Supplemental Information

Lateral Facilitation between Primary Mechanosensory Neurons Controls Nose Touch Perception in *C. elegans*

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

C. elegans Strains

Strains used:
AQ2145 ljEx19[egl-46::YC2.3; lin-15(+)]
AQ2045 ljIs104[cat-1::YCD3]
AQ906 bzIs17[mech-4::YC2.12]
AQ1490 ljEx130[ocr-4::YCD3]

AQ2695 unc-13(e51); ljIs104[cat-1::YCD3]

AQ2126 mec-10(tm1552); ljEx19
AQ2270 mec-10(tm1552); ljEx19; ljEx220[egl-46::mec-10(genomic); unc-122::GFP]
AQ2692 mec-10(tm1552); ljEx381[egl-46::mec-10(cDNA); elt-2::RFP]
AQ2693 mec-10(tm1552); ljEx382[egl-46::mec-10(cDNA); elt-2::RFP]
AQ2694 mec-10(tm1552); ljEx381; ljEx19[egl-46::YC2.3; lin-15(+)]
AQ2697 mec-10(tm1552); ljIs104[cat-1::YCD3]
AQ2698 mec-10(tm1552); ljEx381; ljIs104[cat-1::YCD3]
AQ2699 mec-10(tm1552); ljEx130[ocr-4::YCD3]
AQ2700 mec-10(tm1552); ljEx381; ljEx130 [ocr-4::YCD3]
AQ2481 mec-10(tm1552); bzIs17; ljEx275[mech-4::mec-10(cDNA)]
AQ1413 mec-10(tm1552); bzIs17
AQ2701 mec-10(tm1552); bzIs17; ljEx381

AQ2125 trpa-1(ok999); ljEx19
AQ1491 trpa-1(ok999); ljEx130[ocr-4::YCD3]
AQ2679 trpa-1(ok999); ljEx373[ocr-4::trpa-1(cDNA); unc-122::GFP]
AQ2680 trpa-1(ok999); ljEx374[ocr-4::trpa-1(cDNA); unc-122::GFP]
AQ2696 trpa-1(ok999); ljEx374; ljEx130[ocr-4::YCD3]
AQ2681 trpa-1(ok999); ljEx374; ljEx19[egl-46::YC2.3]
AQ2682 trpa-1(ok999); ljEx374; ljIs104[cat-1::YCD3]
AQ2702 trpa-1(ok999); ljIs104[cat-1::YCD3]
AQ1492 trpa-1(ok999); ljEx115[del-2::trpa-1(cDNA); unc-122::GFP]; ljEx130
AQ2265 trpa-1(ok999); ljEx115; ljEx19[egl-46::YC2.3]
AQ2262 trpa-1(ok999); ljEx19; ljEx119[sra-6::trpa-1(cDNA); unc-122::GFP]

AQ2574 unc-7(e5); ljIs104[cat-1::YCD3]
AQ2683 unc-7(e5); ljEx375[egl-46::unc-7(cDNA); cat-1::unc-7(cDNA); elt-2::RFP]
AQ2684 unc-7(e5); ljEx376[egl-46::unc-7(cDNA); cat-1::unc-7(cDNA); elt-2::RFP]
AQ2685 unc-7(e5); ljEx384; ljIs104[cat-1::YCD3]
AQ2703 unc-7(e5); ljEx19[egl-46::YC2.3]
AQ2707 unc-7(e5); ljEx384; ljEx19[egl-46::YC2.3]
AQ2148 osm-9(ky10); ljEx19[egl-46::YC2.3]
AQ2149 osm-9(ky10); mec-10(tm1552); ljEx19[egl-46::YC2.3]
AQ2274 osm-9(ky10); ljEx19; ljEx222[sra-6::osm-9(genomic); unc-122::GFP]
AQ2294 osm-9(ky10); ljEx19; ljEx235[del-2::osm-9(genomic); elt-2::RFP]
AQ2686 osm-9(ky10); ljEx377[egl-46::osm-9(cDNA); elt-2::RFP]
AQ2687 osm-9(ky10); ljEx378[egl-46::osm-9(cDNA); elt-2::RFP]
AQ2688 osm-9(ky10); ljEx377; ljEx19[egl-46::YC2.3]
AQ2689 osm-9(ky10); ljEx379[ocr-4::osm-9(cDNA); elt-2::RFP]
AQ2690 osm-9(ky10); ljEx380[ocr-4::osm-9(cDNA); elt-2::RFP]
AQ2691 osm-9(ky10); ljEx380; ljEx130[ocr-4::YCD3]
AQ2284 trp-4(ok1605); ljEx19[egl-46::YC2.3]
AQ2266 trp-4(ok1605); ljEx19; ljEx128[dat-1::trp-4(cDNA); unc-122::GFP]
AQ2704 trp-4(ok1605); osm-9(ky10); ljEx19[egl-46::YC2.3]
AQ2705 trp-4(ok1605); ljEx128; ljIs104[cat-1::YCD3]
AQ2706 ljEx383; Ex[dat-1::GFP]

Generation of FLP/PVD cameleon line ljEX19

The egl-46 promoter region was obtained from plasmid TU#307 (Wu et al., 2001), a gift from the lab of Martin Chalfie. A 3 kb HindIII/NotI fragment was fused to cameleon YC2.3 in the vector pPD95.75 (A. Fire). Transgenic lines were obtained by germline injection of a lin-15(n765) mutant strain with the egl-46::YC2.3 plasmid at a concentration of 50 ng/µl along with lin-15(+) genomic DNA (30 ng/µl) as a coinjection marker. Once a stable transgenic line was obtained, the lin-15(n765) allele was then removed by backcrossing to wild-type (N2) animals.

Transgenic rescue lines:

The egl-46 promoter was obtained from plasmid TU#307 (Wu et al., 2001), a kind gift from the Chalfie Lab. Using the primers GGCCCTCTGAAATCAAACG and AGTTCACGCCAGATGCAAGA we PCR amplified a 3 kb fragment which was cloned into a Gateway 3-way P4-P1R donor vector. The ocr-4 promoter we used was a 4.8kb fragment previously described by Kindt et al (Kindt et al., 2007b). A 3 kb sra-6
promoter fragment cloned into a Gateway P4-P1R vector was obtained in the form of plasmid MGW1.2, a gift by E. Busch from the de Bono group. The 5’ end of the promoter is defined by the sequnce 5’-CTTTTAGATATAATAAATCGAAATTG-3’ while the 3’ end is defined by the sequence 5’-GGCAAAATCTGAAATAATAATATTAAATTC-3’ adjacent of the start site of sra-6.

A 2.4 kb del-2 promoter was amplified using the following primers 5’-GATTTGACATCTAGTAAATTG-3’ and 5’-GCTGCAATTGAGTAGAATATTGAGTAG-3’.

A dat-1 promoter of length 0.8 kb was amplified. The 5’ sequence was the following 5’-CTCTGAAATGTTTCTAGTCGTTTTTG-3’ and 5’-GGCTAAAAATTGTTGAGATTCG-3’ adjacent to the start site. A 4.4 kb fragment of mec-10 genomic DNA was amplified using Phusion High-Fidelity DNA Polymerase F-530S from adult genomic DNA using the primers 5’-GTACAAAATTTCTAGTACTGTTTTTTG-3’ and 5’-GGCTAAAAATTGTTGAGATTCG-3’. A 6.3 kb osm-9 genomic DNA fragment was amplified from adult genomic DNA using the primers 5’-ATGGGCCTGTTGAGTTGCGAACC-3’ and 5’-AAGAAAAAAAATTTACAAAAAAATTAG-3’. We used a 5.6 kb trp-4 cDNA clone (Kindt et al., 2007a), which was amplified from adult C. elegans RNA using the primers 5’-CCAGATGAATCCCCACACCTCCC-3’ and 5’-AAGGGGTTATGCTAAAAACTAGTAGGTACTGC-3’. We obtained a 2.8 kb osm-9 cDNA clone described in (Colbert et al., 1997), a gift from the Bargmann lab.
A *trpa-1* 3.6 kb cDNA fragment was amplified from a plasmid generated previously (Kindt et al., 2007b) using the primers 5’-ATGTCGAAGAAATCATTAGG-3’ and 5’-TCAGTTATCTTTCTCCTCAAGT-3’. A 2.2 kb *mec-10* cDNA was amplified using Qiagen OneStep RT-PCR Kit from an RNA library using the primers 5’-ATGAATCGAAACCCGCGAATG-3’ and 5’-TCAATACTCATTTGCGACGATTTC-3’. In order to generate rescue plasmids, we used the MultiSite Gateway Three Fragment Vector Construction Kit (Invitrogen) to fuse promoter, ORF and an *unc-54* 3’UTR. All fragments generated by PCR were sequenced after cloning, and any mutations that were detected were rectified using the site-directed mutagenesis kit: QuickChange Lightning Site-Directed Mutagenesis Kit, from Stratagene.

We injected the *mec-10*(tm1552); *ljEx19*[egl-46::YC2.3] worms with egl-46::mec-10(*genomic*) at 80 ng/µl with *unc-122::GFP* at 25 ng/µl to generate array *ljEx220*[egl-46::mec-10].

To generate arrays *ljEx381* and *ljEx382* we injected *mec-10*(tm1552) with egl-46::mec-10(cDNA) at 75 ng/µl and elt-2::RFP at 25 ng/µl. Two independent arrays were obtained and tested for rescue of the behavioral defect; one of these was subsequently crossed into the various calcium indicator strains that we used for imaging. To genotype the animals that came out of the crosses we used primers in the introns flanking the *tm1552* deletion. The “promoter only” controls for the egl-46 promoter involved injection into a *mec-10*(tm1552) background in two different combinations. The first was egl-46 promoter at 80 ng/µl with *unc-122::GFP* at 25 ng/µl and the other was egl-46 promoter at 80 ng/µl with 25 ng/µl elt-2::RFP.
The sra-6::osm-9 construct was injected in osm-9(ky10); Ex19[egl-46::YC2.3] at 50 ng/µl with unc-122::GFP at 20 ng/µl to generate array ljEx222. Similarly, the del-2::osm-9(genomic) clone was injected at 75 ng/µl together with elt-2::RFP at 25 ng/µl in osm-9(ky10); Ex19[egl-46::YC2.3] to obtain array ljEx235. To obtain cDNA rescue of osm-9 in FLP, osm-9(ky10) animals were injected with egl-46::osm-9(cDNA) at 80ng/µl with 25 ng/µl of elt-2::RFP. Two independent arrays ljEx377 and ljEx378 were obtained and ljEx377 was subsequently crossed into the various calcium indicator lines used for calcium imaging. To genotype the animals that came out of the crosses we used primers in the introns flanking the ky10 point mutation to generate a PCR fragment which was subsequently sequenced with the primer CTAGGTGGAGGGCTGATTA. In order to obtain the rescue cDNA arrays ljEx379 and ljEx380 of osm-9 in the OLQ neurons we injected osm-9(ky10) animals with 90 ng/µl [ocr-4::osm-9(cDNA)] and 25 ng/µl elt-2::RFP. To image from the rescue lines we crossed ljEx380 with osm-9(ky10) Ex[egl-46::YC2.3]. The “promoter only” controls for the egl-46 promoter involved injection into an osm-9(ky10) background at 80 ng/µl with elt-2::RFP at 25 ng/µl and for the ocr-4 promoter at 90 ng/µl with 25 ng/µl elt-2::RFP. The “promoter only” controls were injected as follows: del-2 promoter was injected at 75 ng/µl with elt-2::RFP at 25 ng/µl , egl-46 promoter involved injection of the promoter at 80ng/µl with 25 ng/µl of elt-2::RFP and the ocr-4 promoter at 90ng/µl with 25 ng/µl of elt-2::RFP. All empty promoters were injected in an osm-9(ky10) background.

The ocr-4::trpa-1(cDNA) construct was injected at 80 ng/µl together with unc-122::GFP at 25 ng/µl to create ljEx373 and ljEx374. ljEx374 was subsequently crossed into the various calcium indicator lines used for calcium imaging. trpa-1(ok999); ljEx115[del-
2::trpa-1(cDNA); unc-122::GFP] and trpa-1(ok999); ljEx119[sra-6::trpa-1(cDNA); unc-122::GFP] carrying animals (previously described in (Kindt et al., 2007b)) were crossed into the ljEx19[egl-46::YC2.3] to image from the FLP neurons. To genotype the animals that came out of the crosses we used primers in the introns flanking the ok999 deletion. The “promoter only” controls were injected as follows: the del-2 promoter was injected at 75 ng/µl with unc-122::GFP at 25 ng/µl and the ocr-4 promoter at 80 ng/µl with 25 ng/µl of unc-122::GFP. All empty promoters were injected in a trpa-1(ok999) background.

To determine whether FLP and RIH nose touch defects in trp-4(ok1605) mutant worms were rescued by expression of trp-4 in dopaminergic neurons we crossed trp-4(ok1605); ljEx19 and trp-4(ok1605); ljIs104 with ljEx128[dat-1::trp-4(+); unc-122::GFP] respectively, a previously described array carrying a trp-4 cDNA expressed specifically in dopaminergic neurons (Kindt et al., 2007a). The “promoter only” control was injected as follows: dat-1 promoter was injected at 20ng/µl with unc-122::GFP at 25 ng/µl in a trp-4(ok1605) background.

The unc-7(e5) allele was crossed with ljIs104[cat-1::YCD3] to image from RIH and with ljEx19[egl-46::YC2.3]. To generate rescue lines for unc-7 we co-injected egl-46::unc-7(cDNA) and cat-1::unc-7(cDNA) transgenes at a final concentration of 50 ng/µl each along with 25 ng/µl elt-2::RFP. Two independent arrays were obtained, ljEx375 and ljEx376, and ljEx375 was crossed into the appropriate imaging lines. In order to genotype the animals that came out of the crosses we used primers in the introns flanking the e5
point mutation to generate a PCR fragment which was subsequently sequenced. The “promoter only” controls were injected as follows: *cat-1* promoter was injected at 50 ng/µl with *elt-2::RFP* at 25 ng/µl, the *egl-46* promoter at 50 ng/µl with 25 ng/µl of *elt-2::RFP and cat-1;elg-46* at a final concentration of 50 ng/µl with 25 ng/µl *elt-2::RFP*. All empty promoters were injected in an *unc-7(e5)* background.

To generate the *dat-1::GFP* we injected the construct at 25 ng/µl with 25 ng/µl *unc-122::GFP*.

**SUPPLEMENTAL REFERENCES**


SUPPLEMENTAL FIGURES

Figure S1: Supplemental data for touch-evoked calcium imaging experiments.  A.

Effect of cameleon transgenes on nose touch behavior. The cameleon imaging lines were assayed for their behavioural response to nose touch as described in methods. Fraction of animals responding is shown; error bars indicate SEM. 100 wild-type animals and 50 animals from each cameleon line were tested.  B. Protocols for nose touch and harsh touch stimulation. Shown are images of animals receiving the nose touch or harsh head touch stimulus used in calcium imaging experiments. Worms were glued to the tip of the nose without covering the mouth on a 2% agarose pad and are subsequently immersed in extracellular saline. Two types of mechanical stimulation were delivered. A nose touch stimulus which consisted of a 1 second gentle (buzz) stimulus at the edge of the nose of the worm with a final displacement of 8 µm. A harsh head touch stimulus was delivered in a more posterior position near the terminal bulb of the pharynx. The probe was displaced a total of 24 µm into the worm’s head.
A) Nose touch avoidance behavior

B) Nose touch

harsh head touch stimulus

harsh head touch pre-stimulus

8 µm

24 µm
Figure S2: FLP heat responses in wild-type and mutant animals. A-C. Averaged calcium responses to heat shock stimulus. Each trace represents the average percentage change in R/R₀ for the indicated genotype, where R is the fluorescence emission ratio at a given time point and R₀ is its initial value. The green bar indicates a temperature of 20°C and the red bar indicates a period of stimulation at a temperature of 35°C. 18 wild-type, 10 mec-10, 10 osm-9 and 10 osm-9; egl-46::osm-9 animals were imaged for these experiments. D. Scatter plot of peak calcium responses for each genotype. Statistical significance (*** p < .0005) is according to the Mann-Whitney rank sum test.
Ratio Change, % (YFP/CFP)

wt

osm-9

osm-9; egl-46::osm-9

A) FLP heat response

B) 20°C 35°C 20°C

mec-10

20°C 35°C 20°C

25%

50sec

25%

50sec

25%

50sec

D) FLP heat response

Ratio Change, % (YFP/CFP)

wt osm-9 egl-46::osm-9 mec-10

*** ***
Figure S3: *egl-46::mec-10* does not rescue the *mec-10* touch defect in ALM neurons.

A-D. **Averaged calcium responses to gentle body touch in the ALMs.** Animals expressing cameleon in the ALM gentle body touch neurons were given a 1 second buzz stimulus on the anterior body as described (Suzuki et al., 2003). Each red trace represents the average percentage change in $R/R_0$ for the indicated genotype, where $R$ is the fluorescence emission ratio at a given time point and $R_0$ is its initial value. 11 animals per genotype were imaged. **E. Scatter plot of peak calcium responses for each stimulus.** The red line indicates mean ratio change; the error bars indicate SEM.
wild-type

mec-10(tm1552); egl-46::mec-10

mec-10(tm1552); mec-4::mec-10

10%

10sec

A) B)

C) D)

E) ALM gentle body touch

Ratio Change, % (YFP/CFP)

rescue promoter
mec-10(+)
neurons

- egl-46 mec-4
- FLP ALM
- PVD PLM
- AVM
Figure S4: Effects of genetic background on expression levels of cameleon and promoter::GFP arrays. Mean fluorescence intensity was measured for the indicated neuronal cell body using a Zeiss LSM 510 Meta confocal microscope with a 40x objective. Images were exported as single TIFF files and fluorescence intensity was quantified using ImageJ. For cameleon lines the same strains used in imaging experiments in the main text were used. At least 5 animals of each genotype were analyzed.
A) *egl-46::YC2.3* intensity, FLP

B) *ocr-4::YCD3* intensity, OLQ

C) *dat-1::GFP* intensity, CEP

D) *cat-1::YCD3* intensity, RIH
Figure S5: *sra-6::osm-9* rescues ASH-mediated osmotic avoidance behavior. Wild-type, *osm-9(ky10)*, and *osm-9(ky10); sra-6::osm-9(genomic)* animals were tested for escape behavior evoked by 1M glycerol as described (Hilliard et al., 2002). Each data point represents 50 worms assayed on three independent days. Error bars show S.E.M. *** indicates a significant difference between mutant and rescued strains according to the chi-square test (p < 0.001).
Osmotic avoidance behavior

Fraction responding

- Wild-type buffer
- osm-9 buffer
- osm-9; sra-6::osm-9 buffer
- Wild-type glycerol
- osm-9 glycerol
- osm-9; sra-6::osm-9 glycerol

**Note:** The graph shows the fraction responding under different osmotic conditions.
Figure S6: *trpa-1* acts cell-autonomously in OLQ to promote nose touch response.

Worms expressing cameleon in OLQ neurons were immobilized to the nose with glue and covered in extracellular saline containing 2 mM serotonin. A glass probe delivered a gentle buzzing stimulus to the nose. A-D Average traces in OLQ in response to 2\textsuperscript{nd} nose touch stimulation (5 min after the first stimulation) in (A) wild-type, (B) *trpa-1*, (C) *trpa-1* with RIH ablated and (D) *trpa-1; ocr-4::trpa-1*. (E) Scatter plot quantifying the 2\textsuperscript{nd} OLQ nose touch responses in wild-type (n=14), *trpa-1* (n=14), and *trpa-1; ocr-4::trpa-1* (n=13).
A) OLQ
wild-type

C) OLQ
trpa-1; ocr-4::trpa-1

B) OLQ
trpa-1

D) OLQ repeated nose touch

Ratio Change, % (YFP/CFP)

wt  trpa-1(ok999)

rescue promoter
trpa-1(+) cells
ocr-4
OLQ
Figure S7: Effect of innexin mutations on nose touch responses.  

**A.** Averaged responses of RIH neurons of *unc-7* innexin mutant and rescued animals to nose touch stimulation. Each solid trace represents the average percentage change in R/R₀ for 14 (wild-type, red trace) or 10 (*unc-7(e5)*, green trace) individual recordings. Gray shading indicates SEM of the mean response. Scale bars are indicated. None of these genotypes visibly altered the morphology of FLP or RIH or the expression patterns of the cameleon transgenes. 

**B.** Averaged responses of FLP neurons of *unc-7* innexin mutant and rescued animals to nose touch stimulation. Each solid trace represents the average percentage change in R/R₀ for 10 (wild-type, red trace) or 10 (*unc-7(e5)*, green trace) individual recordings. 

**C.** Scatter plot of peak calcium responses. In addition to the genotypes in panels A and B, 14 animals were imaged for RIH nose touch and 10 animals were imaged for FLP nose touch using *unc-7; egl-46::unc-7; cat-1::unc-7* rescue lines carrying the *cat-1::YCD3* and *egl-46::YC2.3* arrays. Statistical significance (***p < .0005*) is according to the Mann-Whitney rank sum test. 

**D.** Effect of *unc-7* innexin mutations on nose touch-evoked escape responses. 100 animals of each genotype were scored for reversals following nose touch stimulation. Statistical significance (p < 0.005) is according to the Student’s t test. 

**E.** Scatter plot of peak RIH calcium responses to harsh touch. 10 animals were imaged for each genotype. Statistical significance (***p < .0005*) is according to the Mann-Whitney rank sum test.
A) Nose touch

RIH

unc-7(e5)

unc-7; egl-46::unc-7; cat-1::unc-7

B) Nose touch

RIH

unc-7(e5)

unc-7; egl-46::unc-7; cat-1::unc-7

C) RIH nose touch

FLP nose touch

D) Nose touch avoidance

Fraction responding

E) RIH harsh head touch

Ratio change, % (YFP/CFP)
Figure S8: Empty vector controls for unc-7 nose touch rescue.  **A.** Scatter plot of responses to nose touch in the RIH neuron. **“Empty”** plasmids containing only the indicated promoters were injected into unc-7; cat-1::YCD3 animals at the same concentrations as the rescue constructs in Figure S7. 9 animals were imaged for each control strain. **B. Nose touch escape behavior**  **“Empty”** plasmids containing only the indicated promoters were injected into unc-7 animals at the same concentrations as the rescue constructs in Figure S7. 100 animals were assayed on 3 independent days. Error bars indicate SEM.
A) RIH nose touch response

Ratio Change, % (YFP/CFP)

unc-7(e5)

empty promoter transgene

none  egl-46  cat-1  egl-46, cat-1

B) Nose touch avoidance

Fraction responding

wt  unc-7(e5)

empty promoter transgene

none  none  egl-46  cat-1  egl-46, cat-1