Inducible and titratable silencing of Caenorhabditis elegans neurons in vivo with histamine-gated chloride channels

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Recent progress in neuroscience has been facilitated by tools for neuronal activation and inactivation that are orthogonal to endogenous signaling systems. We describe here a chemical-genetic approach for inducible silencing of Caenorhabditis elegans neurons in intact animals, using the histamine-gated chloride channel HisCl1 from Drosophila and exogenous histamine. Administering histamine to freely moving C. elegans that express HisCl1 transgenes in neurons leads to rapid and potentiating inhibition of neural activity within minutes, as assessed by behavior, functional calcium imaging, and electrophysiology of neurons expressing HisCl1. C. elegans does not use histamine as an endogenous neurotransmitter, and exogenous histamine has little apparent effect on wild-type C. elegans behavior. HisCl1-histamine silencing of sensory neurons, interneurons, and motor neurons leads to behavioral effects matching their known functions. In addition, the HisCl1-histamine system can be used to titrate the level of neural activity, revealing quantitative relationships between neural activity and behavioral output. We use these methods to dissect escape circuits, define interneurons that regulate locomotion speed (AVA, AIB) and escape-related omega turns (AIB), and demonstrate graded control of reversal length by AVA interneurons and DA/VA motor neurons. The histamine-HisCl1 system is effective, robust, compatible with standard behavioral assays, and easily combined with optogenetic tools, properties that should make it a useful addition to C. elegans neurotechnology.

Significance

Inducible silencing of neurons is a powerful method for studying the functions of neural circuits. We describe a system for inducible silencing of Caenorhabditis elegans neurons, based on transgenic C. elegans nematodes engineered to produce the inhibitory Drosophila histamine-gated chloride channel (HisCl1) in specific neurons. The activity of HisCl1-expressing neurons is acutely and reversibly silenced by exogenous histamine, which C. elegans absorbs from the media in addition to completely silencing neurons, the histamine-HisCl1 system can be used to titrate neural activity, demonstrating quantitative relationships between neural activity and behavior. The histamine-HisCl1 system is complementary to other methods of monitoring and manipulating neuronal activity, providing opportunities for combinatorial control of circuits.

Author contributions: N.P. and C.I.B. designed research; N.P., Q.L., and A.G. performed research; N.P., Q.L., and A.G. analyzed data; and N.P. and C.I.B. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1400615111/-/DCSupplemental.

Chemical genetics, neuronal silencing, neural circuits, avoidance behavior
demonstrate rapid, dose-dependent effects on behavioral and neuronal activity.

Results

Histamine Rapidly and Reversibly Paralyses Animals Expressing HisCl1 in Neurons. Full-length cDNAs encoding Drosophila HisCl1 and HisCl2 were cloned under a panneuronal promoter and injected individually or together into C. elegans. The resulting transgenic animals were healthy, fertile, and able to move in the absence of exogenous histamine. However, when placed on agar plates containing histamine, animals expressing HisCl1 or a mix of HisCl1 and HisCl2 (HisCl1/2) ceased locomotion and became insensitive to prodding within 2–3 min. Half-maximal paralysis occurred at 6.3 mM exogenous histamine for HisCl1 and 0.6 mM histamine for HisCl1/2; animals expressing HisCl2 alone were less sensitive (Fig. 1A). These values are about 1000-fold higher than those required to activate HisCl channels expressed in cultured cells (17). Animals expressing HisCl1 or HisCl1/2 were fully paralyzed by 10 mM histamine within 10 min (Fig. 1B).

To determine the time course of histamine effects, animals were placed in microfluidic arenas that allow precise delivery and removal of chemical stimuli to free-moving animals (21). The half-time to paralysis of HisCl1-expressing animals in 10 mM histamine was ∼1–2 min (Fig. 1C and Movie S1). Animals expressing HisCl1 were paralyzed with similar kinetics on agar plates containing 10 mM histamine, and remained paralyzed for at least 24 h in histamine (Fig. 1D). Similarly, the cell-specific strains described below did not recover during prolonged histamine exposure. In microfluidic arenas, the half-time to recovery after terminating a 4 min pulse of histamine was 4–6 min, with animals expressing higher levels of HisCl1 recovering more slowly (Fig. 1C). Recovery half-times on agar plates increased as a function of the time animals had been exposed to histamine, and could be as long as 2 h (Fig. 1E). Following recovery, animals were immediately responsive to a second histamine exposure (Fig. S14).

Exploration and locomotion assays demonstrated minimal effects of 10 mM histamine on wild-type animals in the absence of HisCl transgenes. Wild-type animals had a similar slow locomotion speed on a bacterial lawn, and fast locomotion accompanied by a transient increase in reversals and omega turns upon removal from the lawn, regardless of the presence of histamine (Fig. S1 B and C). These assays were also used to ask whether panneuronal expression of HisCl1 or HisCl1/2 caused defects in the absence of histamine. Whereas animals expressing HisCl1 had normal exploration and locomotor behaviors, animals expressing HisCl1/2 had lower reversal frequencies and a slower basal locomotion speed (Fig. S1 B and C). This result suggests that HisCl1/2 forms channels that are spontaneously active or activated by an endogenous C. elegans ligand, so this combination was not pursued further (15, 22).

Histamine-HisCl1 Inhibits Functional Activation of C. elegans Neurons. To directly determine the properties of HisCl1 channels in C. elegans neurons, we performed electrophysiological recordings in dissected animals expressing HisCl1 under the AVA-selective rig-3 promoter. When histamine was applied under current clamp conditions to AVA neurons expressing HisCl1, the membrane potential rapidly equilibrated to approximately -45 mV, the predicted equilibrium potential for chloride, whereas there was no effect on control AVA cells not expressing HisCl1 (Fig. 2A). Administration of histamine under whole-cell voltage clamp resulted in concentration-dependent outward currents that peaked rapidly, equilibrated, and terminated upon histamine removal (Fig. 2B and C). A dose–response curve in AVA yielded an EC50 of 6.2 μM and Hill coefficient mH (1.8) for HisCl1 currents similar to reported values for HisCl1 in Drosophila cells and Xenopus oocytes, as well as similar kinetics (opening t1/2 ~25 msec, recovery t1/2 ~1 s) (17) (Fig. 2C). Thus, HisCl1 functions as a canonical histamine-gated chloride channel in the plasma membrane of C. elegans neurons. The ∼1000-fold higher EC50 and slower kinetics for behavioral effects of histamine on intact HisCl1-expressing C. elegans are likely due to pharmacokinetics of histamine absorption from the medium, as observed for other biogenic amines (23).

The functional effects of histamine and HisCl1 in intact animals were determined by calcium imaging of the nociceptive ASH sensory neurons. ASH neurons respond to aversive stimuli such as high osmolarity glycerol with depolarization and rapid calcium transients generated by G protein- and lipid-regulated transient receptor potential vanilloid (TRPV) channels (24, 25). These calcium transients can be measured with genetically encoded calcium indicators such as GCaMP3 (24, 26, 27). Histamine strongly reduced glycerol-elicited calcium transients in ASH neurons expressing HisCl1 (ASH:HisCl1), sparing a much smaller, slower response (Fig. 2D). In control experiments, histamine did not affect ASH calcium transients in wild-type animals, and ASH:HisCl1 responses were normal in the absence of histamine (Fig. 2D and Fig. S24). The residual response of ASH:HisCl1 cells in histamine was lost in osm-9 TRPV mutants but spared in egl-19 (L-type calcium channel, partial loss of function) and unc-68 (ryanodine receptor) mutants (Fig. 2E and Fig. S2 B and C). These results suggest that ASH calcium transients initiated by osm-9 TRPV channels are propagated by
Histamine-HisCl1 silences C. elegans neurons. (A) Representative membrane potential traces of AVA neurons exposed to 10 μM histamine (HA) for 500 ms, recorded under current clamp. (B) Representative membrane potential (Left) and current (Right) traces of AVA:HisCl1 cells exposed to 10μM histamine for 5 s. (C) Current in AVA:HisCl1 neurons as a function of histamine concentration, fit to the Hill equation. Each point is the average of three to five traces; error bars indicate SEM. Voltage was clamped to -60 mV in B (Right) and C. (Inset) Sample current traces, colors matching concentrations in curve. (D–F) ASH calcium responses to a high osmolality 1 M glycerol stimulus (gray), measured using GCaMP3 and averaged over 6–13 animals. Individual traces are shown in Fig. S2. (D) Wild-type (WT) and HisCl1-expressing ASH neurons (ASH:HisCl1) in the presence or absence of histamine (HA) (Inset), osm−9 (y101), egl-19 (n522), and unc-68 (p540) ASH neurons expressing HisCl1, in the presence or absence of histamine. (F) Hyperpolarization of RIC neurons with an RIC:HisCl1 transgene does not affect glycerol-induced calcium transients in ASH.

Histamine-HisCl1 Has Cell Type-Specific Behavioral Effects. The general utility of the histamine-HisCl1 system was explored by expressing HisCl1 in representative neurons involved in avoidance behaviors (29). When C. elegans encounters a repellent, it typically pauses its sinusoidal forward movement, reverses for one or more body bends, makes an acute turn in which its head touches its tail in an omega shape, and then moves forward in a different direction (Fig. 3A, Figs. S3 A–C, and Movie S2). Two stimuli that elicit this behavior are osmotic stimuli sensed by ASH sensory neurons and light anterior touch sensed by ALM and AVM neurons. ASH, ALM, and AVM synapse directly or indirectly onto interneurons called the backward command neurons, which in turn synapse onto DA and VA motor neurons that drive reversals. ASH also synapses onto a class of interneurons called AIB that drives omega turns and reversals (Fig. 3B).

To examine the possible spread of histamine-induced hyperpolarization through gap junctions, we monitored ASH responses to glycerol in a strain expressing HisCl1 in RIC, an ASH gap junction partner (28). ASH calcium responses and behaviors of RIC:HisCl1 animals were unaffected by histamine, suggesting that gap junctions did not propagate histamine-HisCl1 silencing under these conditions (Fig. 2F and Fig. S2 D–F).

Expression of HisCl1 in the DA and VA motor neurons attenuated reversals in the presence of histamine (Fig. 3D and Fig. S3 D and E), in agreement with ablations and genetic studies of DA and VA motor neurons (29, 32). These animals differed from animals in which AVA was silenced in two respects. First, when presented with stimuli, instead of pausing, these animals crumpled into an abnormal posture, suggesting an attempt to perform a reversal, but an inability to execute it (Movie S2). Second, after several seconds, they recovered forward movement without performing an omega turn (Fig. 3 E and F). These results suggest that VA and DA motor neurons are required for omega turns, unlike AVA interneurons. A role for DA and VA motor neurons in omega turns supports the emerging hypothesis that feedback and signaling among motor neurons regulates movement patterns (33).

Expression of HisCl1 in the RIC interneurons resulted in a histamine-dependent reduction of reversal length during glycerol avoidance, but did not affect reversals during touch avoidance (Fig. 3D and Fig. S3 D and E). These results suggest that ASH signals partly through AIB interneurons, in agreement with its synaptic wiring and ablations (28, 31). Instead of reversing, animals responded to aversive stimuli with a pause in locomotion, followed by an omega turn with a slightly delayed onset (Fig. 3 E and F, Fig. S3F, and Movie S2). Silencing AVA also affected spontaneous forward locomotion speed, which was slowed by about 50% in AVA:HisCl1 animals on histamine (Fig. 3C). These results suggest that glycerol and anterior touch initiate parallel avoidance pathways that pause forward locomotion, stimulate reversals, and stimulate omega turns, and that the reversals require AVA activity but pauses and omega turns do not. The role of AVA in forward speed was unanticipated, but was also observed in quantitative laser ablation experiments [forward speed after AVA ablation 0.06 +/- 0.01 mm/s, mock-ablated controls 0.14 +/- 0.02 mm/s (SD)].

Expression of HisCl1 in the AIB interneurons resulted in a histamine-dependent reduction of reversal length during glycerol avoidance, but did not affect reversals during touch avoidance (Fig. 3D and Fig. S3 D and E). These results suggest that ASH signals partly through AIB interneurons, in agreement with its synaptic wiring and ablations (28, 31). Instead of reversing, animals responded to aversive stimuli with a pause in locomotion, followed by an omega turn with a slightly delayed onset (Fig. 3 E and F, Fig. S3F, and Movie S2). Silencing AVA also affected spontaneous forward locomotion speed, which was slowed by about 50% in AVA:HisCl1 animals on histamine (Fig. 3C). These results suggest that glycerol and anterior touch initiate parallel avoidance pathways that pause forward locomotion, stimulate reversals, and stimulate omega turns, and that the reversals require AVA activity but pauses and omega turns do not. The role of AVA in forward speed was unanticipated, but was also observed in quantitative laser ablation experiments [forward speed after AVA ablation 0.06 +/- 0.01 mm/s, mock-ablated controls 0.14 +/- 0.02 mm/s (SD)].

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and motor neurons in the transformation of sensory inputs into motor outputs.

**Titration of Histamine-HisCl1 Suggests That AVA and DA/VA Activity Encodes Reversal Length in a Graded Manner.** Aversive stimuli of different types or strengths can yield graded reversal behaviors, implying a mechanism for converting input strength to proportional behavioral output (34). Thus, touch-induced reversals were consistently longer than reversals induced by glycerol (Fig. 3A), and increasing the osmolarity of a glycerol stimulus resulted in a smooth increase in reversal lengths (Fig. 4A and B). Electrophysiological studies have demonstrated that AVA membrane potential responds in a graded manner to inputs, suggesting that its activity may be graded (34, 35). However, the quantitative relationship between AVA activity and reversal length and duration has not been characterized. Therefore, we assayed touch-induced reversals as a function of graded histamine inhibition of AVA. Reversal length distributions in response to light touch shifted gradually with histamine concentration, with an EC50 for reversal length that was 10-fold lower than the EC50 for a complete reversal failure (0.4 mM vs. 4 mM; Fig. 4C). Reversal-length distributions in the transition zone (0.5 mM, 1 mM histamine) were well fit by single distributions and poorly fit by linear combinations of the 0 mM and 10 mM distributions, suggesting that AVA activity directs a graded reversal response (Fig. 4D).

The DA and VA motor neurons are the major output of AVA that drive reversals. Exposing DA/VA neurons expressing HisCl1 to increasing histamine levels recapitulated the gradual decrease in reversal length distributions observed with AVA (Fig. 4E and F). Thus, behavioral functions of motor neurons as well as interneurons responded continuously to inhibition by histamine-HisCl1. These behavioral results are consistent with electrophysiological results showing that graded stimulation of *C. elegans* motor neurons results in a graded change in postsynaptic currents in muscle (36).

**Discussion**

Our results indicate that the histamine-HisCl1 system can quickly and robustly silence *C. elegans* neurons. Calcium imaging, electrophysiology, and behavioral data indicate that HisCl1 has little effect on *C. elegans* neurons in the absence of histamine, and that histamine has little effect on wild-type *C. elegans* neurons in the absence of HisCl1. These features make the histamine-HisCl1 system an attractive method for inducibly silencing *C. elegans* neurons.

Acute silencing of sensory neurons, interneurons, and motor neurons with HisCl1 caused behavioral defects that resembled those observed after neuronal ablation, and revealed additional neuronal functions (Fig. 4G). A major advantage of genetic systems such as HisCl1 over ablations is the ability to test large numbers of animals, providing more ability to detect quantitative effects. For example, previous work showed that AIB is required for most spontaneous reversals and omega turns but did not reveal its more subtle effect on touch avoidance or locomotion speed (30). This system is also well suited for inducing perturbations of multiple neurons in one experiment, which may be used to characterize functional relationships systematically using epistasis or quantitative network analysis (37).

The chemical genetic method described here is a useful complement to optogenetic approaches, with different advantages and disadvantages. The major strengths of optogenetics are its millisecond time resolution and rapid reversibility. The effects of histamine administration developed more slowly over a few minutes, as quickly as one could reasonably expect an exogenous chemical to reach internal neuronal tissues. The weakest aspect of this system was the slow recovery of behavior after prolonged incubation in histamine, which could be as long as 2 h. It is possible...
that neuronal physiology is disrupted after prolonged silencing, requiring new gene expression for recovery, or it may be that endogenous tissues or intracellular fluids store histamine after it is absorbed.

For assays that do not require fine temporal precision, some features of the histamine-HisCl1 system may favor its use. First, its effects are stable over long durations, allowing prolonged neuronal inactivation that is difficult to achieve with existing optogenetic silencers. Second, the system is orthogonal to useful light-activated reagents like GCaMP and Channelrhodopsin, so light and chemicals can be used to monitor and manipulate the same neurons at the same time, or to manipulate several neurons independently in the same experiment. Finally, histamine is compatible with nearly any behavioral assay or screen, because it is apparently behaviorally inert, and can simply be added to the media at the beginning of the experiment.

Our initial success with a variety of neuronal cell types and promoters suggests that the HisCl1 channel is relatively robust to expression level and cellular context. The ~6 pS single-channel conductance of HisCl1 is typical of the cys-loop family of ligand-gated channels, whose single-channel conductances range from 5 to 100 pS (17). By contrast, the single-channel conductance of light-activated channels used for optogenetics falls in the 0.25–1 pS range, and therefore optogenetic proteins require high expression levels that are not always achievable (38). Optogenetic hyperpolarization using chloride and proton pumps can cause problems by altering intracellular ion concentrations (39); the use of passive chloride channels should not raise this concern. However, in the special cases in which chloride is depolarizing rather than neutral or hyperpolarizing, passive chloride channels such as the HisCl1-histamine system may be activating rather than inhibitory (40).

Ligand-activated chloride channels dominate inhibition in normal neuronal circuits, and the parallel to physiological inhibition is an attractive feature of using this strategy in experiment design. We considered the possibility of indirect HisCl1 silencing via gap junctions, but did not observe such effects between the coupled ASH and RIC neurons. In addition, HisCl1 silencing of gap junction-coupled AVA and DA/VA cell classes had distinct behavioral effects, suggesting that these neurons could be inhibited independently.

In addition to silencing neurons outright, the HisCl1 system permitted graded control of C. elegans neuronal activity and behavior. Understanding relationships between activity and output is an important step for modeling neural circuits (41), and may be particularly useful for understanding the role of neuromodulation in behaviors, because neuromodulators usually alter the activity level of their targets rather completely silencing them (42).

The rapid access of histamine to C. elegans tissues could be exploited further by developing additional chemical genetic tools for histamine-mediated depolarization (via directed HisCl1 mutagenesis) or GPCR modulation (using mammalian histamine receptors) (13, 43). Histamine-based systems are not ideal for animals that use endogenous histamine as a transmitter, but further exploration of noncanonical transmitters and receptors, such as the odor-activated ion channels of insects, may provide general solutions for chemical genetic manipulations (44).

**Methods**

**Strains and Media.** C. elegans strains were maintained at room temperature on nematode growth medium (NGM) plates, with Escherichia coli OP50 bacteria as a food source. Wild type is Bristol N2. Standard molecular biology and transgenesis methods were used (see SI Methods). Strains are listed in Table S1.

To make NGM-HA plates, histamine (Sigma Aldrich histamine-dihydrochloride, 1 M stock in water) was added to NGM agar at ~45 °C immediately before pouring plates. Histamine-free control plates were poured from the same NGM batch. NGM plates with 10 mM histamine were effective for at least 2 mo when stored at 4 °C. Histamine-containing plates used for dose–response curves were used within 2 d after pouring.

**Behavioral Assays.** For all assays, L4 animals were picked the day before the assay. For assays performed in the absence of food, animals were picked to a food-free plate, allowed to crawl away from the food, and repicked to the assay plate. Glycol and touch avoidance assays were performed after 30 min of starvation (see SI Methods). Paralysis assays were conducted on 20 to 30 animals on a thin OP50 lawn for each data point. Paralysis was defined as no movement or response to prodding with the worm pick.
Electrophysiology. Electrophysiological analysis was performed as previously described, with modifications (36). Briefly, an adult animal was immobilized with cyanocrylate adhesive (Vetbond tissue adhesive; 3M) on a Sylgard 184-coated (Dow Corning) glass coverslip and dissected to expose AVA. Recordings were performed using single-electrode whole-cell voltage clamp (Heka, EPC-10 USB) with two-phase capacitive compensation optimized at rest, and series resistance compensated to 50%. Histamine was delivered with a PicoSpirter III (Parker Hannifin Co.) under active perfusion. For additional detail, see SI Methods.

Calcium Imaging. Calcium imaging was performed as previously described, with minor modifications (26). Briefly, a young adult animal was picked from food and starved for 30 min on an NGM or NEM-PA plate, then loaded into a custom-built PDMS microfluidic device with its nose exposed to buffer under laminar flow. Stimuli were delivered in S-basal buffer with or without 10 mM histamine. Additional details are given in SI Methods.

Statistical Analysis. All statistical analyses were done in MATLAB. Error bars are SEM. Means were compared with bootstrap without replacement or Student t test (both gave similar results), with P values adjusted for multiple comparisons with false discovery rate (FDR) where indicated (45). For additional details, see SI Methods.

ACKNOWLEDGMENTS. We thank J. Gray for the AVA laser ablation results, Y. Xu, P. Kong, and C. Cho for strains, and S. Flavell, D. Albrecht, S. Levy, D. Ventimiglia, Y. Xu, and other members of the C.I.B. laboratory for advice and discussions. C.I.B. is an investigator of the Howard Hughes Medical Institute (HHMI). This work was funded by HHMI and by a grant from The G. Harold and Leila Y. Mathers Charitable Foundation.


Supporting Information

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SI Methods

**Molecular Biology.** HisCl1 was PCR amplified from Berkeley *Drosophila* Genome Project (BDGP) cDNA GH14445 with 5′-caggagagaccttgGCTAGCatggaaccactagca-3′ and 5′-taggatgaagacGTTACCcttagaatgcttgcaat-3′. An unspliced intron in GH14445 was removed by extension overlap PCR using internal primers 5′-CTTCCATGTAGATCGAGTTTATCCCTACG-GTGAAGAGT-3′ and 5′-ATCTTCCAGTGATGGGATAAA-CTTCCGATCATGCG-3′.

HisCl2 was amplified from BDGP cDNA IP02383 with 5′-caggagagaccttgGCTAGCatggaaccactagca-3′ and 5′-taggatgaagacGTTACCcttagaatgcttgcaat-3′ and 5′-taggatgaagacGTTACCcttagaatgcttgcaat-3′. The PCR products were cloned into pSM_SL2_GFP and pSM_SL2_mCherry at the NheI/KpnI sites, and sequenced.

Promoters were inserted at FseI/AscI sites, and included lag-168 [panneuronal (1)], bhl-1 [RIC (2)], rig-3 [AVA (3)], sra-6 [ASH (4)], mec-3 [ALM/AVM (5)], unc-4 [DA/VA (6)], and inx-1 [AIB (7)].

Transgenic animals were generated via standard microinjection protocols, and identified using fluorescent coinjection markers (8).

**Histamine Supplementation.** Stocks of 1 M histamine-dihydrochloride (Sigma-Aldrich) were made with distilled water, sterile-filtered, and diluted into S-basal buffer or NGM agar that had cooled to ~65 °C. Adding histamine-HCl up to 15 mM did not appreciably change the pH of NGM agar or S-basal buffer.

**Glycerol Avoidance and Anterior Touch Avoidance.** Animals were picked from food and starved for 30 min on either NGM-HA or control plates. Typically, 60–90 animals were tested per strain and condition. Only responses initiated within 4 s of the drop or touch were considered positive.

Glycerol drop tests were performed as described (9). Briefly, glycerol was diluted to 1 M in M13 buffer (30 mM Tris pH 7.5, 100 mM NaCl, 10 mM KCl) and drawn into a 10-μL capillary attached to rubber tubing. Drops of fluid were delivered near the tail of a forward-moving animal by mouth pipetting; capillary action drew the fluid up to the nose. Anterior touch assays were performed as previously described; an eyelash attached to a pipette tip was used to stroke a forward-moving animal at or just posterior to the terminal bulb of the pharynx (10).

Reversal lengths were scored by the number of body bends. Reversals that ended before a body bend was completed were scored as 0.5. Omega turns were scored if the animal reoriented >135°, or if the head touched or nearly touched the tail during the turn (11). A stimulus that results in a pause was counted as 0. Omega turns were identified if the animal reoriented >135°, or if the head touched or nearly touched the tail during the turn (11). A stimulus that results in a pause was counted as a positive response, even if it was not accompanied by a reversal or omega turn. Omega latencies were defined as the period from the start of the response (reversal or pause) to omega initiation, and were measured with a stopwatch.

**Exploration and Locomotion Assays.** Locomotion and exploration results were obtained from four to five experiments for each condition, with 20–30 animals per experiment. Young adult animals were transferred to a food assay plate containing a uniform OP50 lawn and allowed to equilibrate for at least 30 min before recording for 10 min. These animals were then transferred to a food-free assay plate (3 min dead time), and recorded for 1 h.

Video recordings were acquired at 3 Hz using Streampix software and a Pixellink camera, and were analyzed with custom software written in MATLAB (Mathworks). Reversals were identified by sharp changes in angular speed (≥75 deg/s). Omega turns were identified by their shape (body-enclosing ellipse eccentricity ≤0.875), and filtered by an angular speed threshold (≥60 deg/s). The error between human and machine scoring of nonforward motion worm frames was similar to the difference between human scorers.

Laser ablations of the AVA neurons were performed by Jesse M. Gray as described (11). Forward locomotion speed was tracked for 30 min after removal from food on agar plates.

**Electrophysiology.** An adult animal was immobilized on a Sylgard-coated (Sylgard 184, Dow Corning) glass coverslip by applying a cyanoacrylate adhesive (Vetbond tissue adhesive; 3M) along the dorsal side of the head region (12). A puncture in the cuticle away from the head was made to relieve hydrostatic pressure. A small longitudinal incision was then made from posterior to the terminal bulb along the glue line to the tip of the nose. The cuticle flap was folded back and glued to the coverslip with GLUture Topical Adhesive (Abbott Laboratories), exposing the nerve ring. The preparation was then treated with 1 mg/mL collagenase (type IV; Sigma) for ~10 s. An upright microscope (Axio Examiner; Carl Zeiss, Inc.) equipped with a 40x water immersion lens and 16× eyepieces was used for viewing the preparation. AVA neurons were identified by mCherry coexpressed with HisCl1 and the position of the cell body. All experiments were performed with the bath at room temperature using single-electrode whole-cell voltage clamp (Heka, EPC-10 USB) with two-stage capacitive compensation optimized at rest, and series resistance compensated to 50%. Borosilicate glass pipettes (BF100-58-10; Sutter Instruments) with resistance (Rg) = 10–15 MΩ were used as electrodes. The pipette solution was (all concentrations in mM) [K-glucanate 120; KCl 15; MgCl2 5; TES 5; CaCl2 0.25; EGTA 5; Na2ATP 5; NaGTP 0.5; Hepes 10; Sucrose 30] (pH 7.3, osmolarity 330 mOsm) and the extracellular solution was [NaCl 140; NaOH 5; KCl 5; CaCl2 2; MgCl2 5; Sucrose 5; Hepes 15; d-Glucose 25] (pH 7.3, osmolarity 330 mOsm). Histamine dissolved in the extracellular solution was filled into a glass pipette with Rg = 2–3 MΩ and spritzed (2.5 psi) onto the cell being recorded with PicoSpritzer III (Parker Hannifin Co.). Bath solution was actively perfused with a gravity perfusion system during all recordings. Experiments were controlled using PatchMaster software (Heka). Analog data was filtered at 2 kHz and digitized at a rate of 100 μs.

**Calcium Imaging Analysis.** Methods were as described (13) except that the thinner unc-68 animals were examined in a device with smaller dimensions for the worm channel: 670 μm (length) × 56 μm (width) × 24 μm (depth). TIFF image stacks were taken at 10 Hz (Andor iXon3 camera, Metamorph software), and a three-way valve (Lee Company) regulated delivery of the 1 M glycerol stimulus to the worm nose. Custom ImageJ (14) and MATLAB scripts were used to identify, track, and analyze the fluorescence of the ASH cell body.

**Modeling.** Dose–response curves were fit to the Hill equation:

\[
Y = \frac{R}{\text{EC}_{50}^{\text{histamine}} n_H} + 1
\]

where \(Y\) is the measured quantity, [histamine] is histamine concentration, \(\text{EC}_{50}\) is the concentration of half-maximal activity, \(n_H\) is the Hill coefficient, and \(R\) is the maximal response (\(R = 1\) if \(Y\) is a fraction).
Kinetic traces were fit to the first-order rate law:

\[ Y = 1 - \exp \left( -\frac{t_{1/2}}{\ln 2} \right) \]  

where \( Y \) is the normalized measured quantity, \( t \) is the time, and the half-time \( t_{1/2} \) is the time for \( Y = 1/2 \). Microfluidic behavior traces and electrophysiology traces were not described well by this model, so \( t_{1/2} \) values for these were estimated as the time required for the half-maximal effect or recovery.

Histamine-dependent reversal length distributions (Fig. 4) were fit individually to the canonical unimodal Gaussian model:

\[ P(x) = \frac{1}{\sigma \sqrt{2\pi}} \exp \left( -\frac{(x - \mu)^2}{2\sigma^2} \right) \]  

where \( \mu \) and \( \sigma \) are from fitting Eq. S2 to the 0 mM distribution, and \( \mu_{10mM} \) and \( \sigma_{10mM} \) are from fitting to the 10 mM distribution, and \( A_C \) is the amplitude of the 0 mM component in the distribution for C mM histamine.

Models were compared with Akaike’s Information Criterion (15).

Fig. S1. Effects of histamine on behavior of wild-type and HisCl1-expressing animals. (A) Animals expressing HisCl1 pan-neurally (5 ng/μL) can be reversibly paralyzed in sequential 10-min histamine treatments. Error bars are SEM for 20–30 animals. (B and C) Control experiments examining exploration behavior after removal from food. The 3-min discontinuity is the assay dead time before recording is started. Error bars are SEM for four to five experiments of 20–30 animals each. (B) Basal locomotion speed is significantly slowed by HisCl1/2 transgenes (***$P < 0.001$), but not by HisCl1 transgenes. (C) Reversal/omega frequencies are reduced in HisCl1/2-expressing animals (*$P < 0.05$), but not in HisCl1-expressing animals. (Inset) Mean reversal/omega frequencies for the interval from 3 to 15 min.
Fig. S2. Individual and average ASH:GCaMP3 responses to 1 M glycerol (gray bar) for each strain/condition; thin lines are individual traces. (A) Traces for data in Fig. 2D. (B) Histamine-free traces for data in Fig. 2E. Note that egl-19(n582) is a partial loss of function mutation that does not eliminate all activity of this essential gene. (C) Traces in the presence of 10 mM histamine for data in Fig. 2E. (D) Traces for RIC:HisCl1 animals (Fig. 2F). (E) Normalized reversal length histograms for 1 M glycerol avoidance by RIC:HisCl1 animals (n = 50–60) in the presence or absence of 10 mM histamine. y axis height is normalized to the largest bin in the histogram; bars add to 100%. Value in the "zero" bin at left represents the fraction of animals that did not reverse. Mean values are color coded in the upper right corner. No significant difference was observed. (F) Fraction of 1 M glycerol stimuli that elicit an omega turn in RIC:HisCl1 animals (n = 50–60) in the presence or absence of 10 mM histamine. No significant difference was observed. Error bars are SEM.
Fig. S3. Detailed characterization of avoidance responses in wild-type and HisCl1-expressing strains. For A and D–F, mean values for each strain and condition are color coded in the upper right corner of histogram plots. (A) Normalized reversal length histograms for wild-type (WT) animals responding to glycerol (red) and anterior touch (blue) stimuli. Anterior touch elicits longer reversals than 1 M glycerol. (B) Fraction of 1 M glycerol and anterior touch stimuli that elicit an omega turn in WT animals. (C) Fraction of 1 M glycerol and anterior touch stimuli that elicit any response (pause, reversal, or omega turn) in WT animals. (D and E) Reversal length histograms for avoidance of 1 M glycerol (D) and anterior touch (E) in the presence (red, blue) or absence (gray) of 10 mM histamine. Strains express HisCl1 in the indicated neurons. y axis height is normalized to the largest bin in each histogram; bars add to 100% for all strains tested. Value in the "zero" bin at left represents the fraction of animals that did not reverse. Residual sensory responses in ASH:HisCl1 and ALM/AVM:HisCl1 strains could represent either contributions of other neurons (for 1 M glycerol) or voltage-insensitive signaling functions of ASH and ALM/AVM. (F) Histograms of latency to omega turn for AVA:HisCl1 animals in the presence (red, blue) or absence (gray) of 10 mM histamine, in response to glycerol and light touch stimuli. The slight delay may reflect either a minor role of AVA in omega initiation, or an indirect effect of the slowed locomotion in AVA:HisCl1 animals on histamine. Differences from control means: *P < 0.05; **P < 0.01; ***P < 0.001. For D and E, P values are adjusted by FDR. Error bars are SEM for 60–90 animals.
Fig. S5. Touch-induced reversal lengths in AVA:HisCl1 animals as a function of histamine concentration and time on histamine. Curves are fit to first order kinetics. Error bars are SEM for 60–90 animals. (A) 0.05 mM histamine, (B) 0.1 mM histamine, (C) 0.5 mM histamine, (D) 1 mM histamine, (E) 5 mM histamine, and (F) 10 mM histamine. $t_{1/2}$, half-time to 50% effect at each concentration; $Y$, reversal length at plateau.

Table S1. List of strains

<table>
<thead>
<tr>
<th>Name</th>
<th>Strain designation</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>WT</td>
<td>N2</td>
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<tr>
<td>5 ng/μL HisCl1</td>
<td>CX14370</td>
<td>kyEx4568 [tag-168::HisCl1::SL2::GFP], 50 ng/μL + myo-3::mCherry, 10 ng/μL</td>
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<td></td>
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<tr>
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<tr>
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<td>osm-9 (ky10); klys602 [sra-6::GCaMP3.0, 75 ng/μL + unc-122::GFP, 10 ng/μL]</td>
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<tr>
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<td>unc-68 (e540); klys602 [sra-6::GCaMP3.0, 75 ng/μL + unc-122::GFP, 10 ng/μL]</td>
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<td>klys6563 [pNP471 (rig-3::HisCl1::SL2::mCherry), 50 ng/μL + pNP476</td>
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</tbody>
</table>

(adx-1::HisCl1::SL2::mCherry), 50 ng/μL)
Movie S1. Wild-type (WT) animals and animals expressing HisC1 in all neurons responding to a 4-min pulse of 10 mM histamine in a microfluidic device. Right, fraction of animals paused, synchronized with video. Video is accelerated 20x.

Movie S1

Movie S2. Typical anterior touch responses for wild-type and the indicated HisC1-expressing strains on histamine-containing plates. Video is accelerated 5x.

Movie S2