

Q&A

# Questions for Alysson Muotri: Applying autism tools to Zika

BY JESSICA WRIGHT

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On the face of it, the **Zika virus** has little to do with autism. But for one autism researcher, Zika's effect on fetal development and the brain hits close to home. **Alysson Muotri**, a native Brazilian, had been following the news about Zika in his home country closely. He realized he could use techniques he had honed to study autism to better understand this public health threat.

Muotri and his colleagues infected pregnant mice with the Zika virus and found that the virus crosses the placenta and causes birth defects, such as an abnormally small head size (microcephaly) in the pups. They also showed that the virus kills human stem cells grown in culture.

The team then looked at the effect of the Zika virus on stem-cell structures that **resemble miniature brains**, which Muotri had perfected for his work in autism. In these organoids, different types of neurons develop into layers like those in the human brain. Zika virus infection disrupts this organization, Muotri's team found.

**Cell death:** Clusters of brain cells grown in culture that have been infected with the Zika virus (left) are much smaller than uninfected spheres (right).

Muotri and his colleagues published this work last month in *Nature*<sup>1</sup>, at the same time as two other studies published in *Cell* and *Cell Stem Cell*<sup>2,3</sup>. Together, the three papers presented the first conclusive evidence that Zika infection in a pregnant woman can cause birth defects, including microcephaly, in her child.

We asked Muotri how he uses organoids to better understand the Zika virus' effects on the brain,

and what this work might reveal about autism.

***Spectrum:* What is the advantage of using mini-brains over a mouse model to study the brain?**

**Alysson Muotri:** Organoids, or mini-brains, provide really good insights into human brain development. When stem cells grow in a three-dimensional suspension, instead of flat on a petri dish, they self-organize just as they would in real life. The stem cells that eventually become neurons grow and divide. Then they migrate out to form the layers that make up the cerebral cortex, the brain's outer shell.

Mouse models are also useful, but mouse brains evolved very differently from human brains and thus have limitations for telling us about humans. This is true for any animal model. There are human-specific cells that help neurons migrate during development into the correct layer of the cortex. So a human model is better for recapitulating the early stages of human development.

**S: How closely do these organoids resemble the human brain?**

**AM:** From a gene expression perspective, mature organoids (6 to 8 months old) resemble a first-trimester fetal brain. Based on shape, the cortical layers are neither as well defined nor as complete as they are in real life. We aren't able to reproduce the blood vessels that traverse the brain, for example; nutrients can only reach the center of these organoids by diffusion. As the ball of cells grows, the nutrients take longer to reach the center and the organoid stops growing.

**S: What could the mouse tell you about Zika?**

**AM:** The mouse model showed that if a pregnant woman is infected with Zika, the virus may be able to enter the placenta and cross into the brain of the fetus. This is not an easy task for a virus. The experiment really proved causation. Despite the previous epidemiological and clinical evidence, there was no definitive proof that the Zika virus was sufficient to cause birth defects. Showing that an agent is in the crime scene does not make it guilty. Our results with mice drove the attention to the real cause.

But when it comes to the effect on the brain, mice are actually naturally resistant to infections. In humans, the virus is very aggressive and we see complete destruction of cells across the brain of the child. In the mouse, the effect of viral infection is minimal in several brain regions.

The reason for this phenomenon is that the virus kills human cells rapidly, and much faster than it kills mouse cells. We were able to discern this by looking in the cerebral human organoids.

**S: What did you learn from your Zika experiments that might apply to autism?**

**AM:** We are using both mouse models and human organoids to study the effect on the brain of mutations in autism-linked genes. In some cases, autism mutations that have clear effects on people have little impact on mouse behavior.

So instead of focusing on behavior, we're looking for malformations in the cortex of these mice. And then we're using human brain organoids in parallel to measure the impact of the mutation on human cells. So far, we're seeing that some of the autism mutations that have little effect on mouse behavior significantly alter these human organoids.

The study is also a model for testing the effect of the environment on the developing human brain, and how environmental factors might interact with autism-linked mutations. In a way, Zika is a dramatic example of an environmental factor that affects brain development.

A plethora of autism studies **speculate about environmental factors**: If you live close to a highway, there's reportedly an increased risk of autism, for example. But these associations provide no proof that this factor actually caused autism. We may be able to use these mini-brains to figure out exactly what kind of impact certain environmental influences have on embryonic development.

**S: Are there parallels between the microcephaly seen with Zika infection and that in some children with autism?**

**AM:** That's a really interesting question. In our initial experiments, we infected the mice with a high dose of virus, to make sure we could see an effect. But we're also repeating the mouse and organoid experiments using less virus, and we see a partial effect on the brain.

The findings suggest that there are children in Brazil who lack microcephaly or brain abnormalities detectable by brain scans, but who still have subtle changes to their neurons resulting from prenatal Zika infection. My prediction is that we may see a generation of kids with autism or developmental delays caused by the Zika virus.

**REFERENCES:**

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