Sensory receptor neurons match their dynamic range to ecologically relevant stimulus intensities. How this tuning is achieved is poorly understood in most receptors. The roundworm Caenorhabditis elegans avoids 21% O₂ and hypoxia and prefers intermediate O₂ concentrations. We show how this O₂ preference is sculpted by the antagonistic action of a neuroglobin and an O₂-binding soluble guanylate cyclase. These putative molecular O₂ sensors confer a sigmoidal O₂ response curve in the URX neurons that has highest slope between 15 and 19% O₂ and approaches saturation when O₂ reaches 21%. In the absence of the neuroglobin, the response curve is shifted to lower O₂ values and approaches saturation at 14% O₂. In behavioral terms, neuroglobin signaling broadens the O₂ preference of Caenorhabditis elegans while maintaining avoidance of 21% O₂. A computational model of aerotaxis suggests the relationship between GLB-5-modulated URX responses and reversal behavior is sufficient to broaden O₂ preference. In summary, we show that a neuroglobin can shift neural information coding leading to altered behavior. Antagonistically acting molecular sensors may represent a common mechanism to sharpen tuning of sensory neurons.

The response properties of sensory neurons can be characterized by tuning curves that relate stimulus parameters to the evoked response (1, 2). Some sensory neurons show dynamic ranges that span several orders of stimulus magnitude (e.g., odor concentration), whereas others show remarkably narrow tuning curves. For example, glomus cells of the carotid body show oxygen-evoked responses that are tuned to a twofold to threefold change in O₂ levels at the physiologically appropriate O₂ concentration range (3, 4). How such sharp tuning is achieved is poorly understood.

Neuroglobins are members of the globin family of heme-binding proteins expressed mainly in neurons (5). They have been described throughout metazoa, from cnidarians to man (6). Their physiological functions are unclear. They have been proposed to metabolize reactive oxygen species (ROS), signal redox state, store O₂, control apoptosis, and negatively regulate Gα/i signaling (7). The genome of Caenorhabditis elegans encodes an unusually large family of globins, and many are expressed in neurons (8). One of these, GLOBIN-5 (GLB-5), is expressed in the oxygen sensing neurons URX, AQR, PQR, and BAG, where it accumulates at dendritic endings (9, 10). C. elegans avoids both normoxia (21% O₂) and hypoxia (11). Avoidance of 21% O₂ enables the animal to escape the surface and is mediated by O₂ receptors, most importantly the glb-5-expressing URX, AQR, and PQR neurons (12). Like vertebrate neuroglobins, GLB-5 has the spectroscopic fingerprints of a hexa-coordinated heme iron and rapidly oxidizes to the ferric state in normoxia (9). The glb-5 gene is defective in the domesticated reference strain of C. elegans, N2 (Bristol), but natural isolates encode a functional allele, glb-5(Haw) (9, 10) (Haw refers to Hawaii, the geographical origin of the natural isolate in which this allele was first described). Behavioral and Ca²⁺ imaging studies suggest that functional GLB-5 alters the properties of the O₂ receptors and C. elegans’ O₂ responses (9, 10). However, how GLB-5 alters the representation of environmental information in these neurons leading to behavioral change is unknown.

The URX O₂ receptors exhibit phasic–tonic signaling properties and, in response to changes in O₂ concentration, evoke both transient behavioral responses that are coupled to the rate of change of O₂, dO₂/dt, and more persistent behavioral responses coupled to O₂ levels (8, 13). The transient responses are reversals and turns that allow C. elegans to navigate O₂ gradients. The sustained responses involve persistent changes in the rate of movement that enable feeding animals to escape 21% O₂ or to accumulate in preferred lower O₂ environments. Besides GLB-5, the URX neurons express another putative molecular O₂ sensor, a soluble guanylate cyclase composed of GCY-35 and GCY-36 (guanylate cyclase) subunits (11, 13, 14). These soluble guanylate cyclases have a heme–nitric oxide/oxygen (H-NOX) binding domain that appears to stimulate cGMP production upon binding molecular O₂ (10, 11). Recent work suggests mammalian soluble guanylate cyclases also mediate O₂ sensing in glomus cells of the carotid body, although they do not bind O₂ (15). Here we show that the GLB-5 neuroglobin and soluble guanylate cyclases work antagonistically to confer on URX a sigmoidal O₂ stimulus–response curve that has its steepest slope between 15 and 19% O₂ and begins to plateau as O₂ levels approach 21%. By tuning URX, GLB-5 broadens the range of O₂ environments preferred by C. elegans. Using computer modeling we show that this altered preference can be explained by changes in how URX evokes reversals in response to O₂ stimuli.

**Significance**

Sensory neurons encode environmental stimuli in their electrical activity and alter behavior and physiology by transmitting this information to downstream circuits. Their response properties can be characterized by tuning curves that relate stimulus parameters to neural responses. Tuning curves identify the response threshold, the stimulus features at the tuning curve peak, and high-slope regions that give maximum stimulus discrimination. Here we show that two antagonistically acting molecular oxygen sensors, a neuroglobin and a soluble guanylate cyclase, sculpt a sharp sigmoidal tuning curve in the URX oxygen sensing neurons of Caenorhabditis elegans. By combining experiments with computational modelling, we show that these changes in stimulus-encoding properties broaden C. elegans’ O₂ preference.

Author contributions: S.O., Y.T., and M.d.B. designed research; S.O. and Y.T. performed research; S.O., Y.T., and M.d.B. analyzed data; and S.O., Y.T., and M.d.B. wrote the paper. The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freyly available online through the PNAS open access option.

1To whom correspondence may be addressed. Email: debono@mrc-lmb.cam.ac.uk or shigeoda811.alumni.u.tokyo.ac.jp.

2Present address: Division of Quantitative Biology, Okazaki Institute for Integrative Biosciences, National Institutes of Natural Sciences, Okazaki, Aichi 444-8787, Japan.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1614596114/-/DCSupplemental.
Results

The GLB-5 Allele in Natural Isolates Broadens C. elegans’s O₂ Preference.

Previous studies of glb-5 used a loss-of-function allele that arose in the N2 laboratory strain during domestication (9, 10, 16). This allele, glb-5(N2), is a partial duplication that generates multiple splice isoforms, and it is unclear if it abolishes glb-5 function. We therefore compared animals bearing the glb-5(Haw) functional allele to mutants carrying a predicted null mutation, glb-5(tm5440), which deletes part of the globin domain and introduces a premature stop codon. To analyze how the GLB-5 neuroglobin alters O₂ preference we compared the distribution of glb-5(Haw) and glb-5(tm5440) animals in a shallow 0–21% O₂ gradient in the presence of food. Unless otherwise indicated, in our experiments we used strains defective in the neuropeptide receptor npr-1, because besides harboring a defective glb-5 allele, the N2 laboratory strain has acquired a gain-of-function mutation in npr-1 that confers O₂-sensing defects (11), glb-5(tm5440); npr-1 animals accumulated in a narrow range of O₂ concentrations, between 7 and 10% O₂ (Fig. 1A and B). By contrast, animals bearing the natural glb-5(Haw) allele distributed over a broader range of O₂ concentrations, between 17 and 5% O₂, but still avoided 21% O₂ and hypoxia (Fig. 1A and B). These behavioral data imply that the GLB-5(Haw) neuroglobin changes how O₂-sensing neurons respond in O₂ gradients.

GLB-5 Changes the Dynamic Range of the URX O₂ Sensor. We used the GCaMP6s Ca²⁺ sensor to examine how functional glb-5 alters neural coding of O₂ levels in the URX O₂ sensors (17). The dynamics of the Ca²⁺ responses evoked in URX by a 7–21% O₂ step stimulus did not differ significantly between glb-5(tm5440) and glb-5(Haw) animals (Fig. S1). However, the Ca²⁺ responses to a 7–19% O₂ exponential ramp stimulus differed markedly between these strains (Fig. 1C). In animals expressing glb-5(Haw), Ca²⁺ in URX increased continuously as O₂ levels rose from 7 to 19%. By contrast, the Ca²⁺ responses of glb-5(tm5440) mutants appeared to plateau at ~14% O₂. As expected, the URX neurons did not respond to the O₂ stimulus in animals defective in both glb-5 and the gcy-35 soluble guanylate cyclase (Fig. 1C). This defect could be rescued by expressing wild-type gcy-35 and glb-5(Haw) cDNA selectively in URX (Fig. S2 A and B). Selectively expressing wild-type gcy-35 cDNA but glb-5(tm5440) cDNA that contained a stop codon conferred URX responses that plateaued at ~14% O₂ (Fig. S2A and B). These differences suggest that the GLB-5 neuroglobin changes the dynamic range of URX (Fig. 1C). The effect of glb-5 alleles on the URX Ca²⁺ response was similar whether we imaged animals in the presence (Fig. 1C) or absence of food (Fig. S2 C and D).

To investigate further how the GLB-5 neuroglobin alters neural coding, we delivered different patterns of O₂ stimuli and imaged Ca²⁺ responses in URX. We focused on URX because these sensory neurons are sufficient for several O₂-coupled behaviors including aerotaxis and aggregation (18, 19) (Fig. S3). To plot the relationship between stimulus intensity and URX Ca²⁺ responses we sequentially increased the O₂ stimulus given to the same animal in 2% increments, returning to 7% O₂ between stimuli (Fig. 24). In glb-5(Haw) animals URX neurons showed a higher O₂ response threshold than in glb-5(tm5440) mutants, as well as a
steep sigmoidal O₂ response curve whose half maximum was at much higher O₂ concentrations (Fig. 2 A and B). Selectively expressing a glb-5(Haw) transgene in URX in glb-5(tm5440) mutants conferred a URX stimulus–response profile that closely resembled that of glb-5(tm5440) animals (Fig. 2 A and B). Thus, GLB-5(Haw) cell-autonomously shifts the URX stimulus–response curve toward higher O₂ concentrations (Fig. 2B).

To extend these observations, we examined URX responses to a different set of stimuli in which we increased O₂ levels in 2% steps but varied the starting O₂ concentration and delivered only one stimulus per animal. Again, we observed that the glb-5(Haw) allele shifted the tuning curve of URX such that both the tonic Ca²⁺ levels at the new O₂ concentration and the change in Ca²⁺ normalized to the prestimulus Ca²⁺ level, ΔR/Rₒ (which is a measure of the response amplitude), gradually increased as O₂ approached 19–21% (Fig. 3 A and B). By contrast, in glb-5(tm5440) mutants, ΔR/Rₒ was at a maximum when animals experienced an 11–13% O₂ stimulus (Fig. 3 A and B). Expressing glb-5(Haw) cDNA selectively in URX in glb-5(tm5440) animals was sufficient to confer a glb-5(Haw)–like dose–response curve to this neuron under these stimulation conditions (Fig. 3 A and B).

Step stimulation is used widely to study the properties of sensory neurons, but in their natural environment, C. elegans likely also encounter slowly varying O₂ levels, similar to those encountered by animals in the aerotaxis assay (Fig. 1A). We therefore measured the Ca²⁺ responses evoked in URX by a set of 2% O₂ exponential ramp stimuli. Our results revealed a response pattern similar to that observed for the corresponding step stimulus (Fig. 3 C and D). In animals expressing the glb-5(Haw) allele, URX responses to ramp stimuli increased gradually as O₂ levels increased. By contrast, in glb-5(tm5440) mutants the URX response amplitudes, measured as ΔR/Rₒ, showed a peak response to the 13 → 15% O₂ stimulus and were otherwise similar across the different ramp stimuli we delivered (Fig. 3 C and D). The response property changes conferred by GLB-5(Haw) are therefore robust to different O₂ stimulation patterns. Together, our Ca²⁺ imaging experiments suggest that GLB-5(Haw) alters neural encoding of O₂ stimuli in URX, shifting the dynamic range to higher O₂ concentrations and making it more sharply tuned.

cGMP Signaling in URX. In previous work we used the genetically encoded cGMP sensor cGi500 (20) to visualize cGMP dynamics in the POR O₂-sensing neuron (14). We showed that a rise in O₂ stimulates a tonic rise in cGMP that requires the GCY-35 soluble guanylate cyclase and that the Ca²⁺ influx resulting from gating of cGMP channels feeds back to limit O₂-evoked rises in cGMP by stimulating cGMP hydrolysis (14) (Fig. S4A). We used the cGi500 sensor to examine if GLB-5(Haw) can modulate cGMP dynamics in URX. We could not detect O₂-evoked cGMP responses in the cell body of URX neurons unless we disrupted cng-1, which encodes a cGMP-gated channel subunit required for O₂-evoked Ca²⁺ responses in URX (Fig. S4 A and B). This suggests that URX and POR have similar negative feedback control of cGMP accumulation. The cGMP responses evoked in URX by an exponential ramp O₂ stimulus were comparable in glb-5(tm5440) cng-1 and glb-5(Haw) cng-1 animals under our experimental conditions (Fig. 4 B and C). These results would suggest that GLB-5(Haw) does not alter URX neural coding by modulating cGMP levels, however, we cannot exclude the possibility that measuring cGMP in the cell body does not adequately report cGMP changes in the dendritic ending, where GLB-5, GCY-35/GCY-36, and the cGMP channels are localized.

GLB-5 Effects on URX Behavioral Outputs. How do the changes in URX information coding mediated by GLB-5(Haw) alter motor responses to O₂ stimuli? To address this question, we quantified behavioral responses to a range of O₂ stimuli, focusing on reversal in the direction of movement and changes in speed, both important features of O₂-evoked behaviors (18, 19). Avoidance of high O₂ levels is mediated principally by three sensory neurons, URX, PQR, and AQR, each of which expresses the GCY-35/36 O₂ receptor and GLB-5 (11–13, 18). To selectively study how URX output alters behavior we studied gcy-35(ok769); glb-5(tm5440) mutants that expressed glb-5(Haw) and/or gcy-35 cDNA in URX but not AQR or POR (Methods). Unexpectedly, only O₂ stimuli that evoked intermediate URX Ca²⁺ responses evoked strong reversals in these animals (Figs. 3 A and B and 4 A and B). O₂ stimuli that evoked either small or large URX Ca²⁺ responses failed to evoke reversals (Figs. 3 A and B and 4 A and B). We also observed this relationship between the Ca²⁺ response magnitude and reversals when we expressed glb-5(Haw) selectively in URX, although the O₂ range that evoked the reversals most strongly was different (Figs. 3 A and B and 4 A and B). These data suggest that URX associated circuits include a filter that prevents strong stimulation of URX from inducing reversals. Consistent with this, a 13 → 21% O₂ step stimulus that evoked a large URX Ca²⁺ response did not evoke reversals in animals expressing gcy-35 selectively in URX (Fig. S5).

One important output for the URX O₂ sensors is the RMG interneurons, which are connected to URX by both synapses and gap junctions (21–23). O₂-evoked responses in RMG correlated well with those in URX when we stimulated animals expressing gcy-35 selectively in URX with 13 → 15% and 13 → 21% O₂ steps.
This suggests information transfer from URX to RMG does not explain the nonlinearity in the relationship between URX Ca\textsuperscript{2+} responses and reversals. URX output also stimulated locomotory speed when O\textsubscript{2} levels rose above 17% O\textsubscript{2} in both glb-5(tm5440) and glb-5(Haw) strains (Fig. 4C and D). Together, our results suggest that information from URX is transmitted to both reversal and speed circuits; however, reversals can be evoked by changes in URX activity that evoke modest or no changes in speed.

**A Computational Model for Aerotaxis.** Using our detailed analyses of how URX responds to different O\textsubscript{2} stimuli, we carried out computational modeling experiments to ask if the relationship between URX activity and reversal behavior was sufficient to explain the altered O\textsubscript{2} preference of animals expressing glb-5(Haw).

To build a model for aerotaxis we incorporated the Ca\textsuperscript{2+} imaging data in Fig. 3A and the behavioral data in Fig. 4C (Methods). For simplicity, and because URX is sufficient for C. elegans to show an
O₂ preference (Fig. S3), we excluded other O₂-sensing neurons, including PQR and AQR, in the computational model. The model included a command neuron that randomly generates a reversal; a URX model neuron transmitted a signal to this command interneuron via an interneuron that could act as a differentiator to promote reversal (Fig. S4 and Methods). In the model, URX Ca²⁺ responses to O₂ stimuli were approximated by a nonlinear–linear–nonlinear (NLN) model (Fig. S4). The parameters for the NLN model were estimated from imaging URX responses to 2% step O₂ stimuli in glb-5(tm5440) and glb-5(Haw) (Fig. S7). A single model reproduced URX Ca²⁺ responses to a variety of O₂ changes. As a result of modeling the URX responses, we acquired two sets of parameters, one for glb-5(tm5440) and the other one for glb-5 (Haw). The parameters for steps downstream of URX were common for glb-5(tm5440) and glb-5(Haw) virtual animals.

Having set up our model, we ran in silico aerotaxis experiments in which the position of a worm was represented as a single point (Fig. S8). These experiments showed that worms for which the URX NLN model used parameters obtained for glb-5(tm5440) preferred 7–10% O₂ (Fig. 5 B and C), whereas those using glb-5(Haw) parameters showed broader O₂ preference, with the majority of worms preferring 7–16% O₂ (Fig. 5 B and C). The simulations made by our computational model mirrored the results of aerotaxis experiments (Figs. 1 A and 5 B–D). In our initial computational model, the values for constants in the model interneuron (l) and command neuron (c) were selected arbitrarily (Methods). We therefore examined how changing these parameters over a wide range altered the results of our simulated aerotaxis experiments. For almost all parameter values we tested, the glb-5(tm5440) virtual animals preferred lower and narrower O₂ concentrations than the glb-5(Haw) virtual animals (Fig. S9). The distinct O₂ preferences of the two strains are thus not strongly influenced by the values of these parameters.
To extend our model, we incorporated data on O₂-evoked changes in speed (Fig. 4C and Fig. S10), in addition to O₂-evoked changes in reversals. We found this did not substantially change the performance of glb-5(tm5440) and glb-5(Haw) in virtual aerotaxis assays. Model worms that modulated both reversals and speed in response to O₂ changes distributed similarly to animals that modulated only reversal (Fig. S11). By contrast, model worms in which changes in O₂ influenced only speed distributed almost evenly in a virtual aerotaxis chamber (Fig. S11). Thus, in our model the relationship between URX responses and reversal frequency is sufficient to account for the worm’s O₂ preference in a shallow O₂ gradient.

**Discussion**

The neuroglobin GLB-5 changes how the URX O₂-sensing neurons encode O₂ concentration. URX sensory receptors enable *C. elegans* to avoid and escape 21% O₂. We find that URX neurons combine two putative molecular O₂ sensors, a soluble guanylate cyclase and a neuroglobin, to sculpt a sigmoidal O₂ tuning curve in which the neurons show little Ca²⁺ response to stimuli below 13% O₂, gradually increase their responsiveness above this O₂ concentration, show a sharp increase in responsiveness between 15 and 19% O₂, and approach saturation as O₂ approaches 21%. The neuroglobin GLB-5 imposes the sigmoidal function by inhibiting the O₂-evoked Ca²⁺ response in URX when O₂ levels fall below 21%. When GLB-5 is defective, the URX stimulus–response curve is shifted to lower O₂ levels and approaches saturation at 14% O₂. At a behavioral level, the effects of GLB-5 signaling are to broaden the O₂ environments preferred by *C. elegans* while maintaining avoidance of 21% O₂. If glb-5 is defective, as in the N2 laboratory strain or the glb-5(tm5440) mutant, animals prefer a narrow O₂ range, from 7 to 10%. Animals with functional glb-5 signaling distribute more broadly, from 17 to 5% O₂. It will be interesting to explore if other sensory neurons that exhibit steep sigmoidal tuning curves at defined intensity intervals achieve their properties by combining antagonistic molecular sensors. Studies of O₂ sensing in the glomus cells of the carotid bodies of mammals have implicated multiple O₂-sensing mechanisms that could act together to sculpt O₂ response features (4). Similarly, a range of CO₂/pH-responsive molecules have been identified in mammals, although whether any of the numerous CO₂/pH-responsive cells use a combination of transducers is unclear (24).

Unexpectedly, we find that the relationship between URX Ca²⁺ response (a proxy of O₂ stimulus intensity) and behavioral output is nonlinear. Whereas intermediate stimulation of URX induces animals to reverse, strong stimulation is less effective.

---

**Fig. 5.** A computational model that links O₂-evoked Ca²⁺ responses in URX to behavioral output. (A) Schematic of the computational model. (B) Heat map representing the location of 10000 fictive glb-5(tm5440) or glb-5(Haw) animals in a 7–21% O₂ gradient. Locations are plotted every second. (C) Histograms of the existence frequency of glb-5(tm5440) and glb-5(Haw) in 7–21% O₂ gradient during the last 100 s of the computational experiments shown in B. The fictive URX responses and reversal frequency of these model worms during aerotaxis are shown in Fig. S8. (D) The modified aerotaxis results from Fig. 1A are shown to compare results of computational experiments and those of aerotaxis experimental data.
We have not investigated the neural mechanisms that underpin nonlinear control of reversals by URX. However, the neuroanatomical reconstructions reveal synapses from URX to both AVE interneurons that promote reversals and AVB interneurons that promote forward movement (21, 25) (wormwiring.org), which could be differentially regulated according to URX stimulation.

Several computational models have been constructed to elucidate behavioral mechanisms underlying C. elegans taxis behavior (26–28). These models have been built using detailed observation of animals moving in gradients. A taxis model that incorporates quantitatively measured neural activities has not, however, been reported but is required to understand how neural signals are processed and transformed to behavior. We incorporated URX Ca\(^{2+}\) responses measured using GCaMP6s into a random walk model. These data can be extended into a more detailed model to study neural circuits of C. elegans at a systems level, e.g., incorporating activities of interneurons and motor neurons, to probe how encoded neural information in neural circuits are used to evoke worm behaviors.

C. elegans respond to changes in O\(_2\) by altering both their speed and their reversal behavior. Previous work has shown that worms use a klinokinesis strategy, where frequency of reversal is changed depending on the concentration of stimuli, when aerotaxing in the absence of food (29). This strategy resembles that used by worms chemotaxing to other cues such as salts and odors (26, 30). Our quantitative experiments show that the URX oxygen sensors evoke reversals in response to O\(_2\) stimuli that have only minor effects on speed. Our computational experiments can replicate the results of aerotaxis experiments without incorporating O\(_2\)-evoked modulation of speed. These results imply that modulation of reversal is more important than modulation of speed when C. elegans navigates O\(_2\) gradients. The persistent stimulation of rapid movement when O\(_2\) levels approach 21% may conversely enable animals to escape from the surface when animals cannot detect an O\(_2\) gradient.

How does the GLB-5 neuroglobin alter the Ca\(^{2+}\) responses of neurons at a molecular level? Like mammalian neuroglobin (31), GLB-5 rapidly oxidizes to a ferric form at 21% O\(_2\) (9), suggesting it could participate in ROS or redox signaling. In URX, GLB-5 colocalizes with GCY-35/GCY-36 soluble guanylate cyclases at dendritic endings (10, 16) and could potentially regulate the function of this other heme-binding protein. Our cGMP imaging did not reveal GLB-5-dependent differences in the O\(_2\)-evoked responses of URX. However, the cGMP dynamics we measured in the URX cell body were very slow compared with the Ca\(^{2+}\) response, which implies that we are measuring a highly filtered response compared with the cGMP dynamics pertaining at the cGMP-gated channel. Although we do not exclude a role for GLB-5 in regulating soluble guanylate cyclases, our data suggest that GLB-5 can alter neural responses independently of these molecules.

In summary, we find that a neuroglobin can participate in neural information processing. The C. elegans genome encodes a variety of other neurally expressed globins that may similarly modify neural function (8). It would be interesting to investigate whether neuroglobin alters information processing in vertebrate neural circuits.

Methods

Strains. Animals were grown at 22–23 °C under standard conditions on Nematode Growth Medium (NGM) seeded with Escherichia coli OP50 (32). Strains used are listed in Supporting Information.

Neural Imaging. Immobilized animals. Animals expressing GCaMP6s or eGFP were glued to agarose pads (2% in M9 buffer) using Dermabond tissue adhesive (Ethicon), with the nose and tail immersed in E. coli OP50 unless otherwise indicated. Glued worms were covered with a polydimethylsiloxane (PDMS) microfluidic chamber, as described previously (12), and imaged using a 40× C-Apochromat lens on an inverted microscope (Axiovert; Zeiss) equipped with a Dual View emission splitter (Photometrics) and a Cascade II 512 electron multiplying charge coupled device (EMCCD) camera (Photometrics). The filters used were as follows: GCaMP6s/mCherry, ex480/15 and 565/15 nm, di525/25 and 625/45 nm, em520/30 nm, em630/50 nm, and di656 nm; and YFP-CFP FRET, ex430/20 nm, di450 nm, em480/30 nm, em535/40 nm, and di505 nm. Fluorescent images were captured at 1 frame per second (fps) with 2 × 2 or 1 × 1 binning using MetaMorph acquisition software (Molecular Devices). Data analysis used MATLAB (MathWorks) and Igor Pro (WaveMetrics). All time-lapse imaging data were denoised using binomial smoothing (Gaussian filter).

Delivery of gas stimuli. Humidified gas mixtures of defined composition were delivered using a PHD 2000 infusion syringe pump (Harvard Apparatus). The flow rate was 1.0 mL/min for all ramp stimuli and 2.0 mL/min for all step stimuli. Syringes containing gas mixtures were connected to PDMS chambers via polyethylene tubing and Teflon valves (Automate Scientific). A custom-built frame counter switched the valves at precise time points using transistor–transistor logic pulses from the camera. To create the ramp stimulation, we used backwash air from the outlet of the PDMS chamber. O\(_2\) stimuli in chambers were measured using an O\(_2\) probe (Oxygen Sensor Spots PS53; PreSens).

Behavioral Assays. Aerotaxis assays were performed as described previously (11); animal positions were noted 25 min into the assay. Briefly, rectangular PDMS chambers (33 × 15 × 0.2 mm) connected at either end to syringe pumps delivering the indicated gas concentrations were placed on a 100 worms on a 9-cm NGM agar plate with food (E. coli OP50). The distribution of worms was recorded by counting animals in each of nine equal areas of the chamber.

To measure behavioral responses to step O\(_2\) stimuli, five adult hermaphrodites were placed on NGM plates seeded 36–40 h earlier with 20 μL of E. coli OP50 grown in 2x TY medium. To create a behavioral arena with a defined atmosphere, we placed a PDMS chamber (1 × 1 × 0.2 cm) on top of the worms, with inlets connected to a PHD 2000 Infusion syringe pump (Harvard Apparatus), and delivered humidified gas mixtures of defined composition at a flow rate of 3.0 mL/min. Videos were captured at 2 fps using FlyCapture software (Point Gray) on a Grasshopper camera (Point Gray) mounted on a Leica M165FC stereo microscope. Videos were analyzed using custom-written MATLAB software to calculate instantaneous speed. Individual speed data were binned over 6 s. Reversals were defined as a change in direction of locomotion in the model. The locomotion direction after the reversal was randomly chosen from a uniform distribution (0,2π) because experimentally measured reversals contain turning events. We assumed that the relationship between URX responses and reversal frequency was approximately linear in our model. This applies because animals in the virtual O\(_2\) gradient, like those in a real-life aerotaxis assay, do not encounter large step O\(_2\) stimuli.

Computational Experiments and Modeling. In the computational model, a worm was represented as a single point in a virtual field that represented an O\(_2\) gradient in our experimental 18 mm (W) × 15 mm (L) aerotaxis chamber. We updated the virtual chamber from 3% O\(_2\) at x = 18 mm. The worm moved forward either at constant (0.05 mm/s) or at variable speed. For iterations when speed varied according to O\(_2\) concentration at the animal’s position we acquired parameters for speed by performing curve fitting with a Hill equation using the speed data shown in Fig. 4C (Fig. S11 B and C). The trend in an averaged time series was identified and defined atmosphere, we placed a PDMS chamber (1 × 1 × 0.2 cm) on top of the worms, with inlets connected to a PHD 2000 Infusion syringe pump (Harvard Apparatus), and delivered humidified gas mixtures of defined composition at a flow rate of 3.0 mL/min. Videos were captured at 2 fps using FlyCapture software (Point Gray) on a Grasshopper camera (Point Gray) mounted on a Leica M165FC stereo microscope. Videos were analyzed using custom-written MATLAB software to calculate instantaneous speed. Individual speed data were binned over 6 s. Reversals were defined as a change in direction of locomotion in the model. The locomotion direction after the reversal was randomly chosen from a uniform distribution (0,2π) because experimentally measured reversals contain turning events. We assumed that the relationship between URX responses and reversal frequency was approximately linear in our model. This applies because animals in the virtual O\(_2\) gradient, like those in a real-life aerotaxis assay, do not encounter large step O\(_2\) stimuli.

The dynamics of the sensory circuit were represented by an NLM model. This NLM model consisted of two nonlinear static filters and a linear temporal filter. O\(_2\) stimulation was first converted by the input nonlinear filter, processed by the temporal filter, then converted by the output nonlinear filter. The nonlinear filter f(x) was expressed using a Hill equation,

\[
f(x) = x^n / (x^m + x^n),
\]

where \(x_0\) and \(n\) were the parameters that defined the range and strength of the nonlinearity of the filter, respectively. For convenience, the input and
output nonlinear filter are hereafter denoted as \( f_n \) and \( f_{\text{out}} \) respectively. The linear temporal filter \( K \) has a 361 sample length \( (t = 0, 1, \ldots, 360) \) and satisfies

\[
y = Uk,
\]

where \( U \) and \( y \) are the input and output time courses of the temporal filter, respectively. This typical expression of a temporal filter should be expanded because our dataset has multiple time courses (multidose). If \( O_2 \) concentration is left as \( x \), \( U \) can be written as

\[
U = [U_1, U_2, \ldots, U_{\text{max}}]^	op,
\]

\[
U_{\text{out}} = f_n\left(\begin{array}{c} x_0(0) - t_{\text{max}} \\ x_0(1) - t_{\text{max}} \\ \vdots \\ x_0(t_{\text{max}}) \\ x_0(0) \\ x_0(1) \\ \vdots \\ x_0(t_{\text{max}}) \end{array}\right),
\]

where \( x_0(t) \) corresponds to the \( O_2 \) concentration at time \( t \) of dth step stimulation and \( x_0(t = 0) \) is replaced by \( x_0(0) \). \( y \) can be expressed as

\[
y = [y_1, y_2, \ldots, y_{\text{max}}]^	op,
\]

\[
y_{\text{out}} = [y_0(0) y_1(1) \ldots y_{\text{max}}(t_{\text{max}})]^	op,
\]

where \( f_{\text{out}}(y_0(t)) \) corresponds to the response of the sensory neuron at time \( t \) in response to \( d \)th oxygen step stimulation. The linear temporal filter \( K \) can be obtained by evaluating

\[
K = (U^T U)^{-1} U^T y .
\]

For denoising, singular value decomposition was applied, and the largest 100 components were used. To find the value of parameters of nonlinear filters, the Nelder–Mead simplex optimization method was used, and the sum of the square difference between \( f_{\text{out}}(y_0(t)) \) and corresponding experimental Ca\(^{2+} \) responses of URX were minimized. Because this optimization was done separately for \( \text{gib}-5(\text{Haw}) \) and \( \text{gib}-5(\text{m5440}) \), we obtained two parameter sets for the NLN model.

The interneuron and command neurons were designed as described below. Because the worms show random reversals, the command neuron should be randomly activated. Furthermore, because the basal URX Ca\(^{2+} \) response (i.e., before stimulation of 2% change of oxygen) depends on the basal concentration of \( O_2 \) but basal reversal frequency does not, the model should contain a temporal differentiation functionality. Therefore, the activity of interneuron \( g(t) \) was modeled as

\[
g(t) = f_{\text{out}}(y_0(t)) - \sum_{i=1}^{I} f_{\text{out}}(y_0(t - i))/l,
\]

where \( l \) is a lag constant and was initially fixed as 11 and then varied (Fig. S9). The activity of command neuron is positive when

\[
r < b(1 + g(t)c),
\]

where \( b \) is the basal reversal frequency that is computed from experimental data, \( c \) is the coefficient of the effect of \( O_2 \) stimulation, and \( r \) is a uniformly distributed random number between 0 and 1. \( b \) and \( c \) were fixed to 0.0723 and 0.0009, respectively (reversal frequency per 1 s before a stimulation is given) and 3, respectively; \( c \) was subsequently varied (Fig. S9). Note that the parameters of interneuron and command neuron \( (l, b, \text{and } c) \) are independent of the gib-5 genotype.

ACKNOWLEDGMENTS. We thank the National Bioresource Project (Japan) for strains; W. Schafer and Y. Iino for plasmids; and L. Beets, L. Fenh, T. Tomida, and S. Laughlin for comments on the manuscript. This work was supported by the Medical Research Council (MC, U105178876) and the European Research Council (AdG 269058) (to M.d.B.); the Uehara Memorial Foundation (S.O.); Grants-in-Aid for Young Scientists (B) (26830006 to Y.T.); and Grant-in-Aid for Scientific Research on Innovative Areas (16H01418 “Resonance Bio” and 17H05970) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to Y.T.).

1. Butts DA, Goldman MS (2006) Tuning curves, neuronal variability, and sensory coding. PLoS Biol 4:e92.
2. Dayan P, Abbott LF (2005) Theoretical Neuroscience: Computational and Mathematical Modeling of Neural Systems, Computational Neuroscience Series (MIT Press, Cambridge, MA).
3. Ward JP (2008) Oxygen sensors in context. Biochim Biophys Acta 1771:1-14.
4. López-Barneo J, et al. (2016) Oxygen sensing by the carotid body: Mechanisms and role in adaptation to hypoxia. Am J Physiol Cell Physiol 310:C629–C642.
5. Bursmester T, Hankeln T (2014) Function and evolution of vertebrate globins. Acta Physiol (Oxf) 211:501-514.
6. Bursmester T, Weich B, Reinhardt S, Hankeln T (2000) A vertebrate globin expressed in the brain. Nature 407:520-523.
7. Bursmester T, Hankeln T (2009) What is the function of neuroglobin? J Exp Biol 212:1423–1428.
8. Tillman L, et al. (2011) Globins in Caenorhabditis elegans. JUBMB Life 63:166-174.
9. Persson A, et al. (2009) Natural variation in a neural globin tunes oxygen sensing in wild Caenorhabditis elegans. Nature 458:1030–1033.
10. McGeorge PT, et al. (2009) Quantitative mapping of a drosophila behavioral trait implicates globin variation in C. elegans sensory behaviors. Neuron 61:692-699.
11. Gray JM, et al. (2004) Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. Nature 430:317–322.
12. Bush KE, et al. (2012) Tonic signaling from O2 sensors sets neural circuit activity and behavioral state. Nat Neurosci 15:581–591.
13. Cheung BH, Arellano-Carabal F, Rybicki I, de Bono M (2004) Soluble guanylate cy- clase act in neurons exposed to the body fluid to promote C. elegans aggregation behavior. Curr Biol 14:1105–1111.
14. Couto A, Oda S, Nikolaevo YO, Soltesz Z, de Bono M (2013) In vivo genetic dissection of O2-evoked cGMP dynamics in a Caenorhabditis elegans gas sensor. Proc Natl Acad Sci USA 110:E3001–E3101.
15. Prabhakar NR, Semenza GL (2015) Oxygen sensing and homeostasis. Physiology (Bethesda) 30:340–348.
16. Gross E, et al. (2014) GLOBIN-5-dependent O2 responses are regulated by PDL-19pBR that targets prenylated soluble guanylate cyclases to dendritic endings. J Neurosci 34: 16726–16738.