Supplementary material

Supplementary figure legends

Figure S1 (Supplement to Figure 2): Mutants of *rig-3* show increased GLR-1::GFP in the AVA cell body

GLR-1::GFP puncta were imaged from AVA near the nerve ring. The images on the left show GLR-1::GFP expression in wild type (WT, n=12), *rig-3* mutants (n=13) and a *rig-3*; P*flp-18*::RIG-3 rescue line (n=12). The dot plot on the right shows the quantitative measure of fluorescence intensity as an arbitrary fluorescence unit (AFU). The entire region indicated in the image was taken for fluorescence quantification. The number of dots in the dot plot shows the number of animals observed, with each dot representing the fluorescent intensity from a single animal. The error bars represent SEM. Statistical significance was determined with one-way ANOVA with Bonferroni's multiple comparison test. Significance is represented as "**" $p<0.01$, "***" $p<0.001$ and "ns" for not significant. The statistics above each plot indicate significance with respect to the WT plot.

Figure S2 (Supplement to Figure 3): Individual traces associated with the Calcium imaging experiment

(A) Traces of calcium activity from freely reversing animals recorded for the whole duration of a reversal using GCaMP5 expressed specifically in the AVA command interneuron. The genotypes used include WT and mutant strains (*rig-3*, *glr-1* and *glr-1; rig-3*). In this figure each trace is plotted for each genotype imaged (genotypes are indicated on the top left of each plot). 14 animals were plotted for each genotype. (B) Graph of body-bends per reversal in WT (n=20), *rig-3* (n=10) and *flp-18* (n=20) animals. The error bars represent SEM. Statistical significance was determined with one-way ANOVA with Bonferroni's multiple comparison test. Significance is

represented as "***" $p<0.001$ and "ns" for not significant. The statistics above each plot indicate significance with respect to the WT plot.

Figure S3 (Supplement to Figure 4): Decreased reversals of *npr-5* mutants could be rescued by NPR-5 expression in sensory neurons

(A) Reversal assays were performed for a duration of 5 minutes (min) and plotted as dot plots for WT (n=14), *npr-5* (n=14) and *npr-5*; P*gpa-3*::NPR-5 (n=14) animals. The error bars represent SEM. Statistical significance was determined with one-way ANOVA with Bonferroni's multiple comparison test. Significance is represented as "***" $p<0.001$ and "ns" for not significant. The statistics above each plot indicate significance with respect to the WT plot. (B) Reversal assays were performed for a duration of 5 min and plotted as dot plots for WT (n=14), *npr-5* (n=14) and *npr-5*; P*gcy-5*::NPR-5 (n=14) animals. The error bars represent SEM. Statistical significance was determined with one-way ANOVA with Bonferroni's multiple comparison test. Significance is represented as "*" $p<0.05$, "***" $p<0.001$ and "ns" for not significant. The statistics above each plot indicate significance with respect to the WT plot. (C) Dot plot of reversals from WT (n=17), FLP-18++ (n=12), *glr-1* (13) and FLP-18++; *glr-1* (n=18) animals. The error bars represent $\pm$SEM. Statistical significance was determined with one-way ANOVA with Bonferroni's multiple comparison test. Significance is represented as "***" $p<0.001$ and "ns" for not significant. The statistics above each plot indicate significance with respect to the WT plot.

Supplementary video legends

Video 1-3: Reversals over a period of 15s

(1) Representative video indicated a WT control animal recorded for a period of 15s on a plate without food. (2) Representative video indicated a *rig-3* mutant animal

recorded for a period of 15s on a plate without food. (3) Representative video indicated a *rig-3* mutant *C. elegans* expressing RIG-3 under the *rig-3* promoter. The movement of these animals were recorded for a period of 15s on a plate without food.

Video 4-7: Calcium imaging in the AVA neuron

Representative video recordings from a moving *C. elegans* with GCaMP5 expressed in the AVA command interneuron of the genotypes given below. The circle indicates the AVA neuron that is being recorded and in the top left corner "F" and "R" indicate forward and reverse movement of the *C. elegans*.

(4) WT

(5) *rig-3*

(6) *glr-1*

(7) *glr-1; rig-3*

Supplementary tables

Table S1: List of strains used in this study

Strain	Genotype	Comments
VC2016	*flp-18 (gk3063) X*	CGC strain
RB1330	*npr-1 (ok1447) X*	CGC strain
tm1782	*npr-4 (tm1782) X*	NBRP
CX14394	*npr-5 (ok1583) V*	CGC strain
AX1444	*Pflp-18:*:FLP-18::*sl2*::GFP	From Mario de bono Lab
KP6535	*glr-1 (n2461); rig-3 (ok2156)*	From Josh Kaplan Lab
BAB1541	*flp-18 (gk3063) X*	3X out crossed CGC strain VC2016

BAB1542	*npr-1 (ok1447) X*	3X out crossed CGC strain RB1330
BAB1543	*npr-4 (tm1782) X*	3X out crossed NBRP strain
BAB1544	*npr-5 (ok1583) V*	3X out crossed CGC strain CX14394
BAB1552	*Pflp-18::*FLP-18::*sl2*::GFP; *npr-5 (ok1583)*	This study
BAB1553	*Pflp-18::*FLP-18::*sl2*::GFP; *npr-1 (ok1447)*	This study
BAB1554	*Pflp-18::*FLP-18::*sl2*::GFP; *npr-4 (tm1782)*	This study
BAB1555	*Prig-3*::HA::GLR-1::GFP	From Villu Maricq lab
BAB1556	*Prig-3::* HA::GLR-1::GFP; *rig-3 (ok2156)*	This study
BAB1557	*Prig-3::* HA::GLR-1::GFP; *rig-3 (ok2156); Pflp-18::*RIG-3 (*indEx508*)	This study
BAB1559	*npr-5 (ok1583); rig-3 (ok2156)*	This study
BAB1560	*rig-3 (ok2156); Prig-3::*RIG-3::sl2::wrmScarlet; *Punc-122::*GFP (*indEx508*)	This study
BAB1561	*rig-3 (ok2156); Pflp-18::*RIG-3; *PCFJ90* (*indEx508*)	This study
BAB1562	*rig-3 (ok2156); Punc-17::*RIG-3; *PCFJ90* (*indEx510*)	This study
BAB1563	*Prig-3::*GCaMP5; *PCFJ90* (*indEx511*)	This study
BAB1564	*rig-3 (ok2156); Prig-3::*GCaMP5; *PCFJ90* (*indEx511*)	This study
BAB1565	*glr-1 (n2461); Prig-3::*GCaMP5; *PCFJ90* (*indEx511*)	This study
BAB1566	*glr-1 (n2461); rig-3 (ok2156); Prig-3::*GCaMP5; *PCFJ90* (*indEx511*)	This study
BAB1567	*Pflp-18::*FLP-18::*sl2*::GFP; *npr-5 (ok1583)*; P*gcy-5*::NPR-5; *PCFJ90* (*indEx512*)	This study
BAB1568	*Pflp-18::*FLP-18::*sl2*::GFP; *npr-5 (ok1583)*; P*gpa-3*::NPR-5; *PCFJ90* (*indEx513*)	This study
BAB503	*glr-1 (n2461) III*	3X out crossed CGC strain KP4
BAB501	*rig-3 (ok2156) X*	3X out crossed CGC strain RB1712

BAB1571	*npr-5 (ok1583)*; P*gpa-3*::NPR-5; *PCFJ90* (*indEx513*)	This study
BAB1572	*npr-5 (ok1583)*; P*gcy-*5:NPR-5; *PCFJ90* (*indEx512*)	This study
BAB1573	*Prig-3::* HA::GLR-1::GFP; *rig-3 (ok2156); Prig-3-18::*RIG-3	This study
BAB1570	*Pflp-18::*FLP-18::*sl2*::GFP*; glr-1 (n2461)*	This study
BAB1574	*rig-3 (ok2156) flp-18 (gk3063)*	This study

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Primer Code	Sequence	Comment	Gene
AB37	ACCTTTCGGCTCCGACTTG	WT Forward	*glr-1*
AB38	ACCTTTCGGCTCCGACTTA	Mutant Forward	*glr-1*
AB39	ATTGAAATGACCATACCACC	Common reverse	*glr-1*
AB113	AGGACGGAAATTACCTGTGC	Genotyping Forward External	*flp-18*
AB114	GCTTCGGGAAACGCTCATAT	Genotyping Reverse Internal	*flp-18*
AB115	TTATTCTTTCTTGTCGGGGCC	Genotyping Reverse External	*flp-18*
AB116	ACCTGTCACTTTTACGCCGG	Genotyping Forward External	*npr-1*
AB117	TGATTTCGTTCCAGTTGAACG	Genotyping Reverse Internal	*npr-1*
AB118	GAACCTTCACTTCTCCTGTG	Genotyping Reverse External	*npr-1*
AB119	AGCTGTTGTCTCCTTCCAGG	Genotyping Forward External	*npr-4*
AB120	CGATTTCCGATGAGGAAACC	Genotyping Reverse Internal	*npr-4*
AB121	CACAGCTTCTAATAGGAAAGGG	Genotyping Reverse External	*npr-4*
AB122	GCACGACGAACTGCAAATTT	Genotyping Forward External	*npr-5*

AB123	TCCTTGAGTTTTCTGGGATG	Genotyping Reverse Internal	*npr-5*
AB124	AGGCATTTTTGGAAACGGCG	Genotyping Reverse External	*npr-5*
AB108	ACGCGTCGAC TCTGTCACATACTGCTCGAA	Cloning Forward *SalI*	P*flp-18*
AB109	CCCCCCGGGGTTGCTGTCTAACCCTGAAA	Cloning Reverse *XmaI*	P*flp-18*
AB139	GCGTCGACAAGTGACACCACGCTCACA	Cloning Forward *SalI*	P*rig-3*
AB140	CCCCCCGGGAGCTGTGAAATTTTTAGGCA GT	Cloning Reverse *XmaI*	P*rig-3*
PRS71	CGAAAAGGGGAGCAAACATCG	Genotyping External Forward	*rig-3*
PRS72	ATCTTGATCTCCTCGTCTCCG	Genotyping Internal Reverse	*rig-3*
PRS73	GCAATACCACACTATCTCCTG	Genotyping External Reverse	*rig-3*
AB148	CTAGCTAGCATGGGACGACTACTTGCCAA GAT	Cloning Forward *NheI*	*rig-3* cDNA
AB149	CGGGGTACCTTAGATAAAAAGACAGACAA AAAATAACGTG	Cloning Reverse *KpnI*	*rig-3* cDNA
AB209	ACATGCATGCACAAAGTTTTTAAAAAGTTG TTGATCGG	Cloning Forward *SphI*	P*gpa-3*
AB210	CCCCCCGGGGAAGCACAACTCTAAAAAG CCCA	Cloning Reverse *XmaI*	P*gpa-3*
AB216	ACATGCATGCCGATTGACATTGGTCTTAC ATTTTGAC	Cloning Forward *SphI*	P*gcy-5*
AB217	CCCCCCGGGATTGAAATTCTACTACTTCT GGGGG	Cloning Reverse *XmaI*	P*gcy-5*

Table S3: List of plasmids used in this study

S. No.	Plasmid No.	Plasmid
1	pBAB515	*Prig-3*::RIG-3::sl2::wrmScarlet

2	pBAB516	*Pflp-18*::RIG-3
3	pBAB519	*Pgcy-5*::NPR-5
4	pBAB520	*Pgpa-3*::NPR-5
5	pAG_09	*Prig-3*::GCaMP5 (from Cori Bargmann lab)