BEHAVIORAL METHODS

Breaking down behavior

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To understand a complex problem, it can be helpful to break that problem down into smaller, simpler pieces. That's been a common—if controversial—approach in classical ethology, says João Marques, a post-doctoral fellow at the Rowland Institute at Harvard in Cambridge, MA, especially as researchers try to decipher the underlying connections between the brain and a behavior. In the past, attempts to establish basic units of movement that can be combined into more complex behaviors have relied heavily on the human eye. But do such units actually exist, or is behavior a continuum that cannot be broken down without human bias? While a PhD student in Michael Orger's lab at the Champalimaud Centre for the Unknown in Lisbon, Portugal, Marques wanted to take people out of the equation.

"This was my motivation. I really wanted to know if movements are a continuum," he says, "or if they're made of units, and if that's the case, how many there are." His idea was to develop an automated algorithm that could break down behavior without subjective humans in between. His model of choice to prove the concept was the larval zebrafish, an animal that swims in fairly discrete patterns and bouts of motion. That characteristic makes it a bit easier to determine when a particular movement starts and finishes, compared to other common laboratory species such as *Drosophila*, he explains. Prior work involving people had broken larval zebrafish behavior into seven simpler categories of movement, providing a benchmark to compare against the computer analysis.

To get to that point, the first step was simple, albeit a bit time-intensive: collect a

Eye & tail tracking. Credit: Image adapted from Curr. Biol. **28**, 181–195 (2018)

lot data. Over the course of the first year of the project, he and his colleagues worked out a real-time, high-speed video tracking system that could detect what the zebrafish were up to in a small arena; they then spent three years recording the behavior of 3000 zebrafish as they swam about and responded to as many stimuli as the group could devise. The dataset ultimately contained tens of thousands of continuous behavioral sequences. Each element observed in the fish, from the angles of its tail to the direction of its eyes, was then converted into a data point and assembled in a multidimensional space. From there, it was a matter of grouping each data point into clusters. The problem became a mathematical one, but, as Marques soon found out, one that no one had solved before. "You can understand my frustration after I spent all this time gathering the data that then I couldn't analyze. But we found a way in the end," he says. A few more years later—seven total—Marques had developed Clusterdv (*bioRxiv* doi: 10.1101/224840; 2017), an automated computational method that could appropriately analyze the data clusters from all those hours of zebrafish tracking.

When put to the task, Clusterdv identified all seven movements previously identified in other ethological studies of zebrafish larvae, like J-turns, C-starts, and O-bends, as well as six novel ones, like the spot avoidance turn. That's a total repertoire

of 13 locomotor motions that the zebrafish can combine to perform complex swimming behaviors, such as hunting rotifers. "They have this very particular and very beautiful way of hunting, and it's really difficult to understand what they're doing. However, if you look at the data, by segmenting the data into these bout types, it becomes clear and much easier to understand," Marques says, "They are made of sequences of simple movement."

Marques would now like to create a brain map behind these 13 basic bout types. Not long ago, that would have been a particularly difficult task—to image the entire zebrafish brain with older microscopy methods larvae needed to be restrained, an obvious problem when observing movement is the goal. But last fall, he and colleagues at the Rowland Institute published their work developing a calcium imaging-based method to track single cells in the brains of freely moving zebrafish (*Nat. Methods* **14**, 1107–1114; 2017). That advance means he can observe and determine which of the approximately 100,000 neurons in the zebrafish brain become active with each motion.

The possibilities aren't limited to just zebrafish larvae, Marques explains. In principal, Clusterdv can be applied to any collection of data. "It's very, very, very general," he says; if enough high-resolution tracking data can be generated for an animal, whether it be a mouse, a fly, or any other species, the clustering method can be applied to break down behaviors. "One of the ends of the study was to do this for the entire repertoire of one animal, to show that this was possible," Marques says, "Now people can apply it to their particular questions."

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