JANSSEN RESEARCH & DEVELOPMENT

Rodents are used to test drugs designed to correct cognitive deficits in people with schizophrenia.

DRUG DEVELOPMENT

The modelling challenge

Researchers have made good progress with animal tests for cognition. The next step is to devise a rodent model for drug development.

BY ALLA KATSNELSON

When Patricio O'Donnell started his lab in 1997 at Albany Medical College in New York, schizophrenia research seemed to be moving forward after a long period of stagnation.

For several decades, attention had focused on the idea that the disease was caused by elevated dopamine levels in the brain, particularly in the striatum, a nugget of brain tissue nestled under the cortex. But by the 1990s, the dopamine hypothesis was proving inadequate to fully explain the disease. *In vivo* imaging with computed tomography and magnetic resonance imaging, and data from post-mortem studies in people with schizophrenia, pointed to cortical effects and implicated other neurotransmitter systems, such as glutamate and serotonin. Biologists were also learning to create transgenic mouse models of the disease, providing a set of tools with which to investigate genetic and aetiological factors.

This proliferation of ideas opened the doors to fresh avenues of investigation, as well as new ways of modelling the disease in animals. The classical rodent model for schizophrenia had involved administering amphetamine — which ramps up dopamine levels in synapses and can cause hallucinations and delusions in people — and then measuring behaviours such as hyperactivity or passive avoidance. Researchers then began using other pharmacological agents, such as phencyclidine (PCP) and ketamine, which interfere with the glutamate receptor NMDA, or knocking out specific genes, such as those that code for neurotransmitter receptors.

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O'Donnell was captivated by one particular approach that entails chemically damaging part of the hippocampus in newborn animals. Rodent pups who receive this intervention initially appear normal, but they later become socially withdrawn and overly responsive to stress, and their cortices show some of the physiological changes observed in post-mortem tissue. These findings reflect the now-accepted hypothesis that schizophrenia is a developmental disorder. "What made it attractive to me was that even though the lesion was done really early in development, all these behavioural abnormalities didn't emerge until adolescence," says O'Donnell, now vice-president and head of psychiatry and behavioural disorders at Pfizer Neuroscience in Cambridge, Massachusetts.

Attempts to mimic the symptoms or underlying physiological effects of schizophrenia have multiplied since then. As genome-wide association studies began pinpointing genes associated with the disease (see 'Unravelling complexity', page S6), researchers have knocked out or perturbed those genes in mice. Others have looked at environmental stressors, such as prenatal infections, early social isolation or stress, or lesions, like the ones O'Donnell used in the hypothalamus. And others give animals drugs such as amphetamine, ketamine and PCP. Some researchers have begun combining some of these manipulations — delivering an environmental stressor, for example, to mice lacking a particular schizophrenia-associated gene.

But when it comes to developing drugs, which are the best models? "There are so many risk factors, so many changes — the dopaminergic system, serotonin, GABA, you name it," says Thomas Steckler, a behavioural neuroscientist at Janssen Research and Development in Beerse, Belgium, who works in drug discovery. "We just don't know what best to model."

BEYOND 'ME TOO'

An animal model has two components: the manipulation that mimics some aspect of the disease, and the test — behavioural or otherwise — that measures the deficit that the manipulation produces. Schizophrenia researchers are getting a handle on the latter, but there's much less clarity regarding the former.

For years, schizophrenia drug discovery efforts were aimed at treatments for psychosis and delusions because new compounds could be tested against the two classes of antipsychotics — the only medicines licensed to treat the disease — that were already on the market. Unsurprisingly, that strategy was unproductive. "If you have models and all they are doing is comparing new approaches with what's already there, how can you move forward?" says neuroscientist Bita Moghaddam of the University of Pittsburgh in Pennsylvania. "All

we have done is make 'me too' drugs for the past 40 or 50 years."

But in the past 15 years, studies have shown that the disease has other core features that are at least as important as psychosis: difficulties with working memory, poor attention, and other cognitive symptoms. Drug discovery efforts have shifted accordingly, making some aspects of animal modelling more straightforward. "Modelling psychosis or negative symptoms [such as lack of emotion or motivation] is tremendously difficult, whereas we know an awful lot about cognition and brain systems in animals, and we know a lot of it translates to humans," says Trevor Robbins, a cognitive neuroscientist at the University of Cambridge, UK.

His lab and others have devised behavioural tests of cognitive processes in animals that can be matched to tests of cognitive processes in people with schizophrenia. One test, for example, probes attention by how accurately rodents respond to a brief stimulus in one of five locations in a cage, and how well they combat impulsivity by not responding before the stimulus occurs[1]. Two major collaborative projects aimed at boosting drug discovery efforts for cognitive symptoms of the disease are working on validating these tasks so researchers can be sure they are using comparable procedures and tapping into the right cognitive modalities. In the United States, researchers working within an effort called CNTRICS (Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia), funded by the National Institute of Mental Health, recently agreed on a handful of tasks that most accurately reflect each of six different cognitive or behavioural domains, such as working memory, executive function and motivation[2].

Meanwhile, as part of a €20 million (US$28 million), five-year public–private partnership in Europe launched in 2009, eight key industry players including Janssen, Eli Lilly and Novartis have joined forces to standardize a set of behavioural tasks that can be used in preclinical testing of new drugs. The collaboration, called NEWMEDS (Novel Methods leading to New Medications in Depression and Schizophrenia), had multiple participants do the same tasks to make sure their procedures aligned. "We started off with object recognition," says Mark Tricklebank, a behavioural neuroscientist at Eli Lilly in Surrey, UK, who is coordinating the animal model part of NEWMEDS. "We found that everybody was doing it in a slightly different way," he says. Researchers were using different-sized testing spaces and objects, for example, or testing at different times of the day. It took NEWMEDS several months per task to hammer out shared protocols. There is considerable overlap between the tests used by CNTRICS and NEWMEDS, but because NEWMEDS' goal is industry testing, it focused on touchscreen versions of the tasks.

"We are at a stage where we can reasonably well test our animals in paradigms that we at least think tap into the same cognitive domains" as they do in humans, says Steckler. Solving the other side of the animal model equation, however — how to produce a deficit in an animal that a drug must reverse — will be much tougher, he says.

CAUSES AND DEFICITS

Having such a wide range of manipulations available should provide the means to test biologically based hypotheses, says O'Donnell. One hallmark of the disease, for example, is the loss of a specific population of interneurons in the frontal cortex, a feature that is also present in many animal models. To determine whether targeting this mechanism will ameliorate disease symptoms, drug candidates need to be tested on this particular feature in animal models. But so far, he says, industry tests its drugs largely with older classical models, inducing symptoms with pharmacological agents. "Even though companies are adopting ideas coming from animal work," O'Donnell says, "the use of biology-specific models is not there yet."

That's changing, says Steven Siegel, director of the translational neuroscience programme at the University of Pennsylvania in Philadelphia. He says that industry researchers have reached out to his group and others to discuss potentially useful and reproducible models, and some companies are investing heavily in setting them up.

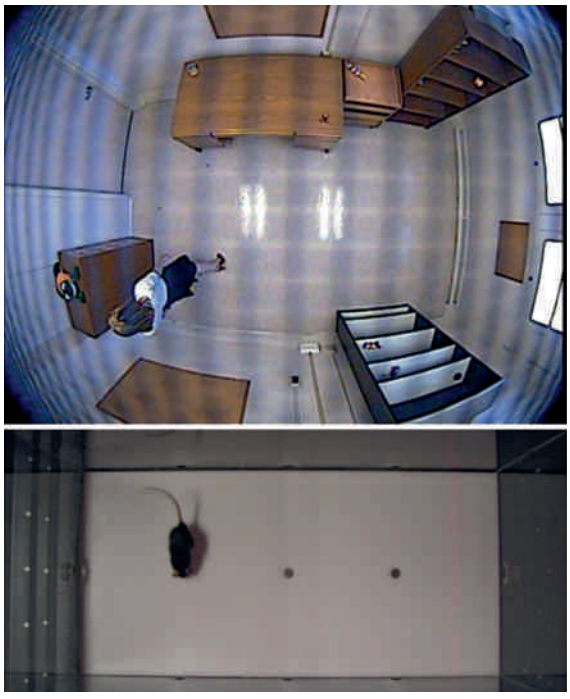
On the flip side, however, industry researchers contend that studies from academia are not always reproducible. Steckler cites the case where a university scientist identifies a cognitive deficit in a mouse in which a schizophrenia risk gene was knocked out. "That's your deliverable in academia; what you do with these data is you publish them," Steckler says. In industry, by contrast, that result must be more repeatable and robust, he says. But there's a catch: with no compound available that improves cognition in people with schizophrenia, it's unclear what to test models against.

In addition to its work on cognitive paradigms, NEWMEDS is trying to tackle the animal models issue from this end. The group has focused on trying to standardize a lesion manipulation in which pregnant rats or mice are injected with methylazoxymethanol acetate, which briefly blocks cell division in the developing embryos. As with hippocampal lesions, in adolescence the animals begin to show many hallmarks of schizophrenia, such as cortical thinning, ventricular enlargement, the loss of a subset of interneurons in the frontal cortex, and cognitive changes.

"The difficulty is diagnosing which animals to include so you have a deficit to correct in the drug trial."

Despite using animals that were bred and injected at the same facility maintaining protocols that are as close as possible among participating companies, the outcome varies — and some animals don't show the expected behavioural changes at all. That creates a problem when it's time to test them a year later. "The difficulty is then accurately diagnosing which animals to include in the drug treatment, so that you can be sure to have a deficit to correct in the pharmacological trial," Tricklebank says. The group is incorporating brain activation and other physiological measures that could also reflect whether a drug is having an effect. Results with genetic models have faced similar challenges, Tricklebank says, but he hopes the group will agree some standardized manipulations before its funding ends later in 2014.

Even if all these issues were resolved, says Tricklebank, there's still a big leap as to whether drug activity in an animal model predicts its activity in humans. Still, validating every step offers the best hope for success. Robbins, the academic leader of NEWMEDS' animal model efforts, agrees. "As far as I'm concerned, we're just at the beginning of a real scientific attack on these issues." ■



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Monitoring the behavioural patterns of humans (top) and mice.

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1. Amitai, N. & Markou, A. *Biol. Psychiatry* **68**, 5–16 (2010).
2. Moore, H. *et al. Neurosci. Biobehav. Rev.* **37**, 2087–2091 (2013).