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The Neural Circuits and Synaptic Mechanisms Underlying Motor Initiation in *C. elegans*

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SUMMARY

C. elegans is widely used to dissect how neural circuits and genes generate behavior. During locomotion, worms initiate backward movement to change locomotion direction spontaneously or in response to sensory cues; however, the underlying neural circuits are not well defined. We applied a multidisciplinary approach to map neural circuits in freely behaving worms by integrating functional imaging, optogenetic interrogation, genetic manipulation, laser ablation, and electrophysiology. We found that a disinhibitory circuit and a stimulatory circuit together promote initiation of backward movement and that circuitry dynamics is differentially regulated by sensory cues. Both circuits require glutamatergic transmission but depend on distinct glutamate receptors. This dual mode of motor initiation control is found in mammals, suggesting that distantly related organisms with anatomically distinct nervous systems may adopt similar strategies for motor control. Additionally, our studies illustrate how a multidisciplinary approach facilitates dissection of circuit and synaptic mechanisms underlying behavior in a genetic model organism.

INTRODUCTION

One of the ultimate goals of neuroscience research is to understand how neural circuits and genes generate behavior. Despite the great diversity of their overall anatomy, the basic building blocks of the nervous systems (i.e., structural motifs/modules of neural networks) display similarity across phylogeny (Reigl et al., 2004; Sporns and Kötter, 2004). As such, genetically tractable organisms have emerged as promising models to decode the neural and genetic basis of behavior (de Bono and Maricq, 2005).

The nematode *C. elegans* possesses complex behaviors ranging from motor, sensory, mating, social, sleep, and drug-dependence behaviors to learning and memory (de Bono and Bargmann, 1998; de Bono and Maricq, 2005; Feng et al., 2006; Liu and Sternberg, 1995; Mori and Ohshima, 1995; Raizen et al., 2008). Interestingly, such a complex array of *C. elegans* behaviors, some of which were once thought to be present only in higher organisms, is mediated by a surprisingly small nervous system with merely 302 neurons and ~7,000 synapses (White et al., 1986). *C. elegans* also represents the only organism whose entire nervous system has been completely reconstructed by electron microscopy (EM) (White et al., 1986). These features in conjunction with its amenability to genetic manipulation make *C. elegans* an attractive model for decoding the neural and genetic basis of behavior. However, even for such a simple model organism as *C. elegans*, it remains largely mysterious as to how the nervous system is functionally organized to generate behaviors.

One of the most prominent behaviors in *C. elegans* is its locomotion behavior (de Bono and Maricq, 2005). Locomotion forms the foundation of most, if not all, *C. elegans* behaviors (e.g., sensory, social, mating, sleep, and drug-dependent behaviors, and learning and memory) because these behaviors all involve locomotion and are, to varying degrees, manifested at the locomotion level. During locomotion, worms often initiate backward movement (i.e., reversals) to change the direction of locomotion either spontaneously or in response to sensory cues (de Bono and Maricq, 2005). Previous work from a number of labs has identified several key components in the neural circuitry that controls the initiation of reversals (Alkema et al., 2005; Gray et al., 2005; Hart et al., 1995; Kaplan and Horvitz, 1993; Maricq et al., 1995; Zheng et al., 1999). In particular, a group of command interneurons (AVA, AVD, and AVE) was found to be essential for the initiation of reversals, as laser ablation of the precursors to both AVA and AVD rendered worms incapable of moving backward (Chalfie et al., 1985). Based on the structural map, these command interneurons receive inputs directly from sensory neurons and also from upstream interneurons (first- and second-layer interneurons), and send outputs to ventral cord motor neurons (A/AS type) that drive reversals (Chalfie

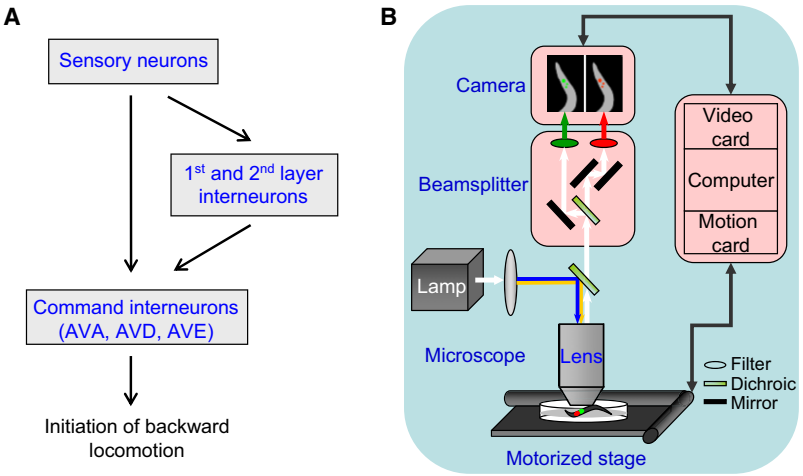

Figure 1. The Current Model of the Locomotion Circuitry that Controls the Initiation of Backward Movement

(A) In this model the command interneurons AVA/AVD/AVE receive input from sensory neurons and interneurons (first layer, AIB/AIA/AIY/AIZ; second layer, RIM/RIA/RIB) and directly synapse onto downstream motor neurons (A/AS, not drawn) to drive backward locomotion.

(B) A schematic drawing of the CARIBN system that enables simultaneous imaging of neuronal activity and behavioral states in freely behaving worms.

See also Figure S1.

et al., 1985; White et al., 1986). Activation of sensory neurons by sensory cues would directly or indirectly excite these command interneurons, leading to the initiation of reversals (de Bono and Maricq, 2005). This constitutes a feed-forward stimulatory circuit (Figure 1A). However, it is not clear whether this circuit, though widely accepted, truly accounts for all of the reversal events seen in this organism.

In this study we applied a multidisciplinary approach to map neural circuits in freely behaving animals. Using this approach, we interrogated the locomotion circuitry and found that our current view of the circuitry needs to be significantly revised. We identified a disinhibitory circuit acting in concert with the command interneuron-dependent stimulatory circuit to control the initiation of reversals. Interestingly, the activity patterns of these two circuits are differentially regulated by sensory cues. Notably, such a dual mode of motor initiation control has also been identified in mammals, suggesting that morphologically distinct nervous systems from distantly related organisms may adopt similar strategies to control motor output. Our study also highlights the value of applying a multidisciplinary approach to dissect the neural and genetic basis of behavior.

RESULTS

Role of Command Interneurons in the Initiation of Reversals during Spontaneous Locomotion

The current model is that the command interneurons AVA, AVD, and AVE, particularly AVA, mediate the initiation of reversals (Figure 1A). As a first step, we imaged the calcium activity of AVA during spontaneous locomotion by expressing in AVA a transgene encoding G-CaMP3.0, a genetically encoded calcium sensor (Tian et al., 2009). DsRed was coexpressed with G-CaMP3.0 to enable ratiometric imaging. To reliably correlate behavior and neuronal activity, we developed an automated calcium imaging system that allows simultaneous imaging of behavior and neuronal calcium transients in freely behaving animals (Figure 1B and Figure S1 available online). We named it the CARIBN (*Ca*lcium *R*atiometric *I*maging of *B*ehaving *N*ematodes) system.

We used the CARIBN system to perform imaging experiments on worms moving on the surface of an NGM (nematode growth media) plate in an open environment without any physical restraint, which is the standard laboratory condition under which nearly all behavioral analyses in *C. elegans* are conducted. Consistent with previous results obtained with a similar system (Ben Arous et al., 2010), we found that AVA exhibited an increase in calcium level during reversals (Figures 2A and 2B), indicating that AVA is involved in controlling backward movement during spontaneous locomotion.

Command Interneurons Are Not Essential for the Initiation of Reversals

To further evaluate the role of the command interneurons AVA/AVD/AVE in reversal initiation, we ablated these neurons individually and in combination. Although worms lacking AVA exhibited a reduced reversal frequency, ablation of AVD or AVE did not result in a notable defect in reversal frequency (Figure 2C), consistent with the view that AVA plays a more important role in triggering reversals than do AVD and AVE (Gray et al., 2005; Zheng et al., 1999). Surprisingly, worms lacking AVA, AVD, and AVE altogether can still efficiently initiate reversals, albeit at a reduced frequency (Figure 2C and Movie S1). These results demonstrate that whereas the command interneurons AVA/AVD/AVE are important for initiating reversals, they are not essential for this motor program. Thus, there must be some unknown circuits that act in parallel to the command interneuron-mediated circuit to regulate the initiation of reversals during locomotion.

RIM Inhibits the Initiation of Reversals, and Its Activity Is Suppressed during Reversals

To identify such circuits, we first examined the wiring pattern of the worm nervous system. RIM, RIA, and RIB are classified as the "second-layer" interneurons that are suggested to act upstream of the command interneurons in the locomotion circuitry (Figure 1A) (Gray et al., 2005). In particular the inter/motor neuron RIM sits at a unique position. It receives input from a number of interneurons and also sends output to downstream head motor neurons and neck muscles (White et al., 1986). Importantly, consistent with previous reports (Alkema et al., 2005; Gray et al., 2005; Zheng et al., 1999), laser ablation of RIM greatly increased reversal frequency (Figure 2C). This