Biophysical *Journal*
New and Notable

Biophysical Society

How to Make a Worm Twitch

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Understanding how the nervous system generates behavior is a long-standing goal of neuroscience. The early nervous system develops alongside other tissues and organs in the course of embryogenesis and is crucial for establishing the organism's early behavioral repertoire, including its ability to perform coordinated movements. The roundworm *Caenorhabditis elegans* has long been an important model system for studying developmental processes and offers several powerful features that aid such investigations, including its rapid embryonic development and its known and invariant cell lineage (1). In addition, the wiring diagram of the *C. elegans* nervous system has been fully reconstructed, which aids investigations of nervous system function. However, although extensive work has been undertaken to better understand the development of the nervous system, very little is known about the dynamics and emerging functional properties of the embryo's developing nervous system and muscles.

Functional imaging with genetically encoded calcium indicators has become an indispensable tool for recording neuronal activity in vivo and elucidating the principles and function of neuronal circuits (2). Such imaging assays benefit from the key capabilities of light microscopy, including the ability to perform quantitative measurements across relatively large spatial scales noninvasively and with high spatiotemporal resolution. Thereby, it is possible to obtain detailed information about the functional state and activity of many neurons in the nervous system simultaneously and under physiological conditions. However, such investigations also frequently face major technical challenges. The *C. elegans* embryo, for example, is fairly sensitive to light exposure and exhibits rapid movements inside its eggshell, which substantially complicate image acquisition. An effective solution to these problems has been established with the iSPIM light-sheet microscope developed by Wu et al. (3).

Over the past decade, light-sheet microscopy has emerged as a powerful method for live imaging in developmental biology (4,5), cell biology (6), and neuroscience (7). The fundamental idea in light-sheet microscopy is to illuminate the specimen with a very thin sheet of laser light from the side while imaging this illuminated volume section with a wide-field detection system. This approach provides intrinsic optical sectioning (i.e., the ability to perform three-dimensionally resolved imaging of a volume) and, furthermore, offers high imaging speeds at high spatial resolution and low light exposure.

In this issue, Ardiel et al. (8) present a skillful and insightful demonstration of light-sheet-based functional imaging of entire *C. elegans* embryos with their iSPIM microscope. The authors very convincingly show that iSPIM is an excellent methodological choice for functional imaging in this model system, considering their microscope's suitability for cover-slip-based sample mounting and its high spatiotemporal resolution during volumetric imaging. Using iSPIM, they reach a volumetric imaging rate of up to 5 Hz for the entire *C. elegans* embryo and achieve a spatial resolution of 0.5/1.5 μm laterally/axially, which is sufficient to systematically record activity in individual neurons and muscles throughout the embryo. The combination of this imaging strategy with computational approaches to untwisting the embryo (9) and tracking calcium dynamics in neurons and muscles through phases of rapid and complex sample motion leads to an impressive analysis of neuronal activity in the early embryo.

Ardiel et al. (8) took advantage of their whole-embryo calcium imaging assay and image analysis methods to identify correlations between activity in neurons and muscles on the one hand and movements of the embryo on the other hand. Thereby, they found that the very first twitching events in the *C. elegans* embryo occur in dorsal muscle bundles and are accompanied by large spreading calcium waves. Detailed quantitative analyses involving the tracking of muscle bundles and behavioral metrics for embryonic movements and rotation furthermore revealed the activity patterns underlying early axial rotation. After this investigation of calcium transients in muscles during

Submitted March 7, 2017, and accepted for publication March 28, 2017.

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Editor: Stanislav Shvartsman.
http://dx.doi.org/10.1016/j.bpj.2017.03.035

myogenic activity and early coordinated movements, Ardiel et al. (8) then utilized iSPIM for brainwide imaging of neuronal calcium dynamics in the freely behaving embryo. The rapid movements of the embryo inside its eggshell raise the question of how these early motor programs are implemented and executed at the cellular level. However, realizing quantitative analyses of calcium dynamics in the nervous system during such phases of fast and complex motion is technically very challenging. Using computational approaches to cell segmentation and tracking as well as their impressive "untwisting software" for straightening the roundworm's body axis (9), the authors overcame these obstacles very effectively. By evaluating the resulting single-neuron activity traces side-by-side with the respective concurrent muscle contractions, Ardiel et al. (8) provide intriguing insights into the functional properties of the embryo's early nervous system. For example, by identifying reversals of the embryo inside the eggshell, they found that these movements are associated with activity in the AVAL and AVAR command interneurons.

The study by Ardiel et al. (8) represents an important step forward in our ability to record nervous system activity at high resolution and throughout entire, rapidly moving organisms. The detailed data on functional activity in developing *C. elegans* embryos collected by the authors suggest many intriguing hypotheses that can now be tested, e.g., by functional perturbation of individual circuit components and by evaluating the impact of these perturbations on network activity and associated behavior.

In the future, it will also be exciting to use light-sheet-based approaches to study the early nervous system of other model systems, including vertebrates such as the zebrafish and higher invertebrates such as *Drosophila melanogaster* (10), which exhibit different body plans, behavioral repertoires, and nervous system architectures. With the availability of such quantitative data and functional investigations in multiple species, it will eventually become possible to elucidate the fundamental rules and key principles underlying development and emergence of function in the nervous system across the animal kingdom.

ACKNOWLEDGMENTS

This work was supported by the Howard Hughes Medical Institute.

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