Nematode Tango Milonguero – the *C. elegans* Male's Search for the Hermaphrodite Vulva

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Abstract

Vulva search behavior corresponds to the first step of mating in *C. elegans* wherein the male recognizes a potential mate through contact and commences a systematic contact-based search of her surface for the vulva. During this 'dance' the male presses his tail genitalia tightly against the hermaphrodite surface and moves backward, modulating tail posture to effect changes in search trajectory. When he senses the vulva he pauses and the copulatory phase of mating begins. External tail sensilla, the rays, induce and guide the male's search by registering hermaphrodite surface cues. Like many other animate interactions (such as predator-prey interactions or intrasexual competition), mating behaviors performed at close quarters requires that participants constantly adjusting their movement with respect to one another on a moment-by-moment basis. So too in *C. elegans* male mating behavior. Design features of the supporting circuitry explain simultaneously the robustness, speed and acuity of the male's behavior and its male-specific nature. Processing at all levels of the circuitry appears to be distributed. Cellular components exhibit both partial redundancy (thus conferring robustness in output) and subtle functional differences (predicted to confer acuity). Surprisingly, gender-shared cell types feature prominently in the circuitry. Male-specific components form sensory pathways that render downstream gender-shared circuits responsive to mate cues, while other male cells act to augment or modify gender-shared cell activity. Overall, the attributes of the vulva search circuitry provide insight into principles guiding the design and operation of circuits supporting dynamic social behaviors expressed by more complex and less tractable animal species.

1. Introduction

Mating behaviors are among the most elaborate displayed among members of the animal kingdom. Their many facets lend themselves to the study of numerous broadly relevant neural processing and behavioral questions. Males and females of a species have different risks, roles and objectives in courtship and the act of copulation. As such their behaviors are finely tuned and specific to each sex, raising the question of how such behavioral dimorphism is encoded at the cellular and molecular level (review by Portman, this issue). Successful consummation of mating behavior typically requires the execution of a complex series of behaviors, progression through which depends on the detection and appropriate temporal sequencing of conspecific cues (Dickson, 2008). Each step in the mating process thus provides an opportunity to ensure that copulation occurs with a worthy (and species-appropriate) partner. Moreover, like predator-prey interactions or intersexual competition (among other behaviors), mating involves interaction between animate protagonists and so the circuits supporting mating behaviors must enable robust but accurate motor responses informed by split second decision-making.. To fully understand the neural processing events that support mating and behaviors with similar attributes requires a detailed knowledge of the circuitry, the neurotransmission mechanisms employed to regulate circuit activity and the ability to interrogate each circuit component experimentally. In most species, such detailed insights are not realistically obtainable because of nervous system complexity and/or relative genetic intractability. The nematode *C. elegans* offers a facile system for exploring many behaviors, mating included. The two sexes, male and hermaphrodite, have simple anatomies that are relatively invariant among members of the same sex. As a consequence it has been possible to determine the lineal origin of every somatic cell and to generate a single cell resolution wiring diagram for the nervous system of each sex (Sulston and Horvitz, 1977; Sulston et al.1980; Sulston et al. 1983;White et al., 1986; Hall and Russell, 1991; Jarrell et al., 2012). Males and hermaphrodites have in common many cell types (gender-shared) generated from homologous lineages in each sex.

Hermaphrodites and males also possess a complement of cell types that are generated only in that sex (male- or hermaphrodite-specific cells). This complement of sex-specific cells is much greater in the male and most of male-specific cells are associated with the genitalia of the tail. With a few exceptions, the gender-shared neural cells of the head region have similar connectivity in the two sexes (Scott Emmons, personal communication). However, many of the gender-shared cells that have processes and/or cell bodies in the male tail are connected to the male-specific cell types concentrated in this region (Jarrell et al., 2012). The recently described male wiring diagram and circuit modules formulated from it (by Jarrell et al., 2012) present compelling circuit candidates for mating behavior. Nevertheless delineation of the circuits used in nature can only be determined experimentally by observing the activity of candidate circuit cells during the behavior and by testing the behavioral impact of manipulating the activity of these cells. Fortunately, *C. elegans* is highly amenable to the genetic and optogenetic approaches necessary to do this and such approaches can be applied in the context of a freely behaving animal (reviewed in Xu and Kim, 2011).

2. C. elegans male mating behavior

The *C. elegans* male employs a sequence of male-specific motor behaviors in his efforts to inseminate. (Barker, 1994; Liu and Sternberg, 1995). When not engaged in mating, males and hermaphrodites alike move through their environment with forward-biased locomotion, making occasional reversals to change direction or to escape noxious stimuli (Fig. 1A; Brenner, 1974; Croll, 1975). Locomotion is powered by propagation of a dorsal-ventral (DV) wave along the anteroposterior (AP) body axis that is regulated by a gender-shared locomotory system. Physical contact between a male and a hermaphrodite may prompt the male to attempt mating, the first phase of which – the vulva search – is the focus of this review. The male places this tail against the hermaphrodite's surface and moves backward along her length (Fig. 1B). The tail bears the male genitalia and several classes of external male-specific sensilla used to guide the

search and copulation. Tail apposition brings these sensilla into direct contact with the hermaphrodite surface and backward movement puts the tail and sensilla at the leading edge of the search. Once the search is initiated, like the Milonguero style of tango, the male and hermaphrodite maintain an intimate contact along much of the body. If the male scans one side of his mate without locating the vulva, he turns, without losing tail contact, and proceeds to scan the other side of his mate until the vulva is located or the partners disengage (Fig 1C). The male-specific ray sensilla are responsible for inducing and modulating search execution in response to hermaphrodite contact cues (Liu and Sternberg, 1995). The location of the vulva is sensed by another male-sensillum - the hook – located on the ventral most side of the tail (Fig. 1C). Hook activity induces the male to stop. The male then prods for the vulva opening using prong-like structures called the spicules. When the opening is identified the spicules insert fully, anchoring the male genitalia to the vulva. The male ejaculates, transferring sperm into the uterus thereby completing the copulation act.

What is the hermaphrodite's part in this mating dance? In contrast to nematode species with male/female sex systems, *C. elegans* hermaphrodites do not chemotax to their mates and their response to a male's mating attempts range from indifferent to evasive - perhaps evoking the (likely incorrect) origin story of Tango as an enactment between an unwilling woman and a gaucho. Interestingly, there is a strong correlation between hermaphrodite non-compliance and whether the hermaphrodite still has a supply of her own sperm (Kleeman and Basolo, 2007; Garcia et al., 2008; Morsci et al., 2011). *C. elegans* hermaphrodites make a limited supply of sperm (~300) and can use it to internally self–fertilize their oocytes (Maupas, 1900). Once this reserve of sperm is depleted hermaphrodites are more docile mating partners. It is quite possible that hermaphrodite behavior has played a significant role in shaping the robustness and acuity of the *C. elegans* male mating circuitry. In this review I will discuss current conceptions of the cellular and molecular underpinnings of vulva search behavior and how circuit design is tailored to deal with the behavior of the *C. elegans* hermaphrodite.

3. The male ray sensilla – the sensory neurons of the vulva search circuitry.

3.1 Ray organization and cellular composition

The ray sensilla are responsible for sensing mate contact and for initiating and guiding the vulva search(Fig. 2). Activation of ray neurons,either by mate contact or artificially using light-gated Channelrhodopson, triggers the two principal motor behaviors of the search: tail apposition and backward locomotion (Liu and Sternberg 1995, Koo et al., 2011). The male tail bears nine bilateral pairs of ray sensilla (numbered 1-9 from anterior to posterior), held in a cuticular sheet called the fan (Fig. 2A, 2B). All rays have a similar cellular composition and organization (Sulston and Horvitz, 1977; Sulston et al., 1980). This is because all rays are generated the same lineage program repeated bilaterally at nine positions along the A-P axis. Specification of this ray lineage program is driven by evolutionarily conserved neurogeneic factors (bHLH proteins) and by conserved sexual development regulators of the Doublesex-MAB-3(DM)-domain family (Zhao and Emmons, 1995; Portman and Emmons, 2000; Ross and Zarkower, 2003; Ross et al. , 2005; Miller and Portman, 2013). Each ray consists of the dendritic endings of two neurons, type A and B. The two dendrites are wrapped in successive layers of a glial-like structural cell, hypodermis and finally cuticle. With the exception of the ray 6 pair, the endings of rays are open such that the tip of the B-neuron is exposed to the environment (The A-neuron ending terminates short of the opening). Individual ray-pair openings are stereotypically directed towards the dorsal, ventral or marginal side of the fan. All rays have the capacity to support vulva search motor behaviors. Together with the distribution of ray openings in both A-P and D-V planes, this functional redundancy creates a large receptive field for sensing mate contact and inducing a response (as discussed in section 3.3), an important property given that contact may alter unpredictably.

The ciliated endings of A and B neurons have distinct morphologies and positioning within the ray (Fig 2A), suggesting these neuron classes may support different sensory modalities. However, while several candidate mechanosensory

and chemosensory receptors have been detected in A and B neurons, firm experimental proof of sensory modalities segregating with ray neuron type is still lacking (Troemel et al.1993; Barr and Sternberg, 1999; Chelur et al., 2000;Barrios et al., 2008;).

3.2 Ray connectivity

Collectively the ray neurons make connections with more than 50 post-synaptic targets (for a detailed discussion of their connectivity see review by Emmons in this issue; Jarrell et al., 2012; Sulston et al., 1980). The cell bodies of all ray neurons and their respective structural cells reside in the left and right lumbar ganglion of the tail (Fig. 2C). Ray neuron axons project anteriorly then ventrally, terminating in the male pre-anal ganglion (PAG). *En route* ray neurons form synapses with each other and with the gender-shared and male-specific muscles of the body wall. However, most connections occur within the pre-anal ganglion. The male PAG is substantially larger and more complex than that of the hermaphrodite, both in terms of cell number and connectivity. In fact, the male PAG neuropil is as complex as the nerve ring of the head. Most of the additional cells in the male PAG correspond to male-specific- sensory, interneurons, motor neurons or cells that can be classified as inter/motor neurons, depending on their circuit assignment. Additionally, the pre-anal ganglion contains the processes of several tail sensory neurons,interneurons and motor neurons (both male-specific and gender-shared) with cell bodies located in more distant ganglia such as head ganglia or the nearby ventral nerve cord. Each ray neuron connects with an overlapping but unique constellation of target cells. As discussed below, this connectivity partly explains similarities and differences in ray- and ray neuron type dependent motor output.

3.3 The rays form a large receptive field of functionally similar but non-identical sensilla

More complex nervous systems employ large fields of sensory cells to extract salient information from the environment and determine the whereabouts

and actions of potential con-specific mates, competitors, prey and predators. Large fields of cells with differing sensitivities and tuned to different stimulus features confer robust response properties (Lewis, 1999; Kawamura and Tachibanaki, 2008; Schwander et al., 2010). The ray sensilla cover a significant region of the male tail, thus providing a large surface area for contact with the hermaphrodite. There is a striking degree of robustness in ray function, such that in the face of significant experimental perturbation males are still able to exercise fine control over tail posture and locomotion in response to moment-to-moment changes in the hermaphrodite contour (or other surface cues). Males with as few as three pairs of ray neurons, of any combination, can sense and respond to contact (Liu and Sternberg, 1995; Koo et al., 2011). Reducing the number of rays results in a reduction in the frequency of search initiation and impaired maintenance of ongoing vulva search behavior. Furthermore, optogenetic stimulation of the neurons within different subsets of rays, using ChR2, reveals that all rays appear to be competent to induce search motor behaviors.

General similarities in the cellular composition and organization of rays may confer robustness, but why then do different ray pairs express different combinations of characteristics in their constituent cells? This differential patterning might impart subtle functional differences among the rays,contributing to behavioral acuity. While all rays can mediate search behavior, some appear to be more effective than others. For example, males with only the anterior rays (1-3) do a better job than males with only rays 4-6 or 7-9 (Liu and Sternberg, 1995; Koo et al., 2011). The neurotransmitter fate and connectivity of ray neurons representtwo features that differ prominently among the rays likely impact circuit output. The A-type neurons of rays 1-4 and 6 synthesize acetylcholine, while those of rays 5, 7 and 9 are dopaminergic (Sulston et al., 1975; Sulston and Horvitz, 1977; Siehr et al., 2011). The B-neurons of rays 1, 3 and 9 make serotonin and all B-neurons express various combinations of FMRF-amide neuropeptides (Loer and Kenyon, 1993; Lints et al., 2004). The ray developmental programs that establish these stereotyped neurotransmitter fates have been studied extensively and include a TGF-beta signaling pathway,

members of the DM-domain family of transcription factors, Hox and Pax-6 transcription factors and the conserved dopamine specification factor AST-1 (Zhang and Emmons, 1995; Ferreira et al., 1999; Lints & Emmons, 1999 Lints et al., 2004, Flames and Hobert, 2009; Siehr et al, 2011).

These differences in transmitter phenotype and connectivity potentially underlay subtle but clear modulations of ray neuron output. For example, artificial stimulation of the A-neurons in different combinations of rays induce subtly different forms of appositional posture. At a finer level of analysis, elegant studies by Correa *et al.* (2012) reveal that the build up of dopamine during unproductive vulva searches (released from the A-type neurons of rays 5, 7 and 9) causes males to abort the search, at least in part through attenuation of appositional posture. These subtle differences in ray contributions to behavioral output could reflect their participation infine-tuning the male's control over his posture and movement during the search process.

3.4 A- and B-type ray neurons have different roles in search behavior

Visualization of ray neuron activity using the calcium sensor GCaMP; Tian et al., 2009 reveals that both A and B-type neurons display similar activity profiles during the search (R. L. and A. Sherlekar, unpublished results). Ray neurons exhibit graded potentials with increases in activity correlating with increases in speed and posture intensity. Initial exposure to the contact stimulus causes a transient decrease in activity followed by high levels of depolarization that increase during turning and that are maintained even when the animal stops at the vulva The significance of this initial rebound depolarization event is not known. One possibility is that it provides a way for the animal to switch between the two behaviors rays are known to control: mate seeking and vulva search behavior. Mate seeking is exhibited by males when hermaphrodites are absent, compelling them to do the unthinkable – leave the food source (reviewed in this issue by Barrios). This leaving behavior is proposed to represent mate seeking because it is extinguished if a mate is provided (Lipton et al. 2004). The rays are required for mate seeking, as males lacking rays (or their A- or B-type ray

neurons), do not leave food devoid of mates (Barrios et al, 2008). Thus, ray activity promotes two connected behaviors: seeking of a mate and mating her once she is located. The puzzleis that mate-seeking and vulva search behavior are characterized by very different movement patterns: the former by forward biased movement, the latter by backward movement. Examination of the state of ray activity under these different conditions might prove informative. Mate contact induces high levels of ray neuron activity, but the activity levels during leaving behavior have yet to be elucidated.

GCaMP-based analyses of ray neuron activity reveal that both neuron types are active during the search, but what are their respective roles? Functionally, A-neuron population activity is critical for all phases of the vulva search and the number and type of A-neurons that are active appears to instruct the degree of body bending. Males lacking A-neurons (due to their specific ablation) have difficulty in controlling posture and maintaining backward movement. Conversely, the bending posture induced by A-neurons is greatly dependent on which and/or how many neurons are stimulated (Koo et al. 2011). As noted above, A-neurons make acetylcholine and dopamine and these can have distinct affects on posture form. B-neurons, by contrast, appear to enhance the robustness of A-neuron modulated outputs, possibly by facilitating the activation of common post-synaptic targets. Males lacking B-neurons need several contact events before initiating the vulva search. However, once begun, the search is conducted with the same efficiency as intact males. The posture generated by artificial stimulation of B-neurons in solitary rays is generally robust, irrespective of which, or how many, B-neurons are stimulated. The neurotransmitter used by B-neurons to regulate search behavior is still an open question, as males unable to synthesis either serotonin or neuropeptides (*tph-1, egl-31* double mutants) can still induce appositional postures when stimulated and do not behaviorally phenocopy B-neuron deficient males in mating assays, as would otherwise be predicted (Koo et al., 2011).

4. Ray-controlled circuits

We now turn to the question of how the circuits that ray neurons control effect search motor behaviors. While hermaphrodites can move backwards and have the capacity to bend their posterior ventrally, these motor behaviors are never sustained as they are in the male during mating. However, the vulva search circuitry recruits many of the gender-shared cells required for the transient expression of these behaviors by the hermaphrodite. In the male, these gender-shared cells are either placed under male-specific sensory control (such as mediated by the ray neurons), or their function is augmented with male-specific cells at the level of motor output (for example, in the cooperative activity of gender-shared and male-specific body wall muscles).

4.1 Posture control circuits

To appose his tail against the hermaphrodite surface, the male produces a ventral tail curl. However, the depth of this bending posture must be dynamically adjusted to accommodate changes in the hermaphrodite's position and contour.. The most dramatic alteration in tail posture during vulva search behavior occurs with the execution of the turn, a demanding maneuver that requires a precisely timed deep ventral bend. Appositional posture is generated by the coordinated contraction of ventral muscles and relaxation of dorsal muscles (Whittaker and Sternberg, 2009). The dorsal muscles are strictly gender-shared and attach to the dorsal body wall and cuticle. The ventral muscles include both male-specific and gender-shared types that are attached the ventral body wall and cuticle (Sulston and Horvitz, 1977). The dorsal and ventral gender-shared muscles are the same muscles used to propagate the sinusoidal wave of locomotion and their involvement in apposition necessarily means that the wave is absent from the tail region while the male is engaged in the mating process. These gender-shared muscles make the dominant contribution to appositional posture. he male-specific muscles enhance postural range and perdurance as males lacking sex muscles have difficulty with turning and with maintaining tail apposition (Loer and Kenyon, 1993; Whittaker and Sternberg, 2009; Koo et al., 2011; Siehr et al,

2011). Possible neurotransmission systems used to regulate tail posture have been uncovered by examining mutants deficient in specific neurotransmitters or by exposing wild type males to exogenous neurotransmitters and their agonists. These studies point to contributions of cholinergic,dopaminergic and GABAergic neurotransmission to the induction and control of mating postures (Whittaker and Sternberg, 2009; Siehr et al., 2011). How is the degree of ventral bending regulated during mating? The observation that ray neuron activity increases as males execute the turn would suggest that the degree of sensory cell activation determines the number or intensity of muscle cell contraction (Koo et al., 2011; R. L. and A. Sherlekar unpublished data). The identity of the neural pathways rays utilize to control muscles is still uncertain, as several possible circuits can be traced (Fig. 3). For example, the rays themselves connect directly to muscles and thus are sensory/motor neurons. There are also several male-specific inter- and inter/motor neurons that ultimately target gender-shared and male-specific body wall muscles (for example PVV and PDB, PDC). Surprisingly, males lacking a large proportion of these interneurons (due to genetic mutations that disrupt their specification) can still bend their tails when rays are artificially stimulated. However, these males exhibit posture defects in the context of mating, particularly during turning (Koo et al., 2011; R. L. and A. Sherlekar unpublished data). Many of the male-specific intermotor neurons may therefore serve to hone the finer details of postural control that enhance the efficiency of mate apposition sub-behaviors.

4.2 Backward movement:

The rays stimulate backward movement by exploiting a gender-shared locomotory system. As detailed below, this control is achieved by placing key command cells in the locomotory system under the control of male-specific neural pathways composed of the ray neurons and male-specific interneurons. What we know about the inner workings of the locomotory system comes from extensive studies in the *C. elegans* hermaphrodite. Simplistically, the gender-shared locomotory system consists of two mutually antagonistic pathways; one

dedicated to forward (FWD) locomotion, the other to backward (BK) locomotion. The FWD and BK pathways each consist of command interneurons located predominantly in the head that control a string of motor neurons arrayed along the animal's ventral nerve cord. These motor neurons, in turn, innervate dorsal and ventral body wall muscles to effect sinusoidal wave propagation and thus movement (White et al., 1976; Chalfie et al., 1985; White et al., 1986; Wicks et al., 1996; Haspel et al. 2010). In the absence of specific sensory cues, males and hermaphrodites move with a forward directional bias, making occasional brief reversals to change direction (Brenner, 1974; Croll, 1975; Pierce-Shimomura et al., 1999; Gray et al., 2005). This movement bias is due to circuit activity that favors the FWD pathway as a consequence of intrinsic system properties (the electrical coupling of certain components; Kawano et al., 2011) and inputs from outside the system (the dis-inhibitory pathway proposed by Piggott et al, 2011).

In the male posterior, command cells of the locomotory system represent a nexus for the broad motor control of vulva search behavior. In both sexes, these key interneurons, whose cell bodies reside in the head, send processes into the PAG where,in the male, they receive direct and heavy inputs from a number of male-specific interneurons that are also postsynaptic targets of the ray neurons.. The rays exert their affect on AVA thorough the male-specific interneuron PVY (Fig. 3). A distinctive feature of this ray-PVY/PVX-AVA connectivity is it's obvious feed-forward properties, with PVY having no reciprocal connections to either the rays or AVA. This is not the case with many other male tail interneurons, which tend to have reciprocal connections with their pre-synaptic partners. Reciprocal connections are predicted to provide reinforcing stimulation (or inhibition) to help maintain circuit activity once induce. As discussed below, the need for reinforcement of this pathway may be met by other inputs to PVY (namely the EF interneurons).

PVY is cholinergic and stimulates AVA through multiple cationic cholinergic receptor subunits (ACR-18, ACR-16 and UNC-29) expressed in the command interneurons (Feng et al., 2006; Sherlekar et al., 2013). The use of

cholinergic neurotransmission to stimulate AVA is atypical as most gender-shared sensory pathways that converge of AVA use glutamate (Hart et al., 1995; Maricq et al., 1996;Chalasani et al., 2007; Ohnishi et al., 2011; Brockie and Maricq 2006). Potentially, the use of cholinergic transmission may confer greater specificity and/or serve to amplify other drive onto AVA,biasing the male's response towards backwards locomotion. Additionally, as several other motor behaviors associated with mating use cholinergic transmission, its use in backing control may serve to coordinate or co-regulate these behaviors. PVX, a second male specific interneuron adjacent to PVY in the male PAG, is also cholinergic and has similar connectivity to PVY, possessing many inputs from the rays and output onto AVA. However, PVX appears to be non-essential for mating and exhibits an ancillary function, enhancing the robustness of PVY-induced reversal in ChR2-based assays (Sherlekar et al., 2013).

Besides direct inputs from the ray neurons, PVY and PVX also receive inputs from the male-specific EF interneurons (EF1-3). The EF interneurons are interesting for a number of reasons. They define a focal point of connectivity in the male tail, forming reciprocal connections with many neurons, ray neurons included. Additionally, EFs are the only male-specific tail neurons that send processes all the way to the nerve ring of the head, and thus represent a potential conduit for integrating and prioritizing mating with other drives. In support of a role in regulating movement, males ablated for EFs pause frequently during the search and, like PVY-ablated males, are unable to sustain their position at the vulva during spicule insertion attempts (A. Sherlekar and R. L, unpublished data). One caveat to interpreting this ablation data is that the lineally related DX neurons are also absent in these EF-ablated males so it is formally possible (though unlikely based on DX connectivity) that loss of DXs may contributed to the behavioral phenotype observed. Their connectivity and ablation phenotype suggest that EF neurons enhance the robustness of backward movement in part by suppressing inappropriate activation of AVB (the command interneuron for forward locomotion) during the search and in part by enhancing ray neuron activation of PVY (Fig. 3). The EFs are electrically coupled

to PVY and have chemical synapse inputs onto the forward command cells of the head, AVBL/R (AVB) (Scott Emmons, personal communication). The rays and EFs have reciprocal connections and these may serve a reinforcing role, acting to sustain circuit activity once initiated.

There is evidence to suggest that the rays could use additional pathways to control movement during mating attempts. From the perspective of behavioral robustness and fine motor control, such distributed processing would seem a wise strategy. The possibility is based on the observation that PVY- and even PVY-EF ablated animals can still reverse, albeit erratically and poorly (A. Sherlekar and R. L., unpublished data). What could these alternative pathways be? Do they also converge on the command interneuron system? Do they act in parallel with the PVY pathway or is their activity only called upon under challenging circumstances? Future studies should be able to address these questions.

4.3 Stopping at the vulva

Upon sensing the vulva the male stops moving backwards and begins his copulation attempt (reviewed in this issue by Garcia). The vulva is sensed by the male-specific hook sensillum, located on the ventral-most surface of the tail (Fig. 2B; Liu and Sternberg, 1995). The hook consists of the sensory endings of two neurons, HOA and HOB. HOA and HOB in many ways resemble the A- and B-type sensory neurons of the rays. HOB's sensory ending is exposed to the external environment, while the HOA sensory ending is located within the sensillum structure. These two neurons have both common and distinct synaptic targets (Sulston et al., 1980; Jarrell et al., 2012) and possibly have partially redundant functions in vulva location behavior (R. L. and A. Sherlekar, unpublished results but *cf.* Liu and Sternberg, 1995). Both neurons come on when the hook contacts the vulva. How their activation causes the male to pause is not well understood. Part of their mechanism likely involves inhibition of PVY. At the moment of vulva detection, PVY activity decreases (R. L. and A. Sherlekar, unpublished results). Pausing at the vulva additionally coincides with

rapid and subtle alterations in tail posture, mediated in part by a major hook neuron target, the post-cloacal sensilla (p.c.s.) (Liu and Sternberg, 1995; Garcia et al., 2001; Liu et al., 2011). Conceivably, adjusting the posterior tail posture may serve to act as an addtional brake (Liu et al., 2011). Perhaps unexpectedly, both EF and PVY are needed for the male to stay at the vulva. While the male is at the vulva, PVY activity returns to levels seen while the male is actively backing. How PVY and the EFs contribute to the vulva penetration step and why their activity does not cause the male to back off the vulva is a puzzle. One possibility is that alterations in tail posture may channel the backward force they generate into breeching the vulva. Alternatively, the vulva detection event could cause PVY and the EFs to alter their circuit affiliations so that their activities now contribute to spicule insert motor behaviors, rather than the locomotory system.

5. Concluding remarks

Male mating behavior is considered the most complex behavior exhibited by *C. elegans*. Vulva search behavior is supported by distributed processing, which is able to confer simultaneously robustness and accuracy. Distributed processing is not limited to the sensory level of these pathways but may extend to other tiers of the network, as elimination of specific circuit components tends to cause a gentle degradation of output rather than complete inhibition. These features have made the delineation of circuits involved in this behavior a challenging task. Particularly problematic questions are the degree to which circuit attributes may homeostatically adjust in the face of experimental manipulation and even whether stochastic differences in neuronal gene expression across wild type individuals are masked by redundant or overlapping circuit properties (Marder, 2011). Moreover, it is important to bear in mind that the rays control at least two behaviors, mate seeking and mating, both of which converge on the locomotory system. How these divergent motor outputs emerge from distinct environmental inputs onto the same sensory target structures is a question that will take time and optogenetics to unravel. Another issue is that current mating behavior assays, which for technical reasons use mildly incapacitated hermaphrodites and

may not reveal the role of cells called upon under more challenging mating conditions. For example, in males the hook neurons form a strongly connected circuit with gender-shared interneuron AVG and forward command cells AVB. However, under current assay conditions this pathway is surprisingly silent (A. Sherlekar and R. L., unpublished data).

While there is still surprisingly little evidence of genetic or circuit homologies in the sexual behaviors of different species, comparative analyses of mating circuits is likely to reveal analogous solutions and common processing principles involved in the rapid modulation of motor outputs in response to the often unpredictable behavior of animate targets. A few interesting themes are, however, beginning to emerge. In *C. elegans*, gender-shared cells contribute to several behaviors that are male-specific. For example, the gender-shared DVA neuron is the source of oxytocin/vasopressin-like peptides that enhance the robustness and coherence of mate searching and mating sub-behaviors by priming activity of the underlying circuitry (Garrison et al., 2012). It is striking that these particular neuropeptides are known to have a critical role in social behavior and mating in vertebrate animals. Gender-shared sensory pathways also provide critical input for mate searching behavior, mate attraction, male-specific odor preference and aspects of vulva search behavior (Lee and Portman, 2007; Liu et al., 2007; White et al 2007; Srinivasan et al., 2008; White et al 2008, Barrios et al., 2012; White and Jorgensen 2012). In these and other examples, gender-shared cells exhibit sexually dimorphic properties that confer the sex-specific behavioral responses (Lee and Portman, 2007; White and Jorgensen 2012). Other behaviors employ sex-specific inputs to regulate gender-shared pathways. In the *C. elegans* hermaphrodite, this circuit design underlies the locomotory patterns associated with egg-laying. In this case, the Hermaphrodite-Specific motor Neurons (HSNs) of the egg-laying system cause a burst of forward acceleration that coincides with egg release via direct regulation of gender-shared locomotory system interneurons (Hardaker et al, 2003). Similarly, in *Drosophila,* the reason males produce a courtship song using wing vibrations, and females do not, is primarily due to male-specific modulation of sex-shared

central pattern generators for wing vibration (Clyne et al. 2008; von Philipsborn et al., 2011; Pan et al. 2012;). In mice, circuitry responsible for male sexual behaviors exist but remain latent in the female brain, suppressed by sensory input from the vomeronasal organ (Kimchi et al., 2007). These various examples, along with our studies of the male mating behavior, provide insight into how new reproductive behaviors might evolve through relatively simple modifications to shared circuitry. The *C. elegans* cell lineage provides some hints as to how male or hermaphrodite-specific inputs or output cells could have arisen during evolution. Many male- and hermaphrodite-specific muscles and inter/motor neurons arise from lineage variations of those that produce functionally-related gender-shared cells (Sulston and Horvitz, 1977; Sulston et al., 1980). Thus, sex-specific cell types are essentially highly derived forms of their gender-shared counter-parts. Cell type evolution and the circuit modifications described above may make prominent contributions to natural variations in behavior that ultimately bring about speciation.

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Figure legends

Figure 1. Male posture and movement patterns during mate seeking and mating. ***A.*** In the absence of mate contact, mate-seeking locomotion has a forward locomotion bias and the sinusoidal body wave driving movement propagates along the full length of the body. ***B.*** The vulva search of mating. In response to mate contact, the male sensory rays (shown in the inset for *B*) induce contact response: tail apposition and backwards movement posture. ***C.*** The rays then modulate tail apposition posture and movement to effect scanning (movement along the hermaphrodite length) and turning (executed at the head or tail). Vulva detection causes the male pauses and prods for the vulva slit opening with his copulatory spicules (not shown).

Figure 2: Structure and organization of the rays

A. Micrograph (DIC) of male tail (ventral view) showing the nine bilateral pairs of rays, the hook sensillum and post-cloacal sensilla (P.C.S.). The yellow circle corresponds to the ray tip region enlarged in *B*. ***B.*** Schematic showing the organization of A and B neuron dendrites in a single ray tip (longitudinal section). ***C.*** Schematic showing cutaway view of male tail (lateral view; anterior is left; posterior is right). Shown is the typical projection pattern of a single ray neuron with the cell body positioned in the lumbar ganglion region (not shown) and the axon projecting to the PAG (purple). The body wall muscles (green) are attached to the dorsal (top) and ventral (bottom) body wall.

Figure 3: Models of the neural circuits controlling the vulva search motor outputs. Cell type and sex-specificity is indicated by the symbol and color, respectively: gender-shared cells (pink), male-specific cells (blue), sensory neurons (triangles), interneuron (hexagons), motor neurons (circles). Color intensity indicates a cell activity state (intense color = high; weak color =low). The arrows indicate the positive action of an activated cell on its postsynaptic target; T-bars indicate inhibitory action. AVA and AVB correspond to the backward and forward command interneuron (CI), respectively. FWD and BK

mns are forward and backward locomotion motor neurons, respectively. ACR-16, ACR-18 and UNC-28 correspond to ionotropic acetylcholine receptors. Neurotransmitters used to regulate posture are likely to include ACh (acetyl choline); DA, dopamine and GABA.