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Drosophila Neurobiology: No Escape from 'Big Data' Science

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Combining a variety of large-scale, data-intensive techniques, a recent study has unraveled the neural pathways involved in *Drosophila* larval escape from a parasitoid wasp invasion.

Some parasitoid wasp species have evolved the fascinating reproductive strategy of attacking *Drosophila* larvae: with their sharp ovipositor, the wasps pierce the fly larva's thick cuticle to lay an egg inside; if successful, the developing wasp larva will consume its prey and eventually a new wasp will emerge rather than a fly [1,2]. To defend itself against this lethal reproductive strategy and avoid being eaten alive, *Drosophila* has evolved a number of clever defense mechanisms [3]. One such mechanism is a remarkable escape behavior to foil the wasp ovipositor penetration. When pain receptors that tile the surface of the larval cuticle are activated by the sting of the ovipositor, the fly larva executes a characteristic corkscrew-like rolling motion [1] that was first described as a 'nocifensive', or pain-triggered defensive escape response against a noxious heat probe [4]. Paradoxically, the larvae roll in the direction of the wasp ovipositor, decreasing the chance of a successful oviposition [2].

In recent years, this intriguing locomotor response has been gradually dissected at the neuronal and molecular level using the growing number of tools available in the fly [1,2,4–7]. These studies have shown that a specific class of dendritic arborization (da) neurons, also called multidendritic (Md) neurons [8–12] is necessary and sufficient for the rolling response [1,2]. The different classes of Md neurons tile the surface of the larval cuticle and play key roles in sensing distinct types of stimuli in the environment of the larva [13,14]. While nocifensive behaviors require class IV neurons [1,2], gentle touch is sensed through a different class of tiling neurons and triggers crawling and turning [14]. Vibrations are perceived by yet another set of so-called chordotonal (Cho) neurons [15,16]. The responses to all these specific sensory cues depend not only on the type of neuron, but also on the molecular machineries involved [12,14,16]. A new paper [17] aims at putting together these elements into a complete circuit controlling the rolling escape response that presumably evolved in fly larvae to escape from parasitoid wasp adversaries.

Building on a recently developed high-throughput screening strategy that integrates different sensory stimuli to dissect escape locomotor circuitry in fly larvae [18], Ohyama *et al.* [17] show that the combination of nociceptive and vibration stimuli acts synergistically to trigger rolling behavior. The authors used a combination of large-scale behavioral screening coupled with silencing and activation of genetic lines, functional imaging and electrophysiological methods, and large-scale electron microscopical reconstructions of the larval nervous system (~10,000 neurons) to identify the postsynaptic targets of the nociceptive (class IV Md) and vibration (Cho) sensory neurons involved in this rolling behavior (Figure 1A). They found that the integration of mechanical pain stimulation detected via Md IV neurons, and vibration detected by the Cho neurons, has a super-additive effect on the rolling escape response.

Ohyama *et al.* [17] went on to characterize a large network of neurons at several levels of the nervous system involved in the rolling escape response. In particular, two output neurons (Goro neurons) were identified that are capable of triggering the rolling behavior, and the neural network connecting the nociceptive and vibration sensitive sensory neurons to the output Goro neurons was largely defined (Figure 1B). The latter network consists of a direct pathway within the nerve cord of the larva where multisensory integration occurs at two stages: first, some of the post-synaptic targets of the sensory neurons (so-called Basin cells) respond to both nociceptive and vibrational stimuli, while others respond only to vibrational stimuli; and second, the Basin cells mediating combined nociceptive/vibrational information and those mediating only vibrational

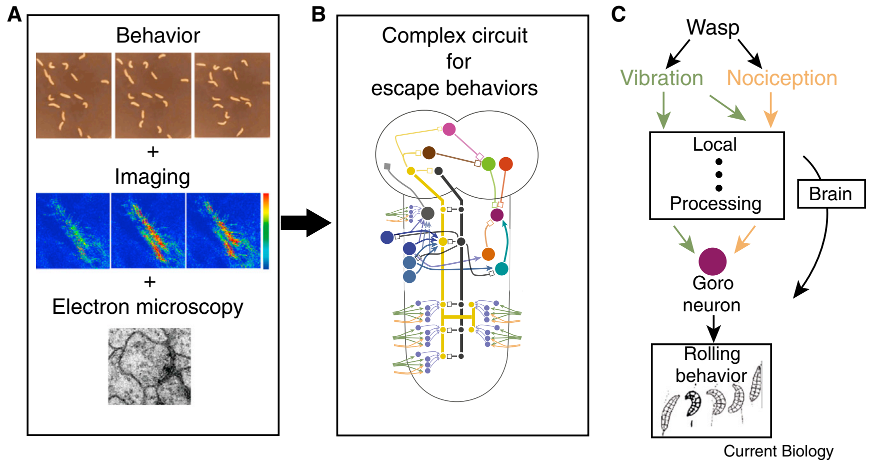

Figure 1. Strategy and summary results of the large-scale approach implemented in Ohyama *et al*. [17].
(A) A combination of behavioral screening of fly larvae, imaging, and electron microscopy led to (B) a detailed circuit diagram of the neurons involved in rolling behavior. (C) This circuit can be broken down into (i) a local pathway that combines multisensory inputs at two stages before converging on command-like (Goro) neurons triggering rolling, and (ii) a brain pathway whose precise role remains to be determined. Panel A (top and middle) adapted from [16] copyright (2013) National Academy of Sciences, USA. Panels A (bottom), B, and C adapted from [17], reprinted by permission from Macmillan Publishers Ltd: Nature, copyright (2015).

information eventually converge onto the Goro neurons. In addition to the nerve cord pathway sketched above, the authors identified a complex multi-stage ascending pathway to the brain that eventually converges back on the same Goro neurons and presumably plays an important role in behavior selection (Figure 1B,C).

This work is a ‘tour de force’ because of the way, in working towards a single goal, it integrates a series of complex techniques: as mentioned above, large scale behavioral screening, functional imaging with neuronal manipulation, and large scale electron microscopy and neuronal tracing. By identifying the pathways that are involved in detection of the nociception and mechanosensory stimuli that initiate the behavior, this work will facilitate the detailed study of the molecular and neuronal mechanisms underlying these two sensory modalities. In addition, it opens the exciting possibility of studying multisensory integration in a model system where complete identification of the neurons and networks involved is possible.

The first key finding of the new work is the demonstration that integration of two types of sensory input — nociceptive mechanical stimuli and vibrations — from different sensory neurons supralinearly enhance the rolling escape response. As mentioned, it was already well established that class IV Md neurons are crucial in mediating nociception, and it was recently shown that larval chordotonal neurons respond preferentially to vibration signals [15,16] and are tuned to the vibration produced by predatory wasps [16]. The second key contribution of Ohyama *et al.* [17] is to map several new components of the circuit, down to the command neurons that are sufficient to trigger larval rolling.

A number of interesting questions are raised by this work. Although the Goro neurons clearly mediate rolling, their silencing decreases, but does not eliminate that behavior. This suggests that other output neurons and presynaptic neural networks are involved in rolling as well. It will be important in the future to apply the techniques developed by Ohyama *et al.* [17] to identify the neuronal element(s) missing from the current picture. It will also be interesting to see whether the missing motor output elements add yet a different layer of sensory input mechanisms. In addition, what type of modulatory control is integrated in the system and at what level does it operate?

Past work has documented the synergistic combination of distinct sensory inputs for action selection, for example in the superior colliculus of the cat [19], but the complexity of the neuronal networks involved has precluded so far a detailed mechanistic investigation of its underpinnings. In this context, a simple model presented by Ohyama *et al.* [17] suggests that convergence of multisensory information at several levels of a neural network is beneficial for enhancing its output tuning selectivity. It will be interesting to see if this can indeed be verified experimentally. In addition, recent multisensory integration studies in mammalian model systems have been emphasizing the optimal combination of cues originating from different sensory modalities [20]: it will be interesting to see if similar parallel results can (or cannot) be established in flies.

This work also raises the question of how multisensory integration is used in natural fly larval behavior. The new findings make ecological sense, because wasps that are trying to lay eggs inside fly larvae presumably produce both vibrational and nociceptive stimuli. Thus, the fly may have evolved an accentuated escape response to the coincidence of these two stimuli. The assay used by Ohyama *et al.* [17] is very effective for large-scale screening, but relies on a strong simplification of the events that a larva may experience naturally. In particular much of the timing and temporal sequence of events like the vibrational sounds emitted by approaching predatory wasps, the touch stimuli and nociceptive stimuli leading to the attack are simplified. A detailed study of how these events occur naturally and how they are related to the many possible escape strategies used by larvae in addition to rolling is likely to facilitate our understanding of how the underlying neural circuits work.

On a sociological level, this work provides a good illustration (in the context of fruit fly biology) of the type of results that could be achieved by 'large scale integrative neuroscience'. Several initiatives both in Europe and the US, like the BRAIN initiative, advocate for renewed integrative efforts in this direction. Clearly, individual neuroscientists will face a choice in the future as to whether they want to be part of large teams pursuing this line of work or whether they want to belong to a small team pursuing an approach based on detailed and more flexible, but also more restricted understanding of nervous system function. Both these options offer exciting lines of research and one is tempted to compare them to what happened in physics, where particle physics has evolved into a large scale enterprise culminating in successes like the discovery of W-, Z-, and Higgs bosons. In contrast, solid-state physics has largely remained a small-scale enterprise, much less known by the broad public although it has fueled a revolution in the semiconductor industry and seeded many of the ideas used to develop particle physics models. Similarly, the cross-fertilization of small team efforts and large data projects in genomics and neuroscience will likely add synergistically to the ongoing revolution to understand the brain.

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