NIH Awards Zylka Lab $6.8 Million to Study Autism, ADHD Environmental Risks

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This eight-year project will focus on the interactions between genetics and environmental exposures that may contribute to neurodevelopmental disorders such as autism and attention deficit disorder.

Although scientists have made significant progress identifying gene mutations linked to neurodevelopmental disorders, such as autism and attention deficit/hyperactivity disorder, they have not investigated to the same extent the environmental factors that might cause these disorders. Mark Zylka, PhD, director of the UNC Neuroscience Center, is one of the few scientists who have studied both aspects. Today the National Institutes of Environmental Health Sciences, part of NIH, awarded his lab $6.8 million to further his team's scientific investigations through a unique three-pronged approach over the next eight years.

First, Zylka's team will identify environmental chemicals and mixtures that target particular pathways important for brain development in fetuses and babies. These pathways were previously implicated in autism from genetic studies, and include synaptic signaling, neuroinflammation, and Wnt/beta-catenin signaling.

For the second part of the NIH study, Zylka and his team will characterize real-world exposures to various chemicals currently in the environment, such as agricultural pesticides and valproic acid, which is used to treat epilepsy, bipolar disorder, and migraine headaches. Zylka's preliminary work showed (see page 2-3) that a commonly used class of fungicides produce gene expression changes in brain cells similar to the changes seen in people with autism and neurodegenerative diseases, such as Alzheimer's and Huntington's disease.

Lastly, Zylka's team will focus on specific gene variants known to be associated with autism. The researchers will use animal models to investigate how susceptible genes influence the level of toxicity in cells and, as a result, lead to neurodevelopmental problems.

"We currently lack a way to systematically evaluate which environmental-use chemicals have the greatest potential to harm the developing brain," said Zylka, the W.R. Kenan Distinguished Professor of Cell Biology and Physiology at the UNC School of Medicine and member of the UNC Autism Research Center executive committee. "The inability to identify these threats before they cause disease represents one of the major public health challenges of our time."

This challenge is particularly relevant to autism, which now affects 1 in 59 individuals in the United States.

Zylka added, "This research project will enable us and others to evaluate real-world risks associated with these chemicals/mixtures, permit future generations to minimize exposure, and help to reduce the prevalence of avoidable neurodevelopmental disorders that are caused or exacerbated by chemical risks."

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https://www.med.unc.edu/neuroscience/nih-awards-zylka-lab-6-8-million-to-study-autism-adhd-environmental-risks/

Could a new class of fungicides play a role in autism, neurodegenerative diseases?

A new UNC School of Medicine study shows how chemicals designed to protect crops can cause gene expression changes in mouse brain cells that look strikingly similar to changes in the brains of people with autism and Alzheimer's disease.

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CHAPEL HILL, NC – Scientists at the UNC School of Medicine have found a class of commonly used fungicides that produce gene expression changes similar to those in people with autism and neurodegenerative conditions, including Alzheimer's disease and Huntington's disease.

The study, published today in the journal *Nature Communications,* describes a new way to home in on chemicals that have the potential to affect brain functions.

Mark Zylka, PhD, senior author of the study and associate professor of cell biology and physiology at UNC, and his team exposed mouse neurons to approximately 300 different chemicals. Then the researchers sequenced RNA from these neurons to find out which genes were misregulated when compared to untreated neurons. This work created hundreds of data sets of gene expression; genes give rise to products, including proteins or RNA.

Zylka's team then used computer programs to deduce which chemicals caused gene expression changes that were similar to each other.

"Based on RNA sequencing, we describe six groups of chemicals," Zylka said. "We found that chemicals within each group altered expression in a common manner. One of these groups of chemicals altered the levels of many of the same genes that are altered in the brains of people with autism or Alzheimer's disease."

Chemicals in this group included the pesticides rotenone, pyridaben, and fenpyroximate, and a new class of fungicides that includes pyraclostrobin, trifloxystrobin, fenamidone, and famoxadone. Azoxystrobin, fluoxastrobin, and kresoxim-methyl are also in this fungicide class.

"We cannot say that these chemicals *cause* these conditions in people," Zylka cautioned. "Many additional studies will be needed to determine if any of these chemicals represent real risks to the human brain."

Mark Zylka, PhD

Zylka, a member of the UNC Neuroscience Center, and his group found that these chemicals reduced the expression of genes involved in synaptic transmission – the connections important for communication between neurons. If these genes are not expressed properly, then our brains cannot function normally. Also, these chemicals caused an elevated expression of genes associated with inflammation in the nervous system. This so-called neuroinflammation is commonly seen in autism and neurodegenerative conditions.

The researchers also found that these chemicals stimulated the production of free radicals – particles that can damage the basic building blocks of cells and that have been implicated in a number of brain diseases. The chemicals also disrupted neuron microtubules.

"Disrupting microtubules affects the function of synapses in mature neurons and can impair the movement of cells as the brain develops," Zylka said. "We know that deficits in neuron migration can lead to neurodevelopmental abnormalities. We have not yet evaluated whether these chemicals impair brain development in animal models or people."

Jeannie T. Lee, MD, PhD, professor of genetics at Harvard Medical School and Massachusetts General Hospital, who was not involved in this research, said, "This is a very important study that should serve as a wake-up call to regulatory agencies and the general medical

community. The work is timely and has wide-ranging implications not only for diseases like autism, Parkinson's, and cancer, but also for the health of future generations. I suspect that a number of these chemicals will turn out to have effects on transgenerational inheritance."

Zylka's group also analyzed information from the U.S. Geological Survey, which monitors county-wide pesticide usage, as well as the Food and Drug Administration and the U.S. Department of Agriculture, which test foodstuffs yearly for pesticide residues.

Of the chemicals Zylka's team studied, only the usage of pyridaben has decreased since 2000. Rotenone use has remained the same since 2000. However, the use of all the fungicides in this group has increased dramatically over the past decade.

Indeed, a study from the Environmental Protection Agency found that pyraclostrobin is found on foods at levels that could potentially affect human biology, and another study linked pyraclostrobin usage to honeybee colony collapse disorder.

The pesticide rotenone was previously implicated in Parkinson's disease through replicated animal experiments and through human epidemiological studies. A separate 2015 UNC study found that Parkinson's disease is much more common in older adults with autism than in older adults without autism.

Previous work has also shown that a single dose of the fungicide trifloxystrobin reduced motor activity for several hours in female rats and for days in male rats. Disrupted motor function is a common symptom of Parkinson's disease and other neurological disorders. The related fungicide picoxystrobin impaired motor activity in rats at the lowest dose tested.

Zylka added, "The real tough question is: if you eat fruits, vegetables or cereals that contain these chemicals, do they get into your blood stream and at what concentration? That information doesn't exist." Also, given their presence on a variety of foodstuffs, might long term exposure to these chemicals – even at low doses – have a cumulative effect on the brain?

Zylka noted that conventionally grown leafy green vegetables such as lettuce, spinach, and kale have the highest levels of these fungicides. But due to each chemical's effectiveness at reducing fungal blights and rust, crop yields have increased and farmers are expanding their use of these chemicals to include many additional types of food crops.

Zylka's team hopes their research will encourage other scientists and regulatory agencies to take a closer look at these fungicides and follow up with epidemiological studies.

"Virtually nothing is known about how these chemicals impact the developing or adult brain," Zylka said. "Yet these chemicals are being used at increasing levels on many of the foods we eat."

This research was funded by three of the National Institutes of Health: the National Institute of Environmental Health Sciences, the National Institute on Neurological Disorders and Stroke, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Mark Zylka, PhD, is a member of the Carolina Institute for Developmental Disabilities and the UNC Lineberger Comprehensive Cancer Center. He was named director of the UNC Neuroscience Center in January and will take over for current director William Snider, MD, in July. Brandon Pearson, PhD, and Jeremy Simon, PhD, were co-first authors on the study. Additional authors from UNC include Eric McCoy, PhD, Giulia Fragola, PhD, and Gabriela Salazar.

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