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Review

Exploratory decisions of the *Caenorhabditis elegans* male: A conflict of two drives

Arantza Barrios*

Cell and Developmental Biology Department, University College London, 21 University St, London WC1E 6DE, United Kingdom

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ABSTRACT

The ability to generate behavioral plasticity according to ever-changing physiological demands and environmental conditions is a universal feature of decision-making circuits in all animals. Decision-making requires complex integration of internal states with sensory context. As a mate searching strategy, the *Caenorhabditis elegans* male modifies his exploratory behavior in relation to a source of food according to recent sensory experience with mates. Information about the reproductive and nutritional status of the male is also incorporated in his choice of exploratory behavior. The study of mate searching in the *C. elegans* male, a genetic model organism with a nervous system of only 383 neurons, provides the opportunity to elucidate the molecular and cellular mechanisms of state-dependent control of behavior and sensory integration. Here I review our progress in understanding the physiological and environmental regulation of the male's exploratory choices – to explore in search of mates or to exploit a source of food – and the neural circuits and neuromodulator pathways underlying this decision.

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Contents

1. Introduction

The biggest challenge facing all animals is to choose what to do when. The prioritization of one behavior over another when the two compete for expression is the foundation of decision-making. Optimal decision-making requires the integration and resolution of conflicting inputs within neural networks as well as modulation of behavioral output by physiological states [1,2]. These are universal features of the neural circuits that drive behavioral choice in all organisms, from nematodes to humans [3].

Decision-making relies on the operational plasticity of neural networks, i.e. the ability of one network to generate more than one behavioral output. The study of small circuits in invertebrate systems has contributed greatly to our understanding of how neural networks produce distinct behavioral outputs. Work on central pattern generators that drive swimming in Tritonia and the leech,

* Tel.: +44 020 76796577.
E-mail address: a.barrios@ucl.ac.uk

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biting in Aplysia and gastric mill movements in crustaceans have revealed the mechanisms by which cellular, synaptic and connectivity properties of neural networks can change to provide output diversity [4]. However, when animals execute behavioral choices that involve the whole organism, such as feeding versus mating or source exploitation versus exploration, the choice is shaped by the physiological demands of the organism. For these decisions, network plasticity needs to incorporate internal drive states that may sometimes be in conflict.

The study of behavioral prioritization in animals with small nervous systems, such as *Caenorhabditis elegans* (only 383 neurons in the male), provides the opportunity to elucidate the cellular and molecular mechanisms by which a whole organismal neural network encodes behavioral decisions according to sensory experience and physiological demands. In this system, one can address with precision and relative ease how neural circuits are modulated by the endocrine system, how previous experience and physiological state regulate the acquisition and processing of environmental stimuli and how behavioral state transitions are generated and terminated at the cellular and molecular level.

The *C. elegans* male needs to explore his environment in search of both food and mates. In the wild, these resources are often available in discrete patches at distant locations. The mate-searching strategy of the *C. elegans* male consists of an exploratory behavior that will take him away from a food source if mates are absent and will keep him at a source of food where he has recently experienced mates. The food-leaving assay exploits the *C. elegans* male's tendency to explore his environment and readiness to leave a plentiful source of food depleted of mates as a measure of the male's drive and motivation to reproduce [5]. Consistent with the idea that male food-leaving behavior is a mate-searching strategy, exploration away from a plentiful source of food only occurs in sexually mature males, not self-fertilizing hermaphrodites or males at larval stages [5]. Furthermore, recent experience with a mate inhibits exploration away from food [5,6].

Food leaving as a mate-searching strategy imposes behavioral prioritization on the male to satisfy competing needs: to explore in search of mates over exploiting a source of food. This exploratory decision is executed through the regulation of specific patterns of locomotion. What need the male prioritizes and what choice he executes depends on his internal nutritional, reproductive and neuromodulatory state and on previous sensory experience of mates. This information needs to be integrated within the neural network for exploration and converge onto the pre-motor interneurons controlling locomotion.

In this review I will present our current knowledge of the mechanisms regulating the male's decision to leave or to stay on food (Fig. 1). I will first provide an overview of the internal physiological signals (reproductive, nutritional and neuromodulatory states) that regulate male exploration. Then I will explain how environmental signals -recent mate experience and food- alter the male's patterns of locomotion to produce exploratory decisions. Finally I will describe the main cellular components of the male's neural network for exploration and how they regulate locomotion based on sensory experience and internal modulatory state.

2. Regulation of exploration by internal states

2.1. Reproductive status

C. elegans is an hermaphroditic species with two genders: males and self-fertilising hermaphrodites, which may also serve as mates for the males. Both males and hermaphrodites explore their environment in search of favorable conditions. Patterns of exploration are sexually dimorphic and are regulated differently in males and

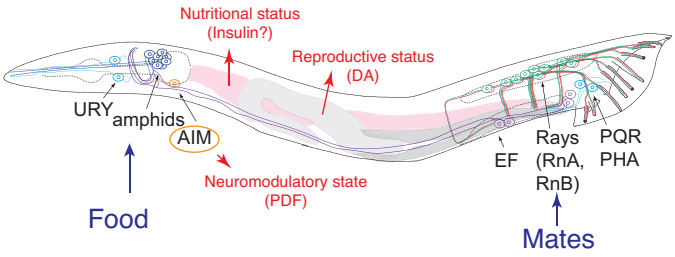

Fig. 1. Physiological and environmental regulation of mate searching. Diagram depicts the tissues of the adult *C. elegans* male that regulate mate searching. Internal physiological signals are shown in red. Environmental sensory inputs are shown in blue. The gonad is shown in gray. The intestine is shown in pink. The neurons that regulate exploratory decisions are labeled: the AIM interneuron, source of PDF neuropeptide in orange; neurons receiving PDF signaling URY, PHA and PQR in light blue; food-sensing amphid neurons in dark blue; mate-sensing ray neurons RnA and RnB in red and green; EF interneurons in purple. DA (dafachronic acid) acts through the nuclear hormone receptor *daf-12*.

hermaphrodites according to their specific needs (reviewed in [7]). One such sexually dimorphic need is associated with their different systems of reproduction. Males, who only have sperm, need to find a mate to reproduce, whereas hermaphrodites, who have both sperm and oocytes, do not. Accordingly, exploration away from a food source as a mate-searching strategy is regulated by signals from the gonad [5,8].

In the male, one of the stimulatory signals for mate searching comes from functional sperm. Surgical ablation or genetic disruption of the male germ line by mutations in *glp-1* (a member of the Notch receptor family required for germ line cell fate specification [9]), *fog-1* (which transforms sperm into oocytes [10]) or *spe-26* (required for spermatid differentiation [11]) decreases food leaving in males [5]. In hermaphrodites however, transforming the germ line uniquely into sperm through gain of function mutations in *fem-3* (a component of the sex-determination pathway required to promote male development [12,13]) or loss of function mutations in *mog-3* (required for oogenesis [14]) does not result in food leaving [5]. With the caveat that hermaphrodite and male sperms are known to have different properties [15], these results suggest that sexual dimorphism is also present in the tissues that receive the signals from the germ line.

Consistent with the idea that exploration away from food is regulated by the need to find a mate, food leaving is displayed by males but not hermaphrodites of other hermaphroditic species, such as *P. pacificus*, and by both males and virgin females of the gonochoristic (one sex per individual) species *C. remanei* and *C. sp. (DF5070)* but not by mated females [5]. These observations might lead one to hypothesize that having one type of gamete stimulates mate searching, whereas the presence of two gametes or fertilized eggs inhibits it. However, this does not appear to be the mechanism by which mate searching is regulated in *C. elegans* since feminizing the germ line and disrupting sperm formation in hermaphrodites by mutations in *fog-1, fem-1, fem-2* or *fem-3*, does not cause them to leave food. Thus, the regulation of sexually dimorphic exploratory strategies is complex and involves tissues other than the germ line.

The mate-searching stimulatory signal induced by the male gonad acts through the nuclear hormone receptor (NHR) *daf-12*. That a NHR is involved in the organization of sexual dimorphism in worms is of particular interest given the fundamental role that NHRs play in the establishment and regulation of sexual dimorphism in vertebrates [16]. Experiments in which temperature-sensitive alleles of *daf-12* were used to disrupt protein function either during development or at adulthood, have demonstrated that DAF-12 has both organizational and activational roles in the regulation of mate-searching behavior [8]. Like all NHRs, *daf-12* has a DNA-binding domain, a ligand-binding

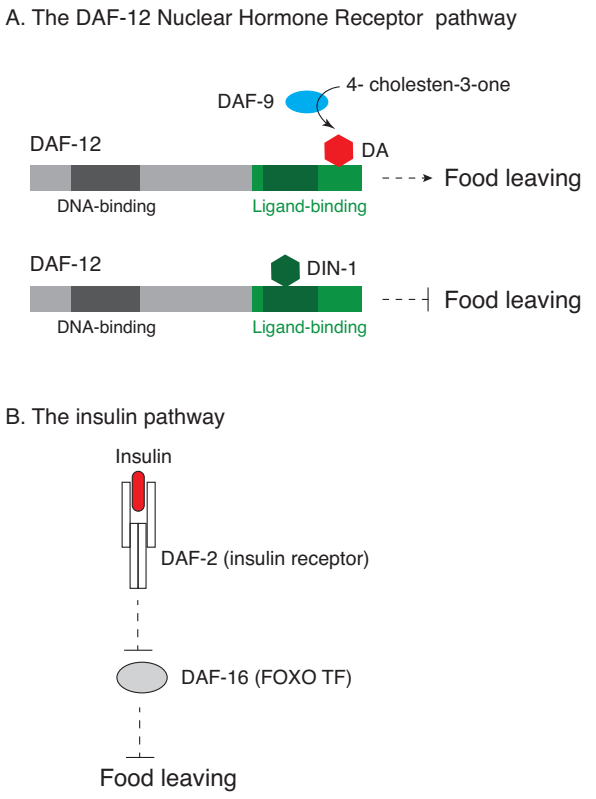

Fig. 2. Genetic pathways for neuroendocrine regulation of male exploration. (A) The nuclear hormone receptor DAF-12 pathway in the regulation of male food leaving. The protein domains of DAF-12 are illustrated in colors: DNA-binding domain (black); ligand-binding domains (light green for activator, dark green for repressor). DA, dafachronic acid (red) is an activator of DAF-12 and food-leaving. DAF-9 (blue) synthesizes DA from its precursor 4-cholestene-3-one. DIN-1 (dark green) is a repressor of DAF-12 and food leaving. (B) The insulin pathway. Upon insulin binding, the insulin receptor DAF-2 stimulates food leaving through repression of the FOXO transcription factor DAF-16.

domain and a domain that binds a co-repressor, DIN-1 [17] (Fig. 2A). Analysis of mate-searching behavior in a battery of mutant alleles that differently disrupt these DAF-12 protein domains has shown that the liganded form of DAF-12 stimulates food leaving, whereas the DIN-1-bound form inhibits food leaving [8]. The *daf-12* ligand dafachronic acid (DA) is metabolized from its precursor 4-cholesten-3-one by the product of the gene *daf-9* [18] (Fig. 2A). *daf-12* and *daf-9* null mutant males display very reduced levels of food leaving and the defects of *daf-9* mutants can be rescued by exogenous exposure to DA [8]. Importantly, and consistent with sexual dimorphism being present not only in the gonad but also in tissues receiving the signals from the gonad, exposure to exogenous DA does not cause hermaphrodites to leave food.

The tissues where *daf-9* and *daf-12* are required for mate searching are not known. However, the male gonad appears to be either the source of DA or to provide an inductive signal to other tissues to produce DA. Gonad ablation in *daf-12* null males does not enhance the mate searching defects of these mutants indicating that the gonad and *daf-12* act in the same pathway. Moreover, the gonad is not required for the increased levels of food leaving displayed by mutants with a ligand-independent form of DAF-12. Also consistent with the idea that the gonad is either the source of DA or the source of an inductive signal for DA production by other tissues, is that exposure to exogenous DA precursor does not rescue the food leaving defects of gonad-ablated males [8]. Tissue-specific rescues of *daf-9* would determine whether DA is produced in the gonad or in other tissues upon induction from the gonad, but these experiments have not been performed.

In summary, the male gonad, through DA, the DAF-12 NHR and functional sperm, conveys the mate-searching stimulatory signals that indicate male reproductive maturity.

2.2. Nutritional status

The male needs to find both food and mates. Therefore, the male's decision to stay at or to leave a source of food depleted of mates depends on the balance of two competing needs: feeding and reproduction. Consequently, changes in the rate at which males leave a source of food may not always reflect changes in reproductive needs but changes in nutritional needs.

Mate-deprived males that have been food starved for 3 h or more remain at a newly located patch of food and do not leave for several hours [5]. After a few hours of feeding, exploration away from food in search for mates resumes at a normal rate [5]. Therefore, the male's behavioral responses to a food source are regulated by nutritional status and reproductive status in opposite ways: the need to feed leads to exploration within a food patch and the need to mate leads to exploration away from the food patch.

The nutritional signal regulating male exploration may be mediated, at least in part, by an insulin-like pathway (Fig. 2B). As in mammals, the insulin pathway acts as a nutritional satiety signal to control several physiological processes and behaviors in *C. elegans* [19,20]. DAF-2 is the *C. elegans* main insulin receptor [21]. Males defective in insulin signaling because of a loss of function mutation in *daf-2* do not leave food [5]. DAF-2 acts via inactivation of the forkhead box O (FOXO) transcription factor DAF-16 [22] and *daf-16* loss of function mutations restore exploration away from food in *daf-2* mutants [5] (Fig. 2B). Although both starvation and disruption of insulin signaling result in lack of exploration away from food, direct evidence of starvation acting through insulin signaling to regulate food leaving is lacking. Investigation into the effects of starvation on exploratory behavior in a *daf-16* mutant background, where insulin signaling is constitutively active, would aid clarification. If starvation acts exclusively through inhibition of insulin signaling, starved *daf-16* males should leave food at normal rates.

The mechanisms by which starvation, and perhaps insulin, suppress male food leaving behavior and promote exploration within the food patch are not known. However, one can envisage several ways by which food responses may be enhanced under a state of nutritional deprivation. For example, chemosensory receptors in food-sensing neurons may be upregulated by increasing transcription or traffic into ciliated sensory endings. Indeed, chemoreceptor expression has been shown to be regulated by signals indicating overcrowding and starvation in *C. elegans* [23]. Another possible level of regulation is through increase in synaptic output from sensory neurons onto downstream interneurons, as has been shown for *Drosophila* food-searching behavior [24,25]. Conversely, under a state of mate deprivation, food responses may be reduced by downregulation of chemoreceptors and/or chemosensory neuron synaptic output.

2.3. Neuromodulatory state

Another mechanism by which internal state can dramatically alter the behavioral responses to one same stimulus is by reconfiguration of neural circuits through the action of neuromodulators. Indeed, from a forward genetic screen for mutant males that do not leave food, the neuropeptide PDF (*P*igment *D*ispersing *F*actor) was identified as a major regulator of *C. elegans* mate-searching behavior [26].

PDF signaling influences decision-making and behavioral prioritization of a sex-specific reproductive behavior (disperse in search for mates) over a gender-shared behavior (exploit a source of food) when the two compete for expression. PDF signaling acts in a

subset of sensory neurons that antagonize the food-sensing circuit to generate a state of arousal for mate searching upon mate deprivation [26] (see Section 4). Mate-deprived *pdf-1* and *pdfr-1* (*pdf receptor 1*) mutant males do not leave food and display enhanced behavioral responses to food. The PDF pathway signals sex-drive state and not nutritional state. *pdf-1* and *pdfr-1* mutant males are not nutritionally deprived since, like wild-type males, they reach satiety-induced behavioral quiescence [19] when feeding in nutritionally high food [26]. Moreover, *pdf-1* mutants reduce their frequency of reversals to start dispersing in response to food deprivation but not in response to mate deprivation, indicating that PDF regulates the circuit that conveys the drive to search for mates [26] (see Section 3.2). Therefore, the lack of exploration away from food by mutants in the PDF signaling pathway reflects a reduction in the reproductive drive and corresponding increase in the relative contribution of the food-searching circuit to the male's network for exploration. Consistent with this interpretation, genetic disruption of the food-sensing pathway in *pdfr-1* mutants restores food-leaving behavior [26].

In addition to mate deprivation failing to stimulate food leaving, *pdf-1 and pdfr-1* mutant males are less efficient than wild-type males at responding to mate contact [26]. A venue worth investigating is whether PDF regulates several of the physiological changes necessary for the efficient execution of mate searching including increased sensory acuity to mate pheromones. PDF acts instructively in the adult male to regulate neural circuit function and it acts as a neurohormone, targeting neurons that are a distance away from the interneuron that is the source of neuropeptide release [26]. Neurohormones are known to coordinate many of the physiological responses necessary to execute a specific behavior. For example, during the fight-or-flight response triggered in vertebrates by the encounter of danger, adrenalin stimulates many of the physiological changes required for this energetically costly behavior, such as glycogenolysis, blood vessel dilation and heart beat rate [27].

Nematocin, the *C. elegans* oxytocin/vasopressin-related neuropeptide [28,29], is another neuromodulator of male exploratory decisions. Mutants in the ligand *ntc-1* or the receptors *ntr-1* and *ntr-2* display reduced food-leaving rates compared to wild-type males [29]. Nematocin also acts extrasynaptically and nematocin signaling mutants display disorganized and uncoordinated mating behavior indicating a global role in the regulation of reproductive behaviors [29].

3. Regulation of exploration by sensory experience

3.1. Sensing a mate on food

As an efficient mate-searching strategy, food leaving is suppressed when mates are found on a food patch [5,6]. Experiencing a mate on food produces a durable change (1 h) in the state of the male and a consequent change in his behavioral responses to food [6,26]. These changes in state and behavior result in exploration being confined to the limits of the food patch [6]. Copulation is not required for this change in male exploratory behavior. Instead, mate experience involves the male contacting and scanning the surface of the hermaphrodite body [6]. Importantly, contact with other males does not suppress food leaving, suggesting that sex-specific pheromone cues trigger mate recognition and consequent changes in exploration [5,6].

Although *C. elegans* males chemotax to and accumulate at sources of ascaroside pheromone blends secreted by hermaphrodites into the environment [30], these pheromones are neither sufficient nor required for the suppression of male food leaving [6]. Males still leave a food patch that has been previously conditioned with mates or with mate secretions, despite secreted pheromones being long lived and still active after several hours [6]. Ascaroside pheromones are produced by a fatty acid oxidation process that involves the enzyme encoded by *daf-22* [30,31]. Males are retained on the food patch after contact experience with *daf-22* hermaphrodites, which do not produce short chain ascarosides (A.B. unpublished), indicating that sex-specific cues other than short-chain ascarosides are responsible for the switch in exploration patterns upon contact experience with a mate.

The contact pheromone has not been chemically isolated but it appears to reside on the cuticle. The role that the cuticle plays in mate recognition has been investigated by exposing males to the preserved carcasses of dead hermaphrodites fixed in paraformaldehyde (PFA) [6]. Upon entering in contact with PFA-fixed hermaphrodites, males initiate a normal mating sequence and this contact experience subsequently suppresses male food-leaving behavior. In contrast, contact experience with PFA-fixed males, like contact with living males, does not prevent the exploring male from leaving the food patch [6]. The nematode cuticle contains a surface coat composed of glycoproteins secreted by glands and hypodermis [32]. Pathogens use this glycocalix as cues for host recognition and attachment [33]. The nematode cuticle glycocalix may also serve as pheromones for mate recognition in a similar manner as cuticular hydrocarbons are employed in arthropods [34]. Cuticular cues may be better indicators of the current presence or absence of mates since secreted ascarosides are long-lived and perdure long after mates have left the spot [35].

3.2. Experience-dependent changes in patterns of locomotion

The decision to leave or to stay at a source of food is executed through the generation of distinct patterns of locomotion. Several parameters of locomotion can be modulated based on sensory experience and internal state: speed, direction and curvature [36–40].

The factor that determines whether a male leaves or stays at a source of food is the probability to change direction upon encountering the edge of the food patch and sensing the immediate loss of food [6] (Fig. 3). Changes in direction can be achieved through the execution of omega turns (180° turns that completely reverse the direction of movement), reversals (backward movement with a change in direction upon resuming forward movement) or asymmetric head swings (slight differences in the amplitude of the head curvature used for steering along a sensory gradient). In contrast, changes in the speed at which a male explores the food patch do not have a significant effect on the probability of leaving [26] (and A.B. unpublished).

Modulation of the frequency of reversals and omega turns upon sensing the loss of food generates two distinct exploratory strategies corresponding to two distinct behavioral states based on previous experience with a mate: local search versus dispersal [26,41,42] (Fig. 3). Local search is characterized by frequent changes in directionality, an exploratory strategy that helps the male locate and return to a recently lost source of stimulus (food and mates) (Fig. 3A and B). Dispersal is characterized by rare or infrequent changes in directionality and high probability of forward runs when the male has completely exited the patch, a strategy that helps the male explore in search of a new source of stimulus (Fig. 3A and C).

Local search events begin when the head of the animal exits the food patch and terminate when the animal fully returns to the food source [43]. These events are very brief in mate-experienced males and increasingly longer in mate-deprived males. The probability of reversing upon encountering the edge of the food patch is nearly 100% in mate-experienced males, resulting in very brief bouts of local search in which the body of the animal does not completely exit the food patch [6] (Fig. 3B). In contrast, well-fed, mate-deprived

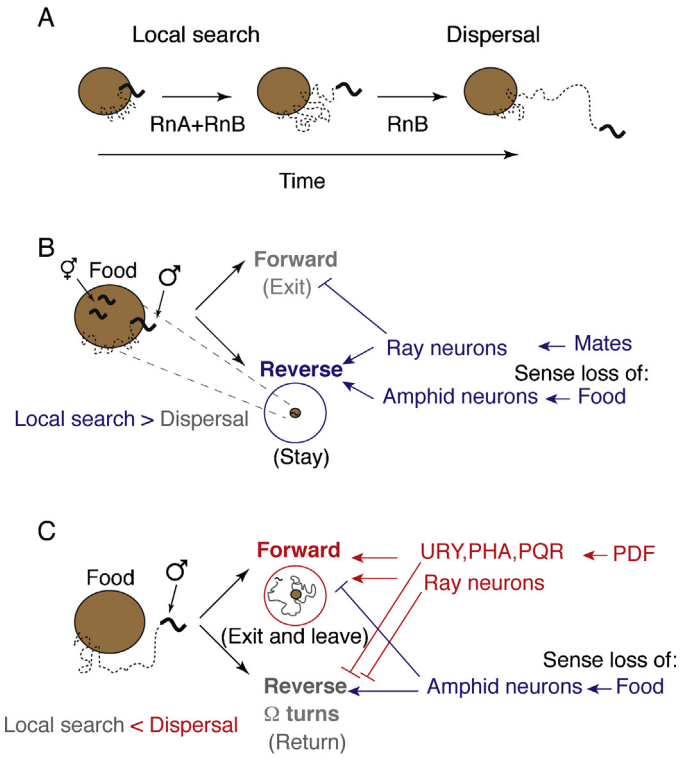

Fig. 3. Regulation of locomotion by sensory experience. Exploration patterns of the male in relation to a food patch. (A) Transition from local search to dispersal. Dashed lines represent exploration tracks. In mate-deprived males, both type A (RnA) and type B (RnB) ray neurons promote full-body exit events. RnB promote the transition from local search to full dispersal. (B and C) Patterns of locomotion generated upon sensing the loss of food in mate-experienced (B) and in mate-deprived (C) males. (B) Reversals at the edge of the food patch are stimulated by amphid neurons and ray neurons in males that have recently experienced mates on food. This leads to brief bouts of local search in which only part of the body exits the patch resulting in exploration being confined to the food patch. In this context, local search is prioritized over dispersal. (C) In mate-deprived males, ray neurons and the sensory neurons that receive PDF neuropeptide signaling stimulate forward runs and inhibit reversals and omega turns stimulated by amphid neurons. This leads to long-lasting full-body exit events, no return to the food patch and exploration away from food. In this context, dispersal is prioritized over local search. PDF (pigment dispersing factor).

males often carry on with forward runs upon encountering the edge of the food patch and this leads, on average, to a frequency of 0.45 full-body exit events per minute spent on the edge of the food patch [6] (Fig. 3C). Full-body exit events can have different time durations depending on whether and when the male changes directionality to return to the food patch. Long exit events with no sharp changes in directionality will lead to dispersal and exploration away from food. The transition from local search to dispersal is regulated by the length of time since last contact-experience with a mate so that within 1 h since last mate experience, male exploration transitions progressively from local search to dispersal [26].

Another possible and intriguing mechanism by which mate-deprived males may leave food, which has not been investigated, is through reverse chemotaxis: changes in trajectory direction against the gradient of food sensation. Indeed, mate-deprived males, when exploring along the edge of the food patch, often produce asymmetric head swings that steer them away from the food source. Similarly, upon exiting the food patch, spontaneous omega turns are rapidly followed by another omega turn to redirect trajectory away from food (A.B. unpublished observations). Reverse chemotaxis may be generated by the intrinsic properties of the male's food-chemosensory neurons. Alternatively or additionally, food-leaving promoting neurons, such as the male rays and PDF receptor-expressing sensory neurons, may antagonize the activity of the food chemosensory neurons at a circuit level by modifying the output of common downstream interneurons (see Section 4).

The different locomotion patterns that contribute to the decision to leave or to stay on food (length of local search bouts, dispersal upon exiting the food patch and possibly reverse chemotaxis) are likely to employ distinct neuronal ensembles and genetic pathways. Therefore, a complete functional map of the male's exploratory decision-making network warrants further and more detailed analysis of locomotion during food leaving.

4. A distributed neural network for male exploratory behavior

In order for the male to produce the appropriate exploratory decision, the internal physiological signals and environmental cues described heretofore need to be processed and effectively integrated within the male's neural network for locomotion. The main neuronal components that convey food, mate and PDF neuromodulation inputs to the network as well as the pre-motor interneurons executing distinct locomotion outputs have been identified.

4.1. Antagonistic regulation of food leaving by food-sensing and mate-sensing circuits

To efficiently explore in search of both food and mates, the male has a distributed neural network for navigation with input circuits dedicated to either food or mate sensation. The relative contribution of each of these circuits to the neural network for locomotion determines whether the male leaves or stays at a source of food.

Gender-shared, food-sensing chemosensory amphid neurons in the head inhibit food leaving by stimulating local search upon sensing the immediate loss of food [6,26,42] (Figs. 3B and 4A). The male-specific, mate-sensing ray neurons in the tail have a dual role and can either stimulate or inhibit food leaving depending on previous experience with a mate. In mate-deprived males, ray neurons stimulate food leaving and dispersal, at least in part, by inhibiting reversals and local search induced by amphid neurons upon sensing the loss of food [6] (Figs. 3C and 4B). Upon contact-experience with a mate, sensed by the rays, ray neurons act in co-operation with amphid neurons to inhibit food leaving by stimulating reversals and local search at the food edge [6] (Figs. 3B and 4A).

The male rays are 9 bilateral pairs of sensilla in the tail, each of which contains a type A (RnA) and a type B (RnB) sensory neuron, which based on ultrastructural morphology, may have distinct sensory modalities [44] (Fig. 1). Ray neurons regulate both male mating and exploratory decisions [6,45]. Experiments in which different subsets of rays were surgically ablated indicate that the 9 pairs of rays act as equivalent groups with redundant functions to stimulate food leaving in mate-deprived males [6]. Although ray-ablated males move normally, their local search bouts are much briefer than those of intact males resulting in significantly fewer full-body exits at the edge of the food patch [6].

Both type A (RnA) and type B (RnB) ray neurons contribute to the stimulation of food leaving in mate-deprived males (Fig. 3A). Killing the RnB neurons by expression of a caspase transgene or disrupting their function through mutations in the polycystin TRP channels *lov-1* or *pkd-2* [46,47] results in males that leave food at significantly slower rates than intact males [6]. The food-leaving defects of males without RnB neurons is distinct from that of males without RnB and RnA neurons. Killing both RnB and RnA neurons results in males that reverse with higher probability at the edge of the food patch producing very brief bouts of local search and very few full-body exits. In contrast, killing RnB neurons alone results in males that produce bouts of local search similar to those of intact males and the

same amount of full-body exits but the transition from local search to dispersal takes longer to occur if it occurs at all [6]. Therefore, RnA neurons can generate some level of exploration away from the food patch in the absence of type B neurons. The contribution of RnA neurons to the regulation of food leaving has been examined by inducing RnA neuron death with a caspase transgene. However, due to incomplete expressivity of the transgene, some RnA neurons still survive. Elimination of most, although not all, RnA neurons alone, has no significant effect on food leaving rates [6] indicating that RnB and/or remaining RnA neurons can compensate and stimulate exploration away from food at wild-type rates. Thus, RnA and RnB neurons have partially redundant and overlapping functions in the stimulation of food leaving and dispersal in mate-deprived males, with RnB neurons having a more essential role (Fig. 3A).

Ray neurons stimulate food leaving, at least in part, by suppressing reversals and omega turns induced by food-sensing amphid neurons upon sensing the loss of food (Fig. 3C). Thus, the food-sensing amphid neurons and the mate-sensing ray neurons act as a push–pull circuit within the network for navigation regulating local search and dispersal respectively in mate-deprived males (Fig. 4B). Consistent with this model, males with loss of function mutations in either the TRPV cation channels *osm-9* and *ocr-2* or the cGMP-gated channel *tax-2*, all of which are required for normal function of food-sensing amphid neurons, disperse upon exiting the food patch and leave food at higher rates than wild-type males [6]. Furthermore, introduction of these mutations in ray neuron-ablated males partially restores food leaving [6]. Ray neurons are likely to antagonize the activity of the food chemosensory neurons at a circuit level by modifying the output of common downstream interneurons.

The probability to reverse or to produce a forward run is ultimate executed by the relative activity of the presumably cross-inhibitory backward and forward pre-motor command interneurons respectively [48,49] (Fig. 4). Thus, the sensory inputs that control food leaving versus retention by regulating reversal probability need to converge onto the pre-motor command interneurons.

4.2. Suppression of food leaving by the mate-sensing circuit

Ray neurons regulate two opposite exploratory decisions depending on context. As well as stimulating food leaving in mate-deprived males, ray neurons are required for sensing mate contact and subsequent inhibition of food leaving in mate-experienced males.

Out of the 9 bilateral pairs of ray sensilla, which occupy distinct topographical locations within the male tail, rays 1–6 are most important for conveying the change in behavioral state and exploratory decision induced by mate-experience. Males missing rays 1 to 6 but with intact rays 7–9 do not completely suppress food leaving upon contact experience with a mate and 50% of males still leave food after mate experience [6]. This is consistent with ray sensilla 1–6 being more efficient than rays 7–9 at controlling the response to contact and scanning of the mate's surface body [45,50], the source of the mate's contact cue regulating male exploratory decisions.

Either RnA or RnB neurons are sufficient to inhibit food leaving upon mate-experience. The low levels of food leaving displayed by males with ablated or functionally disrupted RnB neurons are completely suppressed after contact-experience with a mate indicating that RnA neurons can induce the change in behavioral state and exploratory decision induced by mate experience [6]. Similarly, males with most, although not all, RnA neurons killed (by introduction of a caspase transgene) are fully retained on food after contact with a mate, indicating that RnB or remaining RnA neurons are sufficient to convey the change in exploratory patterns induced by mate experience [6]. This is consistent with the role that RnA and RnB neurons play in mate sensation: RnB neurons initiate the

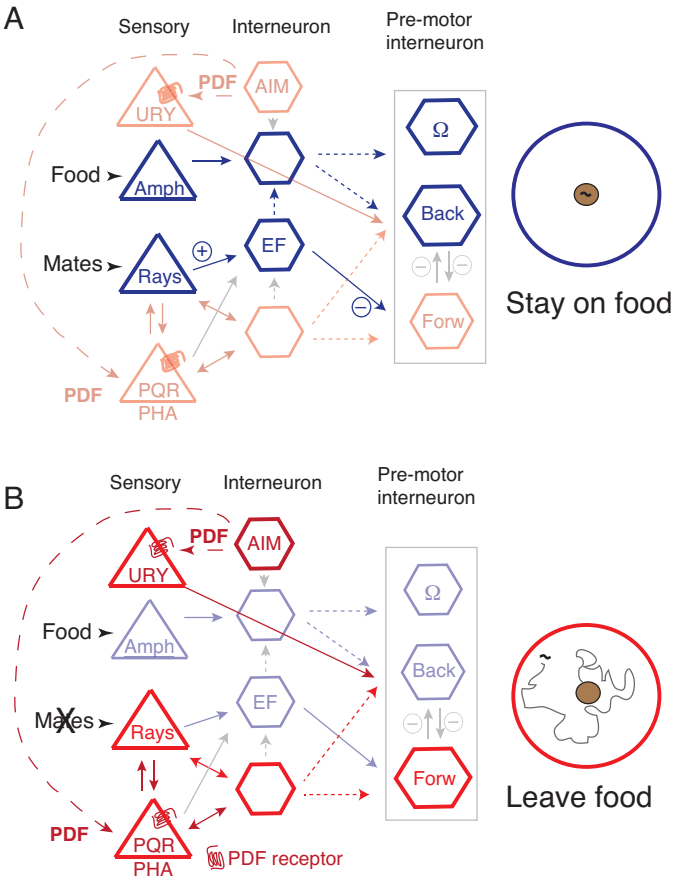

Fig. 4. Two modes of the neural network for exploration. Two different configurations of the male's neural network for exploration are shown: (A) "staying on food" mode; (B) "leaving food" mode. The thick-line, dark-colored neurons represent the circuits that contribute the most to the final exploratory decision in each mode; other neurons (marked in faint-colored, thin lines) may be also active but their activity may be overridden by the highlighted neurons. Triangles and hexagons represent groups of sensory neurons and interneurons respectively. Non-labeled hexagons correspond to several possible candidate interneurons (based on the wiring diagram) not yet functionally implicated in exploratory decisions. Solid arrows represent direct synaptic connections of unknown sign. Long-dash arrows represent neuromodulation. Short-dash arrows represent connectivity (direct or indirect) between unidentified interneurons and the pre-motor interneurons. Amph (amphid neurons); Rays (RnA and RnB ray neurons); Back (pre-motor interneurons driving backward movement); Forw (pre-motor interneurons driving forward movement); Ω (pre-motor interneurons driving omega turns); PDF (pigment dispersing factor neuropeptide). (A) Upon mate sensation, ray neurons may promote staying on food by stimulating EF interneurons. EF interneurons may inhibit the pre-motor interneurons driving forward movement.

mating sequence upon contact with a mate by triggering the "response to contact" step: the placing of the ventral side of the male tail onto the mate's body and initiating the scanning of the mate's cuticular surface [50]; RnA neurons contribute to the "response to contact" step and are required for the maintenance of contact and scanning of the mate's surface and execution of the turns around the end of the mate's body to continue scanning on the other side [50]. In the absence of RnB neurons, RnA neurons can still trigger mate contact and scanning and this is sufficient to induce the change in exploratory behavior from food leaving to retention on food. Conversely, in the absence of most RnA neurons, remaining RnA and RnB neurons can eventually trigger mate contact and retention on food.

Killing all RnB neurons and most RnA neurons renders males unable to leave food when mate-deprived and therefore, the ability of these males to suppress food leaving after mate experience

cannot be directly tested. To circumvent this problem, food leaving can be restored in males with no functional rays by disrupting the function of food-sensing amphid neurons, for example by introducing a loss of function mutation in the TRPV channel *ocr-2*. Ray-ablated *ocr-2* mutant males do not suppress food leaving in the presence of mates, whereas *ocr-2* mutants with intact rays are fully retained [6]. These observations are consistent with the idea that ray neurons are essential for mate sensation and subsequent changes in exploration.

Inhibition of food leaving by contact experience with a mate requires the integration of mate and food cues. The male specific EF interneurons are a likely site of integration. The EF interneurons are the main postsynaptic targets of ray neurons in the pre-anal ganglion and their processes extend within the ventral cord to the nerve ring in the head, where they make synapses with gender-shared sensory circuits [44,51] (Figs. 1 and 4). Ablation of EF interneurons results in males that still leave food after contact experience with a mate [6]. Importantly, EF ablation neither affects the ability of males to initiate and maintain contact and scanning of the mate's body, nor does it affect the ability of males to leave food when mate-deprived [6]. Thus, EF interneurons are required specifically within the circuit that conveys information about mate-experience and produces the decision to stay on food.

In summary, the male network for exploration can be in two different modes or configurations depending on the state of mate deprivation: a configuration in which stimulus-evoked (mate contact) ray neuron activity, acting through EF interneurons, cooperates with the food-sensing circuit to generate the decision to stay on food; and an alternative configuration in which spontaneous or basal tonic ray activity, in the absence of mates, acting through interneurons other than the EFs, antagonizes and overrides the food-sensing circuit to produce the decision to leave food. The latter configuration is dependent on the action of the neuropeptide PDF (Fig. 4).

4.3. Regulation of network state by PDF neuromodulation

The neuropeptide PDF acts on three classes of sensory neurons, URY, PQR and PHA, to stimulate exploration away from food [26]. PQR and PHA are highly interconnected with ray neurons both directly and through common downstream targets [51] (Fig. 4). PDF neuropeptide signaling may change the state of the network for exploration by modulating ray neuron basal activity through PQR and PHA neurons and/or by influencing information flow to common downstream interneuron targets. Consistent with ray neurons contributing, at least in part, to the action of PDF neuropeptide signaling, the increase in food leaving generated by overexpression of *pdf-1* requires intact type B ray neuron activity [26].

It is presently unclear how the neurons upon which PDF neuropeptide acts to stimulate food leaving contribute to male exploration. The neuron PQR is a sensor of internal O_2 levels, which directly correlate with environmental levels [52,53] and may regulate food leaving according to energy requirements and availability. The sensory endings of PHA are exposed to the environment and, based on its connectivity, PHA may be involved in mate sensation [51]. The anatomy of URY suggests it is a sensory neuron of unknown modality and exposed to the internal environment of the animal [54]. In the male, URY is post-synaptic to the male-specific CEM neurons, which sense secreted mate-pheromones [30,55], thus providing a potential regulatory mechanism by which URY contributes to food leaving in the absence of mates.

Although URY, PHA and PQR are gender-shared neurons, in the male they are recruited to male-specific circuits. This sexually dimorphic connectivity may explain why PDF acts instructively only in males to produce a male-specific exploratory strategy despite acting in neurons shared by both males and hermaphrodites [26].

5. Outlook and outstanding questions

The paradigm of food leaving as a mate-searching strategy of the *C. elegans* male provides a powerful system to elucidate the molecular and cellular mechanisms of decision-making under conflicting drives. Although substantial progress has been made toward elucidating main cellular and molecular components of the male's network for exploration and its outputs, as revealed by the present review, there is still considerable knowledge to be gained on the operational logic of this network. A deeper understanding of the mechanisms by which the exploration network changes states, of the signaling properties of the sensory circuits that feed into it, and the mechanisms by which this information is integrated within the network, will likely elucidate fundamental principles of decision-making circuits. Several outstanding questions are of particular interest.

Transitions in behavioral states are determinants of decision outcomes but how state transitions are generated within neural networks is not fully understood. Sensory experience with a mate produces a change in the male's behavioral state and exploratory decision. What are the mechanisms by which mate experience is incorporated into the male's network for exploration? Recent mate sensation and PDF neuromodulation have opposite effects on the state of the network for exploration. Loss of PDF signaling phenocopies the behavioral state induced by mate experience. Conversely, mate contact completely suppresses the increase in food leaving caused by overexpression of *pdf-1* indicating that mate experience acts downstream of *pdf-1* expression. Mate contact may inhibit PDF signaling through three possible mechanisms. First, mate contact may inhibit PDF neuropeptide release from the interneuron AIM, the source of PDF relevant to food leaving. Second, mate contact may modulate the activity of the PDF receptor-expressing neurons PHA, PQR and URY. Modulation may be direct in the case of PHA, whose sensory ending is exposed to the exterior environment and may sense mates, or it may be indirect in the case of PQR and URY, through the activity of pre-synaptic mate-sensing neurons, rays and CEMs respectively. A third possibility may be that mate contact is integrated at the level of the interneurons post-synaptic to both mate-sensing ray neurons and the PDF receptor-expressing neurons PHA and PQR.

Mate experience needs to be incorporated not only within the reproductive arm of the network for exploration but also within the nutritional arm since males are not retained at a spot of mates without food (A.B. unpublished observations). The mate signal does not act through modulation of the food chemosensory amphid neurons directly because mutant males with loss of function mutations in either the TRPV cation channel *ocr-2*, the cGMP-gated channel *tax-2* or the AMPA glutamate receptor *glr-1*, all of which are required for normal function of food-chemosensory circuits, are fully retained on food after contact with a mate [6,26]. This suggests that food may also be sensed through circuits other than chemosensory amphid neurons, perhaps through the dopaminergic mechanosensory neurons [36,41]. Alternatively, or in addition, food signals may be conveyed by internal signals indicating the nutritional status of the male.

Progress toward answering these questions will require knowledge of neural circuit activity within the network in its different states and identification, through forward genetic screens, of additional molecular pathways regulating exploratory decisions and the neurons where they act.

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