NEWS

Songs, squeaks combine to tell story about human language

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Full circle: Researchers are teasing apart an autism-related signaling pathway by studying mice and birds at the same time.

Birdsong is a valuable tool for studying language, and mice are the models of choice for genetic manipulation of behavior. Together, birds and mice can yield unparalleled insights into human language, suggests unpublished research presented Sunday at the **2014 Society for Neuroscience annual meeting** in Washington, DC.

Male zebra finches sing courtship songs that they learn by listening to and imitating others of their species, **much the way humans learn language**. But researchers lack molecular tools to study them.

Mice are easy to manipulate genetically, so researchers can isolate the role of individual genes in producing vocal communication. But mice don't learn their vocalizations the way birds and people do.

Combining studies of the two animal models allowed researchers to probe the reelin signaling pathway, which contains several genes linked to autism.

"The work presented on the poster is a beautiful example of the strengths of comparative work," says **Claudio Mello**, associate professor of behavioral neuroscience at Oregon Health and

Science University. Mello was not involved in the study, but he runs the **Zebra Finch Expression Brain Atlas (ZEBrA)**, a **catalog of gene expression data** for the species.

Area X factor:

When young male birds sing, levels of reelin increase in a part of the brain called Area X, which is found only in males and is a key part of the song circuit. This loop of interconnected brain structures is similar to the neural circuit involved in human speech. The gene encoding reelin, **RELN**, has popped up in some autism genetics studies, and people with autism have **lower levels of this protein** in their brains than controls do.

Singing birds also show more phosphorylation, a marker of protein activation, of a protein called DAB1 in Area X. DAB1, a component of the reelin signaling pathway, has also been **linked to autism**.

Crucially, singing doesn't trigger reelin expression or DAB1 phosphorylation in the ventral striatopallidum, which is not part of the bird's song circuitry. And in female zebra finches, which call but do not learn individual songs, the researchers found no relationship between the frequency of calls and the level of reelin or DAB1 phosphorylation in the brain.

The effect of singing on reelin signaling "seems to be specific to males and specific to the song system," says Elizabeth Fraley, a graduate student in **Stephanie White**'s lab at the University of California, Los Angeles, who presented the poster.

The zebra finches helped the researchers understand how behavior shapes gene expression. To explore how gene expression shapes behavior, they turned to mice.

The researchers recorded ultrasonic vocalizations produced by mice [lacking DAB1 or VLDLR, another member of the reelin signaling pathway. A third set of mice was missing both VLDLR and a third pathway gene, ApoER2.] Mouse pups **emit these high-pitched calls** when they are separated from their mother.

Mice lacking DAB1 call slightly more frequently than controls do when they are 7 days old. At 14 days old, they call a lot more. That's unexpected because typically, ultrasonic vocalizations peak at 7 days of age and then decrease as the pups stop needing their mothers. "We think this may be a delayed communication phenotype," Fraley says.

[The researchers also tested another strain of mice lacking DAB1 using a different genetic engineering technique. These mice also have communication deficits, although the specifics differ from those seen in the first strain: They call less (not more) frequently than controls at 7 days old, and show no difference from controls at 14 days. They don't exhibit the typical decrease in ultrasonic vocalizations that occurs during the second week of life as pups stop needing their

mothers.

This second strain of DAB1 mice also tends to use simpler calls and has a less extensive call repertoire than control mice do. These pups could be at a disadvantage because female mice respond better to pups that call more frequently and emit more complex calls. Similar abnormalities are also present in mice with one copy of the DAB1 gene.]

Mice lacking VLDLR, which has also been **linked to autism in a few studies**, show alterations in their vocalizations, albeit less obvious ones than the DAB1 mice. “A more subtle phenotype is what you would expect,” Fraley says, because the loss of this protein has a mild effect on the overall activity of the reelin signaling pathway.

These mice [call just as often as control mice, but] have a simpler repertoire of vocalizations than controls do. [The researchers also recorded vocalizations of mice lacking both VLDLR and ApoER2. These mice have even more limited calling repertoires than those lacking VLDLR alone.]

[An initial analysis showed that] male and female mice lacking VLDLR emit more short calls, and females make fewer calls of complex types, than do controls. [A larger sample size failed to turn up extensive sex differences in the effects of VLDLR loss. “We found small differences in call amount, and a few differences in repertoire, but none that would make us think one sex was more adversely affected by gene-loss than the other,” Fraley says.]

The researchers plan to circle back to the zebra finches and unravel the cellular mechanisms of reelin expression in Area X. This flexibility is one of the strengths of the two-species model, Mello says. “Information from one organism helps generate hypotheses about mechanisms that can only be tested in the other organism, and vice versa.”

[In the meantime, the researchers suggest that the DAB1 mouse is a potential model of autism. Mice lacking both copies of the gene have severe motor deficits that would make them difficult to study, but those with one intact copy of the gene could shed light on the communication deficits of autism, they say.]

*For more reports from the 2014 Society for Neuroscience annual meeting, please **click here**.*

REFERENCES:

1. Fraley E.R. *et al. Sci. Rep.* **6**, 25807 (2016) **PubMed**