Memory of recent oxygen experience switches pheromone valence in *Caenorhabditis elegans*

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Animals adjust their behavioral priorities according to momentary needs and prior experience. We show that *Caenorhabditis elegans* changes how it processes sensory information according to the oxygen environment it experienced recently. *C. elegans* acclimated to 7% O_2 are aroused by CO_2 and repelled by pheromones that attract animals acclimated to 21% O_2. This behavioral plasticity arises from prolonged activity differences in a circuit that continuously signals O_2 levels. A sustained change in the activity of O_2-sensing neurons reprograms the properties of their postsynaptic partners, the RMG hub interneurons. RMG is gap-junctionally coupled to the ASK and ADL pheromone sensors that respectively drive pheromone attraction and repulsion. Prior O_2 experience has opposite effects on the pheromone responsiveness of these neurons. These circuit changes provide a physiological correlate of altered pheromone valence. Our results suggest *C. elegans* stores a memory of recent O_2 experience in the RMG circuit and illustrate how a circuit is flexibly sculpted to guide behavioral decisions in a context-dependent manner.

neural circuit | experience-dependent plasticity | tonic circuit | oxygen sensing | acclimation

The body comprises multiple highly integrated subsystems working together to sustain life from moment to moment and over long time scales (1). Much of this coordination involves dynamically interacting neural circuits that optimize responses to current circumstances by taking into account sensory input, organismal state, and previous experience (2–8). Circuit cross-talk enables animals to adjust their behavioral priorities to changing environments, e.g., variation in temperature, humidity, day length, or oxygen (O_2) levels (9–13). Whereas some behavioral adjustments can be rapid (14, 15), others develop over time, as animals adapt to changed conditions. How animals store information about their recent environment, and use this information to modify behavioral choices, is poorly understood.

The compact nervous system of *Caenorhabditis elegans*, which comprises only 302 uniquely identifiable neurons (wormwiring.org) (16), provides an opportunity to study the links between prior environmental experience, circuit plasticity, and behavioral change. This nematode is adapted to a life feeding on bacteria in rotting fruit (17). It has sensory receptors for odors, tastants, pheromones, and respiratory gases, as well as temperature, mechanical, and noxious cues (18–21). Despite this simplicity, the mechanisms by which its nervous system marshals information about past and present sensory experience to shape behavioral priorities remain largely mysterious. The anatomical connectome, while valuable (22), is insufficient to explain or predict neuronal network function (23, 24), partly because neuromodulators can dynamically reconfigure and specify functional circuits (25–27).

When ambient O_2 approaches 21%, *C. elegans* wild isolates become persistently aroused and burrow to escape the surface (28–30). This state switch is driven by tonically signaling O_2 receptors called URX, AQR, and PQR (31, 32) whose activity increases sharply when O_2 approaches 21% (28, 30, 33, 34). The URX neurons are connected by gap junctions and reciprocal synapses to the RMG interneurons, and tonically stimulate RMG to promote escape from 21% O_2 (wormwiring.org) (16, 35). URX and RMG are both peptidergic, and at 21% O_2 tonically release neuropeptides (28, 35). Gap junctions connect RMG to several other sensory neurons besides URX, including pheromone sensors (16, 36). Whether information communicated from URX to RMG about the O_2 environment modulates other sensory responses is unknown.

Here, we show that acclimating *C. elegans* to different O_2 environments gradually reconfigures this animal's response to sensory cues. Animals acclimated to 7% O_2 but not 21% O_2 are aroused by CO_2. Pheromones that attract animals acclimated at 21% O_2 repel animals acclimated to 7% O_2. These changes are driven by experience-dependent remodeling of URX O_2 sensors, RMG interneurons, and the ASK and ADL pheromone sensors.

Results

Acclimation to Different O_2 Environments Reprograms CO_2 Responses. *C. elegans* escape 21% O_2, which signals that animals are at the surface, and accumulate at lower O_2 levels, which likely indicate that animals are burrowed (28, 30, 31). We speculated that *C. elegans* gradually change their sensory preferences when shifted between these environments. To test our hypothesis, we first examined responses to CO_2. CO_2 is aversive to *C. elegans*, and, due to respiration, its concentration will typically rise as O_2 levels fall. Animals escaping 21% O_2 will thus often encounter high CO_2, creating conflicting drives that could be ecologically significant. Previous work showed that natural *C. elegans* isolates immediately suppress CO_2 avoidance when O_2 levels approach 21%, due to increased tonic signaling from URX O_2 sensors (37–40). We speculated that not only current but also prior O_2 experience changes *C. elegans*'s CO_2 responses. To test this speculation, we kept wild isolates from California, France, and Hawaii overnight at 21% or 7% O_2, and compared their responses to 3% CO_2 on a thin

Significance

Animals use memories of their recent environment to regulate their behavioral priorities. The basis for this cross-modal, experience-dependent plasticity is poorly understood. *Caenorhabditis elegans* feeds on bacteria in rotting fruit. It monitors O_2 levels, and switches behavioral state when O_2 approaches 21%. We show that *C. elegans*' memory of its recent O_2 environment reconfigures how it processes sensory information. Pheromones that attract animals acclimated to 21% O_2 repel animals acclimated to 7% O_2. O_2 memory is encoded in the activity history of a circuit that continuously signals O_2 levels. This circuit is connected to neurons driving pheromone attraction and repulsion. O_2 experience changes the pheromone responsiveness of these sensors and their postsynaptic targets, correlating with the switch in pheromone valence.

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lawn of bacteria kept at 7% O_2. After halting briefly, animals acclimated to 7% O_2 became persistently aroused at 3% CO_2 (Fig. S1 *A*–*C*), whereas animals acclimated to 21% O_2 showed little change in locomotory activity.

To probe this plasticity, we studied the N2 laboratory strain. Unlike natural isolates, N2 is aroused by 3% CO_2 regardless of prior O_2 experience (Fig. 1*A* and Fig. S2*A*). N2 animals have altered responses to 21% O_2, because output from the RMG interneurons, a major relay of the circuit signaling 21% O_2, is blocked by a hyperactive neuropeptide receptor, NPR-1 215V (35, 36). Natural *C. elegans* isolates have a less active receptor, NPR-1 215F, which does not block RMG output (41, 42). Does this account for altered CO_2 responses? Disrupting *npr-1* caused N2 animals to behave like natural isolates: They were not aroused by CO_2 if previously acclimated to 21% O_2 (Fig. 1*A*). The effects of acclimating *npr-1* mutants to 7% O_2 developed over 16 h and were reversed within 3 h if animals were transferred to 21% O_2 (Fig. S2 *B* and *C*). Selectively expressing NPR-1 215V in RMG interneurons prevented *npr-1* animals from acclimating to 21% O_2—restoring N2-like behavior (Fig. 1*B*). Disrupting the GCY-35 soluble guanylate cyclase, a molecular O_2 sensor in URX required for the URX—RMG circuit to signal 21% O_2 (28, 30, 31, 33, 43) had the same effect (Fig. 1*C*).

The NPR-1 215V receptor isoform inhibits RMG peptidergic transmission (35). This finding led us to speculate that circuitry changes caused by prior O_2 experience might reflect prolonged differences in RMG peptidergic release. To test this possibility, we selectively knocked down the carboxypeptidase E ortholog *egl-21* in RMG using RNAi. Processing of most *C. elegans* neuropeptides depends on EGL-21 (44). RMG knockdown of *egl-21* prevented *npr-1* animals from acclimating to 21% O_2 in the CO_2 assay (Fig. 1*D*). These data suggest that neuropeptide release from RMG is required for *C. elegans* acclimated to 21% O_2 to suppress CO_2-evoked arousal.

Pheromone Valence Changes with Prior O_2 Experience. We studied how prior O_2 experience alters CO_2 responses because the special relationship between these gases has ecological implications. However, *C. elegans* has many CO_2-responsive neurons, complicating analysis of how persistent differences in RMG activity alter the CO_2 circuits (38, 45, 46). Several studies have reported differences in the sensory responses of N2 and *npr-1* mutants that are associated with altered RMG function (35, 36, 47, 48). We speculated that at least some of these behavioral differences could reflect a diminished capacity of N2 animals to acclimate to different O_2 environments due to reduced neurosecretion from RMG.

One such behavior is pheromone preference (36). Select pheromone blends attract *npr-1* hermaphrodites but repel N2 hermaphrodites (36, 49). We replicated these observations using an equimolar 10 nM mix of asc-ωC3 (also called ascaroside C3, ascr#5 and daumone 5), asc-C6-MK (also called ascaroside C6, ascr#2 and daumone 2), and asc-ΔC9 (also called ascaroside C9, ascr#3 and daumone 3) pheromones (36, 50–52), (Fig. 2*A*). We then asked whether acclimating N2 and *npr-1* hermaphrodites overnight in different O_2 environments altered their pheromone response. We assayed animals at 21% O_2. Whereas *npr-1* animals acclimated to 21% O_2 were attracted to the pheromone mix, *npr-1* animals acclimated to 7% O_2 avoided it (Fig. 2*B*). Acclimating N2 animals at different O_2 levels did not alter pheromone avoidance (Fig. 2*B*), recapitulating our observations with CO_2 (Fig. S2*C*). Disrupting *gcy-35* switched the pheromone attraction exhibited by *npr-1* animals acclimated at 21% O_2 into repulsion (Fig. 2*B*). In summary, reducing the activity of O_2 sensing circuitry for prolonged periods of time—either via environmental or genetic manipulation—transforms pheromone attraction to pheromone avoidance.

O_2 Experience Changes Pheromone Responses in ASK Neurons. How does prior O_2 experience switch pheromone valence? The altered behavior must reflect some lasting change in the circuitry that couples sensory detection to motor output. The principal neurons driving pheromone attraction are the ASK ciliated head neurons. Pheromone evokes a decrease in Ca^{2+} levels in ASK (the "ON" response) that quickly returns to above baseline when pheromone is removed (the "OFF" response). The pheromone-evoked Ca^{2+} response in ASK is bigger in *npr-1* animals compared with N2 animals, a difference thought to contribute to the opposite pheromone preference of these strains (36). Does prior O_2 experience change the responsiveness of ASK neurons to pheromones? To test this possibility, we measured pheromone-evoked Ca^{2+} responses in ASK using the ratiometric Ca^{2+} indicator YC3.60. Overnight acclimation at 7% O_2 attenuated the ASK pheromone response in *npr-1* animals to levels found in N2 (Fig. 2 *C* and *D*). Thus, prior O_2 experience alters ASK pheromone responses, commensurate with a change in behavioral preference.

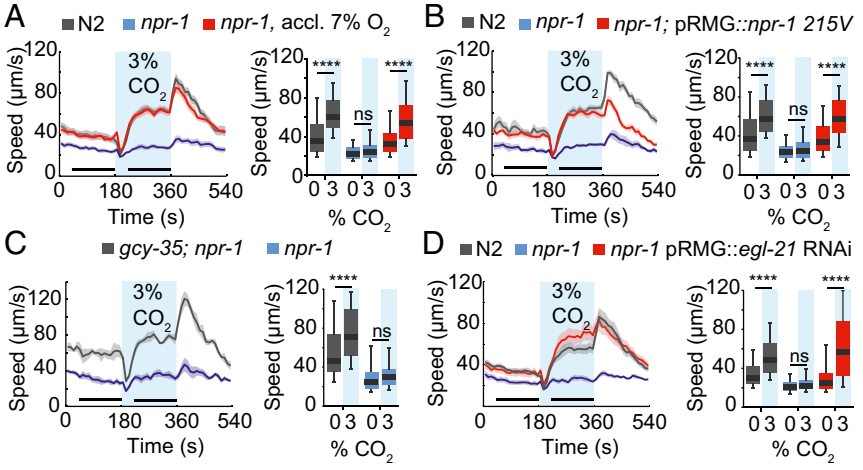

Fig. 1. Recent O_2 experience regulates CO_2-evoked arousal. (*A*) N2 animals and *npr-1* animals acclimated to 7% O_2 persistently increase their speed when CO_2 rises to 3%; *npr-1* animals acclimated to 21% O_2 do not. $n = 247$–302 animals. ****$P < 0.0001$; ns, not significant; Wilcoxon signed-rank test. Animals were assayed on food at 7% O_2. In this and subsequent figures, animals were acclimated to 21% O_2 unless noted otherwise; solid lines indicate the mean and shaded areas, the SEM. Black bars here and throughout indicate intervals used for statistical comparisons; boxplots show the median and the 25th–75th percentiles; whiskers represent 10th–90th percentiles. (*B*) Selectively expressing NPR-1 215V in RMG, (*C*) disrupting *gcy-35*, or (*D*) RNAi knockdown of EGL-21 in RMG prevent *npr-1* animals acclimated to 21% O_2 from suppressing CO_2-evoked arousal. $n = 104$–235.

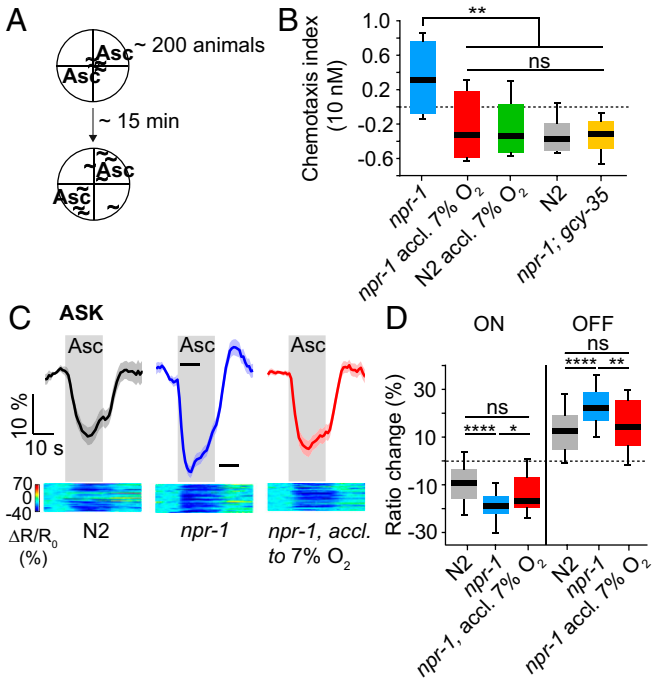

Fig. 2. Pheromone valence changes with prior O_2 experience. (*A*) Quadrant assay for pheromone preference (as in ref. 36). (*B*) Behavioral responses to an equimolar 10 nM mix of C3, C6, and C9 ascaroside pheromones. *npr-1* animals acclimated to 21% O_2 are attracted to the pheromone, whereas siblings acclimated to 7% O_2 avoid it. N2 avoid pheromones irrespective of whether they have been acclimated to 7% or 21% O_2. The soluble guanylate cyclase GCY-35 is required for normal O_2 responses and pheromone attraction in *npr-1* animals acclimated at 21% O_2. $^{**}P < 0.01$; ns, not significant; one-way ANOVA with Tukey's multiple comparisons test. $n = 8$ assays each. (*C*) Previous O_2 experience sculpts pheromone responses in ASK sensory neurons. Acclimation to 7% O_2 reduces pheromone-evoked Ca^{2+} responses in ASK, consistent with altered behavioral preference. The gray shading in this and subsequent figures indicates addition of pheromone. (*D*) Quantification of data shown in *C*. Heat maps in this and all subsequent figures show individual Ca^{2+} responses. $n = 35$–36 animals. $^{****}P < 0.0001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant; Mann–Whitney *U* test.

Peptidergic Feedback Heightens RMG Responsiveness to 21% O_2 After Sustained Exposure to 21% O_2. RMG interneurons are connected to both the URX O_2 receptors and the ASK pheromone sensors via gap junctions (wormwiring.org) (16, 36). A simple prediction made by our data is that the response properties of RMG change when *npr-1* animals are acclimated to different O_2 levels, and this alters the properties of ASK. To explore this prediction, we compared RMG Ca^{2+} responses to a 7–21% O_2 shift in *npr-1* animals previously acclimated to either 21% or 7% O_2. Animals acclimated to 7% O_2 showed significantly smaller RMG responses than those acclimated to 21% O_2 (Fig. 3 *A* and *B*). Thus, prolonged exposure to 21% O_2 augments RMG responses to this stimulus.

RMG responses to 21% O_2 are driven by URX neurons (35). Do the altered RMG properties in animals acclimated to 7% O_2 ultimately reflect reduced input from URX? URX responses were smaller in animals acclimated to 7% O_2 (Fig. 3 *C* and *D*), suggesting that changes in RMG properties at least partly reflect plasticity in URX. However, a causal relationship remains to be proven, because RMG could feed back to alter URX properties following acclimation to 7% O_2.

If we selectively knocked down peptidergic transmission from RMG, by RNAi of *egl-21* CPE, *npr-1* animals acclimated to 21% O_2 failed to increase RMG responsiveness to a 7–21% O_2 stimulus (Fig. 3 *A* and *B*). These data suggest there is a positive feedback loop by which tonic peptidergic signaling from RMG in *npr-1* animals previously kept at 21% O_2 increases RMG responsiveness to 21% O_2. Thus, experience-dependent plasticity in RMG and URX represents a neural correlate of acclimation to different O_2 environments.

Importantly, RNAi knockdown of *egl-21* in RMG also altered pheromone-evoked Ca^{2+} responses in the ASK neurons of *npr-1* animals acclimated to 21% O_2, reducing them to N2-like levels (Fig. 3 *E* and *F*). The physiological effects on ASK and RMG were accompanied by a behavioral switch to pheromone avoidance (Fig. 3*G*). Thus, peptidergic signaling from RMG appears to mediate multiple effects of acclimation to 21% O_2: an increased tonic Ca^{2+} response to 21% O_2 in RMG, a bigger ASK response to pheromone cues, and decreased *C. elegans* avoidance of pheromone.

Communication Between Neurons in the RMG Circuit. The wiring diagram suggests RMG is electrically connected to multiple sensory neurons through gap junctions, including the ASK neurons, the ADL and ASH nociceptors, the AWB olfactory neurons, and the IL2 chemo/mechanoreceptors (Fig. 4*A*) (wormwiring.org) (16). Altered RMG properties may therefore directly influence each of these electrically coupled neurons, and vice versa. Previous studies suggest that the O_2-sensing URX neurons cooperate with the nociceptive ADL and ASH neurons and the ASK pheromone sensors, to promote *C. elegans* aggregation and escape from 21% O_2 (32, 35, 36, 53). However, in the absence of physiological data, it is unclear what information RMG neurons receive or transmit, apart from tonic O_2 input from URX (28, 35). We first asked whether O_2-evoked responses in RMG propagated to ADL and ASK. The wiring diagram suggests ASK and ADL are connected to RMG exclusively via gap junctions. ASK and ADL each showed O_2-evoked Ca^{2+} responses in *npr-1* animals (Fig. S3 *A*–*D*). Conversely, RMG responded to stimulation with the pheromone mix we used to stimulate ASK (Fig. 4*B*); the dynamics of the response are remarkably similar to those observed in ASK, and a robust ON response was typically followed by an increase—or rebound—beyond baseline levels after stimulus removal (OFF response) (Fig. 4*B*). These results lend direct support to a hub-and-spoke model in which multiple sensory inputs are integrated through gap junctions with the RMG hub (36).

NPR-1 215V signaling has been proposed to silence the hub-and-spoke circuit (23, 26). One attractive model is that signaling from the neuropeptide receptor closes RMG gap junctions (36, 49). To investigate this model, we first compared pheromone-evoked Ca^{2+} responses in RMG in N2 and *npr-1* animals, but did not observe any significant differences (Fig. 4 *C* and *D*). We then compared O_2-evoked responses in ASK and also did not observe differences between the two genotypes (Fig. S3 *A* and *B*). By contrast, *npr-1* but not N2 animals displayed a strong O_2-evoked response in ADL neurons (Fig. S3 *C* and *D*). Disrupting GCY-35, the molecular O_2 sensor in URX, abolished the ADL O_2 response in *npr-1* animals (Fig. S4 *A* and *B*). Expressing *gcy-35* cDNA selectively in URX neurons restored the ADL O_2 response. These data suggest the URX O_2 response drives the ADL O_2 response. Sensory responses in ADL require the TRPV1 ortholog OCR-2 (Fig. S4 *C* and *D*) (49). By contrast, the ADL response to O_2 did not require OCR-2, consistent with URX neurons driving the ADL O_2 response and ADL retaining an ability to function as an interneuron in *ocr-2* mutants (Fig. S4 *E* and *F*). Although other interpretations are possible, a simple model to explain our data is that NPR-1 215V signaling in RMG affects different gap junctions differently, inhibiting RMG–ADL communication but having smaller or no effects on the RMG–ASK connection.

O_2 Experience Sculpts RMG and ADL Pheromone Responses. The pheromone attraction mediated by ASK neurons and promoted by RMG signaling is proposed to antagonize pheromone avoidance driven by the ADL neurons in a push–pull mechanism (49). The relative strength of these arms determines the animal's response. We found that acclimating *npr-1* animals to 7% O_2 greatly reduced pheromone-evoked responses in RMG compared with animals kept at 21% O_2 (Fig. 4 *C* and *D*). Thus, acclimation to 7% O_2 weakens both the ASK (Fig. 2 *C* and *D*) and RMG circuit

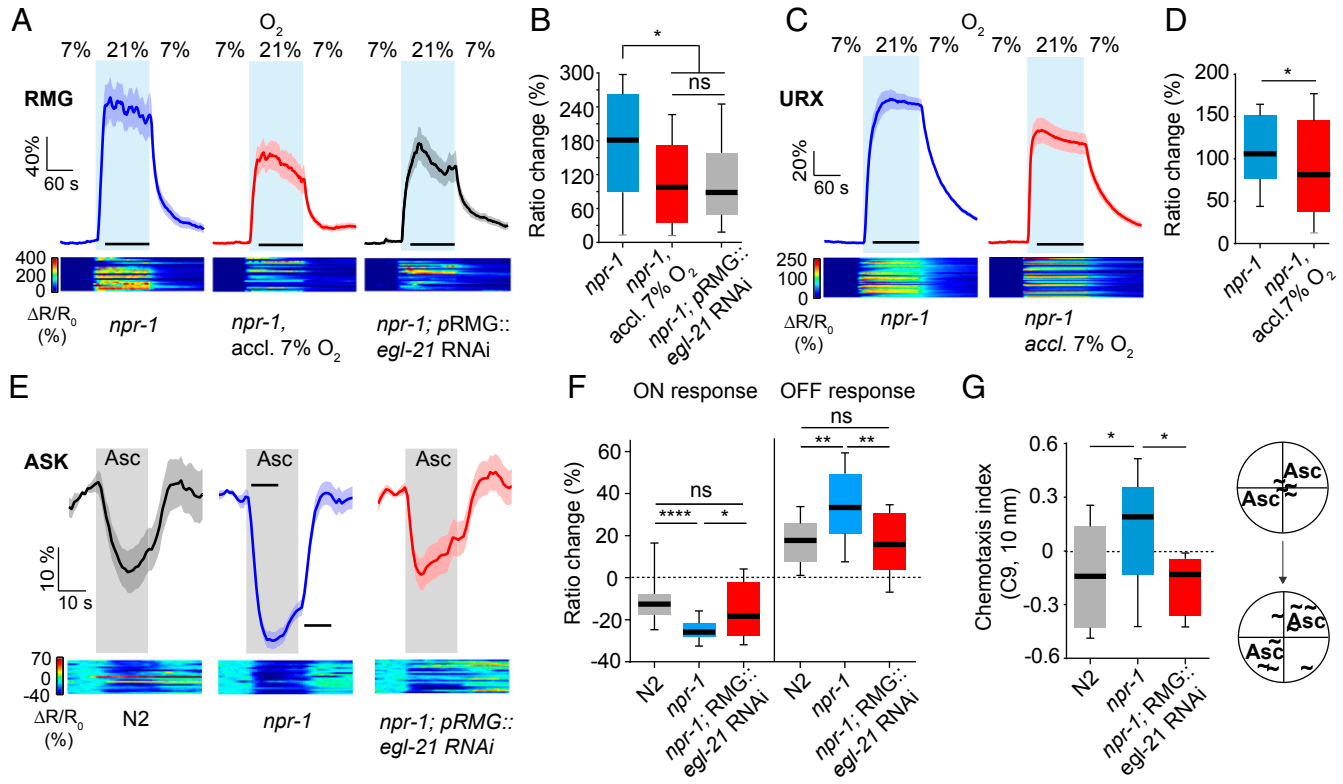

Fig. 3. Peptidergic feedback regulates RMG properties and pheromone preference. (*A* and *B*) Acclimation to 7% O_2, or knockdown of *egl-21*, similarly reduces the RMG Ca^{2+} responses evoked by a 7–21% O_2 stimulus. (*B*) Quantification of data in *A*. $n = 20–21$ animals. $^*P < 0.05$; ns, not significant; Mann–Whitney *U* test. (*C* and *D*) Acclimation to 7% O_2 reduces URX Ca^{2+} responses evoked by a 7–21% O_2 stimulus. (*D*) Quantification of data in *C*. $n = 38–39$ animals. $^*P < 0.05$; Mann–Whitney *U* test. (*E* and *F*) Knockdown of *egl-21* in RMG diminishes pheromone-evoked Ca^{2+} responses in ASK. (*F*) Quantification of data in *E*. $n = 20–21$ animals. $^*P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$; ns, not significant; Mann–Whitney *U* test. (*G*) RNAi knockdown of *egl-21* in RMG prevents *npr-1* animals acclimated to 21% O_2 from being attracted to pheromone. $n = 12$ assays. $^*P < 0.05$; one-way ANOVA followed by Dunnett's multiple comparisons test.

elements that drive attraction to pheromone. Does acclimation to different O_2 levels also alter pheromone responses in ADL? ADL neurons are activated by the asc-ΔC9 (previously known as ascaroside C9), and a drop of this pheromone increases the probability of animals reversing (49). In this behavioral paradigm, the fraction of animals reversing in response to a drop of C9 provides a measure of the pheromone's repulsiveness; this fraction is significantly higher in N2 than *npr-1* animals at low pheromone concentrations (10 nM) (49). We confirmed that N2 animals were more strongly repelled by 10 nM C9 than *npr-1* animals (Fig. 4*E*). We then showed that acclimating *npr-1* animals overnight at 7% O_2 enhanced their avoidance of C9, so that they behaved indistinguishably from N2 (Fig. 4*E*). The avoidance index (A.I.) used in this assay (49, 54) is calculated as [(fraction reversing to pheromone) − (fraction reversing to buffer alone)], and any change in the A.I. could reflect an altered response to the buffer rather than to C9. Consistent with enhanced pheromone avoidance, *npr-1* animals reversed more in response to C9 if they were acclimated to 7% O_2 (Fig. 4*E′*).

Pheromone-evoked Ca^{2+} responses in ADL neurons were previously characterized using 100 nM C9, a concentration that elicits strong and comparable repulsion in N2 and *npr-1* animals (49). By using GCaMP3 var500 we could record ADL responses to 10 nM C9 and assess the impact of previous O_2 experience under conditions similar to those used in behavioral assays. *npr-1* animals acclimated to 7% O_2 showed significantly bigger ADL Ca^{2+} responses compared with siblings acclimated to 21% O_2 (Fig. 4 *F* and *G*). Together our data suggest that acclimation to 7% O_2 simultaneously weakens the ASK and RMG circuit elements that drive attraction to pheromone and strengthens the ADL pheromone response driving repulsion, thereby switching the animal's behavioral choice.

Discussion

Unfavorable environments can evoke slow, sustained changes in behavioral priorities that reflect an altered internal state. The neural mechanisms mediating such integrative, experience-dependent plasticity are poorly understood. *C. elegans* persistently attempts to escape 21% O_2 (28), presumably because surface exposure is unfavorable (30, 31). We find that the O_2 environment experienced recently by *C. elegans* changes the way it processes sensory information. Pheromones that attract *C. elegans* acclimated to 21% O_2 repel animals acclimated to 7% O_2; 3% CO_2 triggers sustained arousal in animals acclimated to 7% O_2 but has comparatively little effect on the speed of animals acclimated to 21% O_2.

A memory of previous O_2 experience arises from prolonged differences in the activity of a tonically active circuit. Exposure to 21% O_2 tonically stimulates the URX O_2 receptor neurons and their synaptic partners, the RMG interneurons. Sustained stimulation of URX and RMG at 21% O_2 increases their signaling at 21% O_2. The reprogramming of RMG requires peptidergic signaling competence in this interneuron. Our data suggest a simple model in which over time, sustained peptide release from RMG at 21% O_2 feeds back to alter RMG properties. In animals kept at 7% O_2 peptide release from RMG is low, disrupting the feedback. In this neural integrator model the time required for animals to acclimate to 7% or 21% O_2 corresponds to hysteresis in the onset or decay of peptide signaling. We previously showed that neuropeptide expression in RMG is positively coupled to neurosecretion from RMG (36), further evidence of a positive feedback loop in this interneuron. Tonically signaling circuits are common in brains. It will be interesting to explore how often such circuits store information about their activity history, and

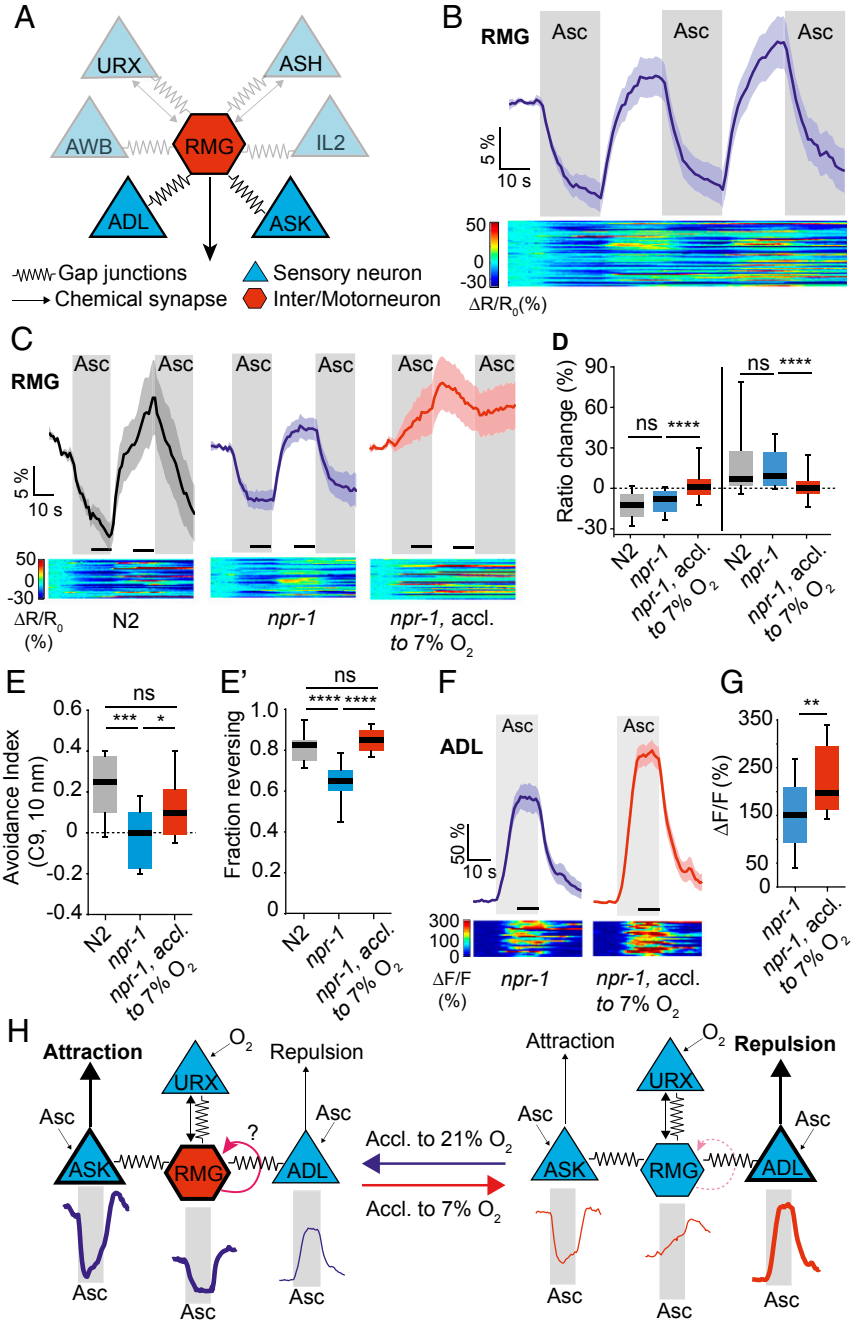

Fig. 4. RMG hub neurons respond to pheromones and alter their response according to recent O_2 experience. (*A*) Circuit showing connections between RMG interneurons and O_2-sensing, nociceptive, and pheromone-sensing neurons. (*B*) An equimolar (100 nM) mix of C3, C6, and C9 ascarosides evokes a decrease in RMG Ca^{2+} in *npr-1* animals acclimated to 21% O_2. $n = 57$ animals. (*C* and *D*) RMG shows robust pheromone-evoked Ca^{2+} responses in both N2 animals and *npr-1* animals acclimated to 21% O_2. Acclimating *npr-1* animals to 7% O_2 alters RMG properties and diminishes both ON and OFF responses to pheromone addition and removal. (*D*) Quantification of data shown in *C*. $n = 36$ animals each. $^{***}P < 0.001$; $^{****}P < 0.0001$; Mann–Whitney *U* test. (*E*–*G*) Acclimation to 7% O_2 enhances ADL pheromone responses and acute pheromone repulsion. *npr-1* animals show decreased avoidance of the C9 ascaroside compared with N2 when grown under standard conditions (~21% O_2), but not when acclimated to 7% O_2. Plotted are the avoidance index (*E*) and fraction of animals reversing (*E'*), in response to a drop of diluted C9 (10 nM) applied to the nose. $n = 260$–280 animals each. $^{*}P < 0.05$; $^{***}P < 0.001$; $^{****}P < 0.0001$; ns, not significant; one-way ANOVA followed by Tukey's multiple comparisons test. (*F* and *G*) The Ca^{2+} responses evoked in ADL by 10 nM C9 pheromone are larger in *npr-1* animals acclimated to 7% O_2 compared with siblings acclimated at 21% O_2. (*G*) Quantification of data plotted in *F*. $n = 23$–24 animals. $^{**}P < 0.01$; Mann–Whitney *U* test. (*H*) Model showing acclimation to different O_2 levels has opposite effects on antagonistic circuit elements promoting attractive and repulsive pheromone responses. This experience-dependent plasticity arises from prolonged changes in the activity state of RMG hub neurons, which receive tonic input from URX O_2-sensing neurons. Sustained peptide release from RMG at 21% O_2 feeds back to alter RMG properties. In animals kept at 7% O_2 peptide release from RMG is low, disrupting the feedback. The time required for animals to acclimate to 7% or 21% O_2 corresponds to hysteresis in the onset or decay of peptide signaling. See also Figs. S3 and S4.

potentially about the animal's experience, by incorporating peptidergic positive feedback loops.

RMG has gap junctions not only with URX, but also with the ASK and ADL pheromone sensors (16, 36). This arrangement suggests that information can be integrated across the circuit (36). We find that ASK and ADL show O_2-evoked Ca^{2+} responses, and moreover, that acclimating animals to different O_2 levels alters O_2 and/or pheromone-evoked responses in each of the URX, RMG, ASK, and ADL neurons. Inhibiting peptidergic transmission from RMG prevents RMG and ASK neurons from changing their pheromone responsive properties in animals acclimated to 21% O_2; it also prevents the experience-dependent switch in pheromone valence. Changes in the pheromone-evoked responses of ASK and ADL neurons are consistent with RMG regulating communication across the network. For example, we speculate that in animals acclimated to 21% O_2 pheromone-evoked responses in ASK may be able to inhibit ADL pheromone responses via RMG, whereas in animals acclimated at 7% O_2 this communication may be less potent. Although this conjecture is plausible, we cannot exclude that the intrinsic properties of several neurons in the circuit are altered by O_2 experience.

We see parallels between our observations and a *Drosophila* study showing that repeated presentation of an aversive shadow cue leads to a persistent change in behavioral state that scales with the number and frequency of the presentations (55, 56). Our findings are also reminiscent of "latent modulation" in the feeding network of *Aplysia*, where the history of activation in some circuit elements has a lasting effect on subsequent responses, most likely by changing neuronal excitability through peptidergic modulation (57, 58).

Why should *C. elegans* reconfigure its sensory responses according to prior O_2 experience? It is tempting to speculate

about a behavioral hierarchy (59) that gives priority to escape from 21% O_2 and that dominates over sensory drives that could hinder escape from the surface. Animals at the surface may gradually suppress their aversion to CO_2 to facilitate escape to low O_2/high CO_2 environments. Once the threat of exposure at the surface recedes, escape from CO_2 again becomes adaptive. In a boom-and-bust species like *C. elegans* (17), pheromones may be repulsive because they predict an unsustainable population density. However, if escaping the surface is more important than avoiding a crowded environment, attraction toward pheromones may be transiently adaptive because crowded environments predict reduced O_2. Irrespective of the precise selective advantage(s), if any, our data reveal a form of cross-sensory and experience-dependent plasticity in *C. elegans*, guiding behavioral choice according to prior O_2 experience.

Materials and Methods

Strains were grown under standard conditions with *Escherichia coli* OP50 food (60). Defined O_2 environments were created using a glove box (Coy Laboratory Products). For detailed information on behavioral assays, Ca^{2+} imaging, molecular biology, and statistics, see *SI Materials and Methods*.

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