Medical Xpress

Competing pathways affect early differentiation of higher brain structures

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A male *Cynotilapa afra* is shown in a tank in the laboratory of Todd Streelman at Georgia Tech. Credit: Gary Meek

Sand-dwelling and rock-dwelling cichlids living in East Africa's Lake Malawi share a nearly identical genome, but have very different personalities. The territorial rock-dwellers live in communities where social interactions are important, while the sand-dwellers are itinerant and less aggressive.

Those behavioral differences likely arise from a complex region of the brain known as the telencephalon, which governs communication, emotion, movement and memory in vertebrates – including humans, where a major portion of the telencephalon is known as the cerebral cortex. A study published this week in the journal *Nature Communications* shows how the strength and timing of competing molecular signals during brain development has generated natural and presumably adaptive differences in the telencephalon much earlier than scientists had previously believed.

In the study, researchers first identified key differences in gene expression between rock- and sand-dweller brains during development, and then used small molecules to manipulate developmental pathways to mimic natural diversity.

"We have shown that the evolutionary changes in the brains of these fishes occur really early in development," said Todd Streelman, an associate professor in the School of Biology and the Petit Institute for Bioengineering and Biosciences at the Georgia Institute of Technology. "It's generally been thought that early development of the brain must be strongly buffered against change. Our data suggest that rock-dweller brains differ from sand-dweller brains – before there is a brain."

For humans, the research could lead scientists to look for subtle changes in brain structures earlier in the development process. This could provide a better understanding of how disorders such as autism and schizophrenia could arise during very early brain development.

The research was supported by the National Science Foundation and published online April 23 by the journal.

"We want to understand how the telencephalon evolves by looking at genetics and developmental pathways in closely-related species from

natural populations," said Jonathan Sylvester, a postdoctoral researcher in the Georgia Tech School of Biology and lead author of the paper. "Adult cichlids have a tremendous amount of variation within the telencephalon, and we investigated the timing and cause of these differences. Unlike many previous studies in laboratory model organisms that focus on large, qualitative effects from knocking out single genes, we demonstrated that brain diversity evolves through quantitative tuning of multiple pathways."

In examining the fish from embryos to adulthood, the researchers found that the mbuna, or rock-dwellers, tended to exhibit a larger ventral portion of the telencephalon, called the subpallium – while the sand-dwellers tended to have a larger version of the dorsal structure known as the pallium. These structures seem to have evolved differently over time to meet the behavioral and ecological needs of the fishes. The team showed that early variation in the activity of developmental signals expressed as complementary dorsal-ventral gradients, known technically as "Wingless" and "Hedgehog," are involved in creating those differences during the neural plate stage, as a single sheet of neural tissue folds to form the neural tube.

Georgia Tech researcher Jonathan Sylvester examines a group of fishes to look for brooding cichlids. After spawning, females hold their embryos in their mouths, so he looks for fish with closed mouths and protruding lower jaws. Credit: Georgia Tech Photo: Gary Meek

To specifically manipulate those two pathways, Sylvester removed clutches of between 20 and 40 eggs from brooding female cichlids, which normally incubate fertilized eggs in their mouths. At about 36 to 48 hours after fertilization, groups of eggs were exposed to small-molecule chemicals that either strengthened or weakened the Hedgehog signal, or strengthened or weakened the Wingless signal. The chemical treatment came while the structures that would become the brain were little more than a sheet of cells. After treatment, water containing the chemicals was replaced with fresh water, and the embryos were allowed to continue their development.

"We were able to artificially manipulate these pathways in a way that we

think evolution might have worked to shift the process of rock-dweller telencephalon development to sand-dweller development, and vice-versa. Treatment with small molecules allows us incredible temporal and dose precision in manipulating natural development," Sylvester explained. "We then followed the development of the embryos until we were able to measure the anatomical structures – the size of the pallium and subpallium – to see that we had transformed one to the other."

The two different brain regions, the dorsal pallium and ventral subpallium, give rise to excitatory and inhibitory neurons in the forebrain. Altering the relative sizes of these regions might change the balance between these neuronal types, ultimately producing behavioral changes in the adult fish.

"Evolution has fine-tuned some of these developmental mechanisms to produce diversity," Streelman said. "In this study, we have figured out which ones."

The researchers studied six different species of East African cichlids, and also worked with collaborators at King's College in London to apply similar techniques in the zebrafish.

As a next step, the researchers would like to follow the embryos through to adulthood to see if the changes seen in embryonic and juvenile brain structures actually do change behavior of adults. It's possible, said Streelman, that later developmental events could compensate for the early differences.

The results could be of interest to scientists investigating human neurological disorders that result from an imbalance between excitatory and inhibitory neurons. Those disorders include autism and schizophrenia. "We think it is particularly interesting that there may be some adaptive variation in the natural proportions of excitatory versus

inhibitory neurons in the species we study, correlated with their natural behavioral differences," said Streelman.

Provided by Georgia Institute of Technology