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Supplemental Information

Neuromodulatory State and Sex Specify Alternative Behaviors through Antagonistic Synaptic Pathways in *C. elegans*

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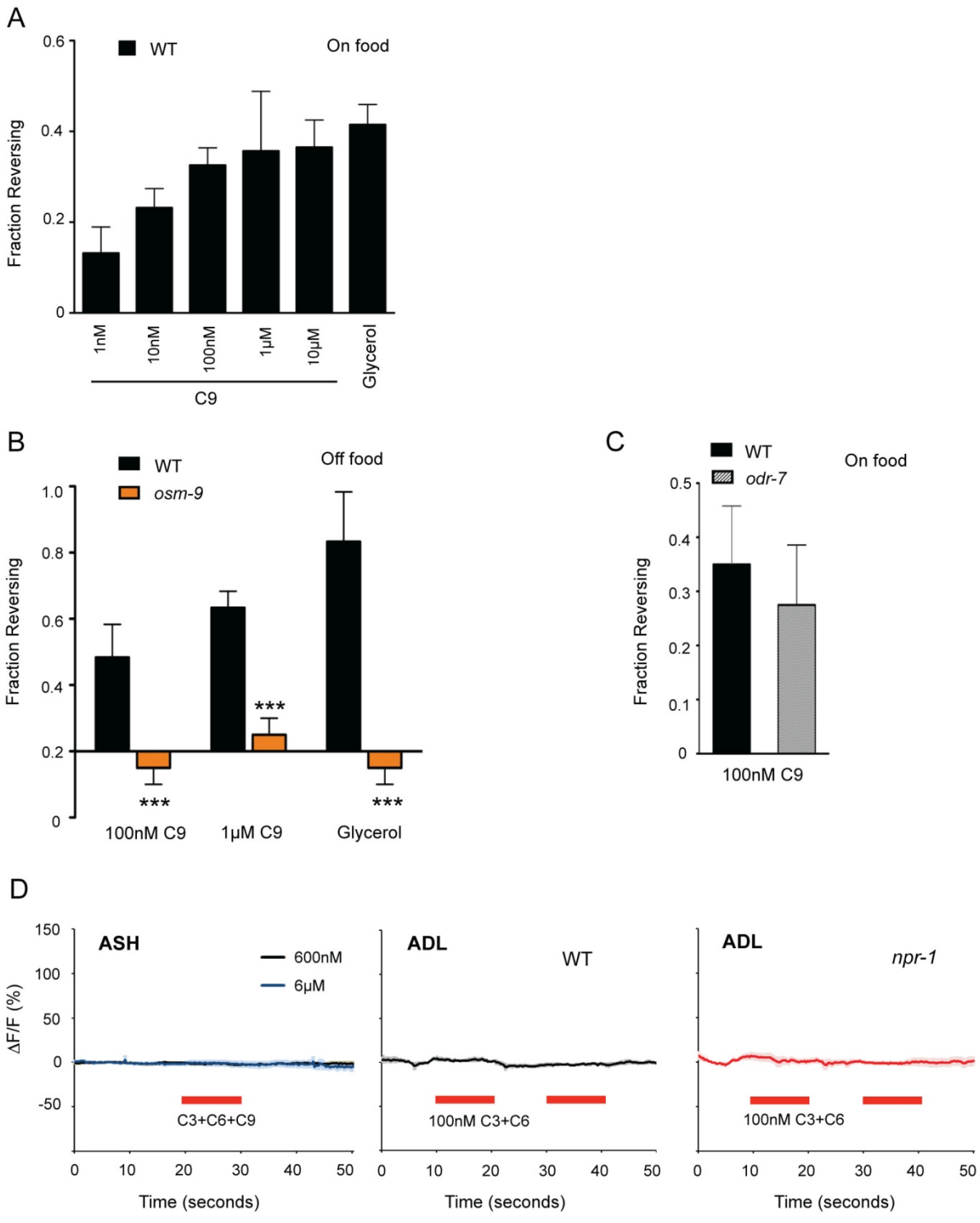

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Figure S1

Figure S1 related to Figure 1. Characterization of C9 avoidance.

A. C9 avoidance at low nanomolar concentrations is enhanced in the presence of food (Compare with Figure 1A).

B. C9 avoidance is abolished in *osm-9(ky10)* mutants. Assays were performed in the absence of food. *** indicates responses different from wild type at $P< 0.001$. Error bars are the SEM. n=20-40 animals each.

C. *odr-7(ky4)* mutants avoid C9. Error bars are the SEM. n=40 animals each.

D. No Ca^{2+} transients are observed in G-CaMP-expressing ASH neurons (left) upon addition of mixtures of 600 nM or 6 μM each C3, C6 and C9 (red horizontal bar), or in ADL neurons of wild-type or *npr-1* hermaphrodites (middle and right) upon addition of a mixture of 100 nM C3 and C6 (red bars). n≥6 neurons each. Shading around the lines represents SEM.

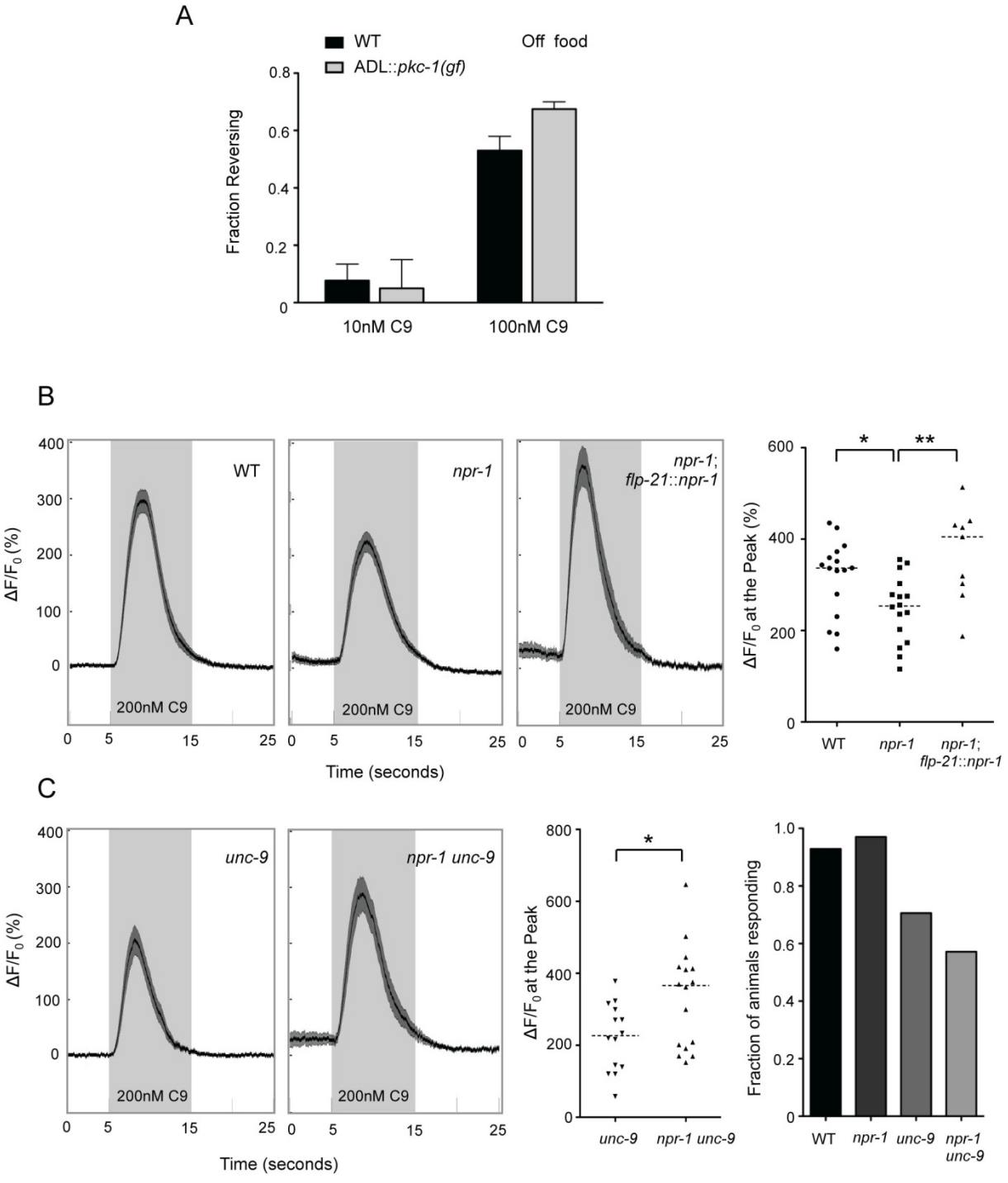

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Figure S2

Figure S2 related to Figure. 2. NPR-1 acts in RMG to modulate ADL C9 responses.

A. *pkc-1(gf)* does not significantly enhance C9 avoidance in wild-type animals in the absence of food (compare *npr-1; pkc-1(gf)* in Figure 2D) or in the presence of food (data not shown). Error bars are the SEM. n=20-100 animals each.

B. *npr-1* expression in RMG restores ADL C9 responses. Wild-type *npr-1* cDNA was expressed from a 4.1 kb *flp-21* promoter fragment that drives expression in RMG and other neurons, but not in ADL (Macosko et al., 2009). The scatter plot represents the percentage change in fluorescence at the peak of 200 nM C9-induced Ca^{2+} responses in ADL neurons. Shading around the lines represents SEM. * and ** indicate responses different from values indicated by brackets at $P<0.05$ and 0.01, respectively. Dotted horizontal lines indicate the median. n≥9 neurons each.

C. *unc-9* is required for the *npr-1*-dependent reduction in ADL C9 responses. The scatter plot represents the percentage change in fluorescence at the peak of 200 nM C9-induced Ca^{2+} responses in ADL neurons of the indicated genotypes. The *unc-9(e101)* allele was used. Dotted horizontal lines indicate the median response. * indicates responses different from values indicated by the bracket at $P<0.05$; note that the direction of change is reversed relative to Figure S2B. Right panel, proportion of ADL neurons that respond to C9 with Ca^{2+} transients; note increased failure rate in *unc-9* mutants. n≥14 neurons each.

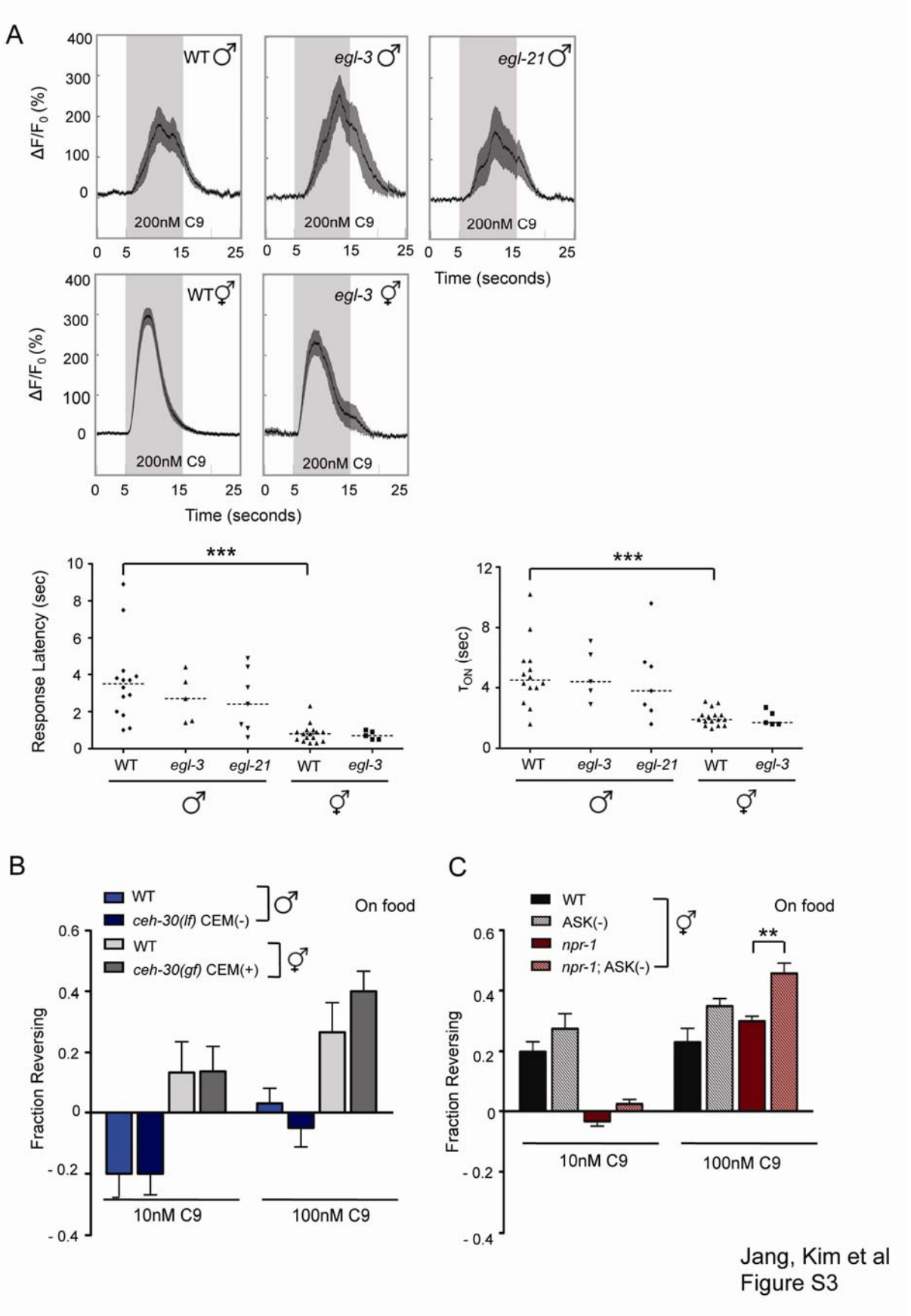
A
WT♂
egl-3♂
egl-21♂
WT♀
egl-3♀
ΔF/F0 (%)
200nM C9
Time (seconds)
Response Latency (sec)
TON (sec)
***
WT
egl-3
egl-21
B
C
On food
ceh-30(lf) CEM(-)
ceh-30(gf) CEM(+)
ASK(-)
npr-1
npr-1; ASK(-)
**
Fraction Reversing
10nM C9
100nM C9
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Figure S3

Figure S3 related to Figure 3. Male responses to C9.

A. C9-induced Ca^{2+} transients in male ADL neurons have increased response latency, which is not dependent on classical neuropeptides. Scatter plots show response latencies (left) and time to half maximum response (τ_{ON}, right) of 200 nM C9-induced Ca^{2+} responses in ADL neurons of males and hermaphrodites of the indicated genotypes. Wild-type males and mutant males with defects in neuropeptide processing exhibit similar C9-induced Ca^{2+} dynamics; the same is true for hermaphrodites. Dotted horizontal lines indicate the median response. Alleles used were *egl-3(ok979)* and *egl-21(n476)*. WT males and WT hermaphrodites are different at $P<0.001$ as indicated by asterisks. No significant difference is observed within males of different genotypes or within hermaphrodites of different genotypes (ANOVA with Tukey test for multiple comparisons). n≥5 neurons each. We were unable to examine the effects of blocking classical neurotransmission on ADL C9 responses using the synaptic vesicle exocytosis mutant *unc-13* (Richmond et al., 1999), because expression of GCaMP3 driven by multiple independent ADL promoters was downregulated in *unc-13* mutants (data not shown).

B. Male-specific CEM sensory neurons do not affect C9 avoidance in the drop test. CEM neurons normally survive in males and die in hermaphrodites, but CEM neurons die in *ceh-30(n4289lf)* males and survive in *ceh-30(n3714gf)* hermaphrodites (Schwartz and Horvitz, 2007). Responses of mutants are not significantly different from the wild-type responses of the same sex. Assays were performed in the presence of food. Error bars are the SEM. n=40-80 animals each.

C. ASK ablation enhances avoidance of 100 nM C9 in *npr-1* hermaphrodites. Assays were performed in the presence of food. ** indicates responses different from non-ablated control at $P<0.01$. Error bars are the SEM. n=40-120 animals each.

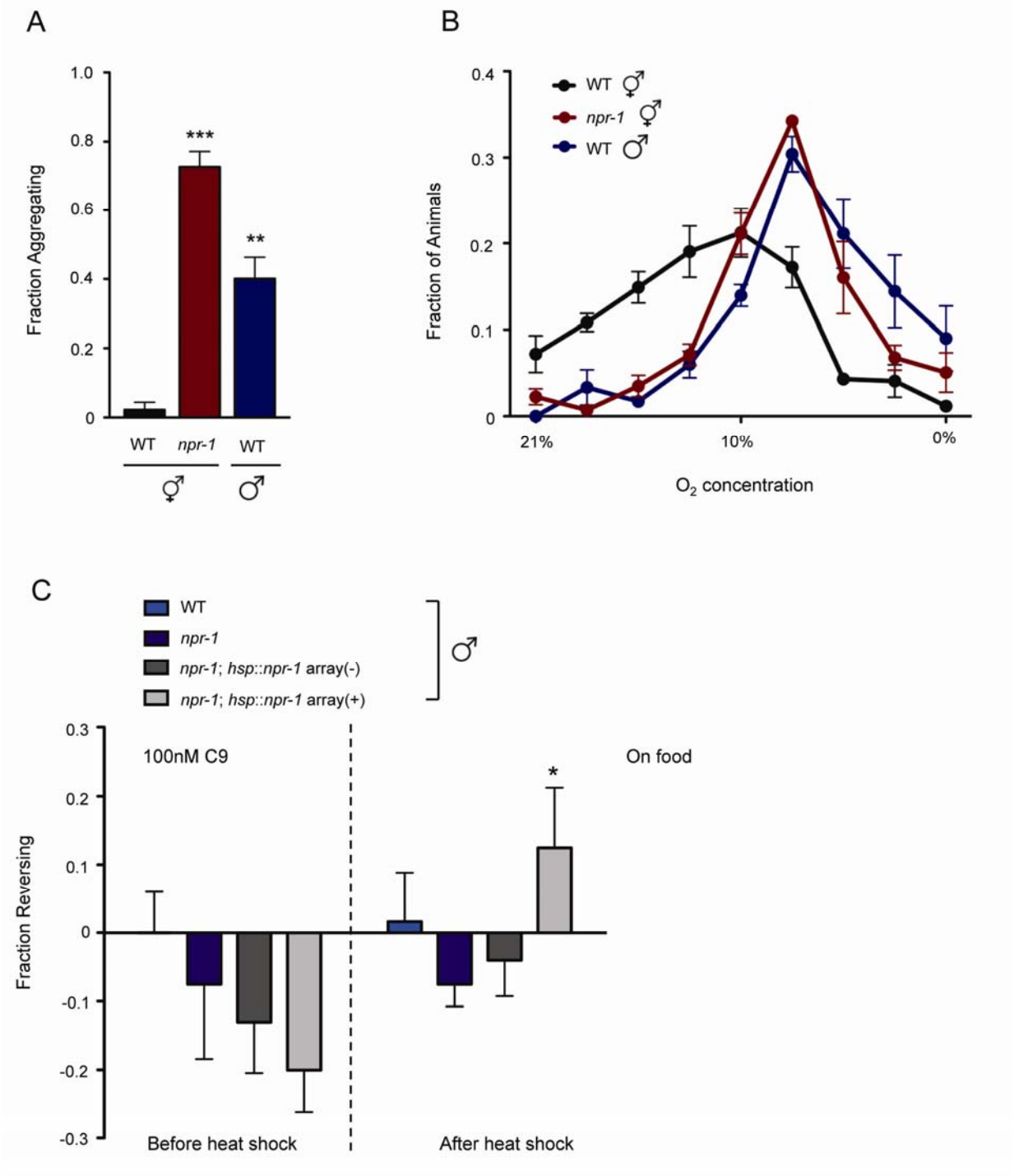

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Figure S4

Figure S4 related to Figure 4. Wild-type males and *npr-1* hermaphrodites exhibit similar aggregation (**A**) and oxygen preference (**B**) behaviors. Aggregation was scored as the percentage of animals touching two or more animals with at least 50% of their body length (de Bono and Bargmann, 1998). 60 animals per assay, n≥3 assays each. ** and *** indicate different from wild-type hermaphrodites at $P<0.01$ and 0.001, respectively. Aerotaxis assays show the distribution of populations of animals in linear oxygen gradients in the presence of food as described (Chang et al., 2006). Hyperoxia avoidance of wild-type hermaphrodites is significantly different from wild-type males or *npr-1* hermaphrodites ($P<0.01$ by ANOVA with Tukey test for multiple comparisons). Responses of wild-type males and *npr-1* hermaphrodites are not significantly different. **C**. Heat shock-driven expression of *npr-1* during the adult stage rescues the C9 avoidance defect of *npr-1* male animals. * indicates responses different from the responses of the same genotype before heat shock at $P<0.05$. Array(-) indicates animals from the *hsp16.2::npr-1*-expressing transgenic strain that have lost the extrachromosomal array, which represent sibling controls for the array(+) animals. Behavioral assays were performed in the presence of food before or immediately after heat-shock at 33˚C for 30 minutes. Error bars are the SEM. n=40-60 animals each.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strains

The wild-type strain used was *C. elegans* variety Bristol strain N2. Alleles used were *ocr-2(ak47), osm-9(ky10)* and *npr-1(ad609)*. Strains expressing *npr-1* or tetanus toxin light chain (TeTx) in RMG were generated using the Cre/*Lox* system as described previously (Macosko et al., 2009). For genetic ablation of ASK, a strain with stably integrated *sra-9*p::m*CaspaseI* was used (*qrIs2*: a gift of Ryuzo Shingai) (Kim et al., 2009). For the heat shock rescue of *npr-1*, a strain with the *hsp-16.2* heat-inducible promoter driving the expression of wild-type *npr-1* cDNA was used (a gift of Mario de Bono) (Coates and de Bono, 2002). Transgenic strains were generated by injecting the experimental plasmid at 30-50 ng/μl together with *unc-122*::*dsRed or unc-122::gfp* as the coinjection marker.

Behavioral assays

20-30 young adult worms grown at 20˚C were transferred to an unseeded NGM plate followed by transfer to assay plates with or without food. Assays were performed in the presence of bacterial food, except for assays in Figures 1A, 1B, S1B and S2A, which were performed in the absence of food. For drop tests in the presence of food, NGM plates were uniformly seeded with OP50 bacteria the day before the assay and grown overnight at 37˚C, followed by incubation at room temperature for at least one hour prior to the assay. For tests in the absence of food, care was taken that no food was transferred to the unseeded assay plate. After 30-60 minutes on the assay plate, a drop of M13 buffer with dissolved pheromone or other chemicals was delivered to individual young adult

worms moving forward using glass capillaries. Responses were scored as reversals if animals initiated backward movements longer than half their body length within 4 seconds. Most reversals initiated immediately and were followed by omega turns. Typically, 10-15% of animals reversed in response to buffer in the absence of food, and 30% in the presence of food. All assays were performed at room temperature on at least two different days. All genotypes were also assayed for their ability to reverse in response to 2M glycerol dissolved in water.

Statistical analysis was conducted by comparing the effect sizes of different genotypes, stimuli, and interactions by ANOVA with post-hoc corrections for multiple comparisons. Effect sizes (shown in figures as fraction reversing) represent the increase or decrease in fraction of animals reversing in response to pheromone, compared to the fraction reversing in response to buffer alone.

For heat shock experiments, animals were assayed for their behavior in the drop test in the presence of food (before heat shock), transferred to a new assay plate with food and incubated at 33˚C for 30 minutes. After heat shock, the assay plate was removed to room temperature, and animals were immediately re-assayed for their behavior in the drop test.

Molecular Biology

1 kb of sequences upstream of *sre-1* was used to drive ADL-specific expression of *pkc-1(gf), TeTx*, and *GCaMP3*. Plasmids driving *ocr-2* genomic DNA under cell-specific promoters were described previously (de Bono et al., 2002). *pkc-1(gf)* and *TeTx* plasmids were described previously (Macosko et al., 2009).

Ca^{2+} imaging

Ca^{2+} imaging experiments were performed using custom-designed microfluidics devices as described (Kim et al., 2009; Macosko et al., 2009; Chalasani et al., 2010). To image males, microfluidic imaging chambers with modified dimensions were used (channel height=25 μm; channel width for body=60 μm, channel width for nose=22 μm). Imaging was performed on an Olympus BX52WI microscope with a 40X objective and a CCD camera (Hamamatsu). Images were processed and analyzed using OpenLab 4.0 software (Improvision), ImageJ (NIH), and custom-written MATLAB (The Mathworks) scripts.

GCaMP3 (Tian et al., 2009) was expressed specifically in the ADL neurons under the *sre-1* promoter, and the behavioral responses of the transgenic strain expressing this construct were verified prior to use in imaging experiments. The same transgenic array expressing *GCaMP3* was examined in wild type and *npr-1* mutant backgrounds. For quantification of fluorescence changes, the cell body area of an ADL neuron was selected as the region of interest and a similar sized area near the cell body was selected as the background. To ensure that fluorescence measurements were performed within the linear dynamic range, only images exhibiting less than 1,000 average fluorescence intensity units in the region of interest and background were used for further analysis. Average fluorescence intensities in the first 5 seconds of imaging were used for normalization. In all cases, control and test genotypes were interleaved over several days of imaging. Animals were pre-exposed to near-UV light for 1 min prior to initiation of imaging; pre-exposure did not affect Ca^{2+} responses in the ADL neurons. *osm-10::GCaMP2.2b-*

expressing transgenic worms were used for ASH imaging, and animals were pre-exposed to near-UV light for 3 min before imaging. The ASH neurons in these animals responded robustly to 10 mM $CuSO_4$ (Sambongi et al., 1999). ASK responses were imaged using animals expressing *sra-9::GCaMP2.2b* (Kim et al., 2009).

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