Neuron
Report

Sensation in a Single Neuron Pair Represses Male Behavior in Hermaphrodites

Jamie Q. White[1] and Erik M. Jorgensen[1,*]
[1]Department of Biology and Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT 84112-0840, USA
*Correspondence: jorgensen@biology.utah.edu
http://dx.doi.org/10.1016/j.neuron.2012.03.044

SUMMARY

Pheromones elicit innate sex-specific mating behaviors in many species. We demonstrate that in *C. elegans*, male-specific sexual attraction behavior is programmed in both sexes but repressed in hermaphrodites. Repression requires a single sensory neuron pair, the ASIs. To repress attraction in adults, the ASIs must be present, active, and capable of sensing the environment during development. The ASIs release TGF-β, and ASI function can be bypassed by experimental activation of TGF-β signaling. Sexual attraction in derepressed hermaphrodites requires the same sensory neurons as in males. The sexual identity of both these sensory neurons and a distinct subset of interneurons must be male to relieve repression and release attraction. TGF-β may therefore act to change connections between sensory neurons and interneurons during development to engage repression. Thus, sensation in a single sensory neuron pair during development reprograms a common neural circuit from male to female behavior.

INTRODUCTION

In most species, males and females display sex-specific behavioral repertoires. Courtship and mating behaviors elicited by pheromones are among the most obvious sexually dimorphic repertoires because they are innate and stereotyped (Stowers and Logan, 2010). What are the neural differences that give rise to different behaviors in each sex? Behavioral differences could be due to differences in the ability of each sex to detect pheromone or to differences in the processing of pheromone sensory information. For example, female mice with an impaired vomeronasal organ exhibit male mating behaviors, suggesting that the underlying neural circuitry is the same in both sexes but only active in males (Kimchi et al., 2007). It may be that females are capable of smelling pheromones that males cannot and that smelling these compounds represses male mating. In this case, the difference is at the level of detection. Alternatively, male flies detect pheromone identically to females (Kurtovic et al., 2007) but possess male-specific ganglia that initiate male courtship behavior (Clyne and Miesenböck, 2008; Kohatsu et al., 2011), even in an animal that is otherwise female (Kimura et al., 2008). Here, both sexes smell the same compound, *cis*-vaccenyl acetate, but male and female higher brain centers generate different responses (Kurtovic et al., 2007). Thus, in this case, the difference is at the level of processing. The two mechanisms are not mutually exclusive. In *Manduca sexta*, transplanting the nascent male sensory apparatus (his antennae) to a female larva induces male development in the female brain, and the adult animal has male behaviors (Schneiderman et al., 1986). The reciprocal switch generates an animal that has female behaviors (Kalberer et al., 2010). In this case, a difference in detection induces sexually dimorphic wiring, resulting in a difference in processing. Behavior that depends only on differences in detection could be easily modulated, for example, by regulating chemoreceptor expression. Behavior that is dependent on sex-specific brain structures must be hardwired during development, and the different contributions of sex-determination pathways and the environment to this hardwiring are not clear. Here, we address these fundamental questions in *C. elegans*, an animal with relatively few sex-specific neurons but a rich sex-specific behavioral repertoire.

C. elegans reproduces both as a self-fertilizing hermaphrodite and by mating between hermaphrodites and males. *C. elegans* hermaphrodites are essentially females that make their own sperm for a short time during development, which they store to later fertilize their own eggs (for review, see Herman, 2005). Hermaphrodites release pheromones that elicit behaviors in both sexes. Hermaphrodite pheromones fall into two broad classes: *daf-22* dependent (Butcher et al., 2009; Pungaliya et al., 2009) and *daf-22* independent (White et al., 2007). The *daf-22* gene encodes a β-oxidase required for the synthesis of a family of small molecules whose distinguishing feature is an ascarylose sugar core (Butcher et al., 2009). The *daf-22*-dependent class of pheromones appears to act as density signals that mediate both development and behavior (Srinivasan et al., 2012). The *daf-22*-independent pheromones elicit robust male-specific attraction; males chemotax to a source of these pheromones and linger, but hermaphrodites do not (White et al., 2007). Behaviors elicited by the *daf-22*-dependent and *daf-22*-independent pheromone classes have different genetic and neural requirements (White et al., 2007; Srinivasan et al., 2008; Macosko et al., 2009; McGrath et al., 2011) and so appear to be distinct. Because *daf-22*-independent pheromones elicit behaviors in males reminiscent of copulation but in the absence of a mating partner, we refer to them as sex pheromones, and the behavior they elicit as sexual attraction (White et al., 2007). As in many species, both sexes are exposed to sex pheromones, but they compel sexual attraction only in males. The mechanism by

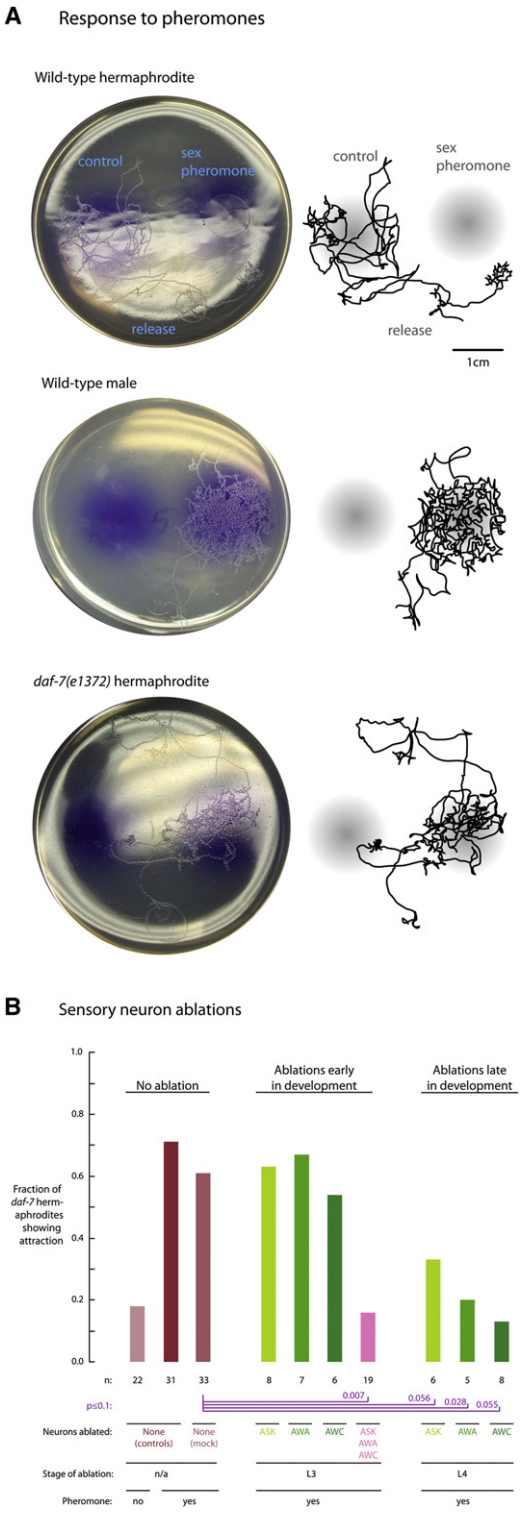

Figure 1. Sex Pheromones Elicit Attraction Behavior in Wild-Type Males and *daf-7* Hermaphrodites; This Behavior Requires the Same Sensory Neurons, and in Both Cases, These Neurons Compensate for One Another

(A) Sex pheromones elicit sexual attraction in wild-type males and *daf-7* mutant hermaphrodites, but not wild-type hermaphrodites. Tracks on agar assay plates (single animal, 3 hr elapsed) show that both wild-type males and *daf-7* mutant hermaphrodites find a spot containing hermaphrodite sex pheromones and linger, but wild-type hermaphrodites do not. Photos of assay plates are on the left; hand tracings are on the right to assist in visualization. Quantitation is shown in Figure S1. (B) Sexual attraction in *daf-7* hermaphrodites requires the core sensory neurons AWA, AWC, and ASK, which compensate for one another. Simultaneous laser ablation in L3 larvae of the ASK, AWA, and AWC pairs of core sensory neurons (six pairs total, pink bar) reduces the attraction behavior of *daf-7* hermaphrodites to a frequency comparable to background. Ablation of each pair singly in L3 larvae (each pair one at a time, green bars) does not appreciably affect attraction (compared to mock-ablated animals). In contrast, ablation of each pair singly in L4 larvae reduces the attraction behavior of *daf-7* hermaphrodites to frequencies similar to the triple L3 ablation. The difference between L3 and L4 ablations is indicative of compensation, as observed for male sexual attraction.

which male-specific sexual attraction behavior is established in *C. elegans* is unknown.

RESULTS

Hermaphrodites Have Latent Male Sexual Attraction Behavior, Generated by a Core Neural Circuit

We surveyed existing *C. elegans* mutants for those with altered sexual attraction and found that *daf-7* mutant hermaphrodites show sexual attraction behavior (Figure 1A). That is, *daf-7* mutant hermaphrodites are attracted to sex pheromones, whereas wild-type hermaphrodites are not. In *daf-7* males, sexual attraction is not detectably altered (see Figure S1 available online). Thus, the absence of DAF-7/TGF-β reveals latent sexual attraction behavior in hermaphrodites.

Sexual attraction requires the same neurons in males and *daf-7* hermaphrodites. Most of the *C. elegans* nervous system is the same in both sexes (Sulston et al., 1983): 294 neurons comprise this core nervous system (out of 302 total in the hermaphrodite). In males, sexual attraction requires the core sensory neurons AWA, AWC, and ASK, and although male-specific sensory neurons normally contribute, these core sensory neurons are sufficient (White et al., 2007; Figure S2). Furthermore, if any of these core neurons are surgically ablated in juvenile males, the remaining neurons compensate and the operated adults express full sexual attraction. As in males, in *daf-7* hermaphrodites sexual attraction requires the core sensory neurons AWA, AWC, and ASK (Figure 1B). Furthermore, as in males, in *daf-7* mutant hermaphrodites, these sensory neurons compensate for one another (Figure 1B). If only one pair is removed late in development (L4 larval stage), the circuit is disrupted and behavior is compromised. However, if only one pair is removed early in development (L3 larval stage), the remaining pairs take over and behavior is not detectably affected, unless all three pairs are removed concurrently. Although other explanations for attraction in *daf-7* hermaphrodites are formally possible, such as altered chemoreceptor expression (Nolan et al., 2002), it is striking that the same distinct set of sensory neurons are required and show the same property of compensation. Given these results, it is likely that the same neural circuit generates sexual attraction in both males and *daf-7* hermaphrodites.

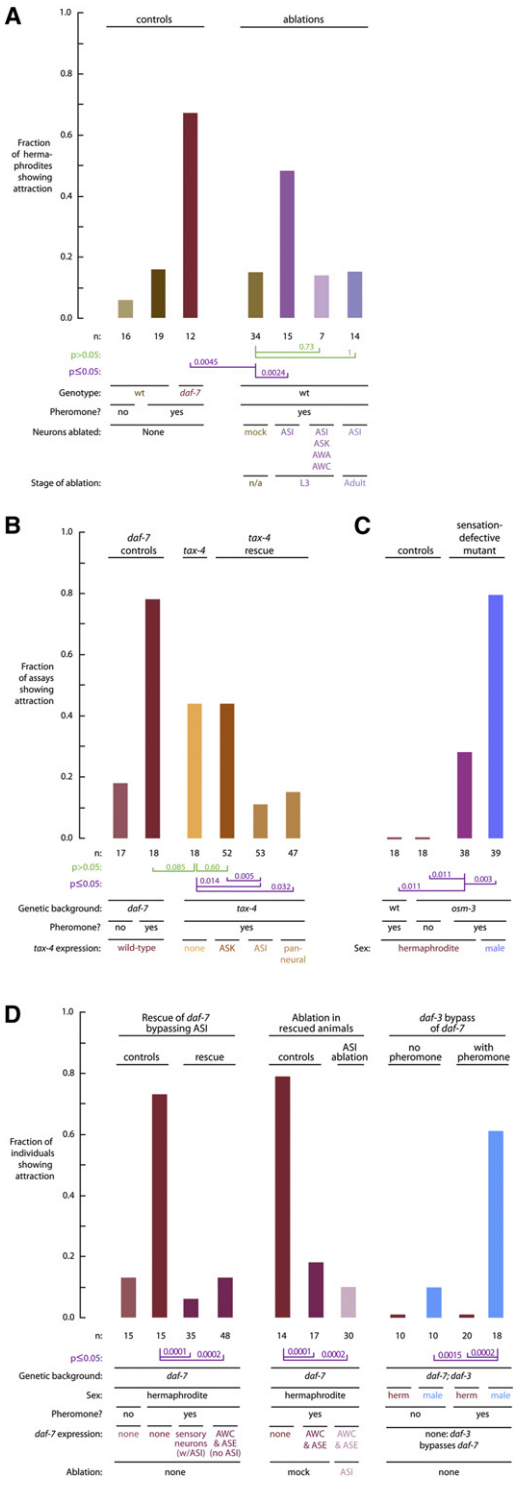

Figure 2. ASI Sensory Activity Is Required to Repress Attraction in Hermaphrodites, but the ASIs Are Bypassed by Restoring DAF-7-Signaling Function Elsewhere

(A) ASI is required for repression of attraction. Ablation of ASI neurons reveals sexual attraction behavior in wild-type hermaphrodites. When ASI neurons are ablated during the L3 larval stage, hermaphrodites exhibit attraction behavior comparable to *daf-7* hermaphrodites and significantly different from mock-ablated animals. Attraction in the absence of ASI requires the ASK, AWA, and AWC sensory neurons, because animals missing all eight neurons have significantly impaired sexual attraction behavior. The ASI neurons must be ablated during development to reveal sexual attraction behavior, because when the ASI neurons are ablated in adult animals, sexual attraction behavior remains repressed. (B) The TAX-4 cGMP-gated channel is required in ASI to repress sexual attraction. *tax-4* mutant hermaphrodites show sexual attraction behavior comparable to *daf-7* hermaphrodites. Expression of *tax-4* in the ASI neurons, but not in ASK, restores full repression of attraction behavior. (C) Sensory cilia must be intact to repress sexual attraction in hermaphrodites. *osm-3* mutants have impaired sensory cilia in a subset of sensory neurons that includes the ASIs (Snow et al., 2004; Signor et al., 1999); *osm-3* mutant hermaphrodites have significant sexual attraction behavior compared either to wild-type hermaphrodites or to no pheromone *osm-3* controls. (D) Restoring DAF-7/TGF-β signaling function elsewhere bypasses ASI. Transgenic expression of DAF-7/TGF-β in the AWC and ASE neurons restores repression of sexual attraction in *daf-7* mutant hermaphrodites. Restored repression is not ASI dependent, because ablation of ASI during the L3 larval stage in transgenic animals does not significantly affect repression. Repression of attraction is restored genetically by the *daf-3* mutation: the absence of DAF-3 activates DAF-7 signaling in target cells independent of DAF-7 (Thomas et al., 1993). Accordingly, *daf-7; daf-3* double mutant hermaphrodites show significantly repressed attraction.

Sensation in the ASI Neurons Releases TGF-β to Engage Repression during Development

The sole source of DAF-7/TGF-β in *C. elegans* is the ASI sensory neuron pair (Ren et al., 1996; Schackwitz et al., 1996). Ablation of the ASI neurons reveals sexual attraction in hermaphrodites (Figure 2A). That is, ASI-ablated hermaphrodites are attracted to sex pheromones, whereas intact hermaphrodites are not. Sexual attraction in ASI-ablated hermaphrodites requires the ASK, AWA, and AWC neurons (Figure 2A). The ASI neurons express a cGMP-gated channel containing the TAX-4 subunit (Komatsu et al., 1996; Coburn and Bargmann, 1996). This channel is required for ASI development and activity (Coburn and Bargmann, 1996; Peckol et al., 1999) but makes only a residual contribution to sexual attraction in males (White et al., 2007). Mutant *tax-4* hermaphrodites show sexual attraction behavior (Figure 2B). Attraction in *tax-4* hermaphrodites is not as consistent as in *daf-7* hermaphrodites, suggesting that—as in males—TAX-4 may also function in cells that promote attraction. Expression of TAX-4 in ASI neurons completely restored wild-type behavior to *tax-4* mutant hermaphrodites (Figure 2B; "wild-type behavior" means that attraction is repressed), but expression in other neurons, such as ASK, did not. Thus, TAX-4 function solely in the ASIs is sufficient to repress attraction. The ASIs are classified as sensory neurons in part because they have dendrites exposed to the external environment (White et al., 1986). Sensory dendrites in ASI require the OSM-3 kinesin to develop properly; *osm-3* mutants have stunted sensory endings (Snow et al., 2004), but OSM-3 is not required in males for sexual attraction (White et al., 2007). *osm-3* mutant hermaphrodites exhibit sexual attraction behavior (Figure 2C), most likely because their ASI neurons are not sensing the external environment and so do not engage repression. Thus, ASI neurons must be (1) present during development, (2) active, and (3) capable of sensing the external environment in order to repress sexual attraction in adult hermaphrodites.

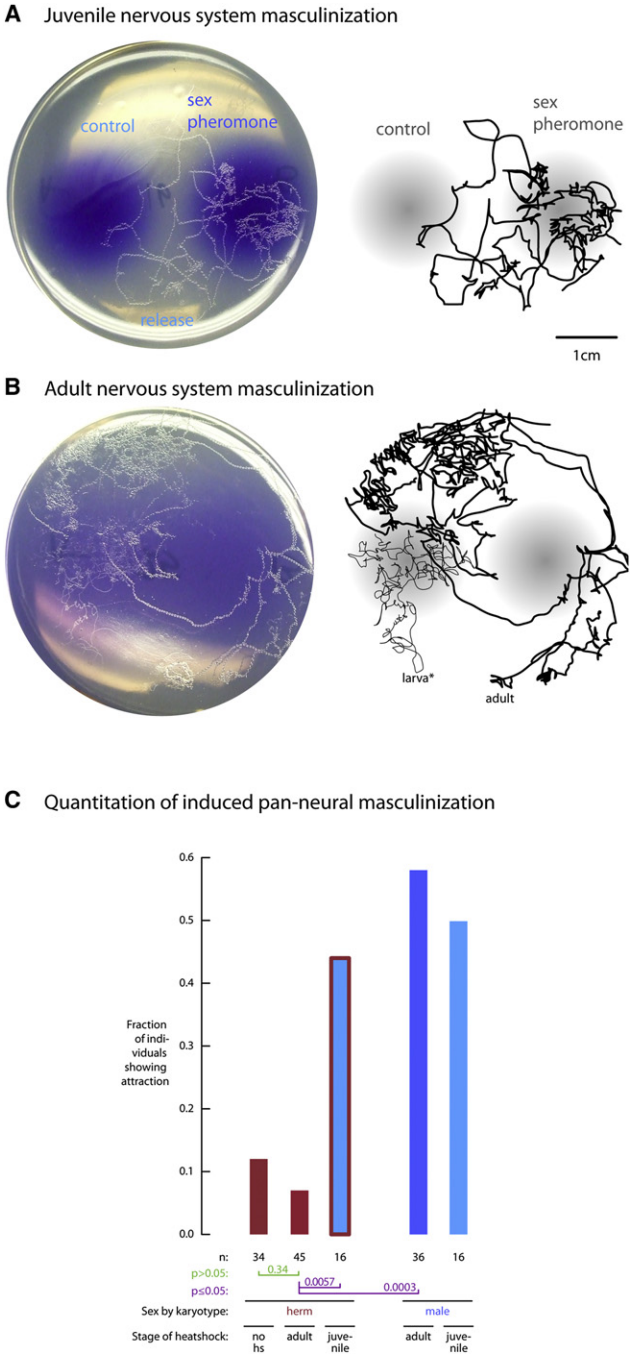

Figure 3. The Nervous System Must Be Sexualized during Development for Sexual Attraction Behavior in Adults

(A) Masculinization of the nervous system of XX animals (physically hermaphrodite) by expression of FEM-3 in the nervous system induced during development ("juvenile") results in adult sexual attraction behavior comparable to control XO animals (physically male). Assay periods were 3 hr. Photos on left correspond to hand-tracings of tracks on right. (B) Masculinization induced during adulthood and assayed a day later results in XX animals comparable to uninduced XX animals and different from juvenile induction. An L1 larva was also present on the plate (*larva) but did not detectably interfere with the behavior of the adult. (C) Quantitation of masculinization of the core *C. elegans* nervous system induced in juveniles and adults.

To repress sexual attraction, the ASI pair could act solely by releasing DAF-7/TGF-β or it could have additional roles. To separate the functions of DAF-7 from the ASI neurons, we experimentally activated TGF-β signaling independent of the ASIs in two ways. First, we expressed DAF-7/TGF-β specifically in the AWC and ASE sensory neurons, but not in ASI, in *daf-7* mutant animals. As expected, DAF-7/TGF-β expression in the AWC and ASE neurons rescues three classic phenotypes of *daf-7* mutants: (1) inappropriate induction of dauer larvae, (2) a dark intestine, and (3) aggregation. Importantly, DAF-7/TGF-β expression in the AWC and ASE neurons also rescues wild-type behavior in *daf-7* mutant hermaphrodites: transgenic hermaphrodites are not attracted to sex pheromones (Figure 2D). Notably, ablation of the ASI neurons has no discernible effect on the attraction behavior of these transgenic hermaphrodites; sexual attraction is repressed regardless of whether ASI is present (Figure 2D). Second, we activated TGF-β signaling using genetics: in a *daf-3* mutant, the absence of DAF-3 function activates the DAF-7 signaling pathway in target cells, independent of DAF-7 (Thomas et al., 1993). Accordingly, *daf-7; daf-3* double mutant hermaphrodites have repressed sexual attraction (Figure 2D). That is, *daf-7; daf-3* hermaphrodites are no longer attracted to sex pheromones. Their brothers, *daf-7; daf-3* double mutant males, exhibit obvious sexual attraction behavior (Figure 2D) comparable to *daf-3* single mutant males (data not shown). Thus, although ASI activity normally modulates expression and release of DAF-7/TGF-β (Chang et al., 2006; Schackwitz et al., 1996), ASI activity may be bypassed either by forcing expression of DAF-7/TGF-β elsewhere or by activating TGF-β signaling. Therefore, the sole role of ASI in repressing sexual attraction is to release DAF-7/TGF-β.

To establish sexually dimorphic behavior, DAF-7/TGF-β could alter how the underlying neural circuit is built, how it is maintained, or how it is modulated. To address these possibilities, we determined when the nervous system must be sexualized to generate sexual attraction behavior. At different times during development, we masculinized the hermaphrodite nervous system using a FLP-ON strategy (Davis et al., 2008). Sexual attraction behavior emerges in adults when the nervous system is switched during development (during the final L4 larval stage or earlier), but not when switched in adults (Figure 3). Consistent with these results, sexual attraction is revealed in adult hermaphrodites only when the ASI neurons are ablated during development (prior to the L4 larval stage or earlier), not when ablated in adults (Figure 2A). The requirement for the ASI neurons during development coincides with the time when they express DAF-7/TGF-β (Ren et al., 1996; Schackwitz et al., 1996). Therefore, DAF-7/TGF-β most likely alters how the sexual attraction circuits are built.

Sexual Attraction Requires Sex Differences in Both Sensory Neurons and Interneurons

To localize neural sex differences required for sexual attraction behavior, we masculinized subsets of neurons in animals that were otherwise hermaphrodites. We focused on the sensory neurons required for sexual attraction behavior (AWA, AWC, and ASK) and the interneurons that comprise their synaptic targets and gap-junction partners (Figure 4A). Because the

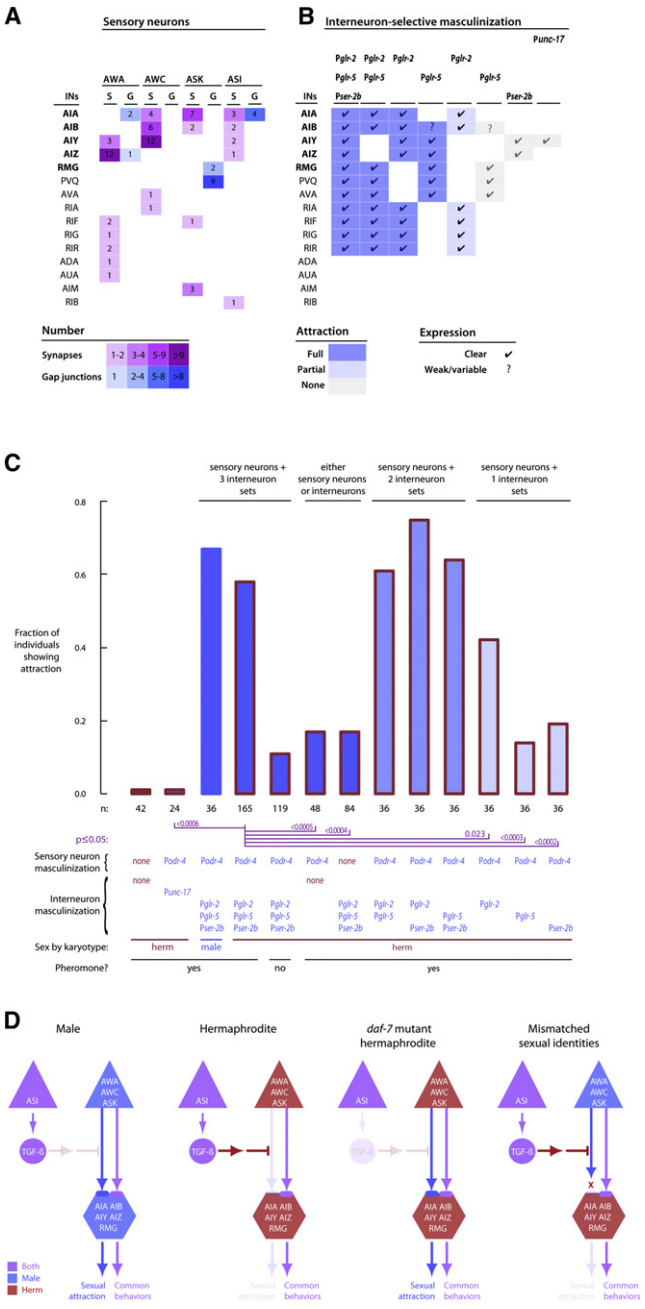

Figure 4. Distinct but Distributed Sets of Sensory Neurons and Interneurons Must Be Sexualized for Expression of Sexual Attraction Behavior

(A) Direct sensory neuron-interneuron connectivity of the sensory neurons required for sexual attraction. The numbers of synapses (S) and gap junctions (G) are from the hermaphrodite wiring diagram (Chen et al., 2006; White et al., 1986); male connections may be different. Additional connections among sensory neurons and among interneurons are not shown. (B) Sensory neurons must be masculinized together with a distributed set of heavily connected postsynaptic interneurons in order for transsexual hermaphrodites to exhibit sexual attraction behavior. The set of P*odr-4* sensory neurons were masculinized together with interneuron sets defined by the P*glr-2*, P*glr-5*, P*ser-2b*, and P*unc-17* promotors. Only the expression patterns of these promotors within the connected interneuron set are shown. Full expression patterns are given in Tables S2, S3, and S4. (C) Masculinization of P*odr-4* sensory neurons together with distinct subsets of interneurons imparts sexual attraction behavior on transexual animals. The graph shows the frequency of sexual attraction conferred by neural masculinization either with the P*odr-4* promotor and none or more of the P*glr-2*, P*glr-5*, P*ser-2b*, and P*unc-17* promotors or the interneuron combination without P*odr-4*. Bars indicate the fraction of individuals exhibiting sexual attraction. Red outlined bars represent worms that are physically hermaphrodite but have been masculinized in the subset of neurons indicated. (D) Model. In males, synaptic connections that enable sexual attraction develop between the AWA, AWC, and ASK sensory neurons and the AIA, AIB, AIY, and AIZ interneurons. In hermaphrodites, DAF-7/TGF-β signaling disables these connections, by acting either directly on the feminine nervous system or indirectly through signals released by another tissue, such as the female gonad. In *daf-7* hermaphrodites, DAF-7/TGF-β signaling is absent, allowing male-type connections. In animals in which the sexual identities of the sensory neurons and interneurons do not match, the feminine neurons respond to DAF-7/TGF-β signaling, repression is engaged, and male-type connections are disabled.

male wiring diagram is incomplete, connectivity information is based on the hermaphrodite wiring diagram (White et al., 1986; Chen et al., 2006). Using cell-selective promotors, we masculinized sets of sensory neurons and interneurons in different combinations (Experimental Procedures; Figure 4B). Hermaphrodites with masculinized AWA, AWC, ASK, and ASI sensory neurons exhibited no detectable sexual attraction (using P*odr-4*, Figure 4C). That is, attraction remained repressed in these animals. Likewise, hermaphrodites in which a broad set of interneurons were masculinized also exhibited no detectable sexual attraction (using a combination of P*glr-2*, P*glr-5*, and P*ser-2b*). In contrast, hermaphrodites in which both sensory neurons and interneurons were masculinized exhibited robust sexual attraction (Figures 4B and 4C, using a combination of P*odr-4*, P*glr-2*, P*glr-5*, and P*ser-2b*), indistinguishable from male controls (Figure 4C) and comparable to masculinization of the entire nervous system using P*rab-3* (White et al., 2007). The particular set of interneurons is important, because masculinizing the P*odr-4* sensory neurons together with P*unc-17* interneurons and motor neurons did not enable expression of sexual attraction; the behavior remained repressed. Thus, both sensory neurons and interneurons must be male for male-specific sexual attraction to emerge. Masculinization of only the P*odr-4* sensory neuron set—which includes both ASI and the sensory neurons required for sexual attraction—is not sufficient. Likewise, masculinization of only an interneuron set—regardless of which—is not sufficient. If either set has a female sexual identity, DAF-7/TGF-β can act—either directly or indirectly—to repress sexual attraction in hermaphrodites.

Interneuron Sex Differences Required for Sexual Attraction Are Distributed

If pheromone sensory input converges on a single interneuron pair that functions, for example, as a modulatory hub (Macosko et al., 2009) or a site of integration (Shinkai et al., 2011), then these neurons might also be the site of the sex differences that account for sexual attraction. To address this, we masculinized a constant set of sensory neurons (using P*odr-4*) in combination with smaller subsets of interneurons. Based on the hermaphrodite wiring diagram (White et al., 1986; Chen et al., 2006), the most heavily connected interneurons that are directly

postsynaptic to the AWA, AWC, and ASK sensory neurons are the AIA, AIB, AIY, and AIZ pairs (Figure 4A). Notably, no single interneuron class is postsynaptic to all three sensory neuron classes. In addition, the RMG interneuron pair modulates signaling from the ASKs via gap junctions (Macosko et al., 2009). A subset that should masculinize all of the AIA, AIB, AIY, and AIZ neurons but not the RMG interneurons (P*glr-2* + P*ser-2b*) fully expressed sexual attraction, comparable to broad masculinization (P*glr-2* + P*glr-5* + P*ser-2b*). That is, repression was not engaged. Subsets that should masculinize only some of the AIA, AIB, AIY, and AIZ neurons and include the RMG neurons also fully expressed sexual attraction (the P*glr-5* + P*glr-2* and P*glr-2* + P*ser-2b* combinations). In contrast, subsets that should masculinize only some of the AIA, AIB, AIY, and AIZ neurons but do not include RMG expressed sexual attraction less frequently (P*glr-2*) or not at all (P*ser-2b*). Conversely, a subset that should masculinize RMG, but not AIA, AIY, and AIZ (and possibly not AIB; P*glr-5*), did not exhibit sexual attraction. Within the framework provided by the hermaphrodite wiring diagram (White et al., 1986; Chen et al., 2006), a straightforward interpretation of these results is that sexual differences in AIA and AIB are most important for sexual attraction, with contributions from AIY and AIZ and possibly modulation by RMG. Independent of the hermaphrodite wiring, it appears unlikely that pheromone sensory input converges on a single interneuron class but instead remains distributed.

Taken together, the neuron-selective masculinization experiments suggest that the AWA, AWC, and ASK sensory neurons and their interneuron partners—most likely the AIA, AIB, AIY, and AIZ neurons—must be male for the animal to display male behavior. A simple model based on these data is that a male-specific constellation of connections among these sensory neurons and interneurons forms during development to generate male-specific sexual attraction (Figure 4D). In this model, hermaphrodites are also capable of developing these connections, but repression either prevents them from being established or subsequently disables them.

DISCUSSION

In general, sex-specific behaviors may be generated by extra circuitry entirely present only in one sex or by modification of circuitry present in both sexes (Stowers and Logan, 2010). In *C. elegans*, there are no additional male-specific neurons in the sex pheromone processing circuitry to account for male-specific sexual attraction, based on two facts. First, the nervous system is fully cataloged in males and hermaphrodites, establishing that there is a core nervous system common to both sexes (White et al., 1986; Sulston, 1983; Sulston et al., 1980; Sulston and Horvitz, 1977). Second, this core nervous system is sufficient for male-specific sexual attraction behavior (White et al., 2007). Therefore, the essential differences that account for male-specific sexual attraction must reside not in the cellular composition, but rather in the properties of the circuit—for example, in connectivity, neural excitability, synaptic strengths, or receptor expression. Under most circumstances, these differences depend on sexual identity set by the somatic sex-determination pathway (White et al., 2007; Figures 3 and 4); however, it is unlikely that DAF-7/TGF-β alters sexual identity. Thus, *daf-7* mutant hermaphrodites possess only neurons with a female sexual identity, yet express the essential differences for generating "male" behavior in the opposite sex.

Because the presence of DAF-7/TGF-β in wild-type hermaphrodites results in the absence of sexual attraction, DAF-7 functions to repress the behavior. However, because males also express DAF-7/TGF-β (Ren et al., 1996), and we have found no manipulation of DAF-7 expression in males that detectably alters sexual attraction, DAF-7 acts only on the feminine hermaphrodite core to repress attraction. That is, female sexual identity is permissive for repression. How might DAF-7/TGF-β repress sexual attraction? In general, DAF-7/TGF-β regulates diverse processes in *C. elegans*, from dauer development (Ren et al., 1996; Schackwitz et al., 1996) to fat metabolism and feeding behavior (Greer et al., 2008). Accordingly, DAF-7/TGF-β signaling culminates in the transcriptional regulation of a wide array of genes (Liu et al., 2004). Furthermore, DAF-7 receptors are widely expressed (Gunther et al., 2000), and their mutant phenotypes do not simply mimic the *daf-7* mutant (Georgi et al., 1990; Estevez et al., 1993; Ren et al., 1996; Gunther et al., 2000). Based on the mechanisms of its other functions in *C. elegans*, DAF-7/TGF-β could act to repress sexual attraction in hermaphrodites either directly or indirectly (Figure 4D). In a direct model, similar to its broad action in dauer development, DAF-7/TGF-β acts on the neurons of the attraction circuit, possibly to disable synaptic connections during development. In an indirect model, similar to its role in feeding (Greer et al., 2008), DAF-7/TGF-β acts on a modulatory cell, which in turn alters the attraction circuit, plausibly via hormones, neuropeptides (Greer et al., 2008), or gap junctions (Macosko et al., 2009). Regardless of the mechanism of repression, DAF-7/TGF-β signaling ultimately alters the attraction circuit but only in hermaphrodites.

Unlike mice (Stowers et al., 2002; Kimchi et al., 2007), repression is set during development and does not have to be maintained by pheromone perception. That is, sexual attraction in wild-type *C. elegans* hermaphrodites cannot be revealed (derepressed) in adults (Figure 2A). The developmental requirement for sensation in ASI to establish repression coincides with the period that the attraction circuit must be masculinized to establish attraction (Figure 3). Plausibly, masculinization during development renders the neurons of the attraction circuit unresponsive to repression. Furthermore, although only a limited subset of cells in the nervous system must be male to generate sexual attraction, both sensory neurons and interneurons must be contemporaneously masculinized. A simple model based on these data is that sexual attraction requires male-type synaptic connections between sensory neurons (most likely AWA, AWC, and ASK) and interneurons (possibly AIA, AIB, AIY and/or AIZ), and that repression interferes with the establishment of these connections (Figure 4D). Thus, our data demonstrate that both sides of a particular constellation of synaptic connections must be functionally sexualized to generate a particular sex-specific behavior.

Although we have not found environmental conditions that lead to the display of sexual attraction in wild-type hermaphrodites, the requirements for properly formed sensory dendrites in ASI (Figure 2C) and for ASI activity suggest that sensation

during development could modulate repression. The ASI neurons modulate behavior in other contexts (Coburn and Bargmann, 1996; Coburn et al., 1998; Peckol et al., 1999; Chang et al., 2006), so it may be that a general task of the ASIs is to integrate information about the environment (such as population density, food availability, p[CO_2], or the presence of sex pheromone) and adjust either the function (Chang et al., 2006) or programming of neural circuits via DAF-7/TGF-β. Mechanisms linking environmental and genetic determinants of behaviors have implications for conceptually similar human conditions such as sexual preference and sexual identity.

EXPERIMENTAL PROCEDURES

Behavior

Sexual attraction assays were as described (White et al., 2007), blind for strain and for pheromone versus control and scored categorically based on track pattern (details in the Supplemental Experimental Procedures). Strains were cultivated at 20°C–22°C. At this temperature, *daf-7* mutants frequently reach adulthood. The data are categorical (attraction or no attraction) and all data are shown. The number of assays for each condition is indicated in each figure.

Statistics

Comparisons were made using Fisher's exact test at 90% confidence with the Bonferroni-Holm correction for multiple comparisons. For comparisons, α was taken at 0.05 unless otherwise indicated. Exact p values after correction are given in each figure.

Laser Ablations

Ablations were performed with a MicroPoint laser system as described (Bargmann and Avery, 1995; White et al., 2007) in L2, L3, or L4 stage larvae or young adults. Operated animals were assayed as 1-day-old adults or after 1 day recovery for adult ablations. Ablations were verified postassay anatomically or by checking for the absence of green fluorescent protein (GFP), if appropriate. ASK and ASI were identified anatomically; other strains contained GFP markers to assist in neuron identification. Strains for ablations are described in detail in Table S1.

Molecular Biology

For neuron-specific expression of TAX-4 or DAF-7, a cDNA encoding either *tax-4* or *daf-7* was placed in an artificial operon also expressing either EGFP or mCherry under the control of a neuron-selective promotor and followed by a generic *unc-54* 3′ UTR. Expression vectors were constructed from the following modules: a 4-1 entry vector containing a cell-selective promotor, a 1-2 entry vector containing a cDNA in an artificial operon, and a 2-3 entry vector containing a generic 3′ UTR from the *unc-54* gene. Promotors used were P*gpa-4* for ASI, P*srg-2* and P*srg-8* for ASK, P*odr-4* for sensory neurons including ASI, P*ceh-36* for AWC-ASE, and P*rab-3* for the entire nervous system. Reported expression patterns and references are given in Table S2.

Inducible Neural Masculinization

To inducibly masculinize the nervous system, we modified the published FLP-ON strategy (Davis et al., 2008). The masculinizing construct contained in order 5′ to 3′: the P*rab-3* promotor, a *let-858* stop cassette marked with mCherry and flanked by FRT sites, EGFP in an artificial operon followed by a *fem-3* cDNA (Mehra et al., 1999), and an *unc-54* 3′ UTR. FLP-recombinase was expressed in a separate construct under the control of the heatshock promotor P*hsp16.41*. In this strategy, heatshock (1 hr at 33°C) induces expression of FLP-recombinase, which excises the stop cassette and mCherry, thus allowing expression of EGFP and *fem-3*. Animals for assays were selected prior to heatshock for no visible EGFP and after heatshock for robust EGFP in the nervous system. Animals were assayed 24 hr after heatshock; EGFP was visible in the nervous system within 4 hr.

Masculinization of Neural Subsets

To masculinize subsets of the hermaphrodite nervous system, we used the Gateway system to fuse different neuron-selective promotors to a standard expression cassette containing *fem-3* cDNA (Mehra et al., 1999) in an artificial operon with mCherry. Masculinized neurons therefore fluoresce red. For sensory neurons, we used P*odr-4*. For interneurons, we used P*glr-5*, P*glr-2*, P*ser-2b*, and P*unc-17*. Reported expression patterns and references are given in Tables S2, S3, and S4.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four tables, two figures, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2012.03.044.

ACKNOWLEDGMENTS

The authors wish to thank the *Caenorhabditis* Genetics Center for strains, Tom Nicholas, Eliott Davidson, Sarah Bodian, Bryan Benham, and Nadja Schäfer for constructing reagents; Michael Ailion, Doug Portman, and Bill Mowrey for unpublished reagents and comments; Villu Maricq, Mike Shapiro, Randi Rawson, and Sean Merrill for comments; and Cori Bargmann, Kaveh Ashrafi, Piali Sengupta, and Kyuhyung Kim for reagents and insight. This work was funded by a National Research Service Award and an American Cancer Society Fellowship to J.Q.W. and NSF grants 0516815 and 0920069 to E.M.J.

Accepted: March 29, 2012
Published: August 22, 2012

REFERENCES

Bargmann, C.I., and Avery, L. (1995). Laser killing of cells in *Caenorhabditis elegans*. Methods Cell Biol. *48*, 225–250.

Butcher, R.A., Ragains, J.R., Li, W., Ruvkun, G., Clardy, J., and Mak, H.Y. (2009). Biosynthesis of the *Caenorhabditis elegans* dauer pheromone. Proc. Natl. Acad. Sci. USA *106*, 1875–1879.

Chang, A.J., Chronis, N., Karow, D.S., Marletta, M.A., and Bargmann, C.I. (2006). A distributed chemosensory circuit for oxygen preference in *C. elegans*. PLoS Biol. *4*, e274.

Chen, B.L., Hall, D.H., and Chklovskii, D.B. (2006). Wiring optimization can relate neuronal structure and function. Proc. Natl. Acad. Sci. USA *103*, 4723–4728.

Clyne, J.D., and Miesenböck, G. (2008). Sex-specific control and tuning of the pattern generator for courtship song in *Drosophila*. Cell *133*, 354–363.

Coburn, C.M., and Bargmann, C.I. (1996). A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. Neuron *17*, 695–706.

Coburn, C.M., Mori, I., Ohshima, Y., and Bargmann, C.I. (1998). A cyclic nucleotide-gated channel inhibits sensory axon outgrowth in larval and adult *Caenorhabditis elegans*: a distinct pathway for maintenance of sensory axon structure. Development *125*, 249–258.

Davis, M.W., Morton, J.J., Carroll, D., and Jorgensen, E.M. (2008). Gene activation using FLP recombinase in *C. elegans*. PLoS Genet. *4*, e1000028.

Estevez, M., Attisano, L., Wrana, J.L., Albert, P.S., Massagué, J., and Riddle, D.L. (1993). The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. Nature *365*, 644–649.

Georgi, L.L., Albert, P.S., and Riddle, D.L. (1990). *daf-1*, a *C. elegans* gene controlling dauer larva development, encodes a novel receptor protein kinase. Cell *61*, 635–645.

Greer, E.R., Pérez, C.L., Van Gilst, M.R., Lee, B.H., and Ashrafi, K. (2008). Neural and molecular dissection of a *C. elegans* sensory circuit that regulates fat and feeding. Cell Metab. *8*, 118–131.

Gunther, C.V., Georgi, L.L., and Riddle, D.L. (2000). A *Caenorhabditis elegans* type I TGF β receptor can function in the absence of type II kinase to promote larval development. Development *127*, 3337–3347.

Herman, R.K. (2005). Introduction to sex determination. In WormBook, The *C. elegans* Research Community, ed. http://dx.doi.org/10.1895/wormbook.1.71.1, http://www.wormbook.org.

Kalberer, N.M., Reisenman, C.E., and Hildebrand, J.G. (2010). Male moths bearing transplanted female antennae express characteristically female behaviour and central neural activity. J. Exp. Biol. *213*, 1272–1280.

Kimchi, T., Xu, J., and Dulac, C. (2007). A functional circuit underlying male sexual behaviour in the female mouse brain. Nature *448*, 1009–1014.

Kimura, K., Hachiya, T., Koganezawa, M., Tazawa, T., and Yamamoto, D. (2008). *Fruitless* and *doublesex* coordinate to generate male-specific neurons that can initiate courtship. Neuron *59*, 759–769.

Kohatsu, S., Koganezawa, M., and Yamamoto, D. (2011). Female contact activates male-specific interneurons that trigger stereotypic courtship behavior in *Drosophila*. Neuron *69*, 498–508.

Komatsu, H., Mori, I., Rhee, J.S., Akaike, N., and Ohshima, Y. (1996). Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. Neuron *17*, 707–718.

Kurtovic, A., Widmer, A., and Dickson, B.J. (2007). A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. Nature *446*, 542–546.

Liu, T., Zimmerman, K.K., and Patterson, G.I. (2004). Regulation of signaling genes by TGFβ during entry into dauer diapause in *C. elegans*. BMC Dev. Biol. *4*, 11.

Macosko, E.Z., Pokala, N., Feinberg, E.H., Chalasani, S.H., Butcher, R.A., Clardy, J., and Bargmann, C.I. (2009). A hub-and-spoke circuit drives pheromone attraction and social behaviour in *C. elegans*. Nature *458*, 1171–1175.

McGrath, P.T., Xu, Y., Ailion, M., Garrison, J.L., Butcher, R.A., and Bargmann, C.I. (2011). Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. Nature *477*, 321–325.

Mehra, A., Gaudet, J., Heck, L., Kuwabara, P.E., and Spence, A.M. (1999). Negative regulation of male development in *Caenorhabditis elegans* by a protein-protein interaction between TRA-2A and FEM-3. Genes Dev. *13*, 1453–1463.

Nolan, K.M., Sarafi-Reinach, T.R., Horne, J.G., Saffer, A.M., and Sengupta, P. (2002). The DAF-7 TGF-β signaling pathway regulates chemosensory receptor gene expression in *C. elegans*. Genes Dev. *16*, 3061–3073.

Peckol, E.L., Zallen, J.A., Yarrow, J.C., and Bargmann, C.I. (1999). Sensory activity affects sensory axon development in *C. elegans*. Development *126*, 1891–1902.

Pungaliya, C., Srinivasan, J., Fox, B.W., Malik, R.U., Ludewig, A.H., Sternberg, P.W., and Schroeder, F.C. (2009). A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *106*, 7708–7713.

Ren, P., Lim, C.S., Johnsen, R., Albert, P.S., Pilgrim, D., and Riddle, D.L. (1996). Control of *C. elegans* larval development by neuronal expression of a TGF-β homolog. Science *274*, 1389–1391.

Schackwitz, W.S., Inoue, T., and Thomas, J.H. (1996). Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. Neuron *17*, 719–728.

Schneiderman, A.M., Hildebrand, J.G., Brennan, M.M., and Tumlinson, J.H. (1986). Trans-sexually grafted antennae alter pheromone-directed behaviour in a moth. Nature *323*, 801–803.

Shinkai, Y., Yamamoto, Y., Fujiwara, M., Tabata, T., Murayama, T., Hirotsu, T., Ikeda, D.D., Tsunozaki, M., Iino, Y., Bargmann, C.I., et al. (2011). Behavioral choice between conflicting alternatives is regulated by a receptor guanylyl cyclase, GCY-28, and a receptor tyrosine kinase, SCD-2, in AIA interneurons of *Caenorhabditis elegans*. J. Neurosci. *31*, 3007–3015.

Signor, D., Wedaman, K.P., Rose, L.S., and Scholey, J.M. (1999). Two heteromeric kinesin complexes in chemosensory neurons and sensory cilia of *Caenorhabditis elegans*. Mol. Biol. Cell *10*, 345–360.

Snow, J.J., Ou, G., Gunnarson, A.L., Walker, M.R., Zhou, H.M., Brust-Mascher, I., and Scholey, J.M. (2004). Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. Nat. Cell Biol. *6*, 1109–1113.

Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H.T., Teal, P.E., Malik, R.U., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2008). A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. Nature *454*, 1115–1118.

Srinivasan, J., von Reuss, S.H., Bose, N., Zaslaver, A., Mahanti, P., Ho, M.C., O'Doherty, O.G., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2012). A modular library of small molecule signals regulates social behaviors in *Caenorhabditis elegans*. PLoS Biol. *10*, e1001237.

Stowers, L., and Logan, D.W. (2010). Sexual dimorphism in olfactory signaling. Curr. Opin. Neurobiol. *20*, 770–775.

Stowers, L., Holy, T.E., Meister, M., Dulac, C., and Koentges, G. (2002). Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science *295*, 1493–1500.

Sulston, J.E. (1983). Neuronal cell lineages in the nematode *Caenorhabditis elegans*. Cold Spring Harb. Symp. Quant. Biol. *48*, 443–452.

Sulston, J.E., and Horvitz, H.R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. *56*, 110–156.

Sulston, J.E., Albertson, D.G., and Thomson, J.N. (1980). The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. *78*, 542–576.

Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.

Thomas, J.H., Birnby, D.A., and Vowels, J.J. (1993). Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics *134*, 1105–1117.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. *314*, 1–340.

White, J.Q., Nicholas, T.J., Gritton, J., Truong, L., Davidson, E.R., and Jorgensen, E.M. (2007). The sensory circuitry for sexual attraction in *C. elegans* males. Curr. Biol. *17*, 1847–1857.

Neuron, Volume *75*

Supplemental Information

Sensation in a Single Neuron Pair Represses Male Behavior in Hermaphrodites

Jamie Q. White and Erik M. Jorgensen

Table S1. Strains

Strain number	Genotype	Source	Notes
N2 Bristol	wild-type	CGC	Laboratory reference strain.
EG2717	*him-5(e1490) V*	White et al., 2007	8x outcrossed against N2 Bristol.
EG2718	*him-8(e1489) IV*	White et al., 2007	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., 2007	*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., 2007	Green AWC and ASE neurons. Routinely used for ablations.

Table S2. Promotors used for sensory neuron and pan-neural expression

		Promotor					
		P*odr-4*	**P*gpa-4***	**P*srg-2***	**P*srg-8***	**P*ceh-36***	**P*rab-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					

Table S3. 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.

Table S4. 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).

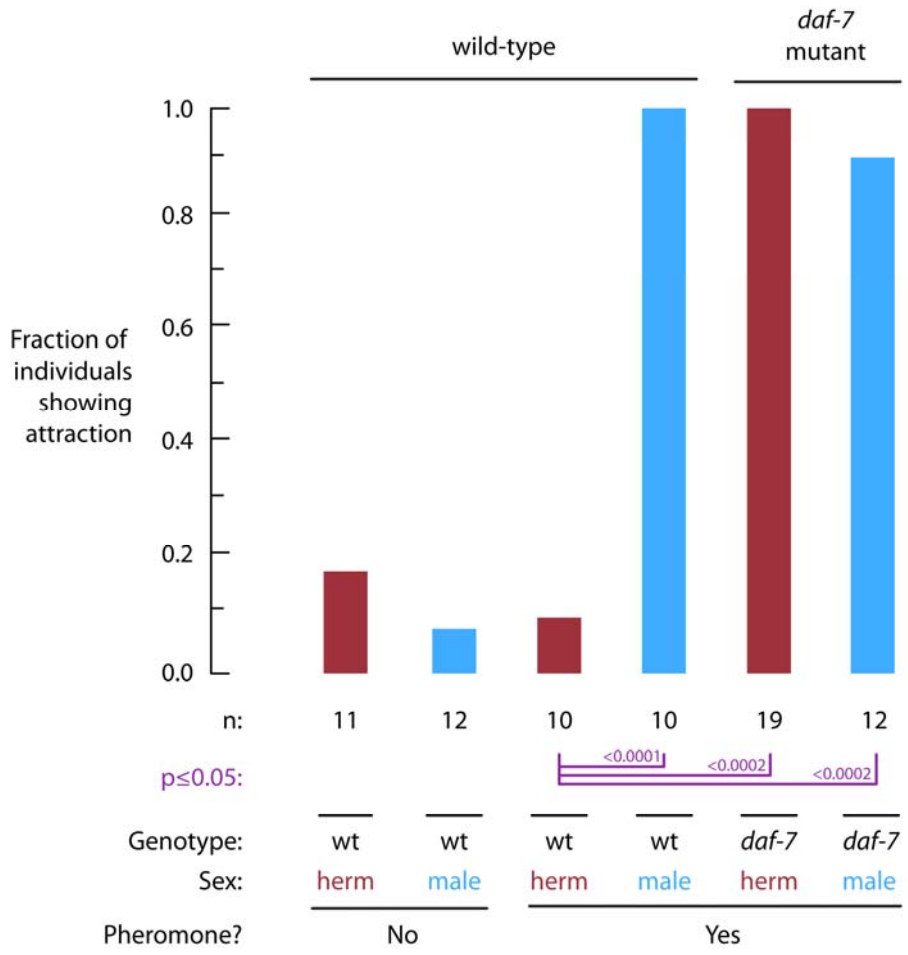

Figure S1. Sex pheromones elicit behavior in *daf-7* mutant hermaphrodites that is similar to males, but not wild-type hermaphrodites. Quantitation of sexual attraction behavior in wild-type males, wild-type hermaphrodites, and *daf-7* mutant hermaphrodites. Wild-type hermaphrodites and males were assayed 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.

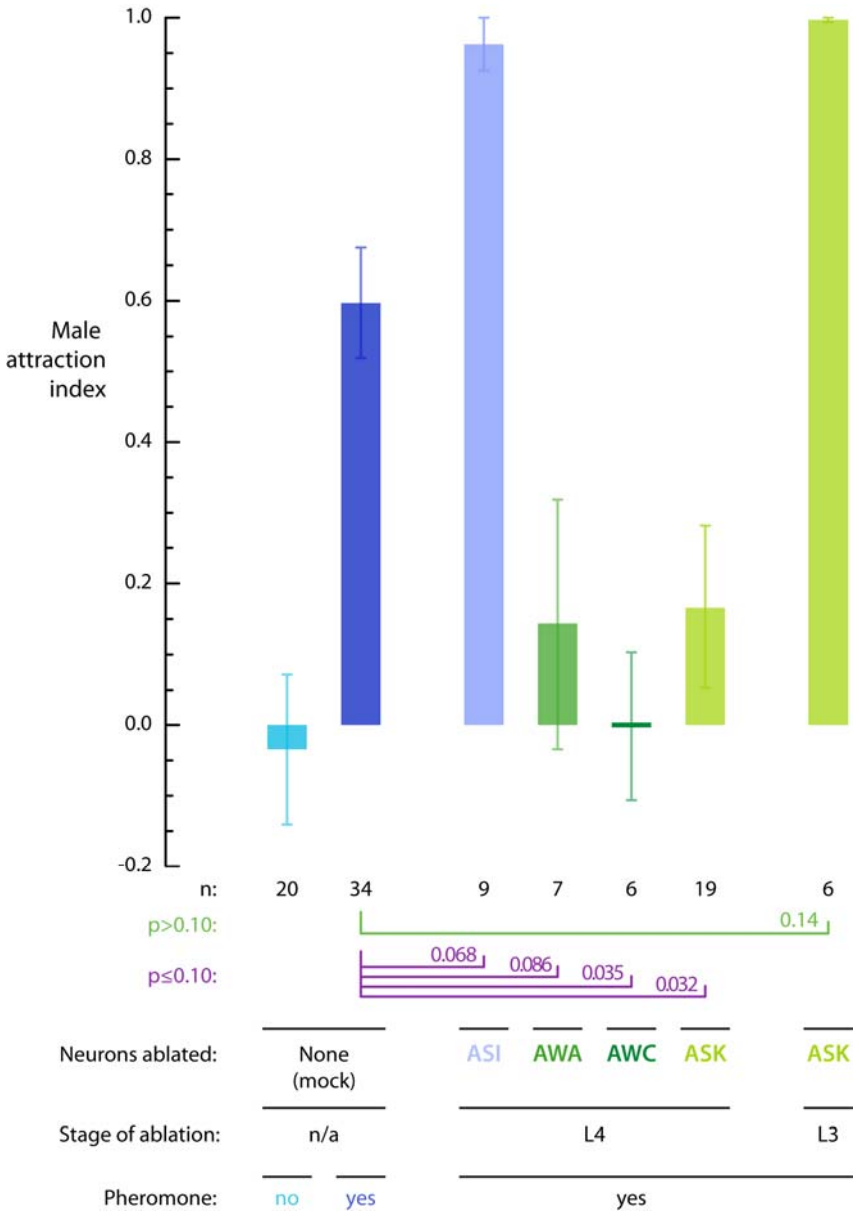

Figure S2. Sexual attraction in males requires the core sensory neurons AWA, AWC, and ASK, which compensate for one another. Laser ablation of each pair (one pair at a time) in L4 larvae shows that the AWA, AWC, and ASK pairs of sensory neurons are required for adult sexual attraction. Ablation of the 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). Error bars indicate the standard error of the mean.

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 Supplemental Figure 2 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 indicate the 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.