Zebrafish study reveals first fine structure of a complete vertebrate brain

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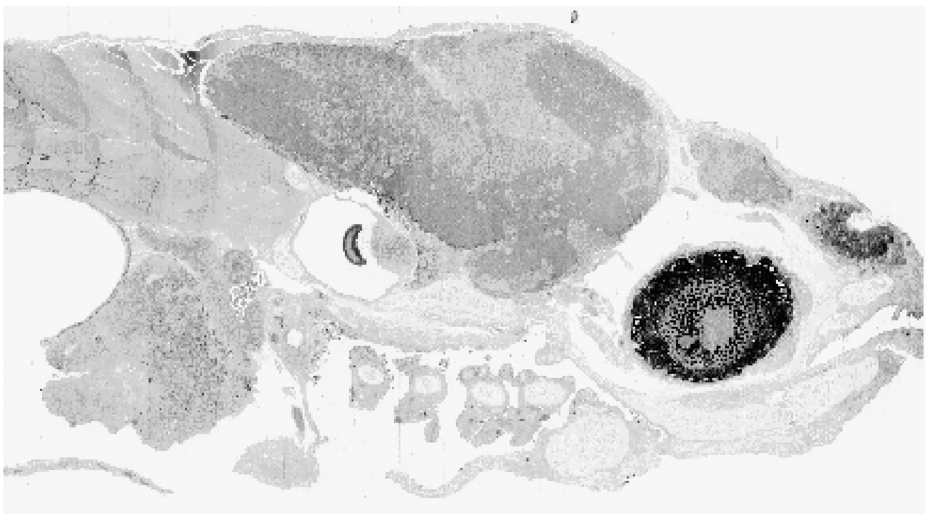

Zebrafish larva head in cross-section. Note this isn't a single electron microscope image, but many thousands of slices seen on edge. The large black circle is the eye, the big darker gray shape at top the brain. You can also see the nose (black crescent at right), the ear (black crescent near center) and several vertebrae (center bottom). Credit: Pittsburgh Supercomputing Center

Every thought, every feeling, every sensation—and every behavioral illness—ultimately depends on how our brains work. Despite decades of stunning advances in imaging the brain and measuring its activity, though, we still don't understand how even a simple vertebrate brain works.

Enter the zebrafish larva. Small and transparent—yet able to swim freely and even hunt small prey—these baby fish have long been studied by researchers to understand how their tiny brains generate behaviors. David Hildebrand, working in the laboratories of Florian Engert and Jeff Lichtman at Harvard University, took this work a step farther, creating electron microscopic images of the zebrafish brain cut into tens of thousands of slices.

With the help of co-author PSC's Art Wetzel, they led an international collaboration that used these images to reconstruct specific nerve cells that spanned nearly the entire larval zebrafish brain. The hope is that this kind of thorough "nano-scale" imaging will make it possible to extract the brain's complete "wiring diagram." While this work has only just begun, it may eventually shed new light on past studies of zebrafish behavior—and point the way toward a better understanding of more complex brains, such as ours.

"Our goal [was] to develop techniques that allow researchers to examine the morphology and circuit connectivity of any neuron in the brain of a larval zebrafish at about five days after fertilization. This is when interesting zebrafish behaviors such as hunting emerge, giving us the opportunity to ask how circuits of neurons parse incoming information from the environment to generate useful behavioral outputs," says David Hildebrand.

To generate image datasets containing all the nerve cells in the zebrafish brain and their many intricate connections, then-graduate-student Hildebrand had to dig deeper than the previous studies. Using a technique developed by Lichtman's laboratory, he cut the front quarter of the zebrafish larva—a total length of 1 millimeter, or about 4 hundredths of an inch—into more than 18,000 slices. Then he used an electron microscope to get images of these slices. The slices, though, are inevitably imperfect, with some varying in thickness and having tears and other defects. To recombine the distorted images to reconstruct the brain in three dimensions, Hildebrand needed advanced automated image registration techniques.

To "un-distort" these images, Wetzel used SWiFT (Signal Whitening image Fourier Transform), software he developed as part of PSC's involvement in the National Center for Multiscale Modeling of Biological Systems. SWiFT gave the scientists the ability to handle distortions and defects stemming from tissue variations, compression of slices, and image distortions caused by the electron microscope's inner workings. Thanks to Wetzel's work, fewer than 1 percent of Hildebrand's slices could not be used in the analysis.

Some 12,500 of the slices contained parts of the brain. The scientists examined these in more detail, collecting a massive 4,900 gigabytes of data in the process—enough to fill five to 10 high-end laptops. They fully or partially traced the path of about 2,500 nerve cells and their axons—the long tails the cells use to connect with other nerve cells. The investigators were able to follow 805 of these nerve cells over the entire length of their axons through the brain. One early finding is that certain nerve fibers on one side (left or right) of the fish brain have twin fibers on the other side. The organization of axons within these nerves on each side followed nearly mirror-image paths. While the scientists don't know exactly what this means yet, they suspect that it may have something to do with a pre-programmed brain development process. This could also be an important clue for a number of inborn behaviors fish follow. It isn't yet clear whether nerve cells in the human brain, which develops slowly and changes greatly throughout life, will have the same degree of left/right symmetry. The collaborators published their initial findings in the prestigious journal *Nature* on May 10, 2017.

"What makes the zebrafish such a spectacular system is that the alternatives in other organisms for deriving wiring diagrams are limited to a tiny, tiny part of a much larger brain, and so don't offer the opportunity to study the full range of an organism's behavior. Nobody previously had dared to think of doing this kind of work in a whole brain," says Florian Engert.

More information: David Grant Colburn Hildebrand et al. Whole-brain serial-section electron microscopy in larval zebrafish, *Nature* (2017). DOI: 10.1038/nature22356

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