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Switching Gears, Structuring the Right Search Strategy

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Nematodes can use local and global search strategies to find food. In this issue of *Neuron*, López-Cruz et al. (2019) unravel a neural circuit mechanism that allows worms to select and switch between these search modes depending on recent experience of food.

Where are my house keys? Who hasn't been in the situation of losing track of something you had sight of just a moment ago? The usual routine that follows is to first search through everything in your immediate surroundings: your pockets, the paper on the desk, behind your laptop, under the chair you're sitting on, in the door! Only after unsuccessfully visiting and revisiting these local spots does it seem worthwhile to look further afield.

Following such a search strategy appears to be an evolutionary conserved pattern of animal behavior (Banks, 1957; Hills et al., 2013). However, it is not understood what determines whether an animal searches locally rather than gallivanting further. Some insight into a neural circuit mechanism that permits the selection of different search strategies now comes from an elegant genetic study of food seeking in the nematode *C. elegans* (López-Cruz et al., 2019). When these worms are removed from food, they engage in search behavior, presumably to relocate the source of food they were previously grazing on. Initially, the animals use local search, signified by repeated reorientations restricted to a small area (Hills et al., 2004). Then, after a time without finding food, they switch to global search, making less frequent reorientations and so exploring the wider environment (Calhoun et al., 2014; Hills et al., 2004). Interestingly, López-Cruz et al. (2019) show that the length of the local search phase depends on the quality of the previously encountered food. Worms search locally for longer if the last food they experienced was high quality and switch more readily to global search if it was poor. This finding suggests that worms retain a representation of food quality that influences the likelihood that they engage in a local versus a global search strategy.

López-Cruz et al. (2019) then unleashed the full power of worm genetics to reveal underlying neural mechanisms. They first performed a small-scale genetic screen of candidate neurotransmitter receptors to gain an entry point. Worms mutant for the metabotropic glutamate receptor MGL-1 showed a specific defect in reorientations during local search, shortly after food removal. To identify relevant neural circuitry for search, López-Cruz et al. (2019) used a clever recombination-based approach to restore expression of *mgl-1* to specific neurons of the small nervous system in an otherwise *mgl-1*-deficient worm. Re-establishing *mgl-1* expression in AIA and/or ADE interneurons restored local search behavior to *mgl-1* mutant worms. MGL-1 is homologous to the mammalian metabotropic glutamate receptor, which couples to inhibitory G proteins, suggesting that glutamatergic input suppresses the activity of AIA and ADE interneurons to promote local search behavior. In support of this model, López-Cruz et al. (2019) showed that defective local search in *mgl-1* mutant worms could be overridden by blocking synaptic release from, or acutely silencing, the AIA and/or ADE neurons. This result implies that active AIA and ADE neurons normally suppress local search behavior and that when worms are taken off food, these interneurons are inhibited to gate the expression of close-range exploratory behavior.

The neural wiring diagram, or connectome, of *C. elegans* combined with the expression pattern of the vesicular glutamate transporter suggested that AIA receives glutamatergic input from chemosensory neurons responsive to food odors and chemicals. ADE, on the other hand, receives glutamate from mechanosensory neurons detecting the texture of food. To test the role of these AIA and ADE afferent neurons, López-Cruz et al. (2019) used CRISPR/Cas9 to edit the locus encoding the vesicular glutamate transporter, so that it could be conditionally removed from individual sensory neurons. However, only disrupting glutamatergic transmission from all mechanosensory and chemosensory input neurons together reproduced the local search defect observed in *mgl-1* mutant worms, lacking the metabotropic glutamate receptor. In addition, acute optogenetic silencing of both the chemosensory and the mechanosensory neurons decreased local search orientations following food removal. These results indicate a redundancy of the food-sensitive inputs that control foraging behavior and demonstrate that local search occurs if the worm perceives changes in either particular food quality, the taste, or the texture. Such potential to detect changes in food composition, together with the finding that food quality affects the duration of local search, suggests that a multimodal food memory guides the optimum foraging strategy.

How does the removal of food dynamically change circuit activity to guide local, then global, search behavior? To address this issue, López-Cruz et al. (2019) focused on the mechanosensory ASK neurons and the downstream AIA interneurons. It was known that spontaneous

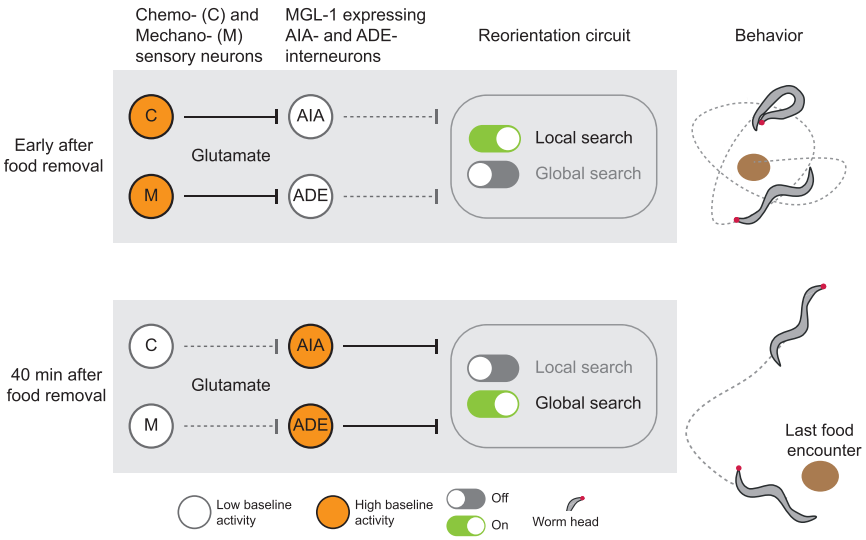

Figure 1. Sensory Control of Search Strategies

activity in ASK chemosensory neurons is enhanced after removing worms from food, or food-related cues (Wakabayashi et al., 2009). Monitoring calcium transients of ASK and AIA interneurons soon after food removal, during the local search phase, and later during the global search phase revealed opposing activity profiles in these neurons. ASK sensory neuron activity increased shortly after food removal and decayed over time. In contrast, AIA neuron activity started low and gradually increased with time, consistent with the inhibitory mode of ASK-released glutamate. Simultaneous recordings showed that ASK excitation and AIA inhibition were often in phase, solidifying a direct relationship between their activities. Perhaps surprisingly, the MGL-1 metabotropic glutamate receptor was not required for the temporal coupling between ASK and AIA activity, but its mutation did disrupt the time-dependent change in AIA activity after food removal. Instead, electrophysiological recordings demonstrated that fast inhibition of AIA involved a glutamate-gated chloride current. Sensory-neuron-released glutamate therefore inhibits AIA neuron responses on two different timescales via at least two distinct glutamate receptors. A fast-acting chloride conductance tightly couples anti-phase AIA activity to that of sensory input neurons, whereas a slower metabotropic MGL-1 receptor-dependent response sustains reduced AIA activity to maintain local search for 10–20 min after food removal.

Together, this comprehensive collection of results supports a model (Figure 1) in which food removal transiently increases the activity of sensory neurons, which leaves the worm with a short-term representation of food. This sensory neuron activity in turn inhibits AIA and ADE interneurons to temporarily switch the downstream circuitry into a configuration that favors the expression of local search behavior. As time passes with the worms unable to find local food, the food representation wanes. AIA and ADE neurons gradually regain their activity, which suppresses local search and gates a transition to global search behavior. In a final set of experiments, López-Cruz et al. (2019) tested this model by using optogenetics to remotely trigger ASK chemosensory neurons during local or global search. Consistent with the circuit being in a different state in the two search modes, the same ASK stimulation produced less of a reorienting effect in worms engaged in global than local search. López-Cruz et al. (2019) proposed that the changed responsiveness of the worm to sensory input could represent a manifestation of a transient memory trace of the previously experienced food, which keeps the worm temporarily in a local search mode. However, they also noted that there must be more elements to the underlying neural mechanisms, because the defective local search of worms lacking sensory-neuron-released glutamate could be restored by blocking neurotransmitter release from AIA and ADE neurons. What might these other signals be? López-Cruz et al. (2019) propose a role for FMRF neuropeptides that are co-released with glutamate from worm sensory neurons. FMRF peptides are related to mammalian NPY and *Drosophila* dNPF, both of which play a role in promoting hunger-dependent food-seeking-related search behavior (Burnett et al., 2016; Krashes et al., 2009) and can work over longer timescales. Some of these network architectures, like that indicated to control search in the worm, also involve hierarchical inhibition (Krashes et al., 2009; Nieh et al., 2015; Perisse et al., 2016). In addition, dopamine provides a key network layer controlling memory-related behavioral expression in the fly (Krashes et al., 2009). It will therefore be interesting to determine whether dopamine release from ADE interneurons plays a role in selecting local or global search behaviors (Hills et al., 2004).

In closing, this impressive work from López-Cruz et al. (2019) exemplifies the incisive experimental power of combining targeted genetic intervention with study of a numerically compact nervous system. It provides fascinating insight into how small neural assemblies can orchestrate and select between different experience-related food search strategies. There is certainly more to do. It will be interesting to investigate how the duration of search strategies is modulated by prior food quality, how the history and relative value of food experience is represented, and whether sensory properties of food alter search strategy in combination with the nutritional value of the food and the internal state of the animal. Uncovering mechanisms that integrate these levels of control of search behavior in the worm may unveil basic principles of how moderately more complex brains allow some animals to find their keys.

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Microglia Sculpt Sex Differences in Social Behavior

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Microglia are increasingly recognized as developmental sculptors of neural circuits. In this issue of *Neuron*, VanRyzin et al. (2019) demonstrate a novel mechanism by which endocannabinoids drive microglia to phagocytose newborn astrocytes in the medial amygdala of male rats, promoting sex differences in social play behavior.

Sex is a biological variable that influences the expression of many social behaviors across the animal kingdom. These behaviors include adult reproductive behaviors, such as mating, parental behavior, and aggression, but also behaviors that emerge earlier in development, such as adolescent social play behavior. The neonatal testosterone (T) surge, which occurs in male rodents around the day of birth, organizes many of these sex differences in social behavior. However, much less is known regarding the mechanisms by which changes in T lead to changes in the neural circuitry that supports social behavior. Microglia, the resident macrophages of the brain, exhibit sex differences in their morphology, suggesting sex differences in their behavior, during the perinatal period (Schwarz et al., 2012). Moreover, they play a critical role in the refinement of neural circuits, e.g., within the developing visual system (Stevens et al., 2007; Schafer et al., 2012). Only very recently have microglia been demonstrated to play a role in the developmental organization of *social* circuits and, thereby, to impact the behaviors those circuits subserve (Kopec et al., 2018). In this issue of *Neuron*, VanRyzin et al. (2019) provide the first evidence for a mechanistic pathway by which the neonatal T surge drives microglia to differentially sculpt social circuits in males as compared to females.

VanRyzin et al. (2019) began with the interesting observation that male rats have higher numbers of phagocytic microglia (defined as having one or more phagocytic "cups" at the distal ends of processes) than females in the amygdala between birth and postnatal day 4 (P4), but not later in development. They observed no sex differences in the total number of microglia or in other aspects of microglial morphology, i.e., ramification. To query the contents of the phagocytic cups and address the question of what microglia are eating, VanRyzin et al. (2019) used Imaris-reconstructed immunohistochemistry. In both males and females, the majority of phagocytic cups contained the nuclei of newly proliferating cells, suggesting that these microglia were engulfing newborn cells. Concordantly, only a small minority of cups contained a marker for apoptotic cells.

Together, these findings suggest that male microglia phagocytose higher numbers of newborn cells in the developing amygdala than female microglia. Indeed, this is in line with previous work from the McCarthy lab demonstrating that males have fewer newborn cells (BrdU-labeled) in the amygdala at P4 than females. Moreover, treatment of female pups with endocannabinoid receptor agonists at P0 and P1 masculinized both newborn cell number and the expression of social play behavior (Krebs-Kraft et al., 2010). Based on the above findings, VanRyzin et al. (2019) hypothesized that neonatal T programs microglia to induce sex differences in social play behavior by phagocytosing newborn cells. They also hypothesized that this process is mediated, at least in part, by endocannabinoid signaling.

Next, VanRyzin et al. (2019) investigated whether neonatal T treatment in females