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## Authors
Rengarajan, Sophie
Yankura, Kristen A
Guillermin, Manon L
et al.

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# Feeding state sculpts a circuit for sensory valence in *Caenorhabditis elegans*

Sophie Rengarajan[a], Kristen A. Yankura[a], Manon L. Guillermin[a], Wendy Fung[a], and Elissa A. Hallem[a,1]

[a]Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095

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**Hunger affects the behavioral choices of all animals, and many chemosensory stimuli can be either attractive or repulsive depending on an animal's hunger state. Although hunger-induced behavioral changes are well documented, the molecular and cellular mechanisms by which hunger modulates neural circuit function to generate changes in chemosensory valence are poorly understood. Here, we use the $CO_2$ response of the free-living nematode *Caenorhabditis elegans* to elucidate how hunger alters valence. We show that $CO_2$ response valence shifts from aversion to attraction during starvation, a change that is mediated by two pairs of interneurons in the $CO_2$ circuit, AIY and RIG. The transition from aversion to attraction is regulated by biogenic amine signaling. Dopamine promotes $CO_2$ repulsion in well-fed animals, whereas octopamine promotes $CO_2$ attraction in starved animals. Biogenic amines also regulate the temporal dynamics of the shift from aversion to attraction such that animals lacking octopamine show a delayed shift to attraction. Biogenic amine signaling regulates $CO_2$ response valence by modulating the $CO_2$-evoked activity of AIY and RIG. Our results illuminate a new role for biogenic amine signaling in regulating chemosensory valence as a function of hunger state.**

*C. elegans* | carbon dioxide | sensory valence | biogenic amines | starvation

To appropriately respond to their environments, animals must detect external chemosensory stimuli and respond to these stimuli in the context of their internal needs. The integration of external stimuli with internal state establishes a framework for ethologically relevant behavior. A critical aspect of an animal's internal state is its hunger state. The responses to many chemosensory cues depend on hunger state (1), and some chemosensory cues can be either appetitive or aversive as a function of hunger (2). For example, humans perceive some food-associated odors as appetitive only when hungry (3, 4). However, little is known about the molecular and cellular mechanisms that modulate neural circuit function to generate feeding-state-dependent changes in the valence of a chemosensory stimulus. *Caenorhabditis elegans* is a powerful genetic model for elucidating the molecular and cellular mechanisms that regulate chemosensory behaviors as a function of feeding state. Despite its small nervous system, *C. elegans* exhibits complex behavioral responses to a wide range of chemosensory stimuli, and many of these responses are altered by changes in feeding state (5, 6). Moreover, *C. elegans* has an extensive genetic toolkit and is easily amenable to quantitative behavioral analysis (6). Thus, *C. elegans* is a uniquely tractable system for addressing how chemosensory circuits are modulated by feeding state.

One of the sensory behaviors of *C. elegans* that can be modulated by feeding state is the response to carbon dioxide ($CO_2$). $CO_2$ is an ambiguous sensory stimulus for *C. elegans* that can signal either favorable environments, such as bacterial food or mates, or unfavorable environments, such as predators, pathogens, or overcrowding (6–8). Consistent with this ambiguity, $CO_2$ can be attractive, repulsive, or neutral for *C. elegans* depending on its life stage, recent experience, and internal state (6, 9–15). For example, well-fed *C. elegans* adults are repelled by $CO_2$ when raised at ambient $CO_2$ (~0.038%), but are attracted to $CO_2$ when raised in a high-$CO_2$ environment (2.5%) (14). In addition, while well-fed animals raised at ambient $CO_2$ are repelled by it, starved animals raised at ambient $CO_2$ no longer exhibit repulsion (9, 10). At the cellular level, $CO_2$ chemotaxis is mediated primarily by the BAG sensory neurons, although other sensory neurons also contribute (9, 11, 16–18). Four pairs of interneurons—AIY, AIZ, RIA, and RIG—operate downstream of BAGs to mediate $CO_2$ response (13, 14).

Here, we show that hunger alters $CO_2$ response valence. Food deprivation results in a gradual shift from $CO_2$ repulsion to $CO_2$ attraction, and this shift is reversed upon refeeding. At the circuit level, this transition is mediated by a change in the $CO_2$-evoked activity of RIG and AIY. At the molecular level, it is mediated by opposing biogenic amine signals. Our results identify a role for biogenic amines in regulating chemosensory valence as a function of hunger state.

## Results

**$CO_2$ Response Valence Changes During Starvation.** Whereas well-fed *C. elegans* adults previously had been found to avoid $CO_2$ (9–12, 16), we found that starved adults are attracted to $CO_2$ in a chemotaxis assay (Fig. 1*A* and *SI Appendix*, Fig. S1). Refeeding starved animals restores $CO_2$ avoidance (Fig. 1*A*). The response of *C. elegans* to $CO_2$ therefore provides a system for understanding the mechanisms by which hunger regulates chemosensory valence. We first investigated how hunger shapes the behavioral response to $CO_2$ by comparing $CO_2$-evoked behavior in animals deprived of food for varying lengths of time. We found that $CO_2$ response valence shifts over the course of hours during starvation (Fig. 1*B*). $CO_2$ attraction in starved animals was observed across a wide range of $CO_2$ concentrations (Fig. 1*C*). Thus, the change in $CO_2$

### Significance

**Hunger regulates many animal behaviors. In particular, some chemosensory stimuli can be attractive or repulsive depending on the feeding state of an animal. The neural basis for hunger-dependent changes in chemosensory valence is poorly understood. Here, we show that the response of *Caenorhabditis elegans* to $CO_2$ depends on hunger. $CO_2$ shifts from repulsive to attractive during starvation. This valence change results from modulation of the $CO_2$ circuit by opposing biogenic amine signals. Dopamine promotes repulsion in well-fed animals, whereas octopamine promotes attraction in starved animals. Biogenic amine signaling alters the $CO_2$-evoked activity of interneurons in the $CO_2$ circuit, resulting in hunger-dependent changes in $CO_2$ response valence. Our results illustrate a role for biogenic amine signaling in determining chemosensory valence.**

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The authors declare no conflict of interest.

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[1]To whom correspondence should be addressed. Email: ehallem@ucla.edu.

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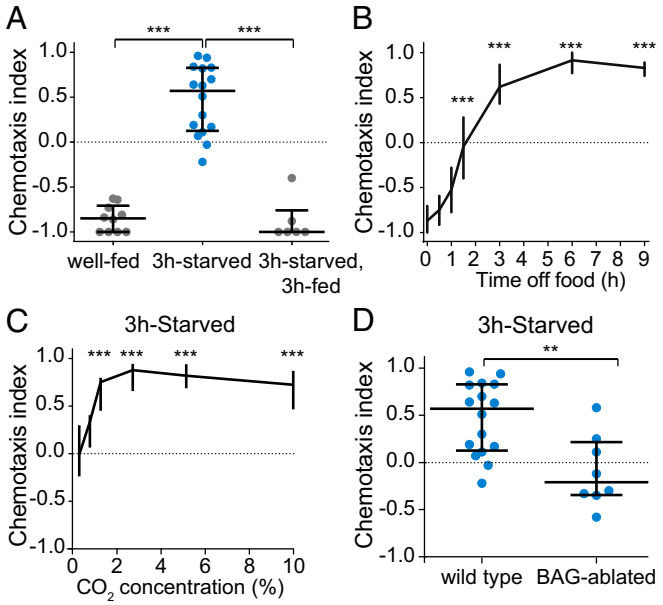


**Fig. 1.** $CO_2$ response valence shifts during starvation. (*A*) Well-fed animals are repelled by $CO_2$, and 3-h-starved animals are attracted to $CO_2$. Refeeding 3-h-starved animals for 3 h restores $CO_2$ repulsion. $n$ = 6–16 trials per condition. ***$P$ < 0.001, Kruskal–Wallis test with Dunn's posttest. (*B*) $CO_2$ response valence shifts from repulsion to attraction over the course of 3 h. $n$ = 10–70 trials per condition. ***$P$ < 0.001, Kruskal–Wallis test with Dunn's posttest. (*C*) 3-h-starved animals are attracted to $CO_2$ across a wide range of concentrations. $n$ = 6–16 trials per condition. ***$P$ < 0.001, Kruskal–Wallis test with Dunn's posttest. (*D*) 3-h-starved BAG-ablated animals do not respond to $CO_2$, indicating that BAG is required for $CO_2$ attraction. $n$ = 8–16 trials per genotype. **$P$ < 0.01, Mann–Whitney $U$ test. Responses are to 10% $CO_2$ except where concentrations are indicated (*C*).

response valence induced by hunger is reversible and relatively concentration-independent.

**$CO_2$ Circuit Interneuron Activity Is Modulated by Starvation.** We then asked how starvation modulates the $CO_2$ microcircuit. Starvation could regulate valence by acting on the sensory neurons at the level of $CO_2$ detection, or it could act downstream at the level of interneurons or motor neurons. To determine where feeding state is first integrated in the $CO_2$ microcircuit, we tested whether starvation modulates the activity of the $CO_2$-detecting BAG neurons. BAG previously had been shown to be required for both avoidance and attraction in the context of well-fed animals cultivated in high- and low-$CO_2$ environments (14). We found that starved animals lacking BAG do not respond to $CO_2$ (Fig. 1*D*), indicating that BAG is required for $CO_2$ attraction in starved animals. We then examined the $CO_2$-evoked activity of BAG and found that it is similar in well-fed and starved animals (*SI Appendix*, Fig. S2). Thus, starvation regulates $CO_2$ response downstream of the BAG calcium response.

We next examined whether starvation regulates $CO_2$ response by modulating the activity of interneurons directly downstream of BAG. $CO_2$ response is mediated by four interneuron pairs downstream of BAG: AIY, AIZ, RIA, and RIG (13, 14). Three of these pairs—AIY, RIA, and RIG—regulate $CO_2$ response valence in animals raised at high vs. low $CO_2$ (14). To determine whether the same interneurons regulate $CO_2$ response during starvation, we screened strains in which each pair of interneurons was genetically ablated (14). We found that two of these interneuron pairs, RIG and AIY, regulate $CO_2$ response during starvation. Whereas wild-type animals were neutral to $CO_2$ after 1.5 h of food deprivation, RIG-ablated animals were attracted to $CO_2$ (*SI Appendix*, Fig. S3). Thus, RIG promotes $CO_2$ avoidance during the early stages of food deprivation. In contrast, AIY-ablated animals failed to shift to $CO_2$ attraction after 6 h of food deprivation, suggesting that AIY promotes attraction during starvation (*SI Appendix*, Fig. S3). Together, these results suggest that RIG and AIY act antagonistically and on different timescales to regulate $CO_2$ response as a function of feeding state. However, AIY ablation is known to alter navigation behavior in other contexts (19, 20), and general navigation deficits may contribute to changes in $CO_2$ chemotaxis during starvation. RIA-ablated animals showed a normal shift to $CO_2$ attraction during starvation, suggesting that RIA is not required for starvation-dependent modulation of $CO_2$ response (*SI Appendix*, Fig. S3).

To determine how RIG and AIY regulate $CO_2$ response during starvation, we monitored their $CO_2$-evoked activity in well-fed and starved animals. RIG showed $CO_2$-evoked excitatory responses in well-fed but not in 6-h-starved animals; 1.5-h-starved animals showed an intermediate response or no response (Fig. 2). In the case of AIY, well-fed animals showed consistently inhibitory responses to $CO_2$ but starved animals showed two categorically different responses to $CO_2$: excitatory and inhibitory (Fig. 3). The responses of AIY in starved animals are probabilistic, such that excitatory and inhibitory responses were observed with approximately equal frequency (Fig. 3). $CO_2$-evoked excitatory responses in AIY previously had been shown to promote $CO_2$ attraction in a different context (14), suggesting that the excitatory activity of AIY during starvation promotes $CO_2$ attraction. Together, our calcium imaging and behavioral

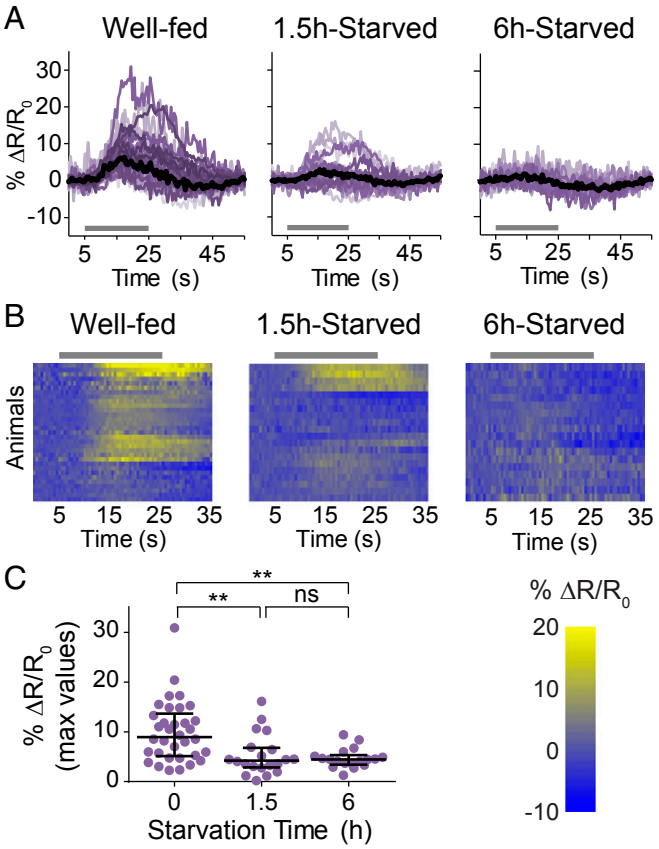


**Fig. 2.** Starvation suppresses the $CO_2$-evoked activity of RIG. (*A*) Colored lines depict individual traces, and black lines depict medians. (*B*) Each row represents the response of an individual animal. Responses are ordered by hierarchical cluster analysis. (*A* and *B*) Gray bars indicate the timing of the $CO_2$ pulse. (*C*) The dot plot shows maximum values of % $\Delta R/R_0$ for each animal; lines show medians and interquartile ranges. $n$ = 17–18 animals per condition. **$P$ < 0.01, Kruskal–Wallis test with Dunn's posttest. Responses are to 10% $CO_2$.

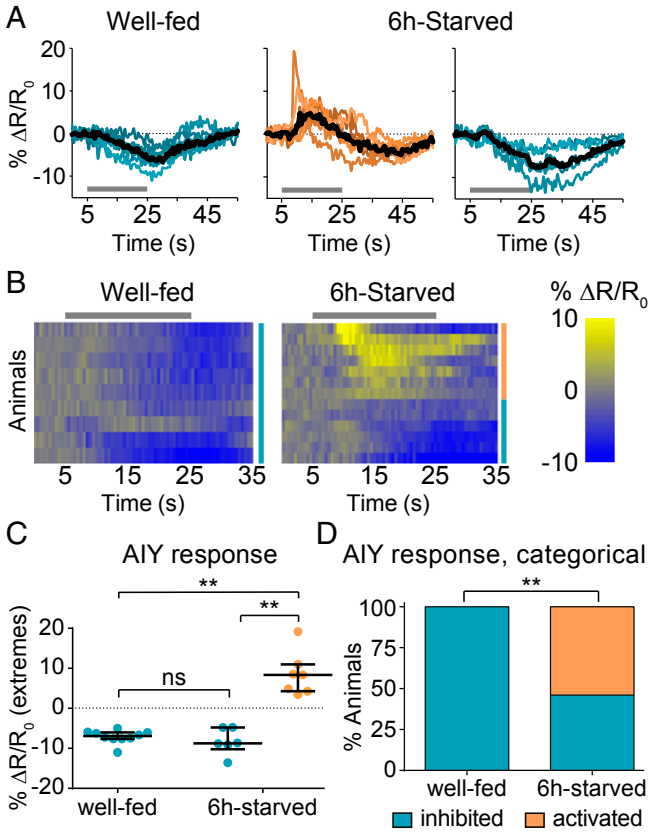


**Fig. 3.** Starvation results in probabilistic $CO_2$-evoked activity in AIY. AIY shows inhibitory responses in well-fed animals but both excitatory and inhibitory responses in 6-h-starved animals. (*A*) Colored lines depict individual traces, and black lines depict medians. (*B*) Each row represents the response of an individual animal. Responses are ordered by hierarchical cluster analysis; orange and blue coding indicates excitatory and inhibitory responses, respectively. (*A* and *B*) Gray bars indicate the timing of the $CO_2$ pulse. (*C*) The dot plot shows maximum values (for excitatory responses) or minimum values (for inhibitory responses) of % $\Delta R/R_0$ for each animal; lines show medians and interquartile ranges. (*D*) The categorical plot depicts the percentage of excitatory and inhibitory responses. $n = 9$–13 animals per condition. $^{**}P < 0.01$, Kruskal–Wallis test with Dunn's posttest (*C*) or Fisher's exact test (*D*). ns, not significant. Responses are to 15% $CO_2$.

data suggest that the excitatory activity of RIG promotes $CO_2$ repulsion in well-fed animals and regulates the timing of the valence switch, whereas the probabilistic $CO_2$-evoked excitatory activity of AIY promotes $CO_2$ attraction during starvation. Thus, starvation regulates the $CO_2$ circuit at least in part by modulating interneuron activity.

**AIY Calcium Activity Reflects Behavioral Robustness.** Like starved animals, animals raised with food in a high-$CO_2$ environment are attracted to $CO_2$ (14). However, a direct comparison of $CO_2$ attraction in these two sets of animals revealed that $CO_2$-cultivated animals show more extreme $CO_2$ attraction than do starved animals (*SI Appendix*, Fig. S4 *A* and *B*). While both groups of animals migrated toward $CO_2$, $CO_2$-cultivated animals gathered directly under the $CO_2$ source (*SI Appendix*, Fig. S4*A*). Thus, the $CO_2$ attraction of starved animals represents a less extreme behavioral state than that of animals cultivated with food at high $CO_2$. This behavioral difference may reflect a risk-benefit calculation in starved worms, as they weigh the ethological ambiguity of a $CO_2$ stimulus with the uncertainty of food availability. This ambiguity is not faced by worms cultivated at high $CO_2$ in the presence of food. In these animals, the positive association between a high-$CO_2$ environment and food may result in stronger attraction. To investigate the neural mechanisms that underlie these differences in behavioral sensitivity to $CO_2$, we compared the $CO_2$-evoked activity of AIY in starved vs. $CO_2$-cultivated animals. While starved animals raised at ambient $CO_2$ showed a roughly equal proportion of excitatory and inhibitory responses in AIY, well-fed animals raised at high $CO_2$ showed primarily excitatory responses (*SI Appendix*, Fig. S4 *C*–*E*) (14). In addition, the excitatory responses of AIY in $CO_2$-cultivated animals were larger than those in starved animals (*SI Appendix*, Fig. S4 *D* and *E*). Thus, the decreased variability and increased amplitude of AIY responses correlate with increased behavioral robustness. These results suggest that AIY activity may control behavioral sensitivity to $CO_2$.

**Dopamine Promotes $CO_2$ Avoidance in Well-Fed Animals.** We next investigated the neuromodulatory mechanisms that regulate $CO_2$ response valence as a function of feeding state. Across species, many hunger-dependent changes in sensory behavior are mediated by biogenic amines (1, 21). In *C. elegans*, biogenic amines play important roles in signaling the presence or absence of food (1, 21). We therefore investigated whether biogenic amine signaling regulates $CO_2$ response across feeding states. We first explored a potential role for dopamine by assaying the $CO_2$-evoked behavior of *dat-1::ICE* animals, which contain a genetic ablation of dopaminergic neurons due to expression of the human caspase ICE under the control of the promoter for the

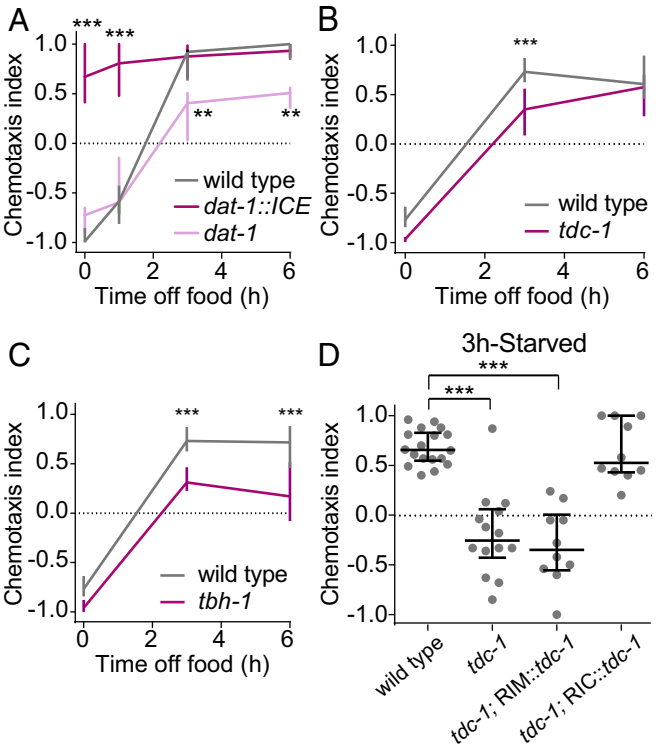


**Fig. 4.** Biogenic amine signaling regulates $CO_2$ response valence during starvation. (*A*) Dopamine promotes $CO_2$ avoidance. Wild-type, *dat-1::ICE*, and *dat-1* animals were food-deprived for 0–6 h. $n = 8$–14 trials per genotype and condition. $^{**}P < 0.01$ and $^{***}P < 0.001$, two-way ANOVA with Sidak's posttest. (*B* and *C*) Octopamine signaling promotes $CO_2$ attraction. Loss of both tyramine and octopamine signaling (*B*) or only octopamine signaling (*C*) delays the shift to $CO_2$ attraction. Wild-type, *tdc-1*, or *tbh-1* animals were food-deprived for 0–6 h. $n = 6$–14 trials per genotype and condition. $^{***}P < 0.001$, two-way ANOVA with Sidak's posttest. (*D*) Restoring *tdc-1* function to 3-h-starved *tdc-1* mutants in octopaminergic RIC neurons but not in tyraminergic RIM neurons restores $CO_2$ attraction. $n = 10$–18 trials per genotype. $^{***}P < 0.001$, one-way ANOVA with Dunnett's posttest. For all graphs, lines show medians and interquartile ranges. Responses are to 10% $CO_2$.

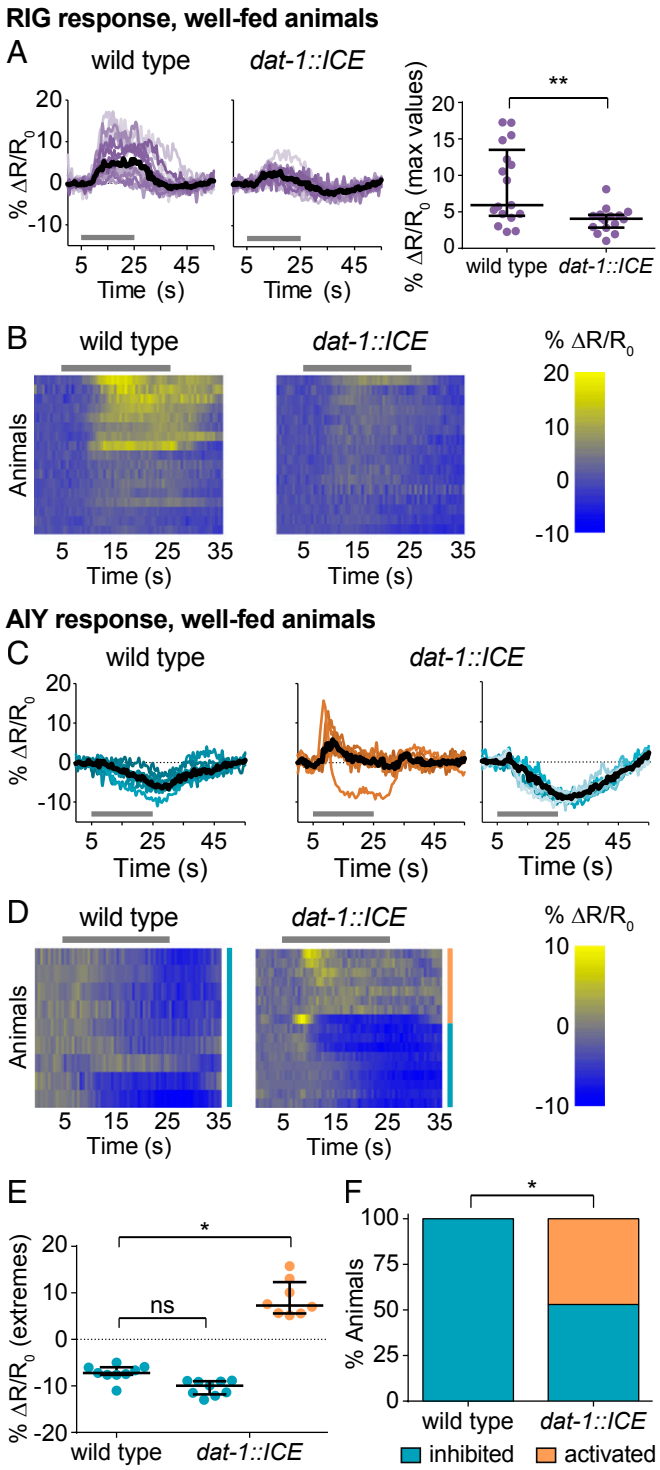


**Fig. 5.** Dopaminergic signaling acts on RIG and AIY to promote $CO_2$ avoidance. (*A* and *B*) The $CO_2$-evoked activity of RIG is attenuated in well-fed *dat-1::ICE* animals compared with wild-type animals. $n$ = 16–17 animals per genotype. $^{**}P < 0.01$, unpaired Student's $t$ test with Welch's correction. (*C*–*F*) Well-fed *dat-1::ICE* animals show more excitatory and fewer inhibitory $CO_2$-evoked responses in AIY compared with well-fed wild-type animals. $n$ = 9–17 animals per genotype. $^{*}P < 0.05$, Kruskal–Wallis test with Dunn's posttest (*E*) or $\chi^2$ test (*F*). (*A* and *C*) Colored lines depict individual traces, and black lines depict medians. (*B* and *D*) Each row represents the response of an individual animal. Responses are ordered by hierarchical cluster analysis. (*D*) Orange and blue coding indicates excitatory and inhibitory responses, respectively. (*A*–*D*) Gray bars indicate the timing of the $CO_2$ pulse. Dot plots depict maximum (for *A* and excitatory responses in *E*) or minimum (for inhibitory responses in *E*) values of % $\Delta R/R_0$ for each animal. Lines in dot plots show medians and interquartile ranges. Data for wild-type animals (*C*) are also shown in Fig. 3. Responses are to 10% (*A* and *B*) or 15% (*C*–*F*) $CO_2$.

dopamine transporter gene *dat-1* (22, 23). We found that these animals are attracted to $CO_2$ regardless of feeding state (Fig. 4*A*). In contrast, starved animals with increased dopamine signaling resulting from loss of the *dat-1* gene (23) showed reduced attraction (Fig. 4*A*). These results suggest that dopaminergic signaling promotes $CO_2$ avoidance in well-fed animals. To further confirm that dopamine drives $CO_2$ avoidance, we administered dopamine exogenously to animals for 30 min before assaying their $CO_2$ response. We found that dopamine treatment restored $CO_2$ avoidance in *dat-1::ICE* animals (*SI Appendix*, Fig. S5*A*). In addition, dopamine treatment eliminated $CO_2$ attraction in wild-type animals deprived of food for 3 h, although it did not result in $CO_2$ avoidance (*SI Appendix*, Fig. S5*B*). Thus, dopamine regulates $CO_2$ response valence by promoting $CO_2$ avoidance in well-fed animals. We then screened well-fed animals lacking individual dopamine receptors (21, 24) in a $CO_2$ chemotaxis assay. However, these mutants responded normally to $CO_2$ (*SI Appendix*, Fig. S6), suggesting that multiple dopamine receptors act redundantly to regulate $CO_2$ response.

**Octopamine Promotes $CO_2$ Attraction in Starved Animals.** We next investigated the roles of tyramine and octopamine in regulating $CO_2$ response. Tyramine and octopamine are invertebrate neurotransmitters that are analogous to vertebrate epinephrine and norepinephrine, respectively (25). Animals lacking the tyrosine decarboxylase gene *tdc-1*, which is required for both tyraminergic and octopaminergic signaling, showed a delayed shift from $CO_2$ avoidance to attraction during starvation (Fig. 4*B*). We then tested animals lacking the tyramine β-hydroxylase gene *tbh-1*, which is required for the conversion of tyramine into octopamine. The *tbh-1* mutants showed a delayed shift to attraction (Fig. 4*C*), demonstrating a specific role for octopamine in promoting $CO_2$ attraction. Within the nervous system, *tdc-1* is expressed in the RIM motor neurons and the RIC interneurons, while *tbh-1* is expressed only in the RIC interneurons (21). Thus, RIM is tyraminergic and RIC is octopaminergic. To further confirm a role for octopamine in regulating $CO_2$ response, we performed a rescue experiment in which *tdc-1* function was restored to *tdc-1* mutants in either RIM or RIC (26). Restoring *tdc-1* function in RIC but not RIM was sufficient to restore normal $CO_2$ attraction in starved animals (Fig. 4*D*). Thus, $CO_2$ response in starved animals is primarily regulated by octopamine, although we cannot exclude a secondary or redundant role for tyramine. Transiently silencing *tdc-1*-expressing neurons in adult animals using the histamine-gated chloride channel HisCl1 (26, 27) resulted in reduced $CO_2$ attraction in starved animals (*SI Appendix*, Fig. S7), suggesting that the effects of octopamine on $CO_2$ response result from real-time modulation of the $CO_2$ circuit. These results suggest that $CO_2$ response valence is regulated by opposing biogenic amines. In well-fed animals, dopamine signaling drives $CO_2$ repulsion; in starved animals, octopamine signaling drives $CO_2$ attraction.

*C. elegans* has three octopamine receptors: *ser-3*, *ser-6*, and *octr-1* (21). The *ser-6* mutants, but not the *ser-3* or *octr-1* mutants, showed reduced $CO_2$ attraction when starved (*SI Appendix*, Fig. S8*A*). The *ser-6* gene is expressed in a subset of head neurons, including the AWB olfactory neurons and the SIA interneurons (28, 29). We found that restoring *ser-6* function to *ser-6* mutants either in all *ser-6*-expressing neurons, in AWB only, or in SIA only was sufficient to restore normal $CO_2$ attraction to starved animals (*SI Appendix*, Fig. S8 *B* and *C*). These results suggest that SER-6 can function in multiple head neurons to regulate $CO_2$ response, perhaps through secretion of a shared neuropeptide from these neurons that acts extrasynaptically on the $CO_2$ circuit.

NEUROSCIENCE

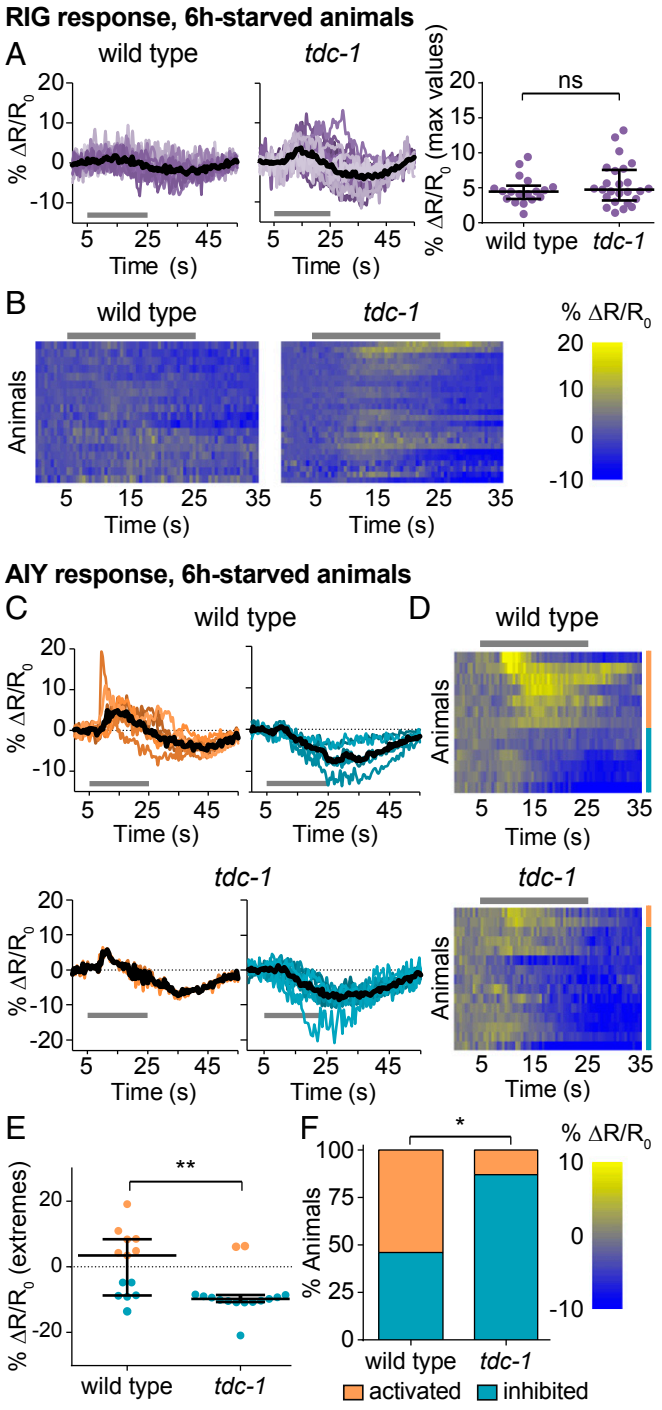


**Fig. 6.** Octopaminergic signaling acts on AIY but not RIG to promote $CO_2$ attraction. (*A* and *B*) $CO_2$-evoked activity in RIG is suppressed in 6-h-starved wild-type and *tdc-1* animals. ns, not significant ($P = 0.4870$), Mann–Whitney *U* test. $n = 18$–25 animals per genotype. (*C*–*F*) 6-h-starved *tdc-1* animals show more inhibitory and fewer excitatory $CO_2$-evoked responses in AIY than 6-h-starved wild-type animals. $n = 13$–15 animals per genotype. $^*P < 0.05$, $^{**}P < 0.01$, Mann–Whitney *U* test (*E*) or $\chi^2$ test (*F*). (*A* and *C*) Colored lines depict individual traces, and black lines depict medians. (*B* and *D*) Each row represents the response of an individual animal. Responses are ordered by hierarchical cluster analysis. (*D*) Orange and blue coding indicates excitatory and inhibitory responses, respectively. (*A*–*D*) Gray bars indicate the timing of the $CO_2$ pulse. Dot plots show maximum (for *A* and excitatory responses in *E*) or minimum (for inhibitory responses in *E*) values of % $\Delta R/R_0$ for each animal; lines in dot plots show medians and interquartile ranges. Data for wild-type animals (*A*) are also shown in Fig. 2; data for wild-type animals (*C*) are also shown in Fig. 3. Responses are to 10% (*A* and *B*) or 15% (*C*–*F*) $CO_2$.

**Biogenic Amine Signaling Modulates Interneuron Activity.** We next asked how biogenic amine signaling acts on the $CO_2$ circuit to regulate $CO_2$ response valence. We first examined how dopamine modulates the $CO_2$ circuit in well-fed animals to promote $CO_2$ avoidance. The BAG neurons of well-fed wild-type and *dat-1::ICE* animals showed similar $CO_2$-evoked activity (*SI Appendix*, Fig. S9 *A* and *B*). In contrast, the $CO_2$-evoked activity of RIG was decreased in well-fed *dat-1::ICE* animals relative to wild-type animals, suggesting that dopamine enhances the excitatory response of RIG to $CO_2$ (Fig. 5 *A* and *B*). In addition, AIY in well-fed *dat-1::ICE* animals showed a decreased frequency of inhibitory responses and an increased frequency of excitatory responses, suggesting that dopamine promotes an inhibitory response in AIY (Fig. 5 *C*–*F*). These results suggest that dopamine promotes $CO_2$ avoidance by modulating interneuron activity. Loss of dopaminergic signaling causes the $CO_2$-evoked responses of interneurons in well-fed animals to more closely resemble those in starved animals, suggesting that decreased dopaminergic signaling during starvation promotes the shift from $CO_2$ avoidance to $CO_2$ attraction.

We then asked how octopamine modulates the $CO_2$ circuit in starved animals to promote $CO_2$ attraction. We found that octopamine, like dopamine, regulates the $CO_2$ circuit downstream of $CO_2$ detection by BAG (*SI Appendix*, Fig. S9 *C* and *D*). In addition, the RIG neurons of starved *tdc-1* animals resembled those of starved wild-type animals in that they did not show $CO_2$-evoked activity (Fig. 6 *A* and *B*). In contrast, the AIY neurons of starved *tdc-1* animals showed predominantly inhibitory responses, suggesting that octopamine promotes an excitatory response in AIY (Fig. 6 *C*–*F*). These results suggest that octopaminergic signaling promotes $CO_2$ attraction by modulating AIY activity, loss of octopamine causes the $CO_2$-evoked responses of AIY in starved animals to more closely resemble those of well-fed animals, and increased octopaminergic signaling during starvation promotes the shift from $CO_2$ avoidance to $CO_2$ attraction.

We previously showed that $CO_2$ response valence is also regulated by neuropeptide signaling (14). NLP-1 dampens $CO_2$ repulsion in animals cultivated under low-$CO_2$ conditions, whereas FLP-16 dampens $CO_2$ attraction in animals cultivated under high-$CO_2$ conditions (14). We found that these neuropeptides also regulate $CO_2$ response valence during starvation: both *nlp-1* and *flp-16* mutants showed a slightly delayed shift from repulsion to attraction during starvation (*SI Appendix*, Fig. S10). Thus, neuropeptide signaling appears to act in concert with biogenic amine signaling to regulate $CO_2$ response valence. The mechanisms by which neuropeptide signaling modulates $CO_2$ circuit function remain to be determined.

## Discussion

We have demonstrated that $CO_2$ response valence is modulated by hunger such that the behavioral response to $CO_2$ shifts from avoidance to attraction during starvation. This shift may reflect an internal risk-benefit analysis. *C. elegans* feeds on aerobic bacteria, which emit $CO_2$ (30); thus, $CO_2$ may indicate the presence of a food source. At the same time, both pathogens (8) and predators (31, 32) emit $CO_2$, making $CO_2$ an ambiguous and inherently risky sensory cue. Starvation often occurs during periods of environmental uncertainty, when *C. elegans* must forage for food at the expense of encountering predators and pathogens. $CO_2$ response valence may shift during food deprivation as animals prioritize food seeking over predator evasion. Increased risk taking during starvation has been observed in many animals, including humans (33–37). Thus, hunger regulates risk-taking behaviors across animal phyla.

We have shown that starvation modulates the $CO_2$ circuit by altering the $CO_2$-evoked activity of RIG and AIY. RIG shows $CO_2$-evoked excitatory responses in well-fed animals, but this activity is suppressed during starvation (Fig. 2). In contrast, AIY shows probabilistic $CO_2$-evoked responses, and starvation state determines the distribution of these responses (Fig. 3). AIY responses in well-fed animals are inhibitory; those in starved animals are both excitatory and inhibitory, with excitatory and inhibitory

responses occurring at roughly equal frequencies (Fig. 3). These results suggest that the increased frequency of AIY excitatory responses promotes $CO_2$ attraction during starvation.

A comparison of the functional state of the $CO_2$ circuit in starved animals raised at ambient $CO_2$ versus well-fed animals raised at high $CO_2$ (14) demonstrated that, although both sets of animals are attracted to $CO_2$, well-fed animals at high $CO_2$ show more extreme $CO_2$ attraction (*SI Appendix*, Fig. S4 *A* and *B*). The reduced behavioral robustness seen in the starved population may be a mechanism for counterbalancing increased risk taking in an uncertain environment by ensuring that some members of the population survive. Furthermore, the $CO_2$-evoked activity of AIY differs in the two cases. Starved animals raised at ambient $CO_2$ show probabilistic AIY responses that can be either excitatory or inhibitory, while well-fed animals raised at high $CO_2$ show consistent excitatory responses (*SI Appendix*, Fig. S4*C*) (14). Thus, behavioral robustness correlates with the probabilistic activity of AIY. Probabilistic AIY activity has also been observed in response to thermal stimuli and found to correlate with behavioral drive (38), suggesting that AIY may play a similar role in regulating behavior across sensory modalities.

We have shown that different biogenic amines play opposing roles in regulating $CO_2$ response during starvation. Dopamine promotes avoidance in well-fed animals, while octopamine promotes attraction in starved animals (Fig. 4). Both dopamine and octopamine regulate $CO_2$ response valence by modulating interneuron activity (*SI Appendix*, Fig. S11). Dopamine modulates the activity of both RIG and AIY (Fig. 5 and *SI Appendix*, Fig. S11), whereas octopamine modulates the activity of AIY but not RIG (Fig. 6 and *SI Appendix*, Fig. S11). Whether the biogenic amines act directly on RIG and AIY, or indirectly on other neurons that modulate RIG and AIY activity, remains to be determined. Neuropeptide signaling also regulates $CO_2$ response during starvation (*SI Appendix*, Fig. S10), but whether neuropeptide signaling similarly alters RIG and AIY activity remains to be determined as well. Finally, other neurons not tested here may also contribute to changes in the $CO_2$ circuit during starvation.

In summary, we have demonstrated a role for biogenic amine signaling in regulating chemosensory valence during starvation. All animals navigate through rapidly changing environments, and neural circuits must be dynamically sculpted by current internal state to drive appropriate behaviors. Thus, similar mechanisms of circuit modulation may operate in other organisms to drive internal-state-dependent changes in chemosensory valence.

## Materials and Methods

$CO_2$ chemotaxis assays and calcium imaging were performed as previously described (14). Statistical analysis was performed using GraphPad Prism Version 6.07. For detailed information on all methods, see *SI Appendix, Materials and Methods*.

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