamics and locomotion (Schröder et al., 2020; Liang et al., 2020; Boissonnet et al., 2023). We, however, show that resulting changes in pupil size and locomotion behavior by manipulating the histaminergic system were not always consistent with the effects on LGN or RGC visual responses (Figure 3). Therefore, the observed changes in their visual responses were unlikely to be associated with behavioral modulation by histamine. This in turn suggests that histamine may not fully explain the fast modulation of RGCs by arousal or locomotion. Additional mechanisms of retinal modulation should then exist, such as the centrifugal serotonergic system (Schütte, 1995; Repérant et al., 2000; Gastinger et al., 2006). Further studies on the function of the retina as an integrated part of the whole organism, but not as an isolated tissue, will be critical to better understand sensory information processing in awake animals under natural conditions.

Materials and Methods

No statistical method was used to predetermine the sample size. The significance level was 0.05 (with multiple comparison correction where appropriate) in all analyses unless noted otherwise. All experiments were performed under the license 233/2017-PR and 220/2024-PR from the Italian Ministry of Health, following protocols approved by the Institutional Animal Care and Use Committee at European Molecular Biology Laboratory. The data analyses were done in Python and Matlab.

Animals

Animals were housed on a 12h light-dark cycle, with ad libitum access to water and food. All experiments were performed on female mice. A total of 20 wild-type (C57BL6/J; RRID:IMSR_JAX:000664) and 10 hemizygous HDC-cre (Hdctm1.1(icre)Wwis/J; RRID:IMSR_JAX:021198) mice were used for pharmacological and chemogenetic manipulations, respectively.

Surgical procedures

All surgical procedures were performed in animals from 4 to 19 weeks old (9.2 weeks median age). Before the surgery, animals were injected with Caprofen (5 mg/Kg) and then anaesthetized with isoflurane (4% for induction, 1% for maintenance in O_2). Throughout the surgery, the temperature of the animals was kept stable at 37°C using a heating pad (Supertech Physiological). Ointment (VitA-Pos, Ursapharm) was applied on both eyes to prevent them from drying during the surgery, and the animals were placed in a stereotaxic frame (Stoelting 51625). A portion of the scalp was removed to expose the skull and the periosteum was scraped away with a round scalpel to increase adherence of the dental cement. For those mice belonging to the chemogenetic manipulation group, the skull was aligned in order to level bregma and lambda on the same horizontal plane, and a 100 µm craniotomy was performed using a motorized drill attached to the stereotaxic arm. Viral

400 solution (250 nL; AAV9::FLEX-PSAM Y115F,L141F:5HT3 HC; Vector Biosystems, VB773)
401 was injected with an automatic injector pump at 1nL/sec, at the coordinate of AP: -2.6, ML:
402 ±0.8, DV:-5.3. All animals were then implanted with a custom-made titanium head plate,
403 cemented on the skull using a mixture of cyanoacrylate (Loctite 401, Henkel) and dental
404 cement (Paladur, Kulzer). The head plate featured a hole (8 mm diameter), allowing for clear
405 observation of both bregma and lambda. The skull surface was then glazed with a thick layer
406 of cyanoacrylate in order to support the skull with mechanical, atmospheric and biological
407 protection, while still allowing for visual identification of reference points. After the surgery
408 animals were allowed to recover on a heating pad and then housed in individual cages. During
409 the following seven days, the mice were administered with painkillers (Caprofen) diluted in the
410 drinking water at a concentration of 50 mg/mL.

411 In vivo electrophysiology

412 After recovery from the surgery, animals were habituated to head fixation by placing them in
413 the experimental apparatus over the course of one week, twice a day for up to two hours. On
414 the day of the recording, we first placed the subject animal in the recording set up with its head
415 fixed, and determined the electrode penetration path the target area using the robotic
416 stereotaxic system (StereoDrive, NeuroStar). The animal was then briefly anesthetized (for
417 about 5 minutes) and a hole was drilled around the electrode entry point on the skull. After the
418 removal of the anesthesia, an acute silicone probe (Buzsaki32L, Neuronexus; or P2,
419 Cambridge Neurotech) coated with a fluorescent dye (DiI stain, Invitrogen, D282) was lowered
420 at 5 μm/s using the robotic arm until visual responses were found in the target area.

421 After presenting a battery of visual stimuli, we briefly anesthetized the animal and
422 injected 0.9% saline solution alone or that containing chlorphenamine (H1 antagonist, 5
423 mg/kg), ciproxifan (H3 antagonist, 12 mg/kg), or PSEM (5 mg/kg). About 20 minutes after the
424 injection and removal of the anesthesia, we presented the same battery of visual stimuli to the
425 animal for the second recording session.

426 After all the recording sessions, the electrode position was verified histologically. After
427 retracting the silicon probe, the mice were anesthetized (2.5% Avertin, 16 μL/g, intraperitoneal
428 injection) and perfused with paraformaldehyde (PFA; 4% in phosphate buffer solution). The
429 brain tissue was harvested and post-fixed overnight in 4% PFA at 4°C. Coronal sections of
430 the brain tissue (thickness, 150 μm) were then examined under a fluorescence microscope
431 (Leica, LMD7000 with N2.1 filter cube) to visualize the trace left by the DiI stain on the probe.

432 Visual stimulation

433 Visual stimuli were presented as described before (Boissonnet et al., 2023). Briefly, visual
434 stimuli were projected onto a spherical screen (20cm in radius) painted with ultra-violet (UV)
435 reflective white paint, placed 20 cm from the mouse's left eye. A gamma-corrected digital light
436 processing device (DLP, Texas Instruments, DLPDLCR3010EVM-LC) was used as a light
437 source after the green and the red LEDs were replaced with UV (365nm, LZ1-00UV00, LED
438 Engine) and infrared (IR: 950nm, SFH 4725S, Osram) LEDs respectively. The IR light was
439 used as a synchronization signal and was recorded with a photodiode (PDA100A2, Thorlabs).
440 Stimulation was conducted at 60 frames per second (FPS), and covered 73° in azimuth and
441 44° in altitude from the mouse eye position. The maximum light intensity at the eye position
442 was 31 mW/m^2 (15.4 mW/m^2 for UV LED and 15.9 mW/m^2 for blue LED; measured with S121C
443 sensor, Thorlabs), leading to mesopic to photopic conditions. Using QDSpy software (Franke
444 et al., 2019), we presented the following stimuli: a randomly flickering full-field stimulus (5
445 minutes) consisting of a random sequence of black and white frames at 60 FPS; and a black-
446 and-white binary dense noise stimulus (15 minutes), consisting of a 32-by-18 pixels
447 checkerboard, where each pixel followed an independent random sequence at 60 FPS but the
448 overall luminance of the frame was kept constant at the mean value.

449 Electrophysiology data analysis

450 Raw data from the same animal before and after treatment were concatenated and processed
451 as a single recording. Spike sorting was performed with Kilosort2 for semi-automatic cluster

452 detection and Phy for visualization and data curation. In total, we recorded 311 RGCs from 18
453 animals and 421 cells in LGN from 12 mice.

454 For systematically characterizing the visual response properties, we used stimulus
455 ensemble statistical techniques ("reverse correlation" methods; 500 ms window; Δt = 1/60 s
456 bin size) to calculate the linear filter and static nonlinear gain function of the recorded cells in
457 response to white-noise stimuli as described before (Figure 1A; Boissonnet et al., 2023).
458 Briefly, we first obtained the linear filter of each cell by calculating a spike-triggered average
459 (STA) of the stimulus with ±1 being "white" and "black," respectively. As a quality measure, p-
460 value was computed for each time bin against a null hypothesis that the STA follows a normal
461 distribution with a mean of zero and a variance of 1/C, where C is the total number of spikes.
462 As a measure of the cell's response kinetics, we then estimated the peak latency by fitting a
463 difference-of-Gaussian curve to the linear filter; and the spectral peak frequency by the Fourier
464 analysis on the linear filter. Spatial response properties were examined for STAs from
465 "checkerboard" stimulation. In particular, we fitted a two-dimensional Gaussian envelope to
466 the spatial filter at the peak latency (e.g., Supplementary Figure 3B,H), and the receptive field
467 (RF) size was estimated as twice the mean standard deviation (SD) of the long and short axes.

468 The linear temporal filters from full-field white-noise stimuli were used for broadly
469 classifying the response types of RGCs and LGN cells, respectively (Supplementary Figure
470 1). Specifically, we first used t-Distributed Stochastic Neighbor Embedding (t-SNE) to map the
471 STAs onto two-dimensional space, and then used K-means++ algorithm for heuristically
472 categorizing the responses into the following six types: fast ON, ON, slow ON, fast OFF, OFF,
473 and slow OFF.

474 Static nonlinear gain function of each cell (bin size, 0.1) was computed as: P(response
475 | stimulus) = N(stimulus | response) / N(stimulus) / Δt, where N(stimulus | response) and
476 N(stimulus) are the distributions of spike-triggered stimulus ensembles projected onto the L2-
477 normalized STA and the entire stimulus ensembles, respectively. The ratio of P(response |

stimulus) before and after the treatment was used as a measure of response gain modulation for each cell.

Behavioral data analysis

For all experiments, the mouse's left eye – i.e., the side presented with visual stimuli – was recorded using an IR camera (The Imaging Source, DMK23UV024) to monitor its motion and pupil dynamics at 30-60Hz. Pupil detection was done using a Mask-Region based Convolutional Neural Network (Mask-RCNN), trained as described previously (Boissonnet et al., 2023). Two-dimensional ellipse was fit to the pupil, and the large axis diameter was used as a measure of the pupil size. We then calculated the mean pupil size during the recording sessions, and used the ratio before and after pharmacologic or chemogenetic treatment as a measure of the treatment effects on the pupil dynamics (Figure 3A).

We also monitored the running speed of an animal based on the turning speed of the custom-made rotary treadmill throughout the recordings. We set a threshold at 2 cm/s to detect running behavior. The animals, however, did not run frequently on the treadmill (17 ± 25%, mean ± standard deviation; 30 animals). As a measure of the treatment effects, we thus used a) the ratio of the fraction of the "non-running" period (Figure 3B); and b) that of the average running speed during the recording session before and after pharmacological or chemogenetic treatment (Figure 3C).

Model analysis

To explore how different aspects of neuronal intrinsic properties and output responses are interrelated between each other, we simulated a model neuron's responses to white-noise stimuli using different model parameter values (Figure 4A), and characterized these simulated responses using a linear-nonlinear cascade model as we did for the experimental data.

Specifically, we employed an integrate-and-fire neuron model with Poisson spiking and refractoriness. A model neuron's discrete linear filter (of length n_T = 25) was given by a

trigonometric function with logarithmic scale in time: i.e., $L(i) = \sin[2*\pi*((10^{t_i}-1)/(10^{s_t}-1))]$, where $s_t = 1.5$ is a temporal spacing parameter and $t_i = i*s_t/(n_T-1)$ for $i=0,\ldots,n_T-1$. The spike-generating function V(i) was then modelled by first projecting the white-noise Gaussian stimulus S(i) (of length 1,000,000; normalized to have a maximum value of 1) onto L(i), followed by the addition of baseline B and the multiplication by gain G: i.e., $V(i) = G*(\Sigma_j L(j)*S(i-j) + B)$. For each simulation, we chose the value of B from -0.45 to 0.45 in steps of 0.15; and that of G from 1.2^g with g = -3, -2, -1, 0, 1, 2, 3. The spiking response was then generated by thresholding V(i) (at a random threshold for every time point derived from a uniform distribution from 0 to 1) and refractoriness (given by an exponential filter: $-\exp[-i/s_h]$ for i = 0,…,5 with $s_h = 1$). The output spike trains were then subject to the reverse-correlation analysis as described above to characterize the apparent linear filter and static nonlinearity of the model neuron, much as we did for the experimentally recorded RGCs and LGN cells. Specifically, we focused on the peak latency and frequency of the estimated linear filters and the slope of the estimated static nonlinear gain function, and examine how these features depend on the baseline B and the intrinsic gain G of the model neuron (Figure 4B-E).

Acknowledgments

This work was supported by research grants from EMBL (H.A.). The EMBL Advanced Light Microscopy Facility are acknowledged for support in histological image acquisition and analyses, and EMBL IT Support for provision of computer and data storage servers. We thank Dmitry Molotkov, Tom Boissonnet, Vanesa Pelcastre, Sahana Rao, Shabnam Chandel for their help in experiments, and all the Asari lab members for many useful discussions..

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