

NEWS

‘Triple-hit’ study may help explain autism’s male bias

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The absence of an autism-linked gene, combined with exposure to a mock infection in the womb, produces social deficits in mice — but only in males, according to a new study¹.

The results suggest that a ‘three-hit’ model of autism risk — genes, environment and sex — could help clarify the gender disparity in autism. (More than four times as many boys as girls are diagnosed with autism.)

The results, published 7 February in the *Proceedings of the National Academy of Sciences*, provide proof of principle for this new hypothesis.

“This is the first explicit test,” says study leader **Donald Pfaff**, professor of neurobiology and behavior at Rockefeller University in New York City. His study looked at mice lacking **CNTNAP2**, a leading autism candidate. “There will have to be many other [studies] with other mutated autism-related genes and other forms of stress,” he says.

The new model adds sex to the traditional ‘two-hit’ models of autism risk, which look at interactions between genes and the environment. “One of the novel things of this paper is considering sex to be a hit,” says **Larry Young**, chief of behavioral neuroscience at Emory University in Atlanta, who was not involved in the work.

Muted cries:

The researchers exposed pregnant mice to lipopolysaccharides (LPS), a class of molecules that mimic a bacterial infection. Pups born to pregnant mice exposed to LPS are known to **show autism-like social deficits**. Molecules released by the maternal immune system in response to infection may affect the developing brain.

Some of the pups in the study lacked a working copy of CNTNAP2. Mice missing CNTNAP2 have **impairments in social behavior** and communication.

When the pups were 3 days old, the researchers recorded high-pitched cries, or ultrasonic vocalizations, that each pup made when separated from its mother. These cries may **mirror the communication problems seen in autism**.

Mice that have one of the three hits — that is, are either male, were exposed to mock infection in the womb or lack CNTNAP2 — emit fewer ultrasonic vocalizations than do female controls. Those with two of these risk factors emit even fewer cries. And those with three hits — males lacking CNTNAP2 that were born to LPS-exposed mothers — make the fewest cries of all.

“They’ve taken a very complicated design and presented it in a way that is very easy to understand,” says **Jason O’Connor**, assistant professor of pharmacology at the University of Texas, San Antonio. “So I think, conceptually, it’s an important step for the field.” O’Connor was not involved in the work but has studied **CNTNAP2 mutants** exposed to infection in the womb.

Memory mechanism:

Once the mice in the new study reached adulthood, Pfaff’s team noted a change in their social behavior. Mice with zero, one or two hits are eager to sniff and investigate a new visitor to their home cage; their interest in the mouse wanes when it visits again.

Mice with three hits don’t show this pattern. They spend a relatively brief amount of time sniffing a visitor mouse, no matter how many times it has visited.

Mice with the triple hit also have altered gene expression in the **hippocampus**, the brain’s memory center. For example, in the left hippocampus, these mice show dampened expression of a gene for corticotropin-releasing hormone, a molecule involved in the stress response.

Finding a molecular alteration in the brain bolsters the results of the behavioral studies, Young says. The hippocampus and the **stress response** are both implicated in autism.

The three-hit combination may also alter gene expression in other brain regions, Pfaff says. In addition, genes in other pathways may contribute to the mice’s altered social behavior.

REFERENCES:

1. Schaafsma S.M. *et al. Proc. Natl. Acad. Sci. USA* **114**, 1383-1388 (2017) [PubMed](#)