Sensation in a single neuron pair represses male behavior in hermaphrodites

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Supplemental information inventory

Tables 1, 2, 3 and 4 - supports Results and Experimental Procedures
Figures 1 and 2 - supports Results
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Supplemental Table 1: Strains

Strain number	Genotype	Source	Notes
N2 Bristol	wild-type	CGC	Laboratory reference strain.
EG2717	*him-5(e1490) V*	(White et al., 2(	8x outcrossed against N2 Bristol.
EG2718	*him-8(e1489) IV*	(White et al., 2(	8x outcrossed against N2 Bristol.
CB1372	*daf-7(e1372ts) III*	CGC	Reference allele.
EG3702	*daf-7(e1372ts) III; him-5(e1490) V*	Thomas J. Nicholas	For checking male behavior in *daf-7* background. Outcrossed *daf-7* mutant strain.
PY3769	*daf-7(e1372ts) III ; oyIs14[Psra-6::gfp] V ; daf-12(rh61rh411) X*	Piali Sengupta	P*sra-6*::*GFP* off in ASI and ASH; daf-d. Independent *daf-7* mutant strain.
PY1671	*daf-7(e1372ts) III ; oyIs14[Psra-6::gfp] V ; daf-3(mgDf90) X*	Piali Sengupta	GFP in ASH, ASI, PVQ, daf-d.
CB1376	*daf-3(e1376) X*	CGC	*daf-3* suppresses *daf-7*
EG6125 EG6126 EG6127	*daf-7(e1372ts) III ; him-5(e1490) V ; oxEx1478[Podr-4::egfp_daf-7::unc-54UTR, Punc-17::mCherry]*	This study	Rescue of *daf-7* in most sensory neurons (includes ASI).
EG6169 EG6170	*daf-7(e1372ts) III ; him-5(e1490) V ; oxEx1484[Punc-17::mCherry, Ppkd-2::gfp]*	This study	Made to check for male neurons in *daf-7* hermaphrodites.
EG6254 EG6255 EG6256	*daf-7(e1372ts) III ; him-5(e1490) V ; oxEx1496[Pceh-36::egfp_daf-7,Punc-17::mCherry]*	This study	*daf-7* rescue in AWC/ASE.
EG6289 EG6290	*daf-7(e1372ts) III ; him-5(e1490) V ; oyIs48[P(ceh-36)::gfp, lin-15(+)] V*	This study	For ablations in a *daf-7* background.
PR678	*tax-4(p678) III*	CGC	Reference allele. *p678* allele verified by sequencing
EG3701	*tax-4(p678) III ; lin-15(n765ts) X*	(White et al., 2(	*p678* allele confirmed by sequencing.
EG5487 EG5488 EG5489	*tax-4(p678) III ; lin-15(n765ts) X ; oxEx1376[Pgpa-4::tax-4_gfp(S65C),ccGFP,lin-15(+)]*	This study	*tax-4* rescue in ASI.
EG5457 EG5459 EG5482	*tax-4(p678) III ; lin-15(n765ts) X ; oxEx1374[Psrg-2::tax-4_gfp(S65C),ccGFP,lin-15(+)]*	This study	*tax-4* rescue in ASK.
EG5671 EG5672 EG5673	*tax-4(p678) III ; lin-15(n765ts) X ; oxEx1379[Pgpa-9::tax-4_gfp(S65C),ccGFP,lin-15(+)]*	This study	*tax-4* rescue in ASJ.
EG5358 EG5359 EG5360 EG5361	*him-5(e1490) V ; lin-15(n765ts) X ; oxEx1299[Prab-3::>mCherry::let-858>::egfp_fem-3::unc-54UTR, Phsp-FLP, Ppkd-2::gfp(venus), lin-15(+)]*	This study	Heat-shock FLP-ON to masculinize the nervous system.
EG6167 EG6168	*him-8(e1489) IV ; lin-15(n765ts) X ; oxEx1482[Podr-4::tra-2ic::mCherry, lin-15(+)]*	This study	P*odr-4* neurons are red (mCherry) and feminized (*tra-2ic*).
EG4168	*him-8(e1489) IV ; oyIs48[P(ceh-36)::gfp, lin-15(+)] V*	(White et al., 2(	Green AWC and ASE neurons. Routinely used for ablations.

Supplemental Table 2: Promotors used for sensory neuron and pan-neural expression

	Promotor					
	Podr-4	***Pgpa-4***	***Psrg-2***	***Psrg-8***	***Pceh-36***	***Prab-3***
Reference	Dwyer et al., 1998	Jansen et al., 1999	Troemel et al., 1995	Troemel et al., 1995	Lanjuin et al., 2003	Nonet et al., 1997
Class	Sensory	Sensory	Sensory	Sensory	Sensory	Pan-neural
Reported expression pattern	AWA					All neurons
	AWB					
	AWC				AWC	
					ASE	
	ASG					
	ASI	ASI				
	ASJ					
	ASK		ASK	ASK		
	ADF					
	ADL					
	ASH					
	PHA					
	PHB					

Supplemental Table 3: Reported expression patterns of P*glr-2*, P*glr-5*, and P*ser-2b*

P*glr-2* + P*glr-5* + P*ser-2b*	P*glr-2*[a]	P*glr-5*[a]	P*ser-2b*[b]	Notes based on hermaphrodite wiring[c]
AIA	AIA			Ring interneuron.
AIB	AIB	AIB?		Amphid sensory neuron. generally responds to aversive signals.
AIY			AIY	Amphid interneuron. Functions in thermosensation.
AIZ			AIZ	Amphid interneuron. Functions in thermosensation
AVA	AVA	AVA		Command interneuron. Drives backward movement in the locomotory circuit.
AVB		AVB		Command interneuron. Drives forward movement in the locomotory circuit.
AVD	AVD	AVD		Command interneuron. Drives backward movement in the locomotory circuit.
AVE	AVE	AVE		Command interneuron. Drives backward movement in the locomotory circuit.
AVG	AVG			Ventral cord interneuron. Pioneers the right tract of the ventral cord.
AVK		AVK		Ring and ventral cord interneuron.
BDU			BDU	Interneuron, process runs along excretory canal and then into NR via the deirid commissures.
DVA	DVA	DVA?	DVA	Ring interneuron. Functions in mechanosensory integration; sets the activity of the touch circuit.
HSN?		HSN?		Hermaphrodite-specific motor neuron. Die in male embryos.
LUA		LUA		Interneuron. Possible connector between PLM touch neurons.
M1	M1			Pharangeal motor neuron.
PVC	PVC	PVC?		Tail interneuron.
PVQ		PVQ		Tail interneuron. Pioneers the left tract of the ventral cord.
PVT +/-			PVT +/-	Tail interneuron. Projects to ring. Different connectivity in males and hermaphrodites.
RIA	RIA			Ring interneuron.
RIC		RIC		Ring interneuron. Expresses octopamine.
RID			RID	Ring motorneuron.
RIF		RIF		Ring interneuron.
RIG	RIG			Ring interneuron.
RIM		RIM		Head motorneuron.
RIR	RIR			Ring interneuron. Single cell body.
RMDD	RMDD	RMDD		Motor neuron.
RMDV	RMDV	RMDV		Motor neuron.
RME		RME (all)	RME	Motor neuron.
RMG		RMG		Head motorneuron.
SABD		SABD		Motor neuron.
SABVL		SABVL		Motor neuron.
SABVR		SABVR		Motor neuron.
SIAD			SIAD	Motor or interneuron.
SIAV			SIAV	Motor or interneuron.
SIB (all)		SIB (all)		Motor or interneuron.
SMD (all)		SMD (all)		Motor neuron.
URA?		URA?		Head motor neurons. Possibly sensory.
URB		URB		Head motor neurons. Possibly sensory.
URY		URY		Head sensory neuron.
VC		VC		Hermaphrodite specific ventral cord motor neurons.

[a] Brockie et al., 2001; Greer et al., 2008

[b] Tsalik and Hobert, 2003; Greer et al., 2008

[c] Taken from WormAtlas (www.wormatlas.org) and Sulston and Horvitz, 1977; White et al., 1986.

Supplemental Table 4: Reported expression pattern of P*unc-17*

P*unc-17*[a,b]	Notes based on hermaphrodite wiring[c]
AIY	Amphid interneuron. Functions in thermosensation.
ALN	Tail neuron of unknown function.
AS.1-11	Ventral cord motor neuron.
DA	Dorsal A motor neuron.
DB	Dorsal B motor neuron.
HSN	Hermaphrodite-specific motor neuron. Die in male embryos.
I1	Pharyngeal interneuron.
I6	Pharyngeal interneuron.
IL2	Inner labial sensory neuron.
M1	Pharangeal motor neuron.
M2	Pharyngeal neuron. Unknown/redundant function.
M5	Pharyngeal neuron. Unknown/redundant function.
PLN	Posterior neuron. Unknown function.
RMD	Motor neuron.
SAA	Interneuron, possible stretch receptor neuron.
SAB	Motor neuron.
SDQ	Interneuron.
SIAD	Motor or interneuron.
SIAV	Motor or interneuron.
SIB	Motor or interneuron.
SMB	Motor neuron.
SMD	Motor neuron.
URA	Head motor neurons. Possibly sensory.
URB	Head motor neurons. Possibly sensory.
VA	Ventral motor neuron.
VB	Ventral motor neuron.
VC	Hermaphrodite specific ventral cord motor neurons.
Others[a]	

[a]Alfonso et al., 1993

[b]The P*unc-17* expression pattern is based on antibody staining and not completely characterized.

[c] Taken from WormAtlas (www.wormatlas.org) and references (Sulston and Horvitz, 1977; White et al., 1986).

Supplemental Figure Legends

Supplemental Figure 1: Sex pheromones elicit behavior in *daf-7* mutant hermaphrodites that is similar to males, but not wild-type hermaphrodites. Quantitation of sexual attraction behavior in wild-type males, wild-type hermaphrodites, and *daf-7* mutant hermaphrodites. Wild-type hermaphrodites and males were assayed in the absence and presence of sex pheromone, and *daf-7* mutant males and hermaphrodites were tested in the presence of sex pheromone blind for genotype and pheromone *vs.* control as described in the Experimental Procedures. Comparisons use Fisher's exact test with Bonferroni-Holm correction for multiple comparisons. P values for each comparison are given in the Figure. Data shown are from *daf-7* mutant strain CB1372; similar results were obtained from two other independent strains.

Supplemental Figure 2: Sexual attraction in males requires the core sensory neurons AWA, AWC, and ASK, which compensate for one another. Laser ablation of each pair (one pair at a time) in L4 larvae shows that the AWA, AWC, and ASK pairs of sensory neurons are required for adult sexual attraction. Ablation of the ASK neurons in L3 larva results in males with unimpaired sexual attraction. The different outcome of ASK ablation depending on developmental stage (L3 *vs.* L4 larvae) is indicative of compensation, as observed previously for AWA and AWC (White et al., 2007). Ablation of the ASI neurons in L4 larvae shows that they are not required; elimination of the ASI neurons in L4 males may in fact increase the frequency of adult male sexual attraction. Ablations were performed and scored as described (White et al., 2007).

Supplemental Experimental Procedures

Behavior. Assays were at 20-22˚C on 50mm nematode growth-media (NGM) agar plates with a 20µl spot of hermaphrodite-conditioned media (pheromone) and a 20µl control spot of unconditioned media (White et al., 2007). All assays were in the presence of a thin layer of HB101 bacteria for the worm to eat. Unless otherwise noted, assays were of single animals. Assays were scored three independent times over a three hour period and once again after 16 hours for a total of four assessments. Assays were classified categorically as either showing attraction behavior or not based on track patterns; an assay needed to show robust attraction track patterns at least twice during the four blind scorings to classify as showing attraction. Each set of assays was repeated on at least three different days, and contained positive and negative controls in numbers approximately equal to the conditions being tested.

Male sexual attraction in the Supplemental Figure, panel A was scored numerically as in previous work (White et al., 2007), rather than categorically. Assays were of single males. Operated or mock-ablated males were scored 3 independent times, scores for the indicated number of assays were averaged; error bars in the figures show the average standard error of the mean.

Supplemental References

Alfonso, A., Grundahl, K., Duerr, J.S., Han, H.P., and Rand, J.B. (1993). The *Caenorhabditis elegans unc-17* gene: a putative vesicular acetylcholine transporter. Science *261*, 617-619.

Brockie, P.J., Madsen, D.M., Zheng, Y., Mellem, J., and Maricq, A.V. (2001). Differential expression of glutamate receptor subunits in the nervous system of *Caenorhabditis elegans* and their regulation by the homeodomain protein UNC-42. J. Neurosci. *21*, 1510-1522.

Dwyer, N.D., Troemel, E.R., Sengupta, P., and Bargmann, C.I. (1998). Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell *93*, 455-466.

Jansen, G., Thijssen, K.L., Werner, P., van der Horst, M., Hazendonk, E., and Plasterk, R.H. (1999). The complete family of genes encoding G proteins of *Caenorhabditis elegans.* Nat. Genet. *21*, 414-419.

Lanjuin, A., VanHoven, M.K., Bargmann, C.I., Thompson, J.K., and Sengupta, P. (2003). Otx/otd homeobox genes specify distinct sensory neuron identities in *C. elegans.* Dev. Cell *5*, 621-633.

Nonet, M.L., Staunton, J.E., Kilgard, M.P., Fergestad, T., Hartwieg, E., Horvitz, H.R., Jorgensen, E.M., and Meyer, B.J. (1997). *Caenorhabditis elegans rab-3* mutant synapses exhibit impaired function and are partially depleted of vesicles. J. Neurosci. *17*, 8061-8073.

Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A., and Bargmann, C.I. (1995). Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans.* Cell *83*, 207-218.

Tsalik, E.L., and Hobert, O. (2003). Functional mapping of neurons that control locomotory behavior in *Caenorhabditis elegans.* J. Neurobiol. *56*, 178-197.