Supporting Information

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SI Materials and Methods

Strains. The WT strain is the standard N2 Bristol strain. Other strains are listed below in the order in which they appear in the figures: MT18636 nls326[gcy-33::CAMELEON; lin-15AB(n765)]; HA1203 nls25[sra-6::CAMELEON]; FQ100 wls32[wls-1::CAMELEON]; lin-15AB(n765); FQ94 wls38[wls-1::CAMELEON]; lin-15AB(n765); FQ136 tax-4(p678); nls326[gcy-33::CAMELEON]; FQ14 tax-4(p671); nls326[gcy-33::CAMELEON]; PS5883 nls326[gcy-33::CAMELEON]; PS5884 npr-1(ad609); nls326[gcy-33::CAMELEON]; PS6220 daf-11(n47); nls326[gcy-33::CAMELEON]; PS6221 daf-11(e1370); nls326[gcy-33::CAMELEON]; PS6222 daf-7(e1372); nls326[gcy-33::CAMELEON]; FQ31 gcy-33(ok232); nls326[gcy-33::CAMELEON]; PS6227 daf-11(e1372); nls326[gcy-33::CAMELEON]; PS6273 nls323[gcy-33::CAMELEON]; PS6264 gcy-9(n470); PS6265 nls323[gcy-33::CAMELEON]; gcy-9(n470); PS6264 gcy-9(n470); syEx1175[gcy-33::gcy-9; myo-2::dsRed]; PS6265 nls323[gcy-33::CAMELEON]; gcy-9(n470); syEx1175[gcy-33::gcy-9; myo-2::dsRed]; CX11697 kyls356[flp-17::s12GFP, elt-2::mCherry]; kyls358[gbp-5::s12GFP, elt-2::mCherry], which contains a genetic ablation of the BAG neurons; MT18503 wls32[wls-1::CAMELEON]; lin-15AB(n765); PS6227 nls323[gcy-33::CAMELEON]; PS6264 gcy-9(n470); syEx1175[gcy-33::gcy-9; myo-2::dsRed]; PS6265 nls323[gcy-33::CAMELEON]; gcy-9(n470); syEx1175[gcy-33::gcy-9; myo-2::dsRed]; Cylinder plasmid EKL15. Extrachromosomal arrays were integrated by γ-irradiation to create the integrated transgenes nls323[gcy-33::CAMELEON]; PS6227 nls323[gcy-33::CAMELEON]; PS6264 gcy-9(n470); syEx1175[gcy-33::gcy-9; myo-2::dsRed]; PS6265 nls323[gcy-33::CAMELEON]; gcy-9(n470); syEx1175[gcy-33::gcy-9; myo-2::dsRed]; CX11697 kyls356[flp-17::s12GFP, elt-2::mCherry]; kyls358[gbp-5::s12GFP, elt-2::mCherry], which contains a genetic ablation of the BAG neurons; MT18629 nls323[gcy-33::CAMELEON]; lin-15AB(n765); PS6229 nls323[gcy-33::CAMELEON]; RB1780 gcy-33(ok2288); PS927 gcy-33(ok2288); syEx1108[gcy-33::gcy-3; pax-2::gfp]; CB1372 daf-7(e1372); DA2202 daf-7(e1372); adEx2202 [gpa-4::daf-7, rol-6::GFP]; PS5889 nls323[kyIs536]; gcy-33(ok296); FQ19161 gcy-1(tm16530); VC22795 gcy-3(gk1154); FQ1653 gcy-4(dh15); FQ10449 gcy-6(m1449); FQ10991 gcy-7(m901); PS4054 gcy-8(sy664); CX2065 odr-1(n1936); IK212 gcy-12(nj10); VC2440 gcy-14(ok2236); VC2675 gcy-15(gk1102); FQ14516 gcy-17(m516); VC2321 gcy-18(n3047); nT1[flp-1]; +/nT1[flp-1]; RB1909 gcy-19(ok2472); RB1935 gcy-20(ok2588); FQ12364 gcy-22(n3047); IK5977 gcy-23(nj37); gcy-8(sy644); gcy-18(nj38); FQ13000 gcy-25(n3040); RB2622 gcy-27(n3063); FQ12411 gcy-28(n3041); RB626 gcy-37(n3044); MT15933 flp-17(n4894). gcy-9(tm2816) was used for all experiments involving gcy-9 unless otherwise indicated.

Transgene Construction. To generate gcy-33::CAMELEON constructs, 1.4-kb 5¢ to the gcy-33 translational start was cloned into the expression vector pCVG6. pCVG6 is a version of the Caenorhabditis elegans expression vector pPD49.26 containing camel- eon YC3.60 (1) and was kindly provided by Chris Gabel, Harrison Gabel, and Aravindan Samuel (Harvard University, Cambridge, MA). ADL and AWB reporter constructs were made by cloning into pCVG6 4.0- and 3.5-kb sequences 5¢ to the translational starts of 3¢-and 3¢-I, respectively. CAMELEON expression constructs were injected into lin-15AB(n765) animals at 100 ng/µL together with 50 ng/µL of the lin-15 rescuing plasmid EKL15. Extrachromosomal arrays were integrated by γ-irradiation to create the integrated transgenes nls323[gcy-33::CAMELEON]; PS6227 nls323[gcy-33::CAMELEON]; PS6264 gcy-9(n470); syEx1175[gcy-33::gcy-9; myo-2::dsRed]; CX11697 kyls356[flp-17::s12GFP, elt-2::mCherry]; kyls358[gbp-5::s12GFP, elt-2::mCherry], which contains a genetic ablation of the BAG neurons; MT18629 nls323[gcy-33::CAMELEON]; lin-15AB(n765); PS6229 nls323[gcy-33::CAMELEON]; RB1780 gcy-33(ok2288); PS927 gcy-33(ok2288); syEx1108[gcy-33::gcy-3; pax-2::gfp]; CB1372 daf-7(e1372); DA2202 daf-7(e1372); adEx2202 [gpa-4::daf-7, rol-6::GFP]; PS5889 nls323[kyIs536]; gcy-33(ok296); FQ19161 gcy-1(tm16530); VC22795 gcy-3(gk1154); FQ1653 gcy-4(dh15); FQ10449 gcy-6(m1449); FQ10991 gcy-7(m901); PS4054 gcy-8(sy664); CX2065 odr-1(n1936); IK212 gcy-12(nj10); VC2440 gcy-14(ok2236); VC2675 gcy-15(gk1102); FQ14516 gcy-17(m516); VC2321 gcy-18(n3047); nT1[flp-1]; +/nT1[flp-1]; RB1909 gcy-19(ok2472); RB1935 gcy-20(ok2588); FQ12364 gcy-22(n3047); IK5977 gcy-23(nj37); gcy-8(sy644); gcy-18(nj38); FQ13000 gcy-25(n3040); RB2622 gcy-27(n3063); FQ12411 gcy-28(n3041); RB626 gcy-37(n3044); MT15933 flp-17(n4894). gcy-


Fig. S1. BAG neurons in C. elegans. Maximum projection image of a series of confocal micrographs of the paired BAG neurons. BAG neuron cell bodies are located in the head and extend dendrites to the tip of the nose. Anterior is to the left. Arrows indicate cell bodies; arrowheads indicate dendrites.
A. An acute assay for CO$_2$ avoidance

avoidance index = fraction that reverse to CO$_2$ - fraction that reverse to control

B. BAG neurons are required for CO$_2$ avoidance

Fig. S2. BAG neurons are required for CO$_2$ avoidance behavior. (A) An assay for acute CO$_2$ avoidance. The head of a forward-moving worm is exposed to CO$_2$, and the worm is given 4 s to reverse (1). (B) BAG neurons are required for avoidance of a range of concentrations of CO$_2$. Animals that lack BAG neurons do not respond to CO$_2$. BAG$^-$ animals were engineered to express a transgene that specifically kills the BAG neurons ($n = 3$–16 trials for BAG$^-$ animals; data for wild-type animals are from ref. 1).


Fig. S3. Animals expressing gcy-33::CAMELEON respond normally to CO$_2$. These lines contain integrated transgenes that express cameleon YC3.60 specifically in the BAG neurons ($n = 9$–32 trials for each genotype).
**Fig. S5.** BAG neurons are activated rapidly and reversibly by CO₂. (A) Animals were exposed to a 25-s pulse of 5% CO₂. Calcium transients were imaged using cameleon YC3.60 driven by the BAG-specific gcy-33 promoter. Black trace depicts the YFP to CFP ratio over the entire time course of the experiment. Yellow and blue traces illustrate changes in YFP and CFP emissions, respectively (in arbitrary units). The 25-s pulse of 5% CO₂ begins at 75 s and ends at 100 s. (B) An image sequence from the recordings shown in A. Time points for each frame are indicated. The time point at 75 s shows the last frame before the CO₂ pulse, and the time point at 100 s shows the last frame before the end of the CO₂ pulse. Colors indicate the YFP to CFP ratio; the scale is linear, and red corresponds to high calcium.

**Fig. S4.** BAG neurons are activated rapidly and reversibly by CO₂. (A) Animals were exposed to a 25-s pulse of 5% CO₂. Calcium transients were imaged using cameleon YC3.60 driven by the BAG-specific gcy-33 promoter. Black trace depicts the YFP to CFP ratio over the entire time course of the experiment. Yellow and blue traces illustrate changes in YFP and CFP emissions, respectively (in arbitrary units). The 25-s pulse of 5% CO₂ begins at 75 s and ends at 100 s. (B) An image sequence from the recordings shown in A. Time points for each frame are indicated. The time point at 75 s shows the last frame before the CO₂ pulse, and the time point at 100 s shows the last frame before the end of the CO₂ pulse. Colors indicate the YFP to CFP ratio; the scale is linear, and red corresponds to high calcium.

**Fig. S5.** rgs-3 is required in the BAG neurons, and daf-7 is required in the ASI neurons for CO₂ avoidance behavior. (A) rgs-3 mutants do not respond to CO₂, and cell-specific rescue of rgs-3 in the BAG neurons using the gcy-33 promoter restores CO₂ response, suggesting that G protein signaling negatively regulates CO₂ response within the BAG neurons. (B) daf-7 mutants do not respond to CO₂, and rescue of daf-7 in the ASI neurons using the gpa-4 promoter restores acute CO₂ avoidance (n = 10–32 trials for each genotype). (C) Ablation of ASI neurons does not affect CO₂ avoidance behavior, showing that ASI neurons are not required for CO₂ detection (n = 11–22 animals). For all graphs, error bars represent SEMs.

**Fig. S56.** gcy-33; gcy-31 double mutants respond normally to CO₂ (n = 12–32 trials for each genotype).
**Fig. S7.** Transcriptional profiling of embryonic BAG neurons. Embryos containing fluorescently labeled BAG neurons expressing a gcy-33::GFP transgene were dissociated with chitinase and sorted by FACS (1) to isolate BAG neurons. mRNA was extracted from BAG neurons and used to generate cDNA, which was hybridized to a *C. elegans* microarray. Transcripts significantly enriched in BAG relative to all embryonic cells were then identified as described in Materials and Methods.


**Fig. S8.**

**A** gcy-9 mutants do not respond to CO$_2$. 

**B** Intron-exon structure of the gcy-9 gene.

**C** Other guanylate cyclase mutants respond normally to CO$_2$.

**Fig. S8.** gcy-9 mutants do not respond to CO$_2$. (A) gcy-9 mutants do not respond to CO$_2$ across a wide range of concentrations. (B) The intron–exon structure of the gcy-9 gene. The top bracket indicates the tm2816 deletion; the lower bracket indicates the n4470 deletion. (C) gcy-9 mutants do not respond to CO$_2$, whereas other receptor guanylate cyclase mutants respond normally to CO$_2$ ($n = 5$–32 trials for each genotype; wt, wild type).
**Fig. S9.** *flp-17* mutants respond normally to CO₂ (*n* = 12–32 trials for each genotype).

**Fig. S10.** The receptor guanylate cyclases of *C. elegans*. A dendrogram of receptor guanylate cyclases in *C. elegans* (1) and mammals (2). Mammalian guanylate cyclases are highlighted in green. Sequences were aligned with ClustalW. GCY-9 is part of a nematode-specific expansion of the GCY family. The guanylate cyclase family also includes seven soluble guanylate cyclases, GCY-31 to GCY-37 (1), which are not shown here.

Dataset S1. Genes that showed enriched expression in embryonic BAG neurons relative to the aggregate of all other embryonic cells

Dataset S2. Genes that showed enriched expression in embryonic BAG neurons relative to the aggregate of all other embryonic cells, annotated with gene ontology (GO) terms for the biological process, molecular function, and cellular component GO categories