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Neuroscience: A big step forward for motor control in *Drosophila*

Mert Erginkaya and Jan M. Ache*
Neurobiology and Genetics, Theodor-Boveri-Institute, Biocenter, Julius-Maximilians-University of Würzburg, Am Hubland, 97074 Würzburg, Germany
*Correspondence: jan.ache@uni-wuerzburg.de
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Connectomics approaches are fundamentally changing the way scientists investigate the brain. Recently published connectomes have enabled dissection of the intricate motor circuits in the fly's version of the spinal cord on a synaptic level. This has allowed reconstruction of complete sensorimotor pathways in *Drosophila*.

In the supermarket, you've likely never consciously controlled the precise placement of your feet as you approached the fruit section, or pondered the muscle forces needed to pick up a mango. And yet, you have accomplished such demanding motor control tasks all your life. This is possible because your spinal cord does this job for you, while your brain is busy strategizing and asking higher-level questions, like: do I really want mango? Motor control is achieved through the exquisite interplay of descending input from the brain, rhythmically active premotor networks in the spinal cord, and local feedback from sensory organs in and on the limbs[1] — all tailored to coordinating movements required to complete the task at hand. The nervous system of fruit flies, which, as the name suggests, spend a considerable amount of their lives hunting for fruit, needs to solve similar motor control tasks (Figure 1A). In fact, the fly's nervous system is able to orchestrate delicate movements of the body during flight — controlled by four power and 13 steering muscles per wing — and walking — driven by 108 muscles controlling 30 joints across six legs. While the field has made great progress at identifying core motor circuits for specific behaviors[2], it remains difficult to understand how these circuits interact and coordinate with each other.

Two recent papers[3,4] from the Tuthill and Lee labs constitute a major step towards understanding how motor circuits in the fly's version of the spinal cord, the ventral nerve cord (VNC), control leg and wing movements to turn thoughts into action. The first, by Azevedo *et al.*[3], presents the synaptic wiring diagram — a 'connectome' — of a female adult nerve cord (FANC). The second, by Lesser, Azevedo *et al.*[4], uses the FANC connectome to investigate principles of motor circuit organization in the leg and wing control systems. Together with previously published connectomes of the *Drosophila* brain[5–7] and parallel efforts in a male VNC[8], these groundbreaking papers make it possible to trace sensorimotor pathways throughout the entire nervous system.

Azevedo *et al.*[3] applied machine learning tools to analyse over 20 million electron microcopy (EM) images[9], each depicting a thin slice of the VNC: they were able to segment 15,000 neurons, and predict 45 million synaptic connections between them to generate the FANC dataset. Ultimately, however, behavioral output is based on muscle contractions. To understand how motor circuits control movement, it is necessary to map each motor neuron to the muscle it innervates, and each muscle to a specific joint and movement direction — for example, leg extension or flexion. For this purpose, Azevedo *et al.*[3] combined the FANC connectome with an X-ray dataset of a fly leg[10] and high-resolution fluorescence images of motor neurons. The overall result is a comprehensive atlas of the wing

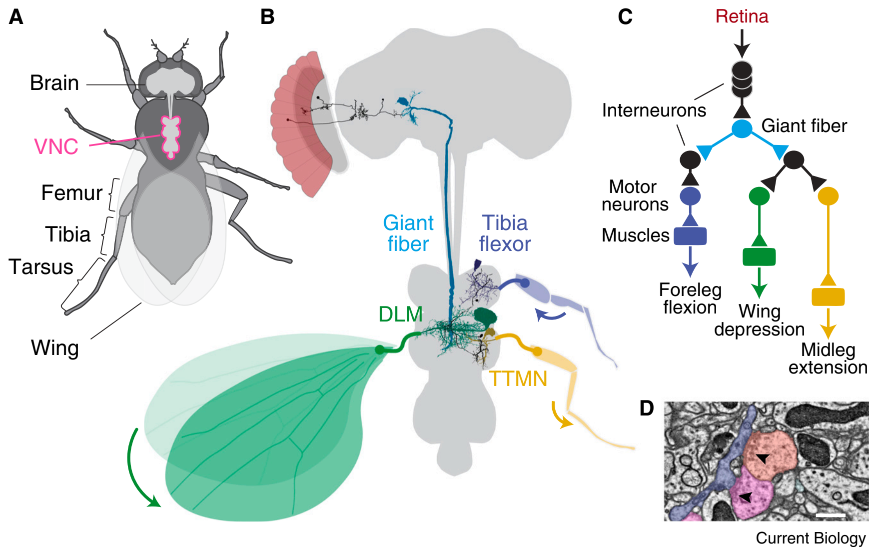

Figure 1. New connectomes link brain circuits to muscles in *Drosophila*.
(A) Schematic of a fruit fly with its central nervous system. The VNC is highlighted. (B) Anatomy of cell types within the giant fiber pathway, assembled using FANC[3] and FlyWire[7,20]. Arrows indicate leg and wing movements during take-off. (C) Connectivity diagram of the giant fiber pathway. Colors as in (B). (D) Section of FANC showing two input neurons (magenta and red) to the tibia flexor (blue). Arrowheads, presynaptic sites. Scale bar, 500 nm. FANC data from Azevedo *et al.*[3], with permission from Springer Nature; FlyWire data from Dorkenwald *et al.*[7] (CC BY-ND 4.0) and Schlegel *et al.*[20] (CC BY 4.0).

and front leg motor neurons, along with the motor circuits controlling them. The morphology and function of individual neurons is highly conserved within insect species, so that this atlas can serve as a blueprint for motor circuits across individuals and datasets.

Using the giant fiber escape pathway as an example (Figure 1B), Azevedo *et al.*[3] illustrate how VNC circuits can control body movements. The giant fiber is the largest descending neuron in the fly nervous system. Once activated, it induces a specific sequence of leg and wing movements controlling escape jumps triggered by an approaching predator[11]. The visual pathway that detects approaching objects and connects to the giant fiber has been characterized and described at synaptic resolution[12] — an effort aided by a full brain EM dataset[5]. In the VNC, the giant fiber connects to motor networks driving midleg extension, foreleg flexion, and wing downstroke. Together, these movements rapidly propel the body into the air[11] — and thus ensure flies aren't touched by humans unwilling to share their mangos. Combining FANC with the brain connectome now allows tracing this entire sensorimotor pathway at the synaptic level — from photoreceptors in the retina to the leg and wing muscles (Figure 1B–D).

In a companion paper, Lesser, Azevedo *et al.*[4] investigate the wiring logic of motor circuits by analysing the inputs of identified leg and wing motor neurons in FANC. They find that premotor circuits are structured into 'motor modules'. These motor modules consist of premotor neurons targeting motor neurons controlling synergistic muscle groups, while avoiding their antagonists. This forms a synaptic basis for muscle synergies (Figure 2A). Some leg motor modules are shaped by premotor neurons providing input to all motor neurons within the module. In these cases, larger motor neurons receive more synaptic input from shared premotor neurons than smaller motor neurons (Figure 2B).

This finding is surprising: the original 'size principle' of motor neuron recruitment, established in vertebrate systems and thought to be a fundamental principle in motor control, assumes equal numbers of inputs to smaller and larger motor neurons[13]. Smaller motor neurons generate less force than larger motor neurons, but are more easily excitable; hence, smaller motor neurons are recruited earlier when receiving the same input. As Lesser, Azevedo *et al.*[4] themselves point out, based on the large number of synaptic inputs they receive, one might assume that the largest motor neurons are the most active, and recruited earlier than small motor neurons in *Drosophila* (Figure 2B). However, the biophysical properties of individual motor neurons

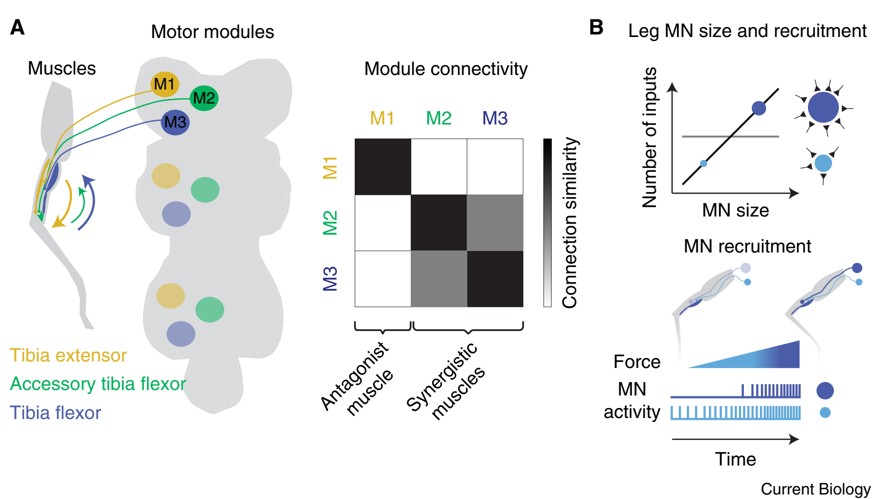

Figure 2. Synergistic organization of leg motor circuits.
(A) Left: schematic of motor modules (M1–3): groups of premotor and motor neurons and the leg muscles they target. Arrows indicate tibia movement direction. Right: schematic connectivity between modules. (B) Top: leg motor neurons (MNs, circles) receive input proportional to their size within each module. Gray line: expected relationship based on the size principle. Bottom: smaller motor neurons are recruited before larger motor neurons during leg flexion.

counteract their input organization, so that this is not the case — as evidenced by prior electrophysiological recordings[14,15]. Thus, leg motor neurons are recruited from small to large, as expected from the original size principle. The proportionality of motor neuron input to size is not observed within wing motor modules, further highlighting differences between the control of legs and wings. As these examples illustrate, connectomes are powerful tools to distill and test hypotheses about the fundamental organization of motor control networks — and they unfold their full potential when accompanied by physiological approaches.

Of course, there is still a lot of uncharted territory in the VNC. The biggest, perhaps, is that anatomical connectomes can only provide a roadmap for activity flow through the nervous system — which road is taken needs to be measured using physiological approaches. For example, neuromodulation can drastically shape functional connectivity in a state and context-dependent way[16]. Moreover, the spatial resolution of FANC is not fine enough to resolve electrical synapses, which play a key role in sensorimotor circuits[2,17]. FANC is accompanied by a similar effort presenting the connectome of a Male Adult Nerve Cord (MANC)[8]. A complete connectome of the fly Brain And VNC (BANC) is also on the horizon, which will facilitate the identification of descending and ascending sensorimotor pathways already underway[18]. Hence, the motor control community will be able to contrast and compare three *Drosophila* VNCs, including male and female versions. This will allow asking questions about sex differences and individuality, for example. In FANC, Azevedo *et al.*[3] managed to connect the neurons of the central nervous system to its body on the output side, by providing a motor neuron atlas and identifying their target muscles. A future upgrade to the connectome would be a similar accomplishment on the sensory side, as has recently become available in the *Drosophila* larva[19]. Thus, it would be possible to build even more powerful connectome-based models for the multitude of sensory contributions to behavioral control.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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