TECHNOLOGY FEATURE

INSIDE THE MINDS OF MICE AND MEN

Monitoring technologies and genetic engineering are producing a growing array of animal models for psychiatric disorders, but researchers are still learning how best to use them.

SAGE LABS

There is no such thing as an autistic rat, but researchers can study human psychiatric conditions by analysing the behaviour of rodents.

BY MONYA BAKER

Anyone familiar with Rett syndrome will recognize the symptoms. Mouse models — animals that carry genetic mutations similar to those that cause the condition in humans — wring their paws, walk awkwardly and learn poorly. Other human brain disorders have animal models, too. Mice with extra copies of the genome regions duplicated in Down's syndrome show motor problems and learning deficits. A developmental disorder known as fragile X syndrome arises when humans lack a working copy of the gene *FMR1*; mice without the gene show learning deficits and hyperactivity similar to the symptoms of the human disorder.

But those are conditions with discrete, recognized causes. Other neurocognitive disorders, such as autism, depression and schizophrenia, have multiple and often mysterious causes, so mimicking them is more complicated. Studies have implicated dozens of genetic variants in producing the disorders, and environmental factors from traumatic life experiences to *in utero* conditions also contribute. Even when researchers have decided which genes or factors to study, it is not always clear how to assess animal models: how can a researcher use a mouse to study diseases diagnosed by hallucinations or an inability to understand figurative language?

FROM BEHAVIOUR TO BIOLOGY

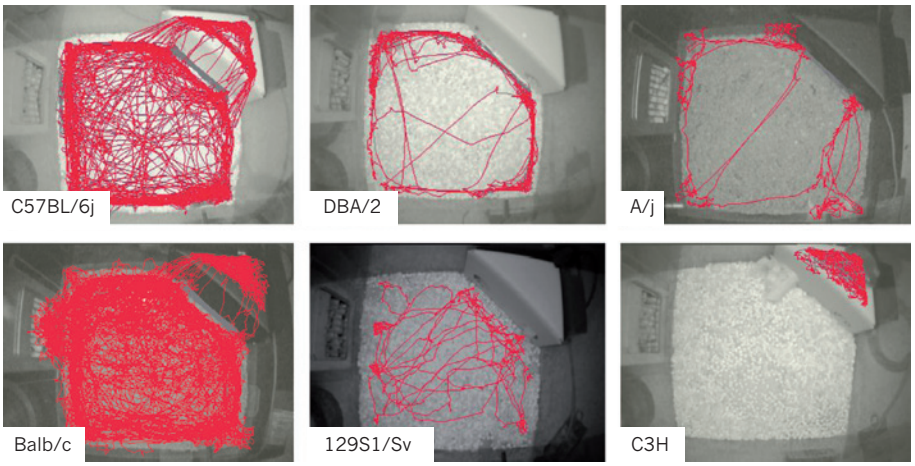
Craig Powell, a neuroscientist at the University of Texas Southwestern Medical Center in Dallas, says that the goal of tweaking genes is

usually to uncover disease mechanisms. "So you make a mouse with a mutation that you know causes autism in humans, and you see that it has behaviours that resemble autism," he says. Powell's next step is to look at slices of the mouse's brain, to see how it differs from normal mice.

At that point, behaviour can help researchers to home in on what really matters. "We might find a hundred things wrong with the brain function, but only one or two cause changes in behaviour, so we want to fix things and see what changes the behaviour," says Powell. In work that has become a touchstone for the field, Mark Bear, a neuroscientist at the Massachusetts Institute of Technology (MIT) in Cambridge, and his colleagues showed that reducing expression of a particular receptor in mice ameliorated the effects of a mutation that causes fragile X syndrome[1]. Several physiological abnormalities were reversed, and engineered mice had fewer seizures and better memories.

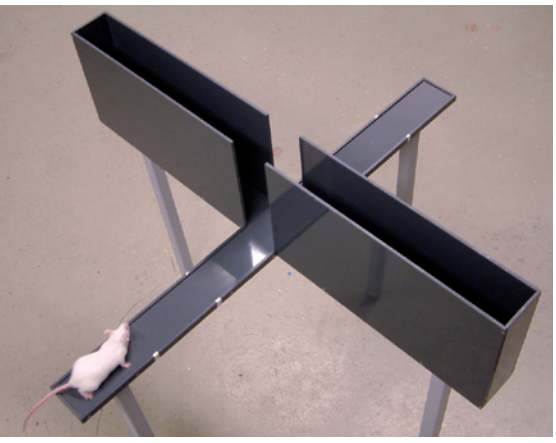
In another example, Adrian Bird, a geneticist at the Wellcome Trust Centre for Cell Biology in Edinburgh, UK, and his colleagues reversed symptoms resembling Rett syndrome in mice[2]. They crafted a version of the defective gene that could be restored to normal activity with a supplement to a mouse's diet, but administered the supplement only after mice carrying the gene began to exhibit symptoms. Activating the gene at this point was expected to have little effect, but the mice showed marked improvement, raising hopes that recovery might also be possible in humans. A similar study[3] into spinal muscular atrophy found that restoring a defective gene's function four days after birth essentially eliminated signs of the disease; doing so ten days after birth had little effect. Such studies could help researchers to predict which patients are most likely to benefit in clinical trials.

Research into behaviour can also probe how genetic variants interact with each other and with environmental factors. In one study published this year[4], wild-type males from five mouse strains were bred with females carrying a mutation that causes symptoms resembling autism and developmental disorders. Tests on the offspring showed that the symptomatic behaviours recurred in only some of the genetic backgrounds. In another study[5], mice from a strain displaying a range of autism-relevant symptoms were reared by and with mice from a strain known for high sociability. The fostered mice showed no social deficits as adults, but other relevant symptoms, such as repetitive grooming, were not reduced. And when a mutant version of *DISC1*, the first gene to be implicated in schizophrenia, is present in mice whose mother's immune system has been stressed during pregnancy, the offspring exhibit symptoms of affective disorders and autism[6]. Without the environmental stressors, they show symptoms of schizophrenia.



B. SPRUIJT/DELTA PHONOMICS

Video tracking of different mouse strains (labelled) reveals that some explore a new cage more than others.



C. TOUMA/MAX PLANCK INST. PSYCH.

Less-anxious mice spend longer in open spaces.

HIDDEN SYMPTOMS

Even the cleverest assays cannot capture some important aspects of human disease, such as the paranoid delusions common in schizophrenia. (In fact, because the models will always be imperfect, most behavioural researchers object to phrases such as 'schizophrenic mice'.) Mikhail Pletnikov, a neurobehaviourologist at Johns Hopkins University in Baltimore, Maryland, is one of many scientists hoping to complement behavioural tests with more readily measured biomarkers. People with schizophrenia exhibit a wide range of behavioural symptoms, but their lateral ventricles — fluid-filled cavities on either side of the brain — tend to be larger than average, so Pletnikov is using brain scans to measure these structures in mice. It is not always necessary to see a behavioural change to probe a diease's biology, he says. "With humility, you can use mice or rats or even worms."

Unexpected behaviours have revealed unanticipated biology. Several years ago, Guoping Feng, a neuroscientist now at MIT, was trying to work out the function of various proteins found on either side of the synapses that connect neurons. Knocking out one such protein, SAPAP3, had no apparent effect on brain function: the mice walked and learned normally[7]. They did, however, seem to have something wrong with their skin: open sores appeared on their faces. After tests showed nothing abnormal, video surveillance revealed that the mice groomed themselves excessively, literally rubbing through their fur. Further work showed abnormalities in a region of the brain linked to obsessive–compulsive disorder (OCD). Although SAPAP3 had not previously been implicated in the condition, drugs that eased OCD symptoms in humans reduced the grooming in mice. Studies[8] of proteins that interact with SAPAP3 revealed that they, too, had links to OCD and autism.

"When in doubt, the experimenter needs to look at what the animal is doing."
Douglas Wahlsten

As human genetic studies reveal gene variants with increasingly smaller impacts on disease, there is increasing demand for new behavioural tests to assess them. Results of assays are highly variable, and they may not measure the most meaningful symptoms, says Jeffrey Mogil, a neuroscientist at McGill University in Montreal, Canada. "We've made a lot of advances in making ever-fancier mice, but at the end of the day the question is, what's your assay and what's your measure and are they relevant?" he says. "The slow link in the chain, the messy link in the chain, has always been the behavioural assays."

Current tests of animal behaviour are blunt tools. The Morris water-navigation task evaluates an animal's cognitive ability by assessing how it learns to use spatial cues to swim to an underwater platform that it can't see. Changes in the animal's performance can be used to measure learning and memory. Tests for anxiety include the open-field test, which measures the time a mouse spends in enclosed spaces or along the edges of its cage;

nervous animals avoid exposed areas. For both, psychiatric drugs effective in humans change the outcomes.

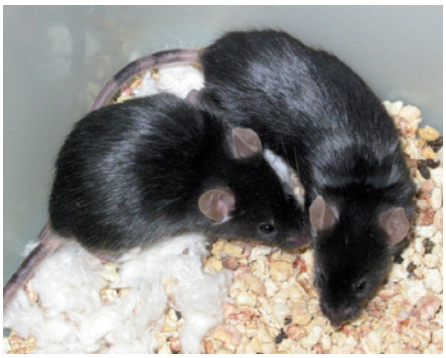
Such tests can be effective at screening new drugs that act by the same mechanisms as existing ones. But they are less useful for conditions for which no effective drugs exist, or for gaining insight into pathology. So researchers are trying to develop tests that capture more-specific components of human disorders.

"We talk more to the clinical researchers," says Jacqueline Crawley, chief of behavioural neuroscience at the US National Institute of Mental Health in Bethesda, Maryland. "There are opportunities for us to sit down and say, 'what do these diseases look like, what is their variability in the real world, and what do you consider the fundamental core symptoms?'"

"An animal model may not be 100% translatable, but maybe 80% is good enough."
Jacqueline Crawley

Crawley's own studies of children with autism inspired her to develop a mouse test for analogous behaviour. "You'll see a group of children without autism playing together and the ones with autism being off to the side, playing with a train or a computer," she recalls. So Crawley designed a task that would assess whether a mouse chose to spend time with a social partner or an inanimate object. The assay is now used in many laboratories.

Clinical tests have inspired other animal counterparts. Mogil and his colleagues produced the mouse-grimace scale for pain assessment[9], based on a scale that used facial expressions to determine pain in infants and other humans incapable of speaking. Mogil believes his scale will prove a more reliable measure of chronic pain than commonly used assays such as the tail-flick test, which measures how quickly a mouse moves its tail out of a beam of light. It should also help researchers to design more-humane experiments. Other scientists have developed a mouse version[10] of the Wisconsin card-sorting test, in which participants are presented with cards displaying, say, three red circles or four blue squares. Once humans recognize that rewards come for, say, matching cards by colour, the reward criteria are changed to matching by shape or number. The test is used to study disorders including autism and schizophrenia. The mouse version relies on scents such as cinnamon and garlic alongside textures such as gravel and cotton balls. Feng is currently evaluating the assay on mouse models of autism.



D. WAHLSTEN/UNIV. NORTH CAROLINA GREENSBORO

The colours of mice and their bedding can confuse video-tracking systems.

Tim Bussey and Lisa Saksida, neuroscientists at the University of Cambridge, UK, have developed a mouse version of a touchscreen interface originally developed for humans and primates. It uses high-contrast images, tailored to mouse eyesight; and instead of pressing a lever or poking its nose into a hole in the wall as in most mouse-testing systems, the animal touches a screen with its nose or a paw. The technology, commercialized by Campden Instruments in Loughborough, UK, last year, could assess many cognitive abilities in rodents; one battery of tests assesses functions typically impaired in patients with schizophrenia, including visual perception,

working memory and pattern-learning[11]. Bussey estimates that the system is now being used in more than 30 labs.

IRONING OUT THE KINKS

Confounding variables are the bane of behavioural testing, says Douglas Wahlsten, a neuroscientist at the University of North Carolina at Greensboro and the author of a handbook on the topic. For example, if a mouse seizes up with fear and stands still in the centre of an open chamber, the time it spends there could be misinterpreted as demonstrating reduced anxiety. And the walls of water-maze tanks are often so high above the water that mice cannot see much of the room, which makes it hard for them to use spatial cues to find the platform.

Genetic manipulation increases the scope for artefacts in the data, because tinkering with genes could alter how mice perform at tasks for reasons that have little to do with the parameters being tested (see 'Assessing the assays'). If learning assessments are based on an animals' ability to associate a sound with a mild electric shock, for example, researchers should make sure that the animals have normal hearing and sensitivity to pain. The biggest confounding variable may simply be moving the mouse from its cage to the area where tests are performed. "It's really rare that a small rodent would be lifted up by another animal and survive," says Laurence Tecott, a neurobiologist at the University of California, San Francisco. "We scare the hell out of the animal, then ask it if it's anxious and how it can learn," he says. "We do that routinely."

To reduce distress, researchers are working on ways not just to observe behaviour, but to do so without physically transporting animals first. In IntelliCage, an observation system from NewBehavior in Zurich, Switzerland, each animal is radiochipped. The system monitors when each animal drinks and eats, and how it performs at various stations in its enclosure.

Tecott, working with colleagues Evan Goulding and Katrin Schenk, has developed an inexpensive system that can monitor animals around the clock[12]. A cage sits on a weight-detecting platform that measures an animal's location 50 times a second. Self-correcting informatics organize the animal's movements into 'bouts' of activity and can keep track of a mouse as it eats, defecates and moves its bedding. As part of the Mouse Phenome Project, an international collaboration to collect phenotypic data on mouse strains used in labs, Tecott is developing a lifestyle database for 16 strains. Even in preliminary results, strains can be distinguished by their distinct patterns of activity.

Tecott has also used his monitoring system on two lines of mice genetically engineered for obesity: *ob*/*ob* mice, which lack the gene to make one of the hormones that regulates appetite; and *htr2c* mutant mice, which lack a receptor for the neurotransmitter serotonin[12]. The mice act like couch potatoes and midnight snackers respectively, says Tecott. The *ob*/*ob* animals eat just slightly more than mice of normal weight, but spend only about one-fifth as much time walking around their cages. The *htr2c* mutants have normal activity and feeding most of the time, but leave their burrows in the middle of their resting periods for a series of snacks. Without automated analysis, such insights into the behavioural components of obesity would be hard to detect.

More-expensive video-tracking systems, already widely used for many behavioural tests, can also be used to monitor animals in their home cages, automatically detecting and categorizing behaviours. As more molecular biologists want to monitor genetically engineered mice, demand for and applications of automated systems are increasing, says Lucas Noldus, chief executive of Noldus Information Technology in

Assessing the assays

Researchers evaluating animal models consider three kinds of validity.

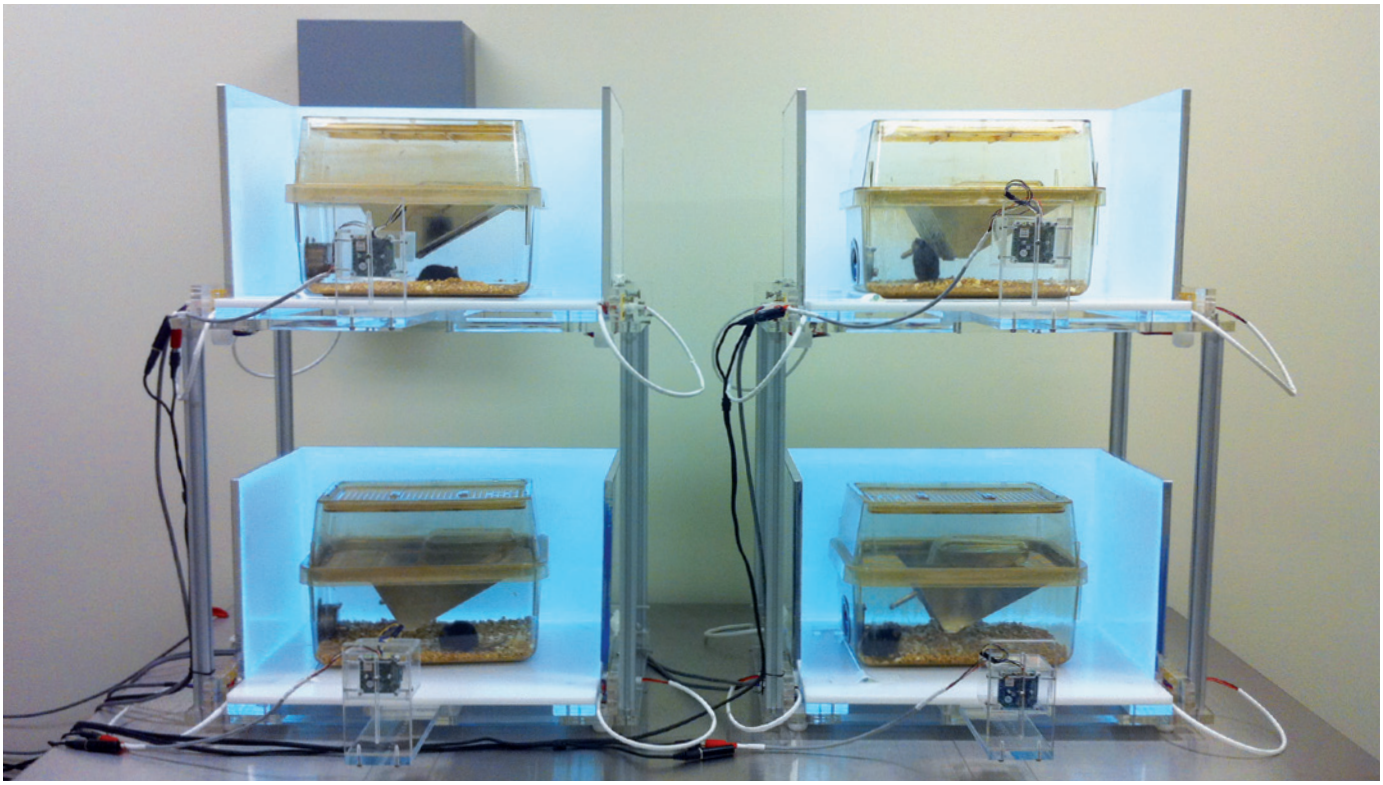
Construct validity means that a test measures what it claims to. In animal models, that means that whatever causes symptoms in the animal is also what contributes to disease in humans. Such validity is relatively easy to achieve when a condition is caused by a single gene, but most are more complicated. Mikhail Pletnikov, a neurobiologist at Johns Hopkins University in Baltimore, Maryland, models schizophrenia by combining genes and environmental stressors. For complex disorders, he says, "we've passed that period where we manipulate one gene to try to understand the whole disease".

Face validity means that a test seems to measure what it needs to, for example that the symptoms in an animal model mirror those in a human. For heart rate or tumour growth, such measures may be straightforward, but for diseases assessed by behaviour, it is considerably more complicated. BTBR mice, a strain used to study autism, avoid interacting with other mice and groom themselves excessively. When BTBR males are exposed to female urine, they do not vocalize and scent-mark as males from other strains do. These traits and others map well onto the diagnostic criteria for autism in humans, which include deficits in interaction and communication, along with repetitive behaviour.

Predictive validity is the extent to which a test predicts a future outcome. In an animal model, the animal should respond to drugs in a way that corresponds to human reactions. For example, antidepressants are sometime evaluated by their effects on the forced-swim test, which measures how long a mouse will try to climb out of a tank of water before giving up. For disorders such as autism, however, there are no effective drugs to serve as positive controls. Even when drugs do exist, the symptoms or mechanisms captured by a single behavioural assay are unlikely to capture everything that is important. M.B.

SAGE LABS

Mice (left) are commonly used as disease models, but rats have more complex and sociable behaviour, so are better suited to modelling neurocognitive disorders.



CLEVER SYS

Video-tracking systems analyse the behaviour of mice in their home cages. Software can capture specific activities, such as grooming and sniffing.

Wageningen, the Netherlands. Noldus describes one of his company's latest systems as "an instrumented home cage that can be configured in a variety of ways, from a very bare cage to very rich stimuli. Depending on the cage conformation you can perform all sorts of tests: an anxiety test with a light spot, or a memory test with automated pellet dispenser."

The monitoring component doesn't interfere with the animal, says Vikrant Kobla, vice-president of business development at Clever Sys in Reston, Virginia. "You're taking the same cage and putting a camera in front of it." His company's systems recognize more than two dozen behaviours, including head bobbing, grooming and standing on hind legs, and the repertoire is expanding. "We have so many modules we've developed that we can adapt it to deal with new behaviours," says Kobla.

CONSTANT VIGILANCE

"If you want a rich detail of measure from many kinds of tests, you really need to use video tracking," says Wahlsten. Nonetheless, he says, accuracy cannot be taken for granted, even for standard tests of animal behaviour. Tracking two animals at once is particularly difficult. It is not uncommon for software to confuse an animal's nose with its tail, for example. Bad lighting wrecks many experiments, particularly if a mouse is moving from a lighter area to a darker one, and different artefacts occur with white, brown, black and patchy mice. Infrared backlighting vastly reduces these problems and is commercially available, but is rarely used, owing to both lack of awareness and the cost of converting existing equipment. But even the best systems require considerable effort to avoid false readings, warns Powell. "It's hard to sum up even in a book what the pitfalls are," he says. "Don't just take the Excel spreadsheet at the end and analyse it. Watch what's going on during the experiment."

Even when behaviours can be observed accurately, a mouse's activity may not be robust or subtle enough to reflect the effects of tweaking a gene or the environment. Rats show more complex behaviours; for example, littermates wrestle with each other, a behaviour that is considered social play. The most established behavioural tests were designed for rats, so many researchers are interested in modelling disease using genetically modified rats. Rats lacking genes implicated in schizophrenia, Parkinson's disease and autism are among the very first strains being produced by Sigma-Aldrich in Saint Louis, Missouri, which began offering a suite of ready-made knockout rats earlier this year. Sigma and other companies also take on custom projects to genetically engineer rats.

Richard Paylor, an autism researcher at Baylor College of Medicine in Houston, Texas, has just begun testing on knockout rats, investigating behaviours such as how the rats interact and vocalize in social situations, and how they respond to social odours. He hopes to report results by the end of the year. It is too early to say anything definitive, he says, but rats should allow finer behavioural assessments than mice. In particular, it may be possible to use them to quantify the effects of potential treatments for social and communication disorders, something that has proved particularly difficult with mice. Rats' larger size also makes it easier to take electrophysiological recordings, brain images and tissue samples.

Paylor predicts that labs working with mice will find it difficult to switch to rats, which are expensive to buy and maintain, needing more space and different equipment. "We will be sort of a test case for labs that may want to study both mice and rats," says Shannon Hamilton, a postdoc in Paylor's laboratory.

But whether testing rats or mice, says Crawley, researchers must remember that the goal of an animal model is not perfection but utility. "An animal model may not be 100% translatable, but maybe 80% is good enough to test for possible treatments." ■

Monya Baker *is technology editor for* Nature *and* Nature Methods.

1. Krueger, D. D. & Bear, M. F. *Annu. Rev. Med.* **62,** 411–429 (2011).
2. Guy, J., Gan, J., Selfridge, J., Cobb, S. & Bird, A. *Science* **315,** 1143–1147 (2007).
3. Lutz, C. M. *et al. J. Clin. Invest.* (in the press).
4. Spencer, C. M. *et al. Autism Res.* **4,** 40–56 (2011).
5. Yang, M., Perry, K., Weber, M. D., Katz, A. M. & Crawley, J. N. *Autism Res.* **4,** 17–27 (2011).
6. Abazyan, B. *et al. Biol. Psychiatry* **68,** 1172–1181 (2010).
7. Welch, J. M. *et al. Nature* **448,** 894–900 (2007).
8. Peça, J. *et al. Nature* **472,** 437–442 (2011).
9. Langford, D. J. *et al. Nature Meth.* **7,** 447–449 (2010).
10. Bissonette, G. B. *et al. J. Neurosci.* **28,** 11124–11130 (2008).
11. Bussey, T. J. *et al. Neuropharmacology* doi:10.1016/j.neuropharm.2011.04.011 (2011).
12. Goulding, E. H. *et al. Proc. Natl Acad. Sci. USA* **105,** 20575–20582 (2008).