Modeling autism

Alla Katsnelson

Autism researchers are making a move from mice to monkeys to help improve model validity. But old challenges remain, and new concerns await.

Helovi/Getty

In a *Cell* paper published in May, a video shows two 5-month-old cynomolgus macaque monkeys in an incubator cage. One of the monkeys ambles calmly to a bowl in the corner, then returns to the middle of the cage. Meanwhile, the second monkey loops across the floor, then along the metal grating of the ceiling, circling through again and again in constant motion.

That's macaque #142004. Her genome, and that of a handful of other macaques in the study, was engineered by researchers to carry a mutation hobbling the gene MeCP2 (ref. 1). In humans, this mutation causes a rare but severe neurological disease called Rett Syndrome. Among its many symptoms, children with Rett syndrome often exhibit autistic behaviors such as repetitive movements and social withdrawal, and the disease is considered a syndromic or single-gene cause of autism. Macaque #142004's tendency to circle through her cage, as well as her reduced social communication abilities, may model some of autism's core features, researchers say.

Macaque #142004 stands unwittingly at the vanguard of a new era of animal models for autism and other brain and psychiatric diseases. For decades, mice have been the dominant model organism in brain research, particularly since tools for knocking out targeted mouse genes were developed in the 1980s. But while

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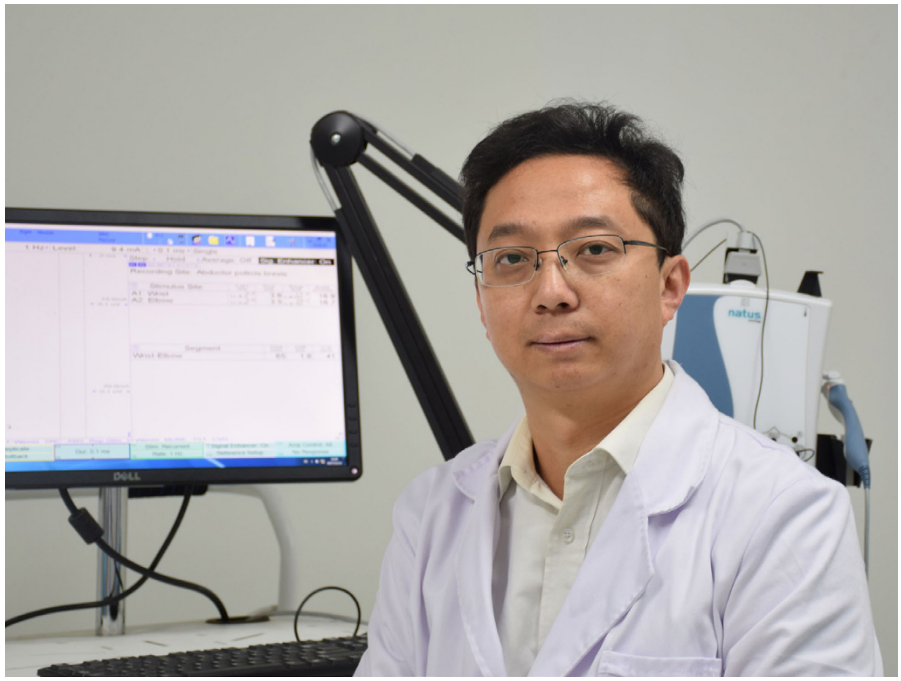
many molecular and cellular aspects of brain function are undoubtedly conserved across mammalian species, researchers are increasingly recognizing the limitations of modeling a behaviorally and socially complex disorder such as autism in the humble *Mus musculus*. The evolutionary distance between rodents and humans may be too big to faithfully recapitulate the complexity of human cognition and behavior, some argue – and recent clinical trial failures of autism drugs that showed promise in mice support the idea[2].

"When we talk about social behavior, there is a huge leap there," says Mar Sanchez, a primatologist studying social behavior and attachment at Emory University. "From everybody in the field, you will hear that there is a critical need for nonhuman primate models so we can really fill in that gap."

New gene editing tools like CRISPR, which can be used in any species, are improving rapidly in ease and precision, opening the door to genetically engineering nonhuman primates. Researchers are quick to point out that macaques like #142004 will never become the primary animal model for autism—that prospect would be both ethically and financially impossible. "But they can supplement for a few critical questions that we know have failed in mice," says Guoping Feng, a neuroscientist who studies both mouse and monkey models of autism at the Massachusetts Institute of Technology.

Even if their use remains limited, however, it is worth asking how exactly such nonhuman primate models are better positioned to succeed where mice have failed, and what factors researchers need to consider in order to maximize the prospects for that success.



Yongchang Chen

MONKEY MAKER | Yongchang Chen, a developmental biologist at Kunming University of Science and Technology in Yunnan, China, has made genetic primate models of several neurological and psychiatric diseases.

A bridge too far?

Modeling complex cognitive disorders is famously difficult. There is no lab test or physiological marker for autism like there is for cancer or for HIV. Brain disorders like autism manifest as behaviors, and while monkey behavior might be closer to humans than rodent behavior, it certainly won't be the same, Feng points out. Additionally, autism's heterogeneity raises distinct challenges. Current diagnostic criteria define the condition's core features as difficulties in social interaction and communication, and repetitive behaviors and restrictive interests. But these features can manifest and recombine in innumerable ways, leading clinicians to proclaim that if you've seen one person with autism… you've seen one person with autism. "I find it is tricky to model in animals what hasn't been well-articulated even in the patients," says Karen Parker, a behavioral neuroscientist developing primate models for autism at Stanford University.

Mouse models of autism are generally based on genetic mutations that cause or strongly raise the risk of the condition in humans. However, these genetically engineered mice often show no difference in behavior from their wild-type siblings, or, the specific phenotype varies based on the genetic strain of the mouse. That makes it difficult to study the gene's role in the social or behavioral effects of the condition—in other words, to close the circle between brain and behavior. Some researchers have started using gene editing technologies to create genetically engineered rats, which are socially more complex than mice, but it's not clear whether they will translate better to humans than mouse studies have.

The trouble with rodents may simply come down to anatomy, says David Amaral, a neuroscientist and primate researcher at the University of California, Davis. "The parts of the brain that are probably most affected in human autism—places like the fusiform gyrus, or the temporal lobe, which participates in face processing, as well as portions of the frontal cortex evolved for social behavior and executive function—are not developed, if present at all, in rodent models," he says. That makes an enormous difference for drug testing, adds Parker. "If the prefrontal cortex is part of a circuit that a drug targets in some way, you are going to see a lot more similarity in response to that drug between two animals that share those anatomical features," she says.

Alongside these anatomical differences, there is also a vast difference in behavioral capacities of rodents compared to primates, says Joseph Garner, an ethologist and neuroscientist at Stanford University. The types of social deficits that burden people with autism are reflected in attributes such as engaging in social play, interacting through eye contact, and possessing what researchers often call theory of mind - the ability to imagine what another member of the

species is thinking, he explains. Two out of three of these attributes are flat-out lacking in rodents, he says. "I think it is fundamentally problematic to try and model the lack of something in an animal that never had it in the first place."

Complicating things further is the fact that human error has muddled the mouse model landscape. Many tests for gauging mouse behavior are used incorrectly, Garner says. Take the three-chambered social approach task, a standard assay for assessing sociability in mice: One chamber is empty, another contains an unfamiliar mouse, and researchers track which of the two stimuli a test mouse favors. The assay presumably mirrors social dysfunction, a core feature of autism, but it completely misaligns how mice and humans experience encounters with conspecifics, Garner says, wryly referring to it instead as "a resident-intruder task". Mice are highly territorial, so when the test mouse enters the unfamiliar mouse's chamber, it's basically asking for a fight. "Autistic kids don't avoid other individuals because they don't want to fight them," he says.

Is "close" close enough?

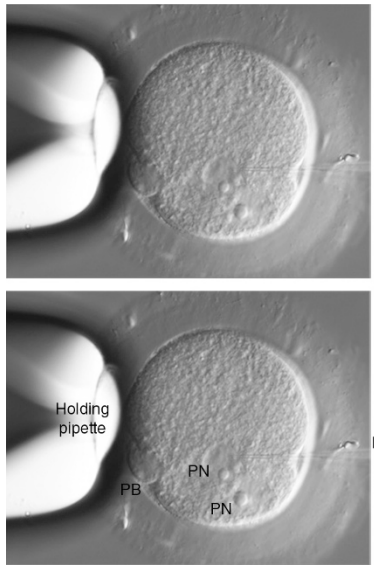
Given these limitations, turning to nonhuman primates makes sense. Already, a handful of genetically engineered monkey models of autism in addition to macaque # 142004 have emerged. Last year, a different team of Chinese researchers reported creating macaques in which MeCP2 was overexpressed rather than knocked out – a mutation that in humans also causes autism symptoms[3]. Those monkeys, too, showed intense repetitive behaviors that researchers compared to those in people with autism. Another group in July published a brain anatomy study of macaques lacking Shank3, a gene encoding a synaptic protein that is mutated in about 1–2% of people with autism[4].

Several other such efforts are in the works. Feng, for example, also created Shank3 mutant macaques with collaborators at the South China Agricultural University in Guangzhou; although the work is not yet published, the animals show some deficits that reflect autism much more closely than do Shank3 mutant mice, he says. And it's not just autism—the first genetically engineered monkey, published in 2008 by researchers at Yerkes National Primate Center in Atlanta, Georgia, was a rhesus macaque model of Huntington's disease[5]. Yongchang Chen, a developmental biologist at Kunming University of Science and Technology in Yunnan, China, who led the creation of macaque # 142004, says he and his colleagues are also developing models for Parkinson's disease, amyotrophic lateral sclerosis, Duchenne's muscular dystrophy, and other neurological conditions. "We have more than 50 live models right now," he says.

Researchers widely agree on the promise of the approach, but it is less clear-cut to say whether the autism models published so far are a success, says Alysson Muotri, a neuroscientist at the University of California, San Diego. Researchers should at least be ready for the possibility that results from studies of genetically engineered monkeys will be at least in some ways as difficult to interpret as those of genetically engineered mouse studies.

Then, there are the challenges of modeling itself. Monkeys recapitulate some aspects of the human mutation, but not others. The MeCP2 knockout monkeys made in Chen's lab show many similarities with Rett Syndrome patients: besides the behavioral effects, they also have disordered sleep and a lower pain sensitivity, for example. But there are also features that patients have but the monkeys do not, he concedes, such as epilepsy and reduced motor activity. "I think there are still some places to improve our monkey models," Chen says. But it's tough to say how much a model must reflect the human disease in order to yield useful data.

Also, although macaques and humans are relatively closely related, behavioral deficits seen when a particular gene is mutated may not be driven by the same neural circuitry in both species. "There's a preponderance of evidence" in terms of anatomical structure, gene expression, and imaging studies, says Parker, that macaque neurocircuitry is broadly similar to that of humans. Here too, however, it's unclear how close the match-up needs to be. Autism is a developmental disorder, which means that the brain changes in people with the condition reflect compensation to some early-life genetic or environmental hit. Even if the circuitry between a normal macaque and human brain is very close, the brain's compensation mechanisms to this early life event that causes the disorder may still differ in the two species.



Guoping Feng Lab

MUTANT MARMOSETS | To perform genetic engineering, CRISPR reagents are injected into the pronucleus of a fertilized marmoset oocyte (zygote).

What's more, it is possible that at least some component of autism is human-specific and wouldn't be seen in any animal model at all, notes Muotri. Recent work points to distinct differences in gene expression and neuronal distribution in the cortex and the brain regions of different primates that may well result in different circuitry[6]. For an even more basic example, macaques and other nonhuman primates have an extra pair of chromosomes compared to humans, pointing to a difference in genomic organization, Muotri says. "It might be, say, that chromatin remodeling agents like MeCP2 work differently."

Wanted: new behavioral assays

To address these uncertainties, researchers will have to pin down how to meaningfully compare social deficits in monkeys with autistic features of humans. That's an issue that researchers are only beginning to work out. "Thinking about what behaviors we are interested in measuring is really important," Parker says.

Parker, who conducts translational autism research in both monkeys and people, is collaborating with Garner and with colleagues at the California National

Primate Research Center at the University of California, Davis, to study monkeys with naturally occurring social impairments within established populations of rhesus macaques at the institution. They aim to develop a battery of behavioral tests with direct relevance to features seen in people with autism, she says. Last year, the group reported that infant macaques that had difficulty recognizing faces and judging aggressive facial expressions in other macaques were much less sociable than normal as juveniles and adults[7]. The tests were able to predict whether the animals would later fall into the less sociable group— much like performance on eye tracking tests is thought to serve as an early indicator of autism in human babies[8].

Other researchers are also looking for autism-like social deficits within the natural variation of behaviors in groups of nonhuman primates. Sanchez collaborates with clinicians at Emory's Marcus Autism Center to study macaque mothers in the Yerkes colony that seem less than capable of reading their offspring's cues. She hypothesizes that these animals' inability to perform this highly-wired behavior reflects a type of social dysfunction similar to that seen in autism. Her team is essentially working backwards from the aberrant behaviors in these monkeys to see if they can identify early predictors of it in infant animals, she says.

Initially, Sanchez and Parker explain, making the match-up between macaque and human social behavior relies on so-called face validity – that is, whether the monkey behavior looks like autism. Mouse researchers have largely moved away from face validity, but because the anatomy and connectivity of the brain is so much more similar between humans and macaques than humans and rodents, assuming a shared underlying mechanism offers a starting point, Sanchez says.

Ultimately, however, genetics will have to ground the research, they say. Genetic factors account for a hefty component of autism risk – recent work suggests the heritability is greater than 80% (ref. 9). And it's clear that similar behaviors can be driven by different pathology: for example, monkeys raised in social isolation in the infamous experiments conducted in the 1970s by Harry Harlow at the University of Wisconsin-Madison, produced repetitive behaviors not unlike those seen in macaque #142004. "We want to show there is at least some shared genetic variants between these socially-impaired monkeys and humans diagnosed with autism," says Parker.

Michael Platt, a neuroscientist at the University of Pennsylvania who studies the natural variation in social interaction among a free-living population of about 1500 macaques in Puerto Rico, is also probing the genetics of socially-challenged animals in that group. "It seems like wherever people find a gene variant or polymorphism that is common in humans, you also see it in monkeys," he says.

His lab first found this overlap in variants related to serotonin signaling, which in humans are associated with multiple psychiatric disorders. These variants also exist in the macaques, and they have behavioral correlates that roughly parallel what's seen in humans[10]. More recently, he is examining a variant of the autism-related gene Shank3. Monkeys with the mutation seem to have different patterns of social behavior than ones with normal copies of the gene, he says. What's more, he adds, whole genome sequencing is revealing an overlap in gene variants identified in the monkeys and those found to raise the risk of autism in humans, though it's still unknown how these variants affect behavior as well as brain structure and function.

From "could we" to "should we"

Closing the loop between behavioral and genetic studies of autism in nonhuman primates may help bring much-needed clarity to the underlying causes of the disease – an undoubtedly worthy goal. But it's also worth thinking through some of the wider implications of embracing this new research paradigm of genetically altering nonhuman primates.

In the years after the technology to genetically engineer mice first appeared, researchers created thousands of different mutant animals. Now, researchers are turning to nonhuman primates specifically because they are more similar to humans – which raises the stakes for these models from an ethical perspective, says Carolyn Neuhaus, a bioethicist at the Hastings Center in Garrison New York. While the approval of animal research protocols depends on the balance of costs to the animal and benefits to society, the harm a monkey might experience from a genetic alteration is difficult to predict. Indeed, Neuhaus argues in a recent publication that such work cannot at present be ethically done[11].

Researchers must undoubtedly be more judicious in deciding which nonhuman primate models to make than they were in creating mice. Yet the criteria for the mutations that should or should not be engineered in monkeys have not been clearly defined. "I don't think people have put enough thought into it – to say, what is the next model that is really going to help us to truly understand the disease," says Feng. He attributes this open-endedness to fact that the field is in the early-days of figuring out the scope and the limits of gene editing. "When the technological feasibility is proven, then we can start to address real questions," he says. "We really need to think very hard. Just because we can generate monkey models doesn't mean we should."

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