

Imaging the Emergence of Behavior

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In this issue of *Cell*, Wan et al. (2019) track comprehensively the development of individual neurons, along with their activity, during zebrafish spinal cord development. They find that mostly motor neurons are the founders of initially small neuronal-activity ensembles, coalescing into larger populations establishing the first motor patterns.

The successful formation of functional circuits during nervous system development is crucial for the survival of animals. Development establishes the gross anatomy of circuits, which is often subsequently refined in an activity- and experience-dependent manner (Crair, 1999). Interestingly, developing circuits exhibit intrinsic, i.e., spontaneous and stereotypically patterned, activity, which was observed in many brain regions such as the spinal cord (SC), the retina, and the cerebellum (Blankenship and Feller, 2010). In motor circuits, such intrinsic activity must ultimately relate to motor patterns for behavior, including hundreds of motor and interneurons. The mechanisms and rules by which intrinsic correlated activity patterns across large neuronal populations emerge are not well understood. Systems-level approaches bring us closer to a better understanding of these events by shedding light on the generation and maintenance of these neuronal population dynamics. In this issue of Cell, Wan et al. (2019) applied a new strategy and successfully combined whole-zebrafishembryo developmental tracking of neurons and single-cell-resolution functional imaging in the SC to be able to observe the very first formations of motorpattern-related activity. For the first time, it was possible to track cells starting from neurogenesis until the formation of motor-like activity patterns across the SC. Thereby, they revealed basic rules by which neuronal population dynamics emerge as well as the relationships between these events and neurogenesis.

From the onset of neurogenesis, Wan et al. (2019) imaged the development of the whole zebrafish embryo by simultaneous multi-view light-sheet microscopy

(Tomer et al., 2012) and tracked the movements of the cells using a nuclear marker. The experimental setup allowed them to coherently continue with the functional imaging of several hundred neurons along the SC using the nuclear-localized pan-neuronal expression of a genetically encoded calcium indicator, GCaMP, by a high-sensitivity light-sheet microscope (Lemon et al., 2015). This powerful combination of two approaches enabled them to observe the emergence of motorrelated neuronal activity patterns while knowing the cellular identities and developmental histories of the members of the ensembles in great detail. Coordinated activity across neurons was quantified by factor analysis, among other measures. Approximately 1 h after individual neurons first exhibited spontaneous activity patterns, the authors could detect local ensembles of just one additional neuron per ensemble being recruited initially (phase I, Figure 1A). Later, these ensembles grew on both sides of the SC (phase II, Figure 1B), and only thereafter, left-right alternating motor-like patterns got established (phase III, Figure 1C).

Interestingly, Wan et al. (2019) found out that the pioneers in the initiation of patterned activity in phase I (Figure 1A) are mostly motor neurons. These leader cells exhibit richer dynamics initially, and then they form local ensembles by recruiting surrounding cells. This is surprising, as interneurons are crucial members of SC central pattern generators (Kiehn, 2016). Moreover, in line with the study of Song et al. (2016) in adult zebrafish, this remarkable finding further demonstrates the role of motor neurons as active functional units in neuronal circuits rather than passive targets of upstream signals.

Furthermore, using SC transection experiments, the authors found out that ensembles can still form partially, even with left-right alternating patterns, but now independently in the remaining segments anteriorly and posteriorly to the lesion sites. They further found that intact middle segments of the SC were crucial to let ensembles grow but that the size of the transectioned segments also mattered; i.e., only ensembles of sufficient size led to complete motor-like patterns. These results strongly suggest that descending inputs to the SC are dispensable for motor-pattern development, which instead starts with a dispersed set of leader cells and then expands through the circuit by coalescence of many local sub-ensembles in a sizedependent manner but without any hierarchy between them.

Furthermore, Wan et al. (2019) collected evidence that inhibitory interneurons (INs) also play crucial roles, though at the later stages. When interfering with glycinergic neurotransmission, phases II and III did not properly develop. Moreover, commissural INs were required for final global left-right alternating patterns (Figure 1C).

The successful coupling of whole-embryo developmental imaging prior to the functional-imaging experiments placed the authors in a unique position with access to birth times, lineage history, and migration tracks of every single neuron they recorded. Thus, they were able to investigate how the developmental features of the cells relate to the formation of patterned activity and found that, for example, birth time and activation time of neurons are highly correlated consistently across fish.



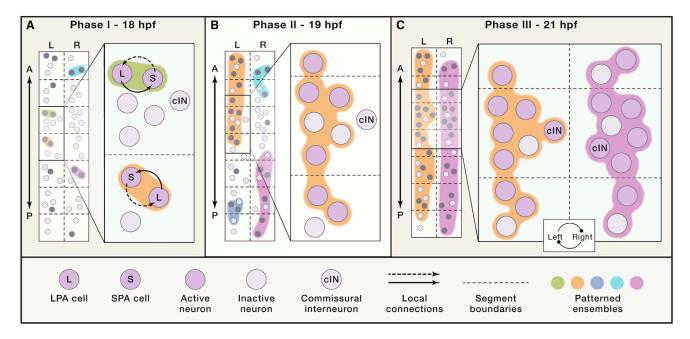


Figure 1. Emergence of Patterned Activity in the Spinal Cord

Schematic visualization of coordinated neuronal population activity through different phases of development in the embryonic zebrafish spinal cord. Left: dorsalview map of the spinal cord. Colored patches indicate different ensembles with active and inactive neurons. Right: close-up view of a single segment. Adapted from Wan et al. (2019). Horizontal dashed lines, segment boundaries; vertical dashed lines, midline; filled and open circles, active and inactive neurons, respectively; A, anterior; P, posterior; L, left; R, right; hpf, hours post fertilization; LPA, long pre-ensemble activity; SPA, short pre-ensemble activity. (A) Phase I indicates the formation of the very first ensembles between neuronal pairs driven by leader LPA cells dispersed through many segments of the spinal cord.

(B) In phase II, neuronal ensembles expand through the recruitment of additional neurons and coalescence of multiple sub-circuits.

(C) Phase III concludes the maturation of the circuit by the recruitment of commissural interneurons, which is necessary for establishing coordination between left and right sides of the spinal cord.

The framework they presented enables future studies to compare many circuits in different regions of the nervous system across individuals and also provides a means to compare similar circuits throughout the development of different organisms. This work therefore opens up exciting avenues for future research. It would be interesting to study the details by which local ensembles get established: What is special about the founder cells; are these specified by genetic programs, or do they emerge from stochastic processes? How are additional neurons recruited; is there a role of gap junctions and/or plasticity mechanisms in chemical synapses? We are looking forward to these and similar studies of other circuits in the brain and

to learning whether similar principles apply there.

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