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Report

Sensory Regulation of *C. elegans* Male Mate-Searching Behavior

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Summary

How do animals integrate internal drives and external environmental cues to coordinate behaviors? We address this question by studying mate-searching behavior in *C. elegans*. *C. elegans* males explore their environment in search of mates (hermaphrodites) and will leave food if mating partners are absent [1]. However, when mates and food coincide, male exploratory behavior is suppressed and males are retained on the food source [1]. We show that the drive to explore is stimulated by male-specific neurons in the tail, the ray neurons. Periodic contact with the hermaphrodite detected through ray neurons changes the male's behavior during periods of no contact and prevents the male from leaving the food source. The hermaphrodite signal is conveyed by male-specific interneurons that are postsynaptic to the rays and that send processes to the major integrative center in the head. This study identifies key parts of the neural circuit that regulates a sexual appetitive behavior in *C. elegans*.

Results

C. elegans Male Mate-Searching Behavior Is Inhibited after Contact with a Hermaphrodite

Mate-searching behavior of the *C. elegans* male is inhibited by the presence of hermaphrodites [1]. How do males detect the hermaphrodite's presence? *C. elegans* males accumulate at sources of hermaphrodite-secreted pheromones [2–4] (Figures S1A and S1B available online). However, hermaphrodite pheromones alone failed to retain males on food when we tested males in the leaving assay [1] (see Experimental Procedures and Figure S1C). This suggests that hermaphrodite cues different from those that cause males to accumulate with them are required for the inhibition of male exploratory behavior.

We therefore examined whether males detect hermaphrodites through physical contact. We placed dead, paraformaldehyde (PFA)-fixed hermaphrodites on the food source and tested their effect on male exploratory behavior. Males were fully retained by fixed hermaphrodites (C_r [coefficient of retention, see Figure 1 legend] = 0.93; for living hermaphrodites C_r = 0.96; no retention C_r = 0) (Figure 1A). Because production and release of hermaphrodite pheromones into the environment is likely to require active metabolism, this result suggests that contact cues alone are sufficient for retention. Furthermore, when contact and mating between the male and the fixed hermaphrodites was blocked by a thin layer of agarose covering the hermaphrodite, males were not retained (C_r = 0.06). These observations suggest that contact is both necessary and sufficient for retention (Figure 1A).

Notably, males can discriminate between PFA-fixed males and fixed hermaphrodites because, just as with living males in parallel experiments, males still left in the presence of fixed males (C_r = 0.30) (Figure 1A). This sexual discrimination is not due to the absence of a vulva in the male because males are fully retained by *lin-39(n1760)* vulvaless hermaphrodites [5] (C_r = 0.93) (Figure 1A). Contact-dependent sexual discrimination suggests that chemical as well as mechanical cues may be detected by the male when it touches the hermaphrodite.

A male may respond to contact with another worm by beginning the mating sequence, pressing its tail against the other worm's body and backing up along it. During the time interval a male is thus responding, it cannot be exploring, and hence this contact response is expected to result in a decrease in the rate of leaving (partial retention). However, contact with a hermaphrodite stops the male from leaving the food altogether. We examined the behavior of males in the presence of hermaphrodites on a food source. We found that the male spends 40.7% ± 5.2% of its time in contact backing along the hermaphrodite body, 32.5% ± 4.0% of its time at the food edge (defined as a ring 1 mm wide at the perimeter of the food), and the remaining 27% of its time exploring other areas of the patch of food (Figures 1B and 1D). Thus the male spends a significant proportion of its time away from the hermaphrodite. This suggests that hermaphrodite contact has some effect on male behavior during periods of noncontact that inhibits exploratory activity.

To examine this possibility, we compared the behavior of a male alone and a male with hermaphrodites during periods of noncontact. This revealed a significant difference in male behavior at the food edge. A male alone spends 78.7% ± 1.0% of its time at the food edge (Figures 1C and 1D). During this time, the male periodically exits the lawn and immediately returns. An exit event was scored when the whole body of the worm came out of the food. A male alone produces on average 0.45 ± 0.08 such exit events per minute spent at the food edge (Figures 1C and 1E). In contrast, when hermaphrodites are present, a male produces only 0.02 ± 0.02 exit events per minute spent at the food edge (Figures 1B and 1E). The suppression of exits was not due to a change in the distribution of lengths of bouts on edge or a consequence of ejaculation (Figures S1D, S1E, and S1F).

The Male-Specific Ray Neurons Regulate Mate-Searching Behavior Both in the Presence and in the Absence of Hermaphrodites

We sought to identify the male sensory neurons responsible for hermaphrodite detection. The male-specific CEM neurons

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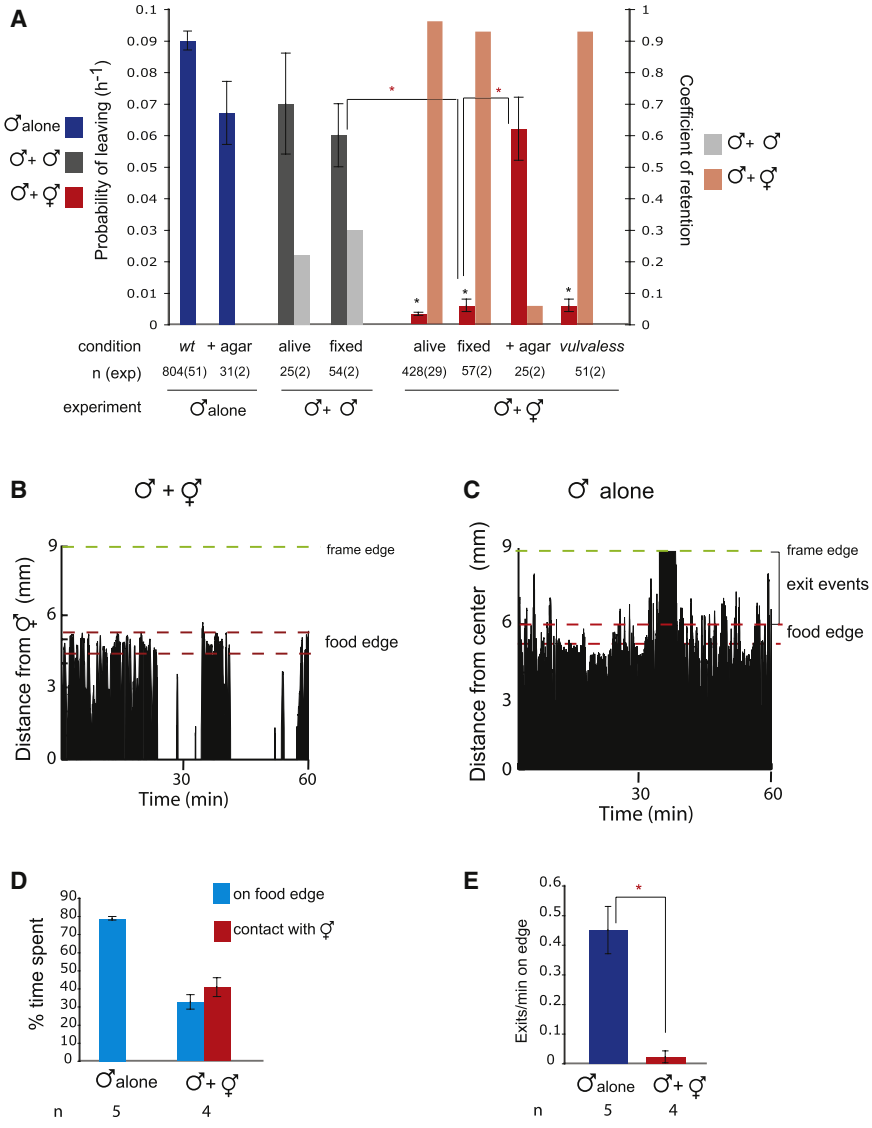

Figure 1. Mate-Searching Behavior Is Inhibited after Contact with a Hermaphrodite

(A) Contact with a hermaphrodite is required to retain males. The hermaphrodite vulva is not required to retain males. P_L values (probability of leaving food per worm per hour) and C_r (coefficient of retention) for males alone or in the presence of agar, with paralyzed living or PFA-fixed males, and with paralyzed living or PFA-fixed hermaphrodites, PFA-fixed hermaphrodites covered in agar, and paralyzed vulvaless (*lin-39 n1760*) hermaphrodites are shown. The number of worms assayed and the number of independent assays is indicated under each bar as "n (exp)." Replicas from living males, fixed males, and fixed hermaphrodites were performed in parallel at the same time on two different days. Fixation was performed in fresh 4% PFA at 4°C overnight and worms were rinsed in PBS before the assay. Assays with PFA-fixed hermaphrodites covered in agar were performed in parallel and compared with assays of males alone in the presence of agar. Retention levels, as the proportion in P_L reduction, are represented with the coefficient of retention, $C_r = [P_L(\text{alone}) - P_L(\text{with hermaphrodites})]/ P_L(\text{alone})$. $C_r = 1$ indicates full retention [P_L(alone) > 0 and P_L(with hermaphrodites) = 0], and $C_r = 0$ indicates no retention [P_L(alone) = P_L(with hermaphrodites)]. Males were fully retained by all hermaphrodites except by those covered in agar, and only partially retained by males. *$p < 0.001$ when P_L values are compared to wild-type males alone; *$p < 0.001$ when P_L values are compared to one another. Error bars indicate SEM.

(B) Male behavior with a hermaphrodite. The position is shown of a single male on a food patch during an hour interval. In the presence of hermaphrodites, a male alternates between bouts of contact and bouts on the food edge. The graph plots a male's distance from one hermaphrodite in the center of the plate (in mm) against time (min). Position was scored every 5 s. The food edge and the frame edge are marked.

(C) Male behavior alone. A male alone spends most of its time on the food edge and beyond. Events scored as exits are indicated by the brackets. The graph plots a male's distance from the center of the plate (in mm) against time (min). The food edge and the frame edge are marked.

(D) Males spend a significant amount of time on the food edge when they are in the presence of hermaphrodites but less time than when they are alone. For each condition, four or five males were analyzed during observation periods of 1 hr and 30 min. Error bars indicate SEM.

(E) Males produce significantly more exit events per minute on the food edge when alone than when in the presence of hermaphrodites. For each condition, four or five males were analyzed during observation periods of 1 hr and 30 min. *$p < 0.003$ compared to one another (t test). Error bars indicate SEM.

in the head are involved in male attraction to hermaphrodite pheromones [3, 4]. Consistent with our observation that hermaphrodite secreted cues do not play a major role in inhibition of male exploratory behavior, retention of males on food by hermaphrodites does not require the CEM neurons (Figure S2). Likewise, examination of touch-insensitive mutants showed that retention does not require the mechanoreceptors present in both males and hermaphrodites (data not shown).

We found that the sensory neurons required to detect the hermaphrodite were the male-specific ray sensory neurons in the tail. Two mutants with defects in the development of the male tail, including the rays, *lin-32(e1926)* and *mab-3(mu15)* [6, 7], displayed significantly reduced male leaving behavior compared to wild-type males and, as previously reported for *mab-3* [8], were strongly defective in retention by hermaphrodites (C_r = 0.40 and C_r = 0.25, respectively) (Figure 2A). The male tail includes four sets of sensilla with putative mechanosensory and chemosensory neurons: the nine bilateral pairs of rays, the spicules, the hook, and the postcloacal sensilla [9] (Figure 2E). To establish which of these sensilla are important for the regulation of mate-searching behavior, we carried out laser ablations of each tail sensillum in wild-type males and tested the operated animals in the leaving assay with and without hermaphrodites at adulthood.

Males without rays 1–6 or without rays 7–9 showed a significantly reduced rate of male exploratory behavior compared to mock-ablated males (Figure 2A). These effects appeared to be additive because ablation of all rays almost completely abolished male exploratory behavior (Figure 2A). Thus, exploratory behavior is induced by the ray neurons.

In the presence of hermaphrodites, males without rays 1–6 displayed partial defects in retention (C_r = 0.6), whereas males

without rays 7–9 were fully retained (C_r = 0.9) (Figure 2A). Due to the extremely low rate at which males with no rays left food, retention could not be meaningfully assessed in these animals. To circumvent this problem we sought a genetic background in which leaving rates are strongly enhanced. The *ocr-2(ak47)* mutation increases the rate at which males leave food, presumably because of decreased food attraction (see below and Figure 4). Thus, in an *ocr-2(ak47)* mutant background, males lacking all rays left food at a high enough rate for retention to be assessed (Figure 2A). In the presence of hermaphrodites, intact *ocr-2* males were fully retained (C_r = 0.96) (Figure 4), whereas in *ocr-2* males lacking all rays, retention was severely impaired (C_r = 0.4) (Figure 2A). Thus, the rays are largely responsible for retention of males by hermaphrodites, although other sensory neurons may contribute.

Disruption of male exploratory behavior and retention by hermaphrodites was specific to the ablation of ray precursor cells. No defects were observed after ablation of the postcloacal sensilla (C_r = 0.96) or the hook (C_r = 0.99) (Figure 2A). Similarly, cauterization of the spicule tips or ablation of the spicule-associated sensory and motor neurons caused no disruption of male exploratory behavior or retention (C_r = 0.94 and C_r = 0.96, respectively) (Figure 2B). Therefore, we conclude that male exploratory behavior is regulated specifically by the rays, which are required both for stimulating exploratory behavior in the absence of hermaphrodites and for retention in their presence.

Both RnA and RnB Neurons Stimulate Male Exploratory Behavior

Each ray sensillum contains two neurons, RnA and RnB, which differ in structure, neurotransmitters, and postsynaptic targets [9, 10] (Male Wiring Project, Albert Einstein College of Medicine, http://worms.aecom.yu.edu/pages/male_wiring_project.htm). To assess the contribution to the regulation of mate-searching behavior made by each neuronal type, we ablated them separately by genetically targeted expression of a caspase (see Experimental Procedures).

RnB-killed males displayed significantly reduced male exploratory behavior compared to wild-type animals and were completely retained by hermaphrodites (Figure 2C). Because of the promoter used to genetically kill RnB neurons, the CEMs and the hook neuron HOB also undergo cell death in these animals [11]. However, the phenotype observed can be attributed to lack of RnB neurons because, unlike ray ablation, lack of CEMs or hook does not result in defects in male exploratory behavior (Figure 2A and Figure S2). We also tested the role of a calcium channel formed by the *pkd-2* and *lov-1* genes, members of the polycystins family of TRP channels, and required for RnB neuron function in mating behavior [11, 12]. *pkd-2*(sy606);*lov-1*(sy582) double mutants displayed the same phenotype as RnB-ablated males (Figure 2C). Thus, RnB stimulation of male exploratory behavior is dependent on the activity of the TRPP channel formed by the PKD-2 and LOV-1 subunits. Retention of RnB-killed and *pkd-2;lov-1* males indicates that RnA neurons are sufficient to detect hermaphrodites and inhibit leaving behavior.

In contrast to males lacking RnB neurons, males lacking RnA neurons displayed normal exploratory behavior (Figure 2C). However, killing both RnA and RnB neurons by the caspase approach caused a higher reduction in exploratory behavior than killing RnB alone and was similar to laser ablation of all rays (Figure 2C). This indicates that RnA neurons also contribute to the stimulation of mate-searching behavior. RnA-killed males were fully retained by hermaphrodites, but because of incomplete death of these neurons in our experimental technique, no conclusion can be drawn about the sufficiency of RnB neurons for retention (Figure 2C).

In order to gain additional insight into the mechanisms by which ray neurons may regulate male exploratory behavior, we compared the behavior of intact males and males with genetically killed ray neurons at the food edge. Compared to intact males, males in which both RnA and RnB neuronal types were genetically killed produced significantly fewer exit events per minute at the food edge when alone (Figure 2D). Moreover, in the presence of hermaphrodites, exits were not completely suppressed (Figure 2D). Thus, ray neurons regulate male behavior at the food edge. In contrast, RnA-killed, RnB-killed, and *pkd-2*(sy606);*lov-1*(sy582) males produced the same rate of exit events/min on food edge when alone as intact males, and these exit events were suppressed by the presence of hermaphrodites (Figure 2D). Normal rate of food exits in backgrounds with a slow leaving rate (RnB killed and *pkd-2;lov-1*) suggests that the rays might alter additional aspects of male behavior.

We compared the locomotion of intact males and males with genetically killed ray neurons on food and off food. We found speed on food to be similar in all males (Figure S3A). In contrast, we observed an increase in high-angle turns (produced either by omega turns or long reversals) when immediately off food in all males with slow rate of leaving behavior (Figures S3C and S3D). These observations suggest that ray neurons, in addition to stimulating food exits, may also inhibit the return to a food source by inhibiting high-angle turns upon exiting food, thereby promoting exploratory behavior away from the food source.

Male-Specific Interneurons Postsynaptic to Ray Neurons Regulate Retention by Hermaphrodites

The male-specific EF interneurons are major postsynaptic targets of A and B ray neurons in the preanal ganglion [9] (Male Wiring Project). EF neurons extend processes along the ventral nerve cord into the nerve ring where they are likely to be pre-synaptic to neurons in the core nervous system (Figure 3F). These properties suggest that EF neurons might carry ray input to the core nervous system to affect male behavior. We removed the EF interneurons and their lineage companions DX interneurons by laser ablation of their precursor cells [9]. Loss of EF and DX interneurons disrupted retention (C_r = 0.6) but not exploratory behavior of solitary males (Figure 3A). The male's tendency to mate was also not affected. There was no decrease in the time the male spent in contact with the hermaphrodite or the average length of contact bouts (i.e., "response to contact" and "backing" steps of mating behavior) (Figures 3C and 3E). This further demonstrates that mating alone cannot account for retention. Furthermore, similar to intact males alone, EF-and-DX-ablated males produced an average of 0.39 ± 0.1 exit events/min on edge in the presence of hermaphrodites (Figures 3B and 3D). These results indicate that male-specific interneurons, most likely the EF interneurons, are required for the behavioral changes in male mate-searching behavior associated with the presence of hermaphrodites.

Amphid Neurons Modulate Male Exploratory Behavior and Contribute to Hermaphrodite Detection

The amphids are the main chemosensory structures present in the head of both males and hermaphrodites [13]. Given

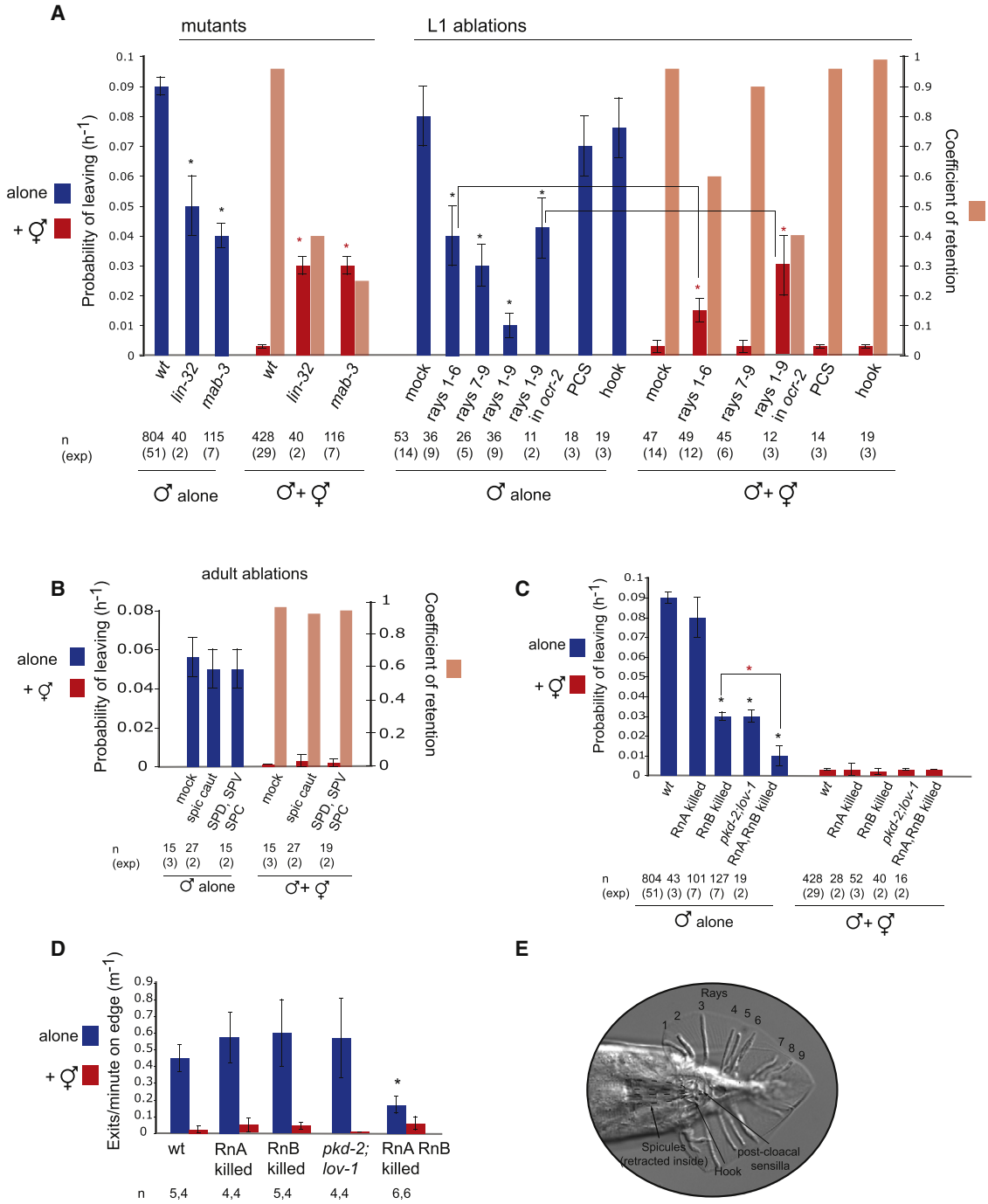

Figure 2. The Male-Specific Ray Neurons Stimulate Male Exploratory Behavior Away from Food and Retention by Hermaphrodites

(A) The rays, but not other tail sensilla, are required to stimulate exploratory behavior and for retention by hermaphrodites. The decrease in retention observed in rays 1–6 ablated wild-type males indicates that activity of rays 1–6 is required to inhibit exploratory activity induced by rays 7–9. This suggests that the exploration and retention signals are two different signals that can be produced by different ray neurons. P_L and C_r values for wild-type, mutant, and operated males alone and in the presence of hermaphrodites are shown. Mutant strains used were *mab-3(mu15)* and *lin-32(e1926)*. Laser ablations of wt and *ocr-2(ak47)* males were performed at the L1 or L2 stage: rays 1–6 were removed by bilateral ablation of V5p L-R and V6p L-R; rays 7–9 were removed by bilateral ablation of Tap L-R; p9p and p10p were ablated for removal of the hook sensillum; and Y was ablated for removal of the postcloaca sensilla. *$p < 0.01$ when P_L values are compared to controls alone (*wt* for mutants and mock-ablated *wt* and *ocr-2* for ablations); the red asterisk represents $p < 0.002$ when P_L values are compared to controls (*wt* or mock-ablated *wt* and *ocr-2* males with hermaphrodites). Error bars indicate SEM.

(B) The spicule neurons are not required for male exploratory behavior or for retention. P_L and C_r values alone and in the presence of hermaphrodites for males with intact, cauterized, or ablated spicule-associated sensory neurons (SPD, SPV, and SPC) are shown. Ablations were performed in young adults in a *syIs33*(P*(gpa-1)*::*gfp*) background so that the spicule sensory neurons could be visualized. Mock animals underwent the same manipulations as ablated animals with the exception of not being shot with the laser microbeam. These manipulations slightly reduced the rate of leaving alone compared to non-manipulated males. Error bars indicate SEM.

(C) RnA and RnB neurons stimulate male exploratory behavior away from food. P_L values, alone and in the presence of hermaphrodites, for males with and without RnA or RnB neurons and *pkd-2*(sy606);*lov-1*(sy582) double mutants are shown. Only males in which all RnB neurons were dead were assayed. RnA

the prominent role of amphid neurons in the regulation of exploratory behavior in relation to food in the hermaphrodite [14–16], we investigated whether they contribute to the regulation of exploratory behavior in relation to food and mates in the male. In particular, we investigated whether male leaving behavior is regulated through modulation of the male's response to food and whether amphid neurons detect hermaphrodite cues required for retention.

We used mutants that disrupt the function of different subsets of amphid neurons, namely mutants in the kinesin *osm-3(p802)*, in the cyclic GMP-gated channel subunit *tax-2(p691)*, and in the TRPV channel subunit *ocr-2(ak47)* [17–19]. In the absence of hermaphrodites, males that are mutant for these genes displayed a greater rate of exploratory behavior than wild-type males, indicating that amphid neurons inhibit male leaving behavior, probably by promoting attraction to food (Figure 4). Thus, a solitary male can still sense food and amphid neurons act antagonistically to ray neurons in the regulation of male exploratory behavior. Consistent with this, killing RnA or RnB neurons in *ocr-2* mutants results in reduction of male exploratory behavior (Figure S4). Similarly, disruption of amphid function in *mab-3* mutants by introducing the *osm-3* mutation results in a significant increase in rate of exploratory behavior (Figure 4).

We then investigated whether amphid neurons regulate retention by hermaphrodites and, if so, whether their effect is because of their function in food sensation or because they function to directly sense hermaphrodite cues. In the presence of hermaphrodites, *ocr-2* and *tax-2* mutant males were completely retained ($C_r = 0.96$ and $C_r = 0.97$, respectively) (Figure 4). This indicates that loss of food attraction does not necessarily result in loss of retention, the presence of hermaphrodites overcoming the sensory deficit in these mutants. In contrast, *osm-3* males, which have defects in male-specific ciliated neurons exposed to the outside including RnB [20, 21], displayed reduced retention ($C_r = 0.66$) consistent with hermaphrodite detection being also weakened (Figure 4). The partial loss of retention in *osm-3* males cannot be explained by the additive effect of reduced food sensation through amphids and reduced hermaphrodite detection through RnB neurons because killing RnB in other food-sensation mutants such as *ocr-2* and *tax-2* does not disrupt retention (Figure S4 and data not shown). Therefore, these results suggest that some gustatory amphid neurons impaired in *osm-3* mutants may sense hermaphrodite cues directly to contribute to retention.

Discussion

In all sexual animals, a drive to reproduce is essential for species survival. We have investigated a sex-specific, appetitive reproductive behavior in *C. elegans* and asked what neurons are responsible for it and how they function. We have found that both the male's drive to explore away from food and its ability to detect hermaphrodites are mediated by the ray sensory neurons, components of the male sexual organ. Both mate-searching behavior and ray-neuron development occur upon maturation of the male to adulthood [1], [9]. Mechanistic coupling of the differentiation of sexual structures with behavior would appear to be a natural way of coordinating diverse events of maturation.

The tendency of a male to explore away from a food source (leaving) depends on the balance of two competing needs: feeding and reproduction. Consistent with this, the rate of leaving is regulated by the opposing activity of food-sensing amphid neurons and sexual ray neurons. In hermaphrodites, amphid neurons are part of the circuit for navigation that regulates exploratory behavior in response to food by modulating the frequency of runs and turns [14–16]. Stimulation of male exploratory behavior away from food by ray neurons is regulated, at least in part, through the stimulation of exit events at the food edge and the inhibition of high-angle turns immediately upon exiting a food source without mates. Our results show that ray neurons do not promote leaving by blocking the food-attraction pathway in amphid neurons. We propose that ray neurons are part of the male navigation circuit that stimulates mate searching independently of food attraction and that together with the amphid neurons allows the male to locate a source of both food and mates.

The presence of mates on the food patch is detected by contact through the ray sensilla. It has previously been shown that rays stimulate the response to contact and backing (along the hermaphrodite's body) steps of mating [22]. We show that although the execution of these steps reduces the time a male spends exploring and reduces the probability of leaving, it is not sufficient to completely retain a male on food. Males execute these steps with both hermaphrodites and males. However, periodic backing along the hermaphrodite body produces a behavioral change in the male for an extended period of time, which correlates with a suppression of exit events at the food edge and prevents the male from leaving. Prior experience has been shown to alter *C. elegans* behavior in several contexts [23, 24].

Because males can discriminate between fixed males and fixed hermaphrodites, we speculate that cuticle-bound chemicals may be detected upon contact to bring about the difference in behavior. Another possibility is that bouts of contact need to be a minimum length of time to cause a lasting effect in the male. This would be consistent with the observation that bouts of contact with hermaphrodites are significantly longer than those with males and that loss of retention due to lack of most rays also reduces the length of contact bouts. In this scenario, ray neurons would act as timers that register length of contact bouts.

Both, response to contact and retention are triggered by the same sensory sensilla, the rays. The circuit for retention, however, includes male-specific interneurons that do not

death was not complete. Strains used are as follows: *bxIs14(*P*(pkd-2)::gfp); bxEx136*[P*(pkd-2)::ICE*+P*(unc-122)::gfp*] for RnB genetic ablations; *bxEx137*[P(*trp-4)::caspase3-NZ*+P*(grd-13)::CZ-caspase3*+P(*elt-2)::gfp*] or *bxEx138*[P(*trp-4)::*ICE+P(*elt-2)::gfp*] for RnA genetic ablations; and *bxEx136; bxEx137* or *bxEx136;bxEx138* for RnA,RnB genetic ablations. *p < 0.001 when P_L values are compared to wild-type males alone; *p < 0.02 when P_L values are compared to one another. Error bars indicate SEM.

(D) Loss of all RnB neurons and most RnA neurons results in a reduction of exit events at the food edge. Bars plot the average of exit events per minute spent on food edge per worm in 1 hr 30 min observation periods alone and in the presence of hermaphrodites; *p < 0.05 compared to intact males alone (t test). Error bars indicate SEM.

(E) DIC photograph of a male tail with the ventral side in focus; posterior is oriented toward the right. All the male-specific sensilla are labeled. Dashed lines indicate the position of the spicules, which remain retracted inside the gubernaculum.

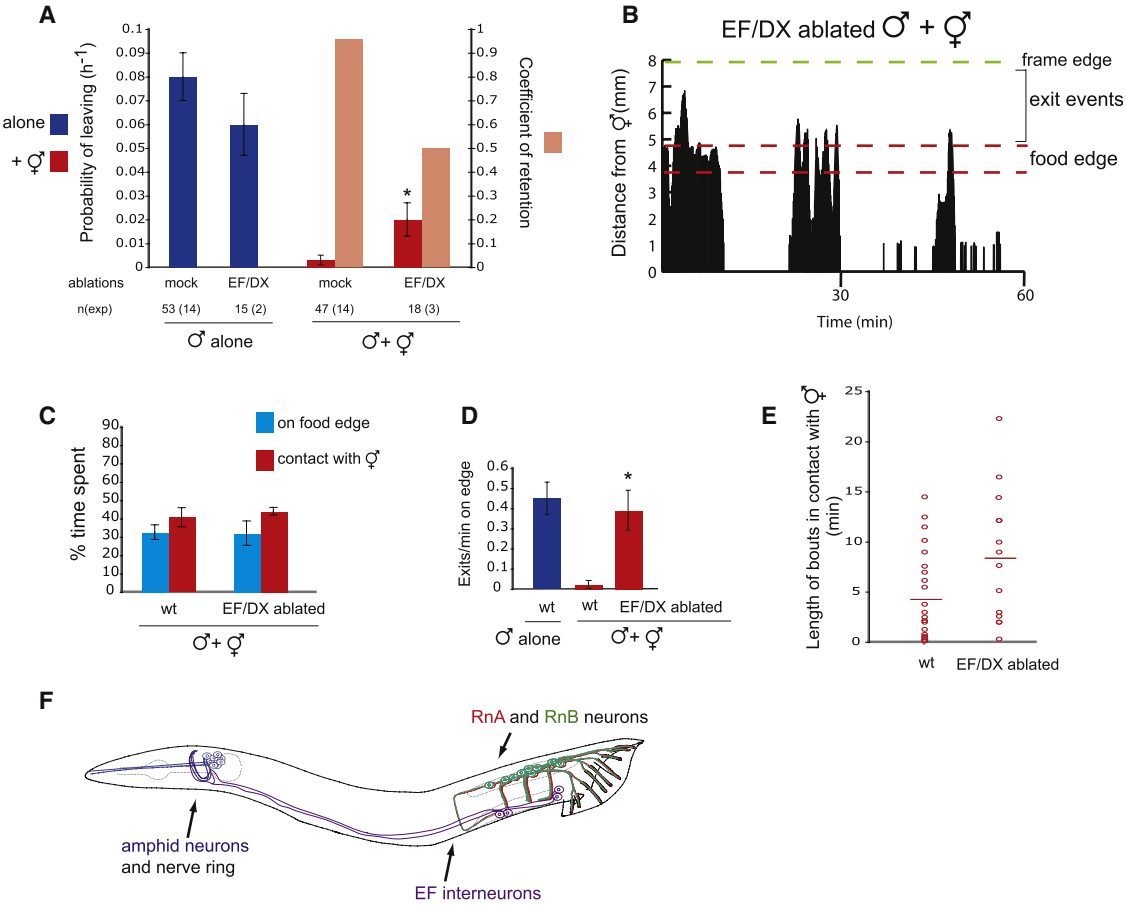

Figure 3. Male-Specific Interneurons Regulate Retention by Hermaphrodites

(A) Loss of EF and DX interneurons results in partial loss of retention by hermaphrodites. P_L and C_r values for intact and operated males alone and in the presence of hermaphrodites are shown. EF and DX interneurons were removed by laser ablation of the U and F cells at L1 stage. DX interneurons are postsynaptic mainly to hook neurons and presynaptic to postcloacal sensilla (PCS) neurons, and may be involved in sperm transfer (Male Wiring Project) [25]. We have shown that, unlike the rays, hook and PCS are not required for retention, making the DX interneurons unlikely candidates for conveying the hermaphrodite signal. EF and DX ablation did not disrupt retention as severely as ablation of all rays. This difference could be explained by the fact that, unlike EF ablated males, males with no rays spend little or no time in contact with the hermaphrodite, and therefore the proportion of time available to explore away from the food lawn is increased in ray ablated males. *$p < 0.001$ when P_L values are compared to mock-ablated males with hermaphrodites. Error bars indicate SEM.

(B) A male without EF and DX interneurons moves beyond the food edge after bouts of contact with a hermaphrodite. The graph plots the male's distance from the hermaphrodites in the center (in mm) against time (min). The food and the frame edge are marked.

(C) EF-and-DX-ablated males spend the same proportion of time on the food edge and in contact with hermaphrodites as intact males. Bars plot the average percentage of time spent on the food edge or in contact with a hermaphrodite per worm for intact and EF-and-DX-ablated males. Same data for wild-type male as in Figure 1D; n = 4 for each condition in 1 hr 30 min observation periods. Error bars indicate SEM.

(D) EF-and-DX-ablated males in the presence of hermaphrodites produce the same frequency of exit events as intact males alone. Bars plot average of exit events per minute spent on the food edge per worm. Same data for wild-type male as in Figures 1E and 2D are shown; n = 4 for each condition (n = 5 for *wt* alone) in 1 hr 30 min observation periods *$p < 0.03$ compared to intact males with hermaphrodites (t test). Error bars indicate SEM.

(E) Bouts of contact with a hermaphrodite are similar in length for EF-and-DX-ablated and intact males. The duration (in minutes) of each contact bout produced by four individual intact or EF-and-DX-ablated males in 1 hr 30 min observation periods is represented by a circle, and the average is represented by a horizontal line.

(F) Male neurons that regulate mate-searching behavior. A drawing of a male worm, anterior is oriented to the left, is shown. Neurons are labeled in colors: A and B type ray sensory neurons in the tail are shown (red and green); EF interneurons postsynaptic to ray neurons in the preanal ganglion (purple) send processes to the nerve ring in the head (integration center); and amphid sensory neurons in the head are shown (blue).

affect response to contact. The EF interneurons are major postsynaptic targets of ray neurons in the preanal ganglion and extend processes into the nerve ring in the head [9] (Male Wiring Project). Thus, EF interneurons may provide a neural substrate for the integration of hermaphrodite signals, sensed by male-specific neurons in the tail, into the male's core nervous system in which food signals are processed. Determination of the molecular mechanisms by which hermaphrodite signals are detected and male exploratory behavior is produced, and the complete identification of the mate-searching circuit, awaits further studies.

Supplemental Data

Supplemental Data include Supplemental Results, four figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/supplemental/S0960-9822(08)01419-X.

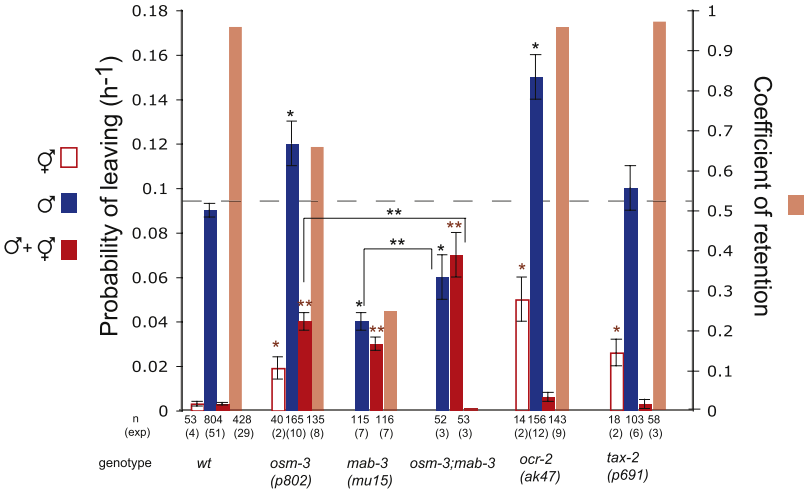

Figure 4. Amphid Neurons Modulate Male Exploratory Behavior and Contribute to Retention

Mutants in food sensation display higher rate of exploratory behavior but are fully retained by hermaphrodites; mutants defective for retention have defects in neurons involved in both food sensation and hermaphrodite detection. Unlike wild-type hermaphrodites, which remain on food, *osm-3(p802), tax-2(p691)*, and *ocr-2(ak47)* hermaphrodites left food at measurable rates, indicative of the role of these genes in food sensation. Examination of *tax-2* and *ocr-2* canonical reporter lines in males revealed no expression in ray neurons [18, 19]. Only *osm-3* is expressed in RnB neurons, as well as in the other male-specific ciliated neurons exposed to the outside [20, 21]. Bars show the P_L and C_r values of hermaphrodites alone, males alone, and males with hermaphrodites for wild-types and mutants in gustatory amphid and ray neurons. The dashed line indicates the rate of exploratory behavior in wild-type males. Strains used are as follows: *wt*, osm-*3(p802)*, *mab-3(mu15)*, *mab-3(mu15);osm-3(p802)*, *tax-2(p691)*, and *ocr-2(ak47)*; *p < 0.001 when P_L values are compared to wild-type males alone; one red asterisk represents p < 0.001 when P_L values are compared to wild-type hermaphrodites; two red asterisks represent p < 0.001 when P_L values are compared to wild-type males with hermaphrodites; **p < 0.03 when P_L values are compared to one another. Error bars indicate SEM.

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