Unlocking Cortical Function in Rett Syndrome

Mouse study identifies vision as potential biomarker for brain function in autism-linked disorder, provides new insight on targeting interventions

by Parizad M. Bilimoria

Team MECP2
The Rett research team in the Hensch and Fagiolini labs, posing in front of a picture of a parvalbumin cell—the type of inhibitory neuron that appears to be hyper-connected in the visual cortex of Mecp2-deficient mice: Takao Hensch, Alex Simon, Mari Nakamura, Annarita Patrizi, and Michela Fagiolini (shown left to right). Patrizi is co-first author on the new study reported in *Neuron*, along with Severine Durand (not pictured here).

A throat culture can tell a doctor if someone has strep throat. A blood test if they have anemia. An X-ray if they have a broken bone. Problems in brain function, by contrast, are much harder to evaluate objectively.

Imagine on top of this challenge that the patient is unable to talk or use sign language. Add to that difficulty walking, seizures, breathing problems, and several other medical issues.

This is precisely the dilemma that doctors and families face when trying to treat and support individuals with Rett syndrome—a sporadically-occurring genetic disorder defined by a pattern of developmental regression in expressive language and fine motor skills, usually occurring in girls between 1 and 5 years of age. When clinical trials are conducted for Rett syndrome, it is difficult for doctors to directly assess the effects of any given treatment on brain function. Even if a patient's cognitive abilities are significantly improved, communication barriers may prevent detection of the improvement.

A new study conducted in mice suggests a novel way to tackle this dilemma. Vision might serve as a biomarker for cerebral cortex function in Rett syndrome, according to this report from the laboratories of Michela Fagiolini, assistant professor of neurology at Harvard University and Boston Children's Hospital, and Takao Hensch, professor of neurology and molecular and cellular biology at Harvard and Boston Children's and director of the Conte Center, published December 20 in *Neuron*.

"The biomarker that we have identified can be measured in a non-invasive way, so you don't have to sedate the patient. It is very rapid, and can be repeated as many times as you want," says Fagiolini, senior author on the study, explaining that vision can be assessed objectively by measuring brain waves in response to a visual stimulus, either as EEG recordings or visually evoked potentials. "With the appropriate infrastructure it could be implemented in a clinical analysis of the patient and might really produce a picture of what is going on at the level of the cortex."

The ability to easily repeat the measurement is particularly important for developmental disorders like Rett, where doctors need a biomarker they can monitor over a long period of time. "Looking at a snapshot of a condition could be very misleading. Instead the trajectory of a condition can help in identifying when and how things go wrong, so you can design an intervention that is tailored to the patient," Fagiolini observes.

Mouse to man

Rett mice are deficient in methyl-CpG-binding protein 2 (Mecp2), the single gene affected in the vast majority of Rett cases, and recapitulate many of the behavioral and physiological symptoms observed in Rett patients. Since Rett syndrome includes some features of autism, these mice are often studied as a monogenic model of autism.

Fagiolini and Hensch's teams discovered that vision in Rett mice—like the established features of Rett in humans–displays a clear developmental regression. This can be demonstrated both through behavioral tests of visual acuity and measurements of brain responses to visual stimuli in the MeCP2-deficient mice.

"There are very few disorders that are defined on the basis of losing skills. So until now all the work on mice which are deficient for Mecp2 has shown some features that are seen in the disorder, but not this, which is the defining feature—a loss of some type of skill," says Walter Kaufmann, director of the Rett Syndrome Program at Boston Children's and one of the biologists who has led efforts to characterize the pathology of Rett since the 1990s, before Mecp2 mutations were even discovered.

"So from the point of view of having identified a loss of function featured in a mouse model, this is a landmark study," Kaufmann observes. While some of the other symptoms of Rett mice do show signs of regression, he explains, that regression is not nearly as "well-defined or catastrophic" as the loss of skills noted in humans.

Another important aspect of the discovery of visual regression in the Rett mice is the fact that this regression is seen in female as well as male mice. This is a key point because many previous mouse studies have focused on males, even though the human disorder primarily affects females. (Mecp2 is an X-chromosome gene, and females have two copies of the X chromosome, while males have only one. So even if one copy of Mecp2 is badly mutated, the other copy presumably makes survival possible in females.)

The vision defects are preceded by altered development of circuits in the visual cortex. Specifically, a population of inhibitory neurons called parvalbumin cells (PV-cells) is hyper-connected. Both this and the visual abnormalities can be prevented by environmental or genetic interventions timed early in development.

These mouse discoveries have led to a collaboration with the Rett Syndrome Program at Boston Children's and the cognitive neuroscience laboratory of Charles Nelson, professor of pediatrics and neuroscience at Boston Children's and Harvard Medical School. Together the team is beginning to study vision in Rett patients. Preliminary results in a small group of patients reveal abnormalities in visually evoked potentials.

"What is now required is a systematic longitudinal study," Fagiolini notes. Already visual assessments have been added as an exploratory outcome measure to a phase II clinical trial expected to start soon at Boston Children's, testing the growth factor IGF-1 as a treatment for Rett.

Vision as a key to the cortex

Studying vision in Rett mice was a bold step. When researchers began the project, proposals to study basic sensory function in autism or autism-related disorders were generally subject to skepticism. And there were no clear reports of visual problems in Rett syndrome—only a few scattered studies on small populations tested at different time points, with variable results.

In general, eye gaze is preserved in Rett patients and may be used as a means of engaging with others. Girls with Rett can definitely see, although it is not clear exactly how well. "Vision is often tested in a way that requires interaction, activity, communication between the doctor and the patient. The issue of not being able to speak makes evaluation difficult... and doctors are also overwhelmed with the other, more devastating and observable aspects of the disease," Fagiolini notes. "Vision is the last thing you think about."

So why did her team choose to study vision?

They wanted a key to the mysteries of cortical development in Rett syndrome, some way to unlock on a circuit level why and how the regression occurs. They knew that Mecp2 regulates plasticity, the ability of the nervous system to change in response to its experiences. And they knew that the balance between excitatory and inhibitory neural activity, known as E/I balance, is disrupted in Rett syndrome as it is in many other neurodevelopmental disorders—one of the clearest signs being the high incidence of seizures in Rett patients.

These observations, coupled with the fact that visual cortex is one of the best studied regions of the brain and Fagiolini and Hensch have already gained insights into some of the molecular mechanisms orchestrating plasticity and E/I balance in the visual system, made vision an attractive way to probe cortical development in Rett syndrome.

What the team found was that E/I balance is disrupted in a complex manner in the visual cortex of Mecp2-deficient mice. The "silence" of the cortex—its failure to respond sufficiently to visual stimuli—originally suggested an excess of inhibition. However, the levels of various inhibitory neuron markers were actually decreased. Parvalbumin, a marker of one subtype of inhibitory neurons, was the only one tested that increased. This immediately suggested to the researchers that interventions simply aiming to increase or decrease inhibition may not be ideal—it may make more sense to target specific subtypes of inhibitory circuits.

Correcting imbalances

The team zoomed in on PV-cells because they believe these cells are crucial for timing critical periods—windows of heightened plasticity in brain development, in which environmental experiences exert a powerful influence on neural architecture. And they hypothesize that critical periods are disrupted in autism. Also, PV-cell abnormalities have been documented in a wide range of mouse models for autism, such that that anything that is discovered about correcting the PV-cell abnormalities in Rett mice could be broadly informative.

In the current study, the hyper-connectivity of PV-cells was revealed by immunofluorescence staining of presumed synaptic sites. Since visual experience promotes the maturation of PV circuits, the investigators tried raising Rett mice in the dark. While this lack of stimulation would delay visual development in normal mice, in Rett mice it seems to compensate for the effect of Mecp2 deficiency—as there is no PV hyper-connectivity and the mice do not undergo visual regression.

Looking for an alternate rescue strategy—one not involving sensory deprivation—the researchers turned to NMDA receptor signaling. NMDA receptors are one category of excitatory neurotransmitter receptors in the brain, composed of different subunits, including the NR2A and NR2B subunits. Brain autopsy studies have long revealed abnormalities in NMDA receptors in Rett syndrome. And PV-cells are known to be exquisitely sensitive to manipulations targeting NMDA receptors.

The team had observed that the balance of NR2A/NR2B levels was abnormal in Rett mice and suspected this might be important for visual regression, in part because visual experience is known to regulate NR2A/NR2B ratios as it does PV-cell maturation. So they tried to correct the NR2A/NR2B ratio, deleting one copy of the gene for NR2A on top of the Mecp2 deletion. This, like raising the mice in darkness from birth, seems to block the PV hyper-connectivity effect and prevent visual regression. However, while the Mecp2 mutant mice raised in the dark remained overall unhealthy, the NR2A/Mecp2 double mutant mice seemed to experience benefits beyond the visual system—including improved survival, a more normal weight, and the absence of a hindlimb clasping problem that's endemic in Rett mice.

While deleting the gene for NR2A would not be practical in humans, there are some drugs that target NMDA receptors. Fagiolini's group is currently testing in mice an antagonist called ketamine, which appears to act somewhat preferentially on PV-cells. Another antagonist called dextromethorphan—an active ingredient in many cough medications—is being tested in clinical trials. Fagiolini notes that more selective agents targeting specific NMDA receptor subunits would be of great use, and developing them might be a good investment for pharmaceutical companies interested in neurodevelopmental disorders. There is already some interest in developing such agents for schizophrenia.

A key within a key

Being a single gene disorder that's relatively easy to model in mice, Rett syndrome has been dubbed a "Rosetta stone" for understanding the molecular pathology of autism. Studying vision in Rett mice is essentially a key within this key—a way to decipher cortical development in Rett syndrome, but hopefully also in autism in general.

So might vision be a biomarker for cortical function in other neurodevelopmental disorders? Or even hearing or touch responses? This remains unknown, but is one hope of the researchers, Fagiolini says.

An interesting development related to this is an anticipated change in the official definition of autism. The new version of the DSM—the standard guide psychiatrists use to diagnose mental disorders, including developmental disorders such as autism—will for the first time list abnormalities in sensory processing as a potential feature of autism in young children. Kaufmann, who serves on the DSM revision committee, explains that sensory symptoms may play a role in the "restricted repetitive behavior" component of the disorder. Autistic children may have enhanced or diminished sensitivity to certain sensory stimuli, or may have an unusual interest in sensory aspects of the environment.

While visual abnormalities could be key to assessing cortical function in Rett syndrome, Fagiolini and Kaufmann both emphasize that vision is actually one of the most preserved functions in Rett patients. Girls with Rett syndrome tend to display, as Kaufmann puts it, "a profound interest in engaging visually," particularly after age 5. Using eye gaze in communication and education is an area of active interest for families, therapists, and teachers working with these girls.

In studying vision in Rett patients, the Boston Children's team may want to explore this fascinating paradox, Kaufmann says—trying to ascertain the relationship between the abnormalities in visually evoked potentials and this strong propensity for visual engagement.

"We're very excited that our basic research in mice has opened up a new avenue for designing targeted therapeutic intervention," Fagiolini says. "We also look forward to learning more about the developmental trajectory of visual function in girls with Rett. We hope that visual assessments might offer physicians and families a window into brain function, perhaps improving how clinical trials are conducted in the future."