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Neurodevelopment: Maintaining function during circuit reconfiguration

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In Brief:

Neural circuits change during development or following experience by adding neurons and rewiring connections, but mapping such processes is technically daunting. A new study traces maturation of an entire circuit in subcellular detail, revealing continuous functionality under gradual structural change.

At 4:50am on September 3rd, 1967, a horn blared, and a loudspeaker announced, "Now is the time to change over!" Remarkably, the next few hours were uneventful, and the next few days were almost accident free. This was the Swedish H-Day in which millions of Swedes followed the campaign song "Håll dig till höger, Svensson", and orderly switched from driving on the left to driving on the right. When systems increase in size or complexity, they face a similar challenge: how to switch from one functioning system configuration to another while

maintaining continuous output and averting catastrophic failure. By far the safest strategy involves a global synchronized switch, as evidenced by the experience of the Swedes on that historic day.

A common characteristic of complex systems is that they start out relatively simple. Therefore, any additions or deletions are constrained by the previous instantiation of the system and the required functionality of the output. A major open question in complexity science is how evolving or developing systems maintain functionality in the face of change. Nowhere is this more apparent than in the development and life-long changes of neural circuits as they incorporate, omit and re-appropriate neurons and connections[1]. As they report in this issue of *Current Biology*, Mulcahy *et al.*[2] approached this question in one of the most thoroughly described nervous systems, that of the nematode *Caenorhabditis elegans*, as its juvenile locomotion circuit develops into its mature configuration. The question of persisting circuit output during neurogenesis and neuronal remodeling has a long history in vertebrate[3,4] and invertebrate[5,6] systems to which this research adds exquisite subcellular details and indeed some surprising revelations and insight.

At hatching, the *C. elegans* locomotion circuit includes only 22 neurons of three classes: two cholinergic classes that innervate dorsal muscle, and one GABAergic class that innervates ventral muscle[7,8] (Figure 1Ai); 53 more neurons of five new neuronal classes are integrated post-embryonically (Figure 1Aii,iii). The more-than-tripling of this circuit raises two overarching questions: how does the asymmetric juvenile circuit generate the alternating muscle pattern needed to drive undulatory locomotion? And how is catastrophic failure avoided during the transition from the juvenile to the mature circuit? The first question was addressed by the authors in another paper[9] in this issue of *Current Biology*: in that paper, they demonstrate that, during

rhythmic undulations (in either the forward or backward direction), cholinergic neurons rhythmically activate one side of the body (the dorsal side) while rhythmic inhibition by GABAergic neurons relaxes opposite (ventral) muscles. This inhibition overrides tonic excitation due to cholinergic spillover onto ventral muscle from descending interneurons (Figure 1Ai).

As to the second question, until now neuroscientists believed the Swedish model: the new post-embryonic infrastructure was put in place within hours of the animal hatching, in the first larval stage[10], and the switch was then assumed to be orchestrated during a sleep state that occurs between the first and second larval stages. But *C. elegans* never ceases to surprise us. Mulcahy *et al.*[2] show that remodeling continues after the sleep state so the circuit must maintain functionality in the awake state as it matures along the body. Far from a global switch, this result points to a complex mechanism that is coordinated globally but is locally staggered in time and space, an even more startling finding for an unsegmented animal.

C. elegans provides significant technical advantages for tracing circuit reorganization over most vertebrates and many invertebrates. First, the nematode, and more so, its larvae, are relatively small and develop with a generation time of three days. Second, with an invariant and fully mapped cell lineage[11,12], anatomical changes can be traced developmentally across different animals at the subcellular and even synaptic resolution[13,14]. Mulcahy *et al.*[2] did just that. They used two state-of-the-art serial electron microscopy methods to reconstruct the neuronal morphology and neural apposition of the locomotion circuit at six different developmental stages before, during and immediately after the circuit is reorganized to the mature configuration. They first confirmed what we already knew about this beautiful three-stage process: neurogenesis occurs early on in a neatly staggered fashion in time and along the body[10]. Next, embryonic neurons and their synapses undergo significant remodeling. Neurons of the GABAergic

embryonic class dramatically reverse their axonal-dendritic polarity, and switch from innervating ventral to dorsal muscles[7]. Remodeling follows a similar spatiotemporal order from head to tail[15]. Finally, neurons grow and form synapses with the rest of the circuit and muscles. So, what's new?

While the broad brushstrokes have been previously described[10,15], Mulcahy *et al.*[2] describe the transition in fine detail and extract several rules of organization. First, they describe the succession of developmental processes: neurogenesis, neurite growth, synaptic formation, and synaptic pruning, in this order, as they occur sequentially and iteratively along the anterior-posterior axis, from head to tail. The staggered transition implies a gradient of maturity, corresponding to the different co-existing transitional states (for example, Figure 1Aii). Second, the process of neurite growth is hallmarked by a growth-cone wrapping around future synaptic partners, other neurons, and non-neuronal tissue. These outgrowths then unwrap, leaving neural tracks in their place, reminiscent of intercellular communication during development in other systems. Finally, for each developing and state-transitioning neuron, presynaptic structures (output) mature before postsynaptic structures (input), presumably preventing aberrant activity and providing a final checkpoint for each functional switch to the mature configuration. If Svensson and the rest of the Swedes in September of 1967 were to (unwisely) emulate the nematode, they would have sounded a succession of loudspeaker announcements, sequentially changing the driving side from the Finnish border in the north to Malmö in the south.

To study the consistency of circuit output, Mulcahy *et al.*[2] complemented the EM reconstruction with calcium imaging and behavior analysis of stereotypic crawling. They demonstrated that the overall phase relationships between muscle activity and body curvature that underlie dorsoventral alternations are comparable before, during, and after the transition

from the juvenile to the mature circuit. Several hypotheses have been suggested[16–19] to explain how dorsal cholinergic and ventral GABAergic inputs to muscles can produce rhythmic alternating contractions in the early juvenile, specifically in a ventral bend. A computational model[16] in which ventral muscles are tonically active and rhythmically inhibited by GABAergic neurons, much like the result here, is now supported by the finding of cholinergic spillover. Most importantly, the continuous and staggered orchestration of the transition, its intricate details, and its extended duration open the field to further investigation of continuous structure-function transitions of neural circuits.

Indeed, among the exciting questions that this study opens up, a few are particularly noteworthy: what remains the function of the lethargus state between the first and second larval stages? How does the robust behavior persist during the transitional state in which the two configurations co-exist along the body? What are the local and global regulators that orchestrate the staggered functional transition, both in the circuit and in the ventral muscles? It will be interesting to determine whether this unsegmented animal nonetheless develops and operates with the same principles of repeating units along the body, as postulated by Haspel and O'Donovan[20]. The growing availability of complete nervous system reconstructions[2,13,14] makes it possible to address more immediate issues as well: What are the baseline levels and distribution of variability of connectivity across individuals[13]? And can we develop mathematical and computational models grounded in more detailed experimental data?
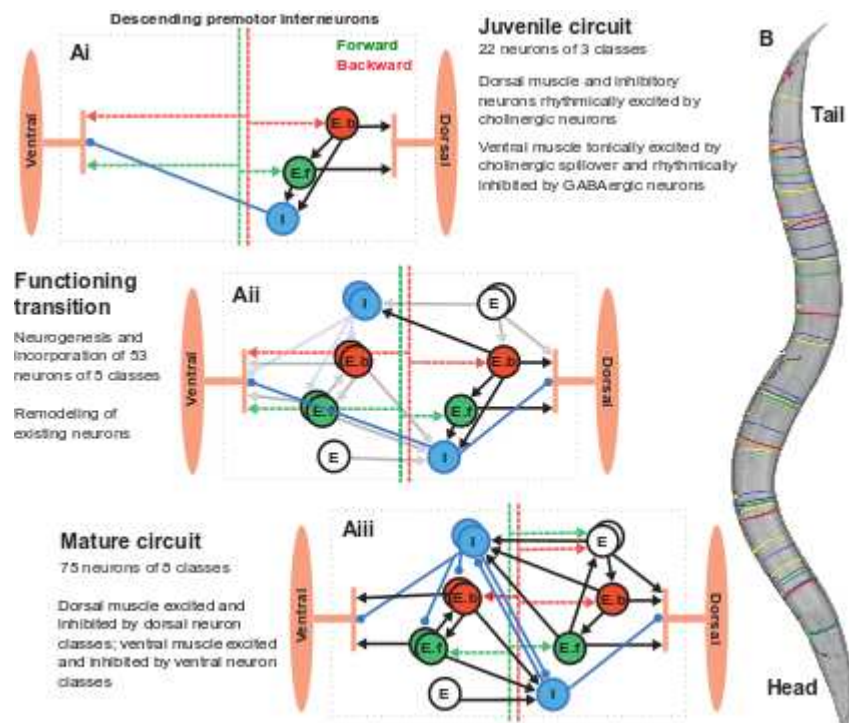
Figure 1. A small, juvenile circuit reorganizes by neurogenesis and synaptic remodeling sequentially and antero-posteriorly.

(Ai) In the juvenile configuration, each of the six units (one shown) along the nematode ventral cord[20] includes only three neurons[9]. Two excitatory neurons, dedicated to either forward (E.f; green) or backward (E.b; red) locomotion, excite dorsal muscle cells to induce a dorsal body bend. They also excite one inhibitory neuron (I; blue) that inhibits the opposing ventral muscles. When not inhibited, ventral muscles contract due to cholinergic spillover from interneurons to induce a ventral body bend. (Aii) The juvenile circuit can function uninterrupted as 53 neurons of five more neuronal classes and their postsynaptic connections are added to each functional unit. (Aiii) In the mature circuit, cholinergic neurons (two ventral and three dorsal classes; E.f, E.b, E) alternately excite ventral and dorsal muscle cells as well as one dorsal and one ventral GABAergic inhibitory classes (I) that inhibit the opposing muscle cells. (B) Schematic showing an adult hermaphrodite (with head pointing down) and undulatory locomotion circuit including neuronal cell bodies (all located in the ventral cord, left) and dorsoventral commissures for some classes of neurons. Each component of the process – neurogenesis, synaptogenesis, synaptic maturation, and pruning – is staggered beginning anteriorly and progressing posteriorly. (Ai,B) adapted from Haspel *et al.*[19].

Taken together, the new results unravel a transition from one functioning neural circuit configuration to another by gradual preparation at the subcellular scale, programmed and orchestrated structural transition at the cellular and circuit scale, and concomitant staggered switching of circuit activity, all without catastrophic failure. Tracing a neural circuit at synaptic resolution for multiple animals and over developmental time is an impressive technical feat that offers a priceless resource to the scientific community, opening new horizons for neural circuit research in this animal model and beyond.

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