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Connectomics: Bringing Fly Neural Circuits into Focus

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Three new studies use a whole adult brain electron microscopy volume to reveal new long-range connectivity maps of complete populations of neurons in olfactory, thermosensory, hygrosensory, and memory systems in the fly *Drosophila melanogaster*.

Neural systems need to organize diverse sensory information to direct appropriate actions and form appropriate memories. Maps of the brain circuits that implement these processes have remained blurry: they lack the resolution and completeness necessary to resolve comprehensive connectivity within and between networks. Three studies [1–3] reported in this issue of *Current Biology* now bring the organization of sensory and memory systems in the brain of the adult fruit fly, *Drosophila melanogaster*, into sharp focus.

Densely Mapping Long-Distance Projections

Mapping complete populations of neurons and synapses is no easy task. Determining precise neuron morphology and synapse locations requires the nanometer resolution currently only afforded by electron microscopy (EM). Because connected brain regions can sit on opposite sides of the brain, EM volumes must be big enough to include them all. Moreover, tracing and proofreading of neural reconstructions is labor intensive (although increasing automation promises to reduce this burden [4]). These challenges have historically constrained connectomics studies in the adult fly, such that mapping often focuses on relatively sparse tracing of long-range projections to a handful of neurons [5] or dense tracing of complete populations without reconstructing long-range projections [6].

The studies of Bates and Schlegel *et al.* [1], Marin *et al.* [2], and Otto *et al.* [3] now provide complete maps of large neural populations and their long-range axonal or dendritic projection patterns in the adult fly. All three trace many new neurons and their synaptic partners from an EM volume of an entire adult female brain [7]. Their findings provide a level of detail about fly circuits that has previously only been available in larvae [8].

Intermingling of the Odor Code

Bates and Schlegel *et al.* [1] investigate the fly olfactory system, focusing on second order projection neurons (PNs) of

the antennal lobe. PNs integrate inputs from olfactory receptor neurons, forming an odor code to send to the lateral horn and mushroom body, the two higher-order olfactory regions in the fly. Compared to the well-known function of the mushroom body in memory and flexible behavior, the role of the lateral horn remains more mysterious. While we know that lateral horn neurons integrate spiking inputs from PNs encoding ecologically related odors [9], a comprehensive understanding of what happens to the odor code in the lateral horn is only starting to take shape [10].

Bates and Schlegel *et al.* [1] have now mapped all 347 olfactory PNs in one brain hemisphere, traced their axons into the lateral horn, and identified many of their synaptic partners. Like many long-range axons, PNs are often described as 'relays', faithfully conveying the odor code so target neurons can perform subsequent computations. As the authors now show, this description is incomplete. Once they arrive in the lateral horn, PN axons begin to mingle with each other like it's their first day in college.

The authors discovered three types of input onto PN axons (Figure 1A). First, they found that nearly all receive input from other PN axons; these connections are selective, organizing PNs into communities tuned to similar odor categories. Second, they discovered that axons receive input from centrifugal feedback neurons carrying information from higher brain areas (including the mushroom body). Third, they found that PN axons receive input from local lateral horn neurons.

What is the function of this axonal cocktail party? Detailed answers will require precise functional studies, but comparisons with other circuits point to some hypotheses. Axo-axonic electrical synapses in the hippocampus are thought to synchronize pyramidal neurons [11]. Because the axo-axonic PN synapses are chemical, they may regulate spike timing on a slower timescale (although the current fly EM data cannot resolve electrical synapses). Feedback connections often provide contextual information, but the precise context encoded by the lateral horn centrifugal neurons is still unclear. Nevertheless, the authors clearly show that PN axons are likely a site of important computation that helps flies choose appropriate behaviors in response to specific odors.

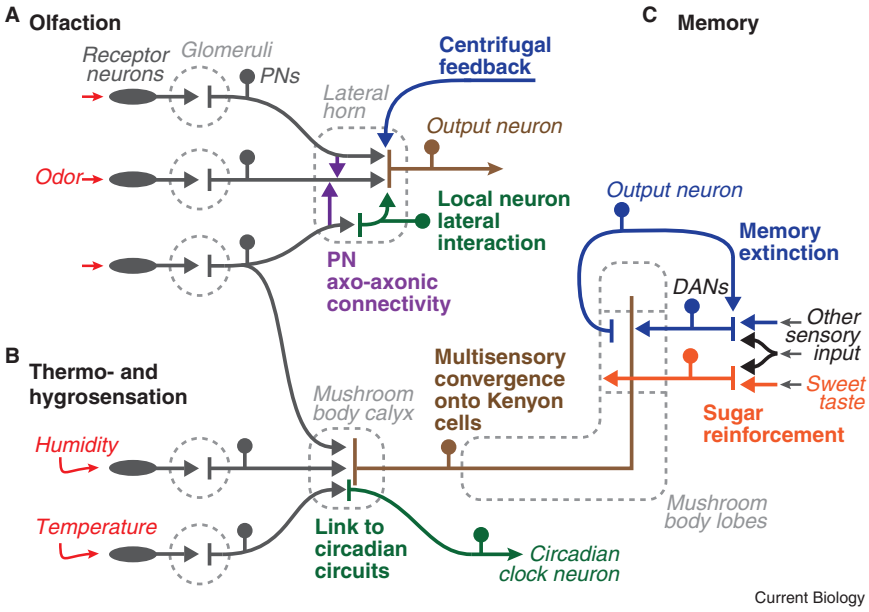

Figure 1. New circuit motifs in olfaction, thermo- and hygrosensation, and memory circuits in the adult fly brain.
(A) Tracing of olfactory circuits reveals that projection neuron (PN) axons intermingle with each other, with feedback neurons, and with local neurons. (B) Tracing of thermo- and hygrosensory circuits demonstrates convergence with olfactory neurons and wiring onto circadian neurons in the mushroom body. (C) Tracing of memory circuits shows diverse connectivity of the dopamine neurons (DANs) innervating individual compartments.

Processing Temperature and Humidity

With their tiny bodies, flies are especially susceptible to overheating and dehydration. Accordingly, they have extensive thermosensory and hygrosensory systems to drive regulatory responses. These systems follow the same basic organization as olfaction: antennal sensory neurons target glomeruli in the antennal lobe, synapsing onto PNs that project into the mushroom body and lateral horn, among other areas [12]. Marin *et al.* [2] now report a complete set of 52 PNs on one side of the brain with predominant input from antennal thermosensory and hygrosensory neurons. This catalogue is a rich resource for guiding physiological investigations into the transformation of this information in the brain.

The authors next investigated downstream connectivity of these PNs specifically in a subdivision of the mushroom body called the lateral accessory calyx (Figure 1B). Many target neurons are Kenyon cells — intrinsic mushroom body neurons — that also receive olfactory inputs. Odor and temperature are often correlated in nature because chemical volatility increases with heat (one reason your garbage smells so bad in the summer). Mixing these modalities in Kenyon cells may improve flies' ability to exploit regularities in their natural statistics, or to form memories of particular odor–temperature combinations.

The authors then show that most of the PN output synapses in the lateral accessory calyx contact neurons other than Kenyon cells. For example, several PN types connect to neurons that control the fly's circadian arousal cycle. This connectivity provides a neural pathway to entrain daily rhythms to temperature, consistent with recent physiological evidence [13]. This is useful because temperature varies systematically throughout the day. The wide integration of thermosensory and hygrosensory information into the brain highlights its importance not just for survival, but also for providing essential context to other functions.

Diversity in Memory Reinforcement Circuits

Flies form diverse odor memories, depending on reinforcement mediated by dopamine neurons (DANs) in the mushroom body. This neuropil segregates into distinct compartments, where distinct DANs contact distinct partners, drive synaptic plasticity with distinct rules, and modulate the activity of distinct output neurons to drive distinct forms of learning [14]. These observations have led to an assumption that compartments form the finest level of DAN organization.

However, behavioral experiments have revealed compartmental multifunctionality: for example, DANs of one compartment can control both positive and negative memories, depending on context [15,16]. How does a single population of DANs perform multiple memory tasks? Otto *et al.* [3] found that this ability is achieved, in part, by a division of labor between DANs targeting the same compartment.

The authors analyzed the morphology of 29 DANs on one side of the brain and sampled 821 neurons that provide input to them. Even for those DANs that innervate the same compartment, there is considerable diversity (Figure 1C). For example, they find that the 20 DANs targeting the compartment called 'γ5' organize into five morphological types and that each type receives its own specialized set of inputs (although some input is shared across all types). In particular, one type gets feedback from the output neurons of the same compartment, while another type gets a specific combination of sweet taste inputs. The authors then silenced each of these two DAN types during learning, finding that the first participates in memory extinction, whereas the second participates in sugar reinforcement.

These experiments show that the multifunctionality of memory can result from a striking division of labor among DANs in the same compartment. In the future, it will be important to understand how within-compartment specializations interact with between-compartment specializations. Intriguingly, a recent investigation of the larval mushroom body has found extensive feedback connections between compartments, and predicts their importance for diverse forms of learning [8]. Other mechanisms may also contribute to the diversity of learning. For example, a temporal division of labor could be achieved through dynamic crosstalk between DANs or their input networks (within which the authors find extensive axo-axonic connectivity). This idea is reminiscent of multifunctional pattern generating circuits in motor systems [17] and could enable the same DANs to engage in different ways at different times to achieve different goals of learning. The anatomical and functional organization reported by Otto *et al.* [3], provides a solid foundation to test these ideas.

From Anatomy to Function

Behavior results from the orchestration of many neural circuits, each performing its own set of computations. In flies, most of what we know about computation comes from the analysis of circuits in so-called 'structured' neuropils (such as the antennal lobe and mushroom body), where modular organization is easy to see under light microscopy. In contrast, most of the brain's neuropil (such as the lateral horn) is unstructured, where modular organization — if it exists — is not immediately obvious [18]. Learning how neural computation controls behavior in the fly will require understanding how circuits of structured and unstructured neuropils work together. Each of the new studies [1–3] provides crucial lifelines for venturing ever deeper into the unstructured frontier of the fly brain.

The impressive detail and coverage of these new connectivity maps should enable new investigations into the links between anatomy and function. For example, it is well known that the relationship between anatomy and function is not one-to-one: a single anatomical motif can perform multiple functions and multiple distinct motifs can perform the same function [19]. How does circuit structure constrain the computations it can perform? In addition, connectivity is not static: learning and experience can re-route long-range connections between brain regions, and even eliminate connections between individual neurons [20]. How does new wiring create new functions? By taking advantage of the resolution afforded by EM, the studies of Bates and Schlegel *et al.* [1], Marin *et al.* [2], and Otto *et al.* [3] lay the groundwork for using the fly to provide new insight into these fundamental questions.

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Cell Division: Switching On ECT2 in a Non-Canonical Fashion

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Determining the site of cell cleavage is crucial for cytokinesis and involves precise activation of the RhoGEF ECT2. A new study demonstrates how a non-canonical interaction of ECT2 with centralspindlin underlies cytokinesis in animal cells, solving a mechanistic conundrum.

Cell division partitions the genetic material and cytoplasmic contents of a mother cell to its daughters. Cytokinesis is the last step of cell division during which the final cleavage occurs (Figure 1A). To divide correctly, animal cells must position the cleavage plane between the segregated pairs of chromosomes in anaphase (Figure 1A). A new study by Gómez-Cavazos *et al.*, published in this issue of *Current Biology*, provides molecular insights into the regulation of cleavage initiation at the cell equator — a major but not fully understood question in cell biology [1].

During animal cell cytokinesis, astral and central spindle microtubules, a region of antiparallel microtubules that forms between the segregated chromosomes, specify the cleavage site by establishing a narrow equatorial zone of RhoA GTPase activity [2–4]. Active RhoA promotes assembly of the actomyosin ring, which then contracts to drive ingression of the cleavage furrow and the completion of cell division [5]. During cytokinesis, the Rho guanine nucleotide exchange factor (GEF) ECT2 converts inactive RhoA–GDP to active RhoA–GTP [5]. A key regulator of RhoA activation by ECT2 at the cell equator is the mitotic kinase PLK1 [2,6–9]. How PLK1 achieves this has been the intense focus of research that has unearthed some puzzling findings and conundrums. The work by Gómez-Cavazos *et al.* now provides an answer.

Centralspindilin is a conserved heterotetramer, composed of kinesin MKLP1 and MgcRacGAP dimers (called ZEN-4 and CYK-4, respectively, in *Caenorhabditis elegans*), that helps build the central spindle [2]. Once chromosomes have fully segregated, the kinase PLK1 accumulates at the central spindle and phosphorylates the amino-terminus of MgcRacGAP, providing an anchoring site for ECT2. ECT2 is present in a soluble inactive form in which the triple BRCT domain module at its amino-terminus interacts with the GEF domain at its carboxyl terminus [10–12]. Binding of the BRCT module to phosphorylated MgcRacGAP at the central spindle is believed to liberate the ECT2 GEF domain from intramolecular autoinhibition. The prevailing model for cleavage furrow formation thus suggests that the central spindle directs the accumulation of activated ECT2 at the adjacent equatorial membrane, where in turn it initiates cytokinesis [3,5] (Figure 1B, left).

However, conflicting data and observations have raised doubts about the model that PLK1 phosphorylation of MgcRacGAP/CYK-4 at the central spindle activates ECT2 to initiate cleavage furrow ingression. MgcRacGAP/CYK-4 and ECT2 are similarly required for RhoA activation and furrow ingression in human cells, *C. elegans*, and *Drosophila melanogaster* [5]. However, ECT2 does not accumulate at the central spindle in *C. elegans* and *D. melanogaster* like it does in human cells [2]. In human cells, a non-phosphorylatable mutant of MgcRacGAP