Behavioral Neuroscience: Crawling Is a No-Brainer for Fruit Fly Larvae

How are stereotyped behaviors organized in a simple nervous system? A new study in the *Drosophila* larva reports that the foraging routine can be performed in the absence of any input from the brain.

Julia Riedl and Matthieu Louis

Everyone has heard the story of a headless chicken running around seemingly unperturbed by the loss of its brain. This anecdote begs the question of how much brain function is required to produce meaningful behaviors. It turns out that the zombie chicken has been chasing many headless insects. As early as 1962, Horridge demonstrated that the ventral ganglia (loosely equivalent to the spinal cord) of cockroaches and locust are sufficient to associate leg positions with an electric shock punishment [1]. In vertebrates and invertebrates, removal of the brain has little impact on the execution of basic motor patterns, even though the resulting behaviors often lack coordination [2,3]. Providing that they stay hydrated, decapitated adult flies will happily stand on their six legs and groom spontaneously or upon touching their mechanoreceptor bristles [4]; application of dopamine receptor agonists on their neck induces grooming and walking [5]; excitation of the giant fiber system makes them jump [6]. Although the fundamental role of the central pattern generators in the ventral ganglia of insects and the spinal cord of vertebrates has long been recognized [7,8], the exact contribution of descending inputs from the brain remains poorly understood. A common feature of the previous studies is the limited precision of surgical ablation of different brain regions, particularly in small insects. As Booker and Quinn [9] wrote in 1981, future progress in the functional dissection of brain regions "will need more sophisticated tools than hot forceps and a razor blade". As they report in this issue of *Current Biology*, Berni *et al.* [10] accomplished this feat in the *Drosophila* larva.

The fruit fly larva harbors a nervous system that contains fewer than 10,000 neurons arranged in three main centers: the central brain, the suboesophageal ganglion and the ventral nerve cord (Figure 1A). In spite of its numerical simplicity, the larval nervous system commands a rich repertoire of locomotor behaviors: larvae crawl forward or backward, cast their head sideways, turn with different amplitudes, roll and twist [11]. In the absence of a sensory gradient, foraging proceeds from the alternation of episodes of peristaltic crawling (runs, Figure 1B) and halts followed by a change in orientation [12,13] — a maneuver called a 'pause turn' by Berni *et al.* [10] (Figure 1B). By exploiting the powerful genetic toolkit of the fruit fly, the authors are the first to produce a 'zombie' larva by silencing the activity of the central brain and the suboesophageal ganglion in a reproducible and reversible way. They found that a temporary inhibition of synaptic activity in the central brain and suboesophageal ganglion does

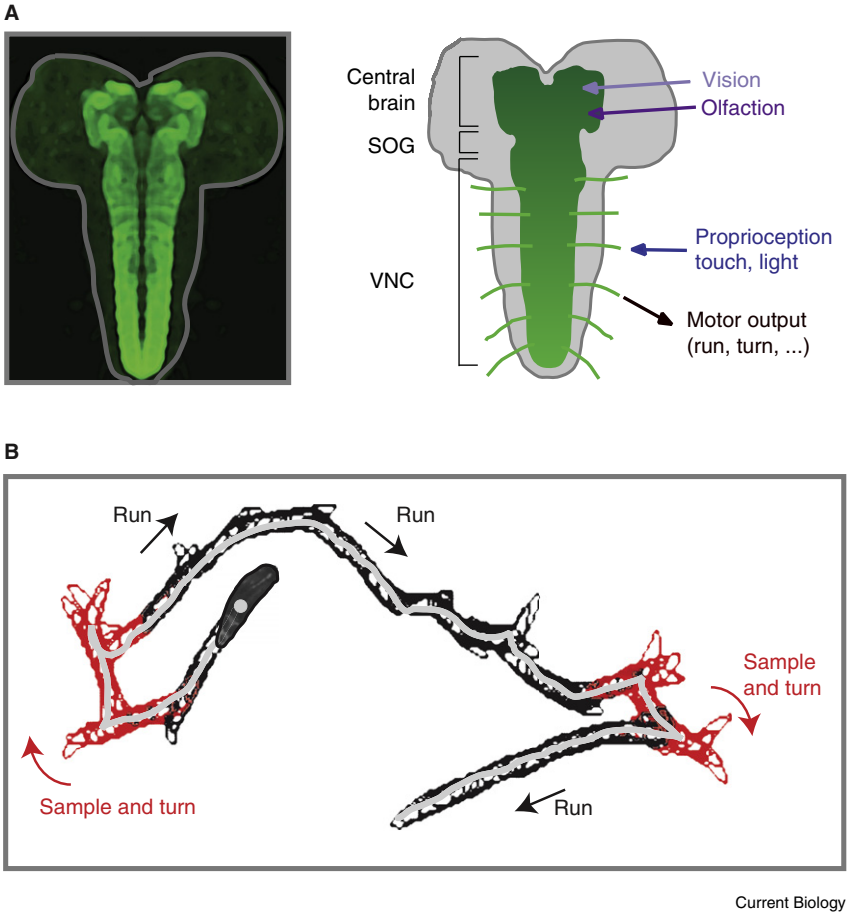

Figure 1. Form and function of the central nervous system of the *Drosophila melanogaster* larva.

(A) Whole mount staining of the central nervous system of a third instar larva. The nervous system is composed of three centers: two symmetrical brain hemispheres (central brain), the suboesophageal ganglion (SOG) and the ventral nerve cord (VNC). The sensory neurons innervating the olfactory system and the main visual organ project in the central brain on the antennal lobe and the optical neuropile, respectively. Afferent proprioceptive and nociceptive neurons located in the larva's body walls project in the VNC. One class of nocipetive neurons has been found to be responsive to light [16]. Efferent motorneurons innervate the body wall muscles through the segmental nerves. (B) Representative trajectory of a wild-type larva engaged in the exploratory routine studied by Berni *et al.* [10]. The series of contour illustrates the position and posture of the larva at a temporal resolution of two frames per second. The trajectory illustrates the alternation between runs (black) and pause turns (red). Tracking was achieved with the freeware described in [20].

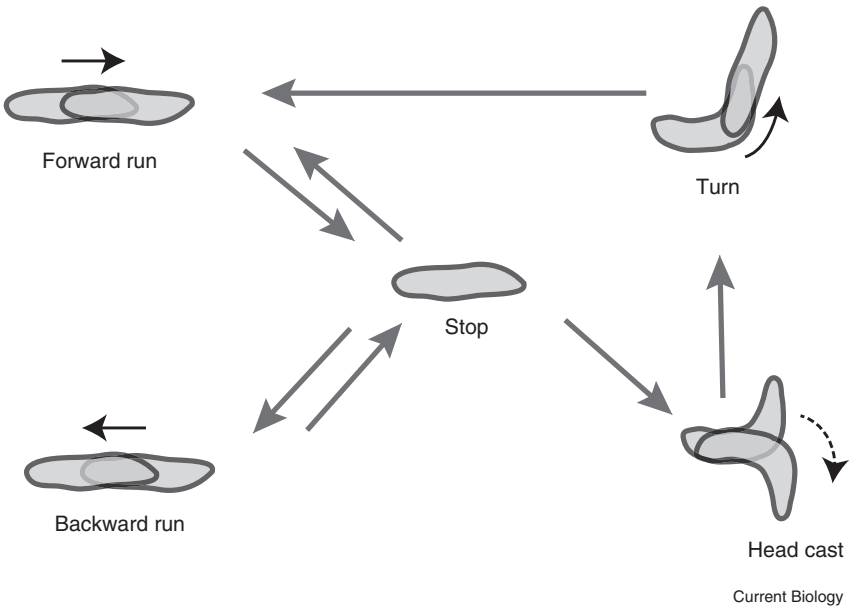

Figure 2. Ethogram associated with the foraging behavior illustrated in Figure 1B.
As discussed in [11,12,14], the main behavioral states displayed by the larva are: forward peristaltic locomotion (run), backward peristaltic locomotion, (stop), lateral head sweeps (casts) and turn. The arrows illustrate the most common transitions between these states during foraging behavior.

not interfere with normal locomotor behavior; detailed quantification of the behavior revealed that neither the number nor the duration of forward and backward waves of peristaltic contractions are changed by such inhibition. By contrast, disrupting neural activity in the ventral nerve cord severely abolishes coordinated peristaltic contractions.

During a typical pause turn, the larva stops, sweeps its head laterally and executes a turn. In the absence of sensory stimulation, pause turns happen spontaneously. In sensory gradients, pause turns are associated with decision points where the larva corrects its direction of motion based on the temporal integration of changes in the stimulus intensity [14]. Berni *et al.* [10] observed that, even in the absence of a functional brain and suboesophageal ganglion, pause turns occur without any significant alteration in the frequency and the amplitude of the turns. To investigate the extent to which the sensory inputs are necessary for maintaining the alternation between runs and turns, the authors went on to silence the central brain, suboesophageal ganglion and all sensory afferent neurons, finding that the loss of afferent inputs affects the frequency and the duration of the waves of peristaltic contraction without abolishing them and that the number of pause turns is reduced without being eliminated.

These observations led Berni *et al.* [10] to hypothesize that neither the central brain, nor the suboesophageal ganglion, nor sensory input is necessary for basic locomotor behavior, but that the brain is important to control the temporal arrangement of behavioral patterns in a purposeful way. To test this idea, the authors studied the orientation behavior of the 'zombie' larvae in response to two different sensory modalities: olfaction and vision. They quantified the ability of larvae to locate the position of a single odor source in an odor-search assay. Wild-type individuals orient towards an attractive odor source and they increase their turning rate in the vicinity of the source [15]. Silencing the brain reduces the number of pause turns and uncouples their occurrence with changes in odor concentration. Consequently, zombie larvae are unable to reach the odor source. This result is consistent with the fact that the olfactory pathway projects in the brain before reaching the ventral nerve cord (Figure 1A). But the body walls of the larva do contain a class of nociceptive neurons that respond to light: these neurons bypass the brain and project directly in the ventral nerve cord [16]. Accordingly, zombie larvae are capable of integrating aversive light stimulation and carrying out successful escape maneuvers. These results suggest that the brain operates as an orchestra conductor that synchronizes coexisting motor programs more than an ON/OFF switch that is necessary for the initiation and the maintenance of any motor response.

With the advent of optogenetics, the ability to reconstruct neural circuits at the level of individual synapses and high-resolution behavioral analysis [12,15,17,18], the *Drosophila* larva offers an excellent opportunity to clarify the relationships between the structure and the function of neural circuits. A solid starting point for this systems-based analysis is an ethogram [19] — a catalogue of the discrete states of behavior displayed by the larva (Figure 2). While each state can be associated with a distinct motor program, every motor program is governed by a subset of neurons converting sensory input and internal states into motor commands. The finding of Berni *et al.* [10] is remarkable in that it shows that the ventral nerve cord harbors more than rigid pattern generator circuits: it can function autonomously to produce coherent behavioral sequences executed in the absence of higher brain input. This increases our appreciation for the level of sensory integration and coordination that takes place within the neural circuits of the ventral ganglia. While much remains to be learned about the circuit processing that underlies spontaneous exploratory behaviors, the findings of Berni *et al.* [10] suggest that the search can be restricted to the ventral nerve cord.

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Membrane Biology: Making Light Work of Lipids

A clever genetic trick allows the lipid composition of the plasma membrane to be manipulated using light, paving the way for new investigations into the many membrane interactions that dictate cell shape, movement and communication.

Gerald R.V. Hammond

Many of the crucial cellular functions occurring at the plasma membrane rely on a small family of phosphorylated lipids, the phosphoinositides. These molecules direct the interactions and activities of a multitude of proteins at the membrane, impacting on many aspects of cellular physiology, from cell division to secretion. Recently, novel 'optogenetic' tools have been developed allowing manipulation of these lipids in living cells that is rapid, reversible and spatially restricted [1], facilitating investigations into the lipids' function with unprecedented temporal and spatial resolution. Indeed, the authors provide some fascinating new insights into the speed with which the lipids can modulate the underlying cytoskeleton.

As the ultimate cellular frontier, the plasma membrane regulates the passage of ions and small molecules, dispatches and receives the vesicular carriers that import and export cargo, and bristles with receptors that relay signals from the environment or the rest of the organism. Crucial to all of these functions are proteins; some embedded within the membrane, such as the channels that carry potassium ions, while others are recruited to the membrane bilayer's inner leaflet, like the adaptor proteins that grab laden cargo receptors, sculpting the surrounding membrane into endocytic vesicles. Many of these proteins require interactions with phosphoinositide lipids, which either activate embedded membrane proteins (like ion channels), or act as an anchor for the recruitment of soluble proteins to the membrane surface (such as endocytic proteins).

Historically, evidence for the participation of phosphoinositides in biological function came from biochemical and pharmacological studies of the lipids' metabolism, *in vitro* studies of protein–lipid interactions, and genetic studies of the kinases and phosphatases that make and degrade them. However, these studies are limited in their capacity to answer crucial questions about the kinetics and specificity of the functional interactions as they occur in living cells. The development of fluorescent biosensors permitted real-time readouts of phosphoinositide dynamics, but experimental manipulation still required either non-specific pharmacological manipulations, or else chronic genetic manipulations such as over-expression or knock-down of an enzyme.

In the last decade, real-time manipulation of phosphoinositide function has become a reality using 'chemical genetics' — the manipulation of protein function using small, cell permeable drugs. Specifically, several groups independently devised a similar technique, which relies on the drug-induced heterodimerization of two proteins [2–5]. One of these proteins is fused to a membrane-targeting motif, often a small plasma membrane-bound peptide. The other is fused to a phosphoinositide-modifying enzyme, stripped of its regulatory domains so that the catalytic core is adrift in the cytoplasm, away from its substrate. On addition of the dimerizing drug, the two proteins dimerize and the enzyme is recruited to its target membrane (Figure 1A). This can lead within seconds to degradation of the phosphoinositide target, such as the dually phosphorylated lipid PIP_2 after recruitment of a phosphatase activity. The system can also be used to elevate lipid concentration, for example by recruitment of a PI 3-kinase that converts PIP_2 to tris-phosphorylated PIP_3. When coupled to a real-time readout of cellular function, the role of the lipid can thus be interrogated *in vivo*. Such studies have already