

## *Translational animal models of autism and neurodevelopmental disorders*

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*Autism is a neurodevelopmental disorder whose diagnosis is based on three behavioral criteria: unusual reciprocal social interactions, deficits in communication, and stereotyped repetitive behaviors with restricted interests. A large number of de novo single gene mutations and chromosomal deletions are associated with autism spectrum disorders. Based on the strong genetic evidence, mice with targeted mutations in homologous genes have been generated as translational research tools. Mouse models of autism have revealed behavioral and biological outcomes of mutations in risk genes. The field is now poised to employ the most robust phenotypes in the most replicable mouse models for preclinical screening of novel therapeutics.*

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### Genetic causes of autism spectrum disorder

**A**utism spectrum disorders were originally diagnosed by Kanner and Asperger in the 1930s.<sup>1,2</sup> However, the diagnostic criteria were not codified until the 1994 *Diagnostic and Statistical Manual of Mental Disorders (DSM)*.<sup>3</sup> Astonishingly high heritability of autism spectrum disorders, reaching 90% concordance for monozygotic twins, as compared with less than 10% concordance for dizygotic twins and siblings, along with a 4:1 male:female ratio of prevalence, quickly led to an major international search for genes causing autism. By assembling large numbers of simplex and familial cases, several research consortia have discovered single gene mutations, rare and common polymorphisms, and epigenetic modifications associated with autism.<sup>4,5</sup> Copy number variants, including duplications of a sequence of genes within defined chromosomal loci, were reported to be relatively common in autism.<sup>6-9</sup> Clearly, autism is not a single-gene disorder.

To parse the role of each of these many genetic abnormalities in the etiology and symptomology of autism spectrum disorders, and in other neurodevelopmental disorders in which autism is concomitantly diagnosed, homologous genetic mutations have been generated in experimental animals. Because the targeted gene mutation technology was perfected in the mouse, mice are currently used throughout biomedical research as the primary model organism for generating transgenic and knockout mouse models of human genetic disorders. *Table I* and the descriptions in this review illustrate a small portion of the wealth of available mouse

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models of autism. In addition, recent advances in generating knockout rats<sup>10</sup> are leading to the development of mutant rat models of neurodevelopmental disorders.

Because concordance for autism spectrum disorder is not 100% between identical twins, whose genomes are presumably identical, environmental and epigenetic causes of autism spectrum disorders are also under investigation. Hypotheses about prenatal exposure to toxicological and immunological insults, and neuroanatomical lesions, have been modeled in mice, rats, and monkeys.<sup>11-18</sup> The challenge now is to understand the consequences of each of these genetic and environmental perturbations, and their interactions. Animal models employing behavioral assays relevant to the specific symptoms offer excellent translational research tools to identify the biological mechanisms underlying the core features of autism spectrum disorder.

## How do we model the behavioral symptoms of autism in mice?

As defined in the *DSM-IV*,<sup>3</sup> the diagnosis of autism requires the presence of at least six symptoms, including a minimum of two measures of qualitative impairment in social interaction, one symptom of qualitative impairment in communication, and one symptom of restricted and repetitive behaviour.<sup>19,20</sup> The *DSM-5* is expected to redefine Autism Spectrum Disorder into two symptom domains: (i) Social interaction and social communication deficits; (ii) Restricted, repetitive patterns of behavior, interests, or activities (<http://www.dsm5.org/ProposedRevisions/Pages/proposed-revision.aspx?rid=94>, January 2011). Associated symptoms that appear in subsets of individuals with autism include seizures, anxiety, intellectual impairment, hyperactivity, hyper-responsiveness and hyporesponsiveness to sen-

	Gene	Protein	Autism-relevant behavioral phenotypes
	<i>Integrin β3</i>	Integrin β3	Lack of preference for social novelty Increased repetitive self-grooming
	<i>Nlgn1</i>	Neuroigin 1	Increased repetitive self-grooming
	<i>Nlgn2</i>	Neuroigin 2	Increased anxiety-like behavior
Synaptic cell-adhesion proteins	<i>Nlgn3</i>	Neuroigin 3	Reduced pup ultrasonic vocalizations Sensory abnormalities
	<i>Ngln4</i>	Neuroigin 4	Reduced sociability and vocalizations
	<i>Neurexin-1α</i>	Neurexin-1α	Increased repetitive self-grooming
	<i>Cntnap2</i>	Contactin-associated protein2	Seizures, reduced social behaviors
	<i>Shank3</i>	Shank3	Mild reduction in social interactions and adult vocalizations
	<i>En2</i>	Engrailed-2	Reduced social behaviors - Impaired learning and memory
Signaling and developmental proteins	<i>Met</i>	Tyrosine kinase/ hepatocyte growth factor receptor	Cognitive deficit
	<i>Foxp2</i>	Forkhead box protein 2	Reduced pup ultrasonic vocalizations
	<i>Pten</i>	Phosphatase and tensin homolog	Reduced reciprocal social interactions - Reduced sociability
	<i>Avrp1</i>	Vasopressin receptor	Impaired social recognition - Reduced reciprocal social interaction Reduced ultrasonic vocalizations
Neurotransmitters and receptors	<i>Cadps2</i>	Calcium-dependent secretion activator 2	Reduced reciprocal social interactions
	<i>Gabbr3</i>	GABA A receptor beta3 subunit	Low sociability - Lack of preference for social novelty Repetitive stereotyped circling behavior
	<i>Oxtr</i>	Oxytocin receptor	Impaired social recognition - Reduced pup ultrasonic vocalizations
	<i>Slc6a4</i>	Solute carrier family 6, member 4 (Serotonin transporter)	Low sociability Lack of preference for social novelty

**Table 1.** Autism-relevant behavioral phenotypes in selected mouse models with targeted mutations in genes associated with autism.<sup>155-169</sup>

sory stimuli, sleep disruption, and gastrointestinal distress.<sup>20-26</sup>

Given that the defining criteria for autism are behavioral, investigations employing mouse models require considerable insight into which specific behaviors in the mouse repertoire are sufficiently relevant to each category of the diagnostic symptoms of autism. Inclusion of behavioral assays relevant to associated symptoms further enhance the heuristic value of animal models of autism spectrum disorders. We and other behavioral neuroscientists have generated a comprehensive set of assays for social interaction, social communication, and repetitive behaviors in mice, to test hypotheses about the causes of autism.<sup>27-45</sup> Social approach, reciprocal social interactions, olfactory communication, ultrasonic vocalizations, motor stereotypies such as circling and vertical jumping, repetitive behaviors such as self-grooming and digging, and perseveration in spatial tasks, are now in routine use for phenotyping mouse and rat models of autism and other neurodevelopmental disorders. Procedures for assaying behaviors relevant to associated symptoms of autism, including neurodevelopmental milestones, cognitive abilities, anxiety-like tendencies, seizures, motor dysfunctions, hyperactivity, responsiveness to sensory stimuli, and altered sleep patterns in mice have been adapted from the available behavioral neuroscience literature.<sup>46</sup>

In each case, we begin with the human endophenotype. In what ways are mouse behaviors similar to a defining feature of autism? Luckily, *Mus musculus* is a social species. Laboratory mice display a social repertoire that includes approach to olfactory pheromones emitted by other mice, approach to familiar and new conspecifics, reciprocal social interactions, ultrasonic vocalizations, communal nesting, sexual and parenting behaviors, territorial scent marking, and aggressive behaviors.<sup>47-49</sup> Standardized methods for scoring adult social approaches, reciprocal social interactions, nesting, sexual interactions, parental behaviors, and aggressive encounters are available in the behavioral neuroscience literature.<sup>28,50-61</sup>

### First diagnostic category

We employ social assays that have been refined from standard tests in the behavioral neuroscience literature.<sup>48,49</sup> These choices are designed to maximize relevance to the types of social deficits specific to autism,

including playing alone with inanimate toys rather than engaging in social interactions, and inappropriate responses to social cues. To quantify tendencies to engage in reciprocal social interactions, each subject mouse is paired with a novel partner mouse, inside a testing arena that permits free interactions over a test session of 10 to 30 minutes' duration. Digital videocameras record the session for later scoring of multiple parameters of social interactions. Ratings are performed by investigators who are blind to the genotype or treatment of the subject mice. Parameters routinely scored include sniffing, following, physical contact, and allogrooming.<sup>58,62</sup> Automated videotracking systems can accurately score some of the simpler elements of social interactions.<sup>34,35,44,63,64</sup>

Tendency to spend time with a novel mouse versus a novel nonsocial object is evaluated in a social approach apparatus (*Figure 1*). This 3-chambered assay, which was developed by our team to provide a simple measure of general sociability,<sup>30</sup> is widely used as an initial, high-throughput test for social deficits in mouse models of autism.<sup>27,32,34,38,41,64-70</sup>

### Second diagnostic category

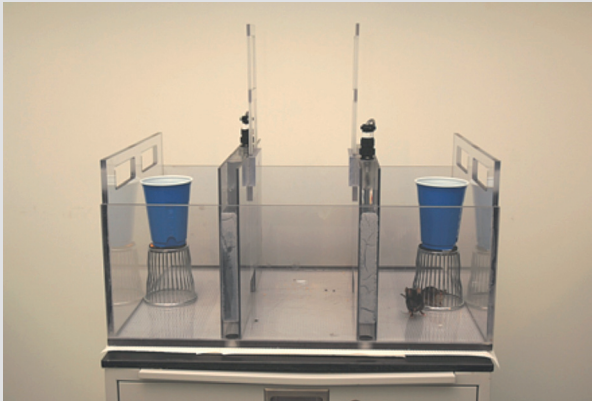
Social communication in rodents is mostly through the emission and detection of olfactory pheromones, and perhaps to a lesser extent, the emission and detection of ultrasonic vocalizations.<sup>40,44,55,57,59,60,71-74</sup> Olfactory communication is assayed by time spent sniffing olfactory stimuli from novel mice, and identification of novel versus familiar mice through olfactory cues.<sup>36,75,76</sup>

Vocal communication is assayed by recording ultrasonic vocalizations emitted during social interactions<sup>77</sup> (*Figure 2*). Number of calls and their properties are subsequently scored by investigators. Software is available for quantifying some of the simpler parameters. Different patterns of ultrasonic vocalizations are emitted by separated pups, adult males interacting with estrus females or urine from estrus females, adult females interacting with each other, and adult male residents in response to an intruder.<sup>72,78</sup>

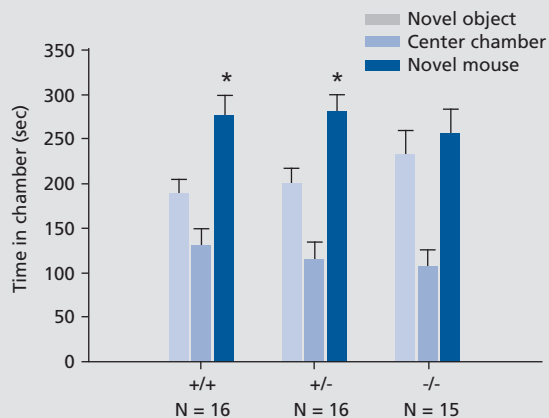
The anatomical and neurobiological substrates of olfactory and ultrasonic communication in mice do not precisely map onto the biological substrates of language and visual social communication in humans. In addition, considerably more work is needed to fully understand the communication value of ultrasonic vocalizations in mice.

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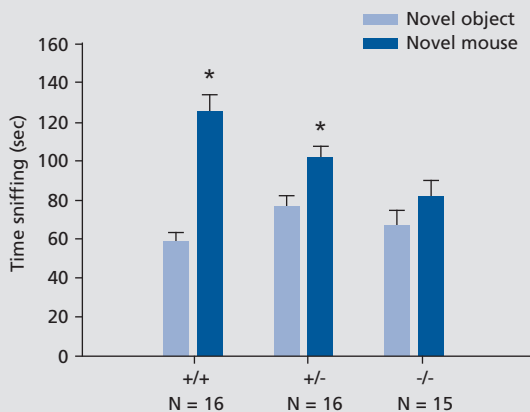
1a



1b Engrailed2 social approach



1c Engrailed2 social approach



At present, the existing tasks offer a reasonable start for discovering mechanistic similarities between species. Face validity of these mouse social interaction and communication tasks to the tendencies of people with autism to engage in less social approach and interaction, and to respond appropriately to complex social cues, remains inferential. We cannot know what a mouse is thinking, feeling, or intending, but only the quantifiable external expressions of those internal states.

### Third diagnostic category

Mice with various genetic mutations exhibit spontaneous motor stereotypies such as circling and vertical jumping, and spontaneous repetitive behaviors such as long bouts of self-grooming and excessive digging in the litter (Figure 3). Assays generally focus on the number of bouts of the behavior, or the cumulative time engaged in the behavior, during a defined test session of 10 minutes or

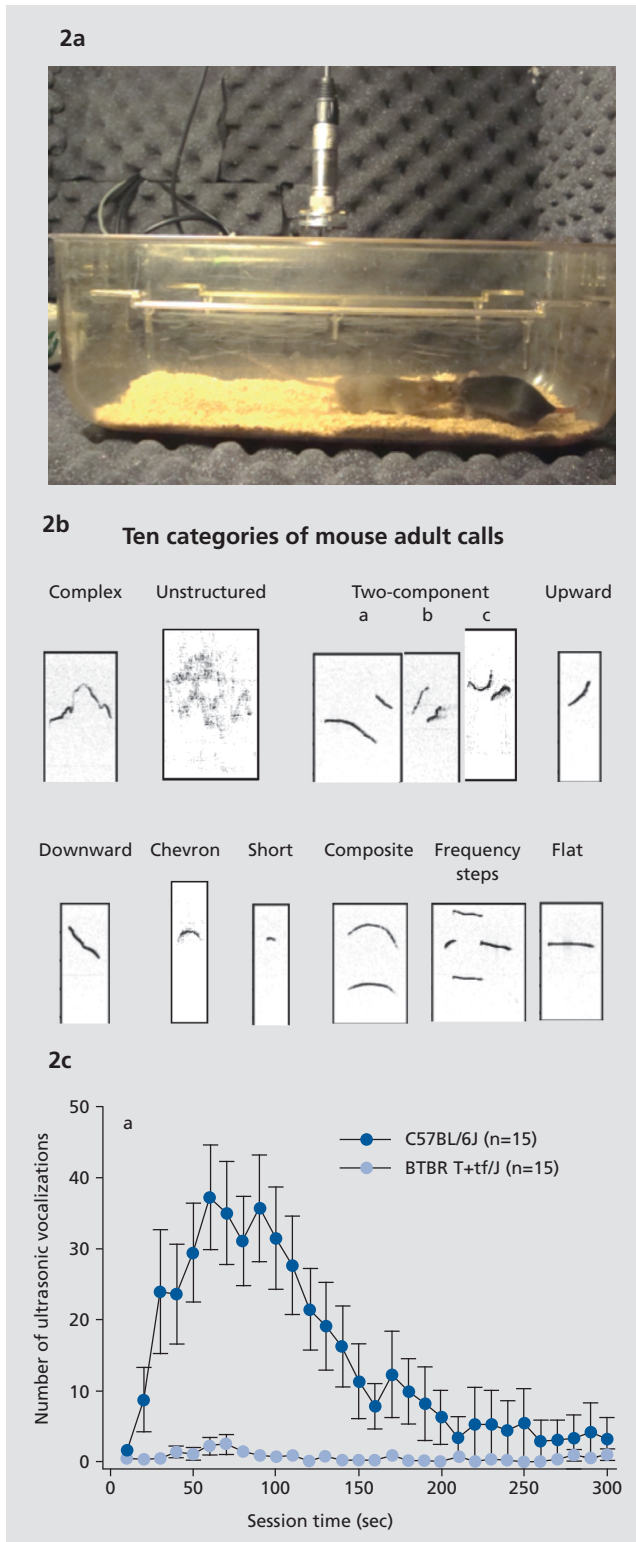
**Figure 1.** (a) Social approach apparatus for assaying sociability in mice.

The subject mouse begins in the empty center chamber of a three-chambered Plexiglas apparatus. A novel object, an inverted wire pencil cup, is placed in one side chamber. A novel mouse, who has not been in visual, olfactory, or tactile contact with the subject, is placed inside an identical wire control cup in the opposite side chamber. Over a 10-minute test session, the automated photocells and software score the amount of time the subject mouse spends in each of the three chambers. The number of entries into each compartment is simultaneously recorded as an internal control for general exploratory activity. Time spent engaged in bone fide social interactions with the novel mouse, and directed exploration of the novel object, are subsequently scored from digital videotapes of the session, by investigators uninformed of the genotype or treatment condition. Photograph by Dr Mu Yang, contributed by the author. (b) Social approach chamber time scores in adult Engrailed2 (*En2*) knockout mice. Normal sociability is defined as more time spent in the chamber with the novel mouse than in the chamber with the novel object, and more time spent sniffing the novel mouse than sniffing the novel object. Wild-type littermate controls and heterozygotes displayed normal sociability on the chamber time parameter, while *En2* null mutants failed to display sociability on this parameter.

Reproduced from reference 170: Brielmaier J, Matteson PG, Silverman JL, et al. Autism-relevant social abnormalities and cognitive deficits in engrailed-2 knockout mice. *PLoS One*. 2012;7:e40914.

(c) Social approach sniff time scores in adult *En2* mice. Wild-type littermate controls and heterozygotes displayed normal sociability on the sniff time parameter, while *En2* null mutants failed to display sociability on this parameter. Time spent sniffing the novel mouse is a more direct measure of social interactions, and usually more sensitive to mutations.

Reproduced from reference 170: Brielmaier J, Matteson PG, Silverman JL, et al. Autism-relevant social abnormalities and cognitive deficits in engrailed-2 knockout mice. *PLoS One*. 2012;7:e40914.



longer.<sup>40,58,65,79-81</sup> Restricted interests, insistence on sameness, and special interests are more challenging diagnostic features of autism to model in mice. Perseveration of spatial habits, such as difficulty in learning a new location of a reinforcer in a T-maze or water maze after the initial learning of a first location, has been employed with some success in mouse models of autism.<sup>82,83</sup>

**Associated symptoms**

Established, standardized tests are available in the voluminous behavioral neuroscience literature for most of the associated symptoms of autism.<sup>46,84-86</sup> Neurodevelopmental milestones are scored from postnatal day 2 to postnatal day 18 on physical attributes such as body length and eye opening, and behavioral attributes such as responses to handling and the righting reflex. Spontaneous seizures are scored with a rating scale or EEG electroencephalography. Learning and memory tests for mice include Morris water maze spatial navigation tasks, contextual and cued fear-conditioned freezing after exposure to an aversive foot-shock, and operant nose-poke and touch-screen reinforcement schedules.

Anxiety-related tests for rodents are primarily approach-avoidance conflicts. Mice are nocturnal. They prefer to be in dimly lit, enclosed environments. The current gold standard for anxiety-like tests for mice is the elevated plus-maze, which consists of two open and two enclosed arms, raised 1 meter from the floor, thus offering the choice between enclosed spaces and a high drop-off ledge.<sup>85</sup> The corroborating light↔dark test consists of

**Figure 2. (a)** Ultrasonic vocalizations are recorded in adult mice engaged in social interactions, using an ultrasonic microphone and specialized software. Photograph by Dr Jennifer Brielmaier, contributed by the author.

**(b)** Ultrasonic vocalization call categories in adult C57BL/6J mice, an inbred strain with normal sociability.

Reproduced from ref 153: Scattoni M, Ricceri L, Crawley J. Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes Brain Behav.* 2011;10:44-56. Copyright © Munksgaard 2011.

**(c)** C57BL/6J adult male mice emit high numbers of ultrasonic vocalizations, while BTBR adult male mice emit low numbers of ultrasonic vocalizations, during a 5-minute session with an estrus female mouse. BTBR T+tf/J (BTBR) is an inbred strain of mice that displays robust, well-replicated social deficits on multiple tasks,<sup>58,62,147</sup> markedly fewer vocalizations during social interaction sessions,<sup>59,153,154</sup> and high levels of repetitive self-grooming and marble burying,<sup>58,62,83</sup> representing the three diagnostic criteria for autism.

Reproduced from ref 154: Wöhr M, Roulet FJ, Crawley JN. Reduced scent marking and ultrasonic vocalizations in the BTBR T+tf/J mouse model of autism. *Genes Brain Behav.* 2011;10:35-43. Copyright © Munksgaard 2011

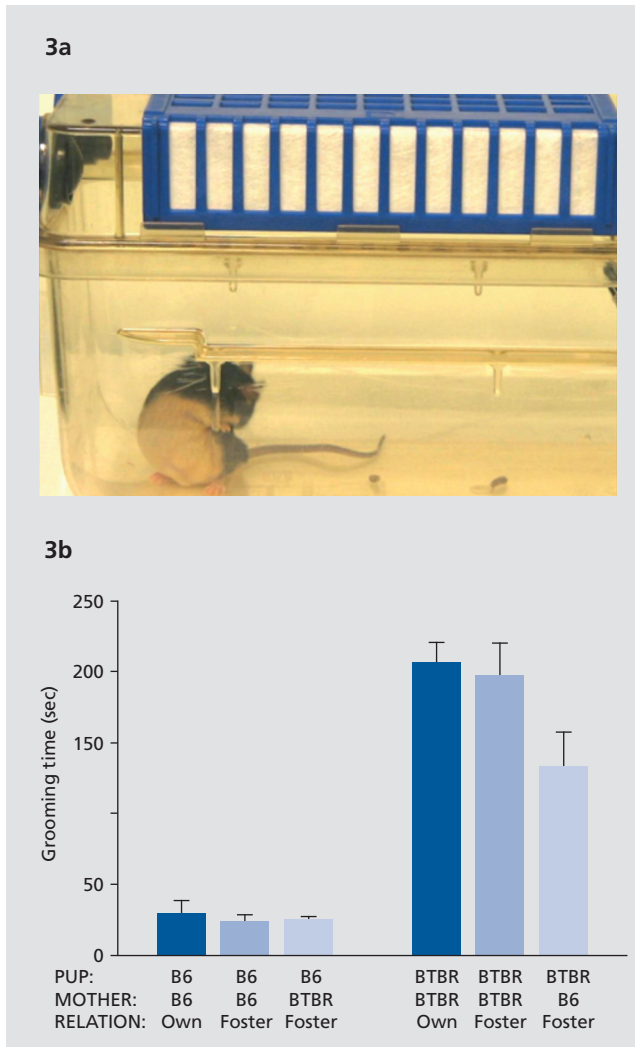
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a two-compartment apparatus in which one chamber is dark and enclosed while the other chamber is open and brightly lit.<sup>87,88</sup> Mice spend more time in the closed arms

of the elevated plus-maze and more time in the dark compartment of the light↔dark apparatus. Excessive anxiety-like traits are interpreted when the preference is unusually high for the closed arms and for the dark compartment. Anxiolytic-like treatment responses are interpreted when mice venture out more frequently into the open arms of the elevated plus-maze and the brightly lit chamber of the light↔dark box.

Responses to sensory stimuli include acoustic startle, olfactory habituation and dishabituation to a series of non-social and social odors, and the hot plate and tail flick thermal tests. Hyperactivity is scored from automated parameters of locomotion in a novel open field. Unusual sleep patterns are scored by observations of the home cage during the daylight sleeping hours and during the nighttime active hours, and/or by EEG recordings.

Optimal animal models should incorporate: (i) face validity, ie, close analogies to the defining features of the human syndrome; (ii) construct validity, ie, the biological dysfunction that causes the human disease, such as a gene mutation or anatomical abnormality; and (iii) predictive validity, ie, responsiveness to treatments that prevent or reverse symptoms in the human disease. The best animal models of autism and related developmental disorders will maximize face, construct, and predictive validities. At present this combination represents a very small subset of the model systems in use, particularly for neurodevelopmental disorders in which no effective therapeutics exist to test predictive validity of the animal model. The selected examples below are designed to illustrate the progress and promise of the mouse modeling approach in autism basic research and therapeutic development. Our goal is to systematically analyze the wealth of emerging animal models of neurodevelopmental disorders, understand the strengths and weaknesses of each, gain basic knowledge about phenotypic outcomes, and employ the best model systems for treatment discovery.



**Figure 3.** (a) Unusually high levels of spontaneous repetitive self grooming, in which the normal pattern of grooming behaviors are present but the bouts of grooming are strikingly prolonged, are measured over a 10-minute session in which the subject mouse is in an empty cage. Digital videos of the session are scored by an investigator uninformed of the genotype or treatment condition. Photograph by Dr Mu Yang, contributed by the author. (b) High levels of repetitive self-grooming are displayed by adult BTBR mice as compared with C57BL/6J mice. In this cross-fostering experiment, self-grooming scores in adults were found to be independent of the strain of the dam that raised the pup. Reproduced from ref 55: Panksepp JB, Jochman KA, Kim JU, et al. Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. *PLoS One*. 2007;2:e351. Copyright Public Library of Science 2007

## How do we discover therapeutics using mouse models?

The unmet medical need for effective treatments for neurodevelopmental disorders is striking. The number of reported cases of autism has risen rapidly over the past decade.<sup>89,90</sup> This rapid rise is largely a function of better diagnostic instruments and public awareness, although possible environmental causes and gene x environment interactions are under investigation.<sup>91-93</sup>

Personal and financial costs are high, to the affected individuals, their families, schools, and health care providers. At present the only effective interventions are intensive behavioral therapies.<sup>26,94</sup> The only pharmacological treatments approved by the US Food and Drug Administration are risperidone and aripiprazole: Risperdol™ and Abilify.™ Their approved use is solely for the associated “irritability,” which includes aggression, self-injury, and tantrums.<sup>95</sup>

A major revelation from the genetic association studies is that the most frequent mutations in autism are in genes that mediate the formation and maturation of synapses, particularly the postsynaptic densities, dendritic spines, and signaling mechanisms downstream from receptors mediating excitatory neurotransmission.<sup>96-100</sup> Pharmacological agents that alter synaptic functions are already available to some extent, and next-generation compounds are under development.<sup>101-104</sup> To evaluate the ability of novel drug treatments to reverse and/or prevent the symptoms of diseases, biomedical researchers often begin by testing exploratory compounds in appropriate animal models. Robust behavioral phenotypes with face validity to autism, in mouse models with construct validity to autism spectrum disorders, hold great promise as preclinical tools for discovering effective treatments for components of autism spectrum disorders.

Because rodents are similar to humans in many aspects of biochemistry, physiology, anatomy, and genetics, mice and rats are routinely employed in biomedical research as translational systems. Compounds that reverse behavioral and biological phenotypes in mouse models of autism offer leads which may be worth pursuing in human clinical trials. However, species differences exist in drug metabolism, alternate biochemical pathways, genetic variants, and toxicology. As in any field of biomedical research employing model systems, 100% predictive validity of efficacy and practicality in humans cannot be expected.

Keeping these caveats in mind, we design rigorous methods to evaluate proposed therapeutic interventions for autism spectrum disorders for their ability to reverse and/or prevent the major phenotypes in mouse models.<sup>29,43</sup> Behavioral pharmacologists test acute and chronic drug treatments, across a dose range, at various time points after administration, and assay for the most robust autism-relevant behaviors, in the strongest mouse models. Examples of some of the successes are described in the next section.

## Mouse models with high translational value

### Cell surface adhesion glycoproteins

Cell surface adhesion glycoproteins are a primary mechanism through which connections of presynaptic axons and postsynaptic dendrites are elaborated in neuronal synapses.<sup>97,105</sup> Mutations in cell surface protein genes have been reported with comparatively high frequency in neurodevelopmental disorders. Individuals with autism have been identified with mutations in *NEUREXIN1*, *NEUROLIGIN3*, *NEUROLIGIN4*, *SHANK2*, *SHANK3*, and *CNTNAP2*. For each of these rare mutations, a small number of individuals with the mutations who meet the diagnostic criteria for autism spectrum disorder has been identified.<sup>106-109</sup> Mice with homologous mutations in these genes are available from several excellent molecular genetics laboratories and from The Jackson Laboratory repository.

### *Shank3* knockout mice

*Shank3* knockout mice present a particularly fascinating example of the importance of the location of the mutation within the gene. The *Shank3* gene includes an ankyrin repeat domain, a PDZ domain, and a Homer binding domain.<sup>110-112</sup> Five distinct lines of *Shank3* knockout mice with mutations at these various sites were generated and phenotyped in the past 2 years.<sup>71,81,113,114</sup> Two lines of *Shank3* knockouts containing the mutation at the ankyrin domain displayed impairments in excitatory neurotransmission and long-term potentiation, but were predominantly normal on standard measures of sociability, with only small genotype differences detected in ultrasonic vocalizations and repetitive behavior.<sup>71,81</sup> Inserting the mutation at the Homer binding site resulted in mice with more social interactions, primarily in the form of aggression, along with mostly normal dendritic spines, reduced long-term potentiation, and enhanced long-term depression.<sup>113</sup> When the mutation was in the PDZ domain, *Shank3* knockouts displayed much more severe phenotypes, including high spontaneous self-grooming resulting in skin lesions, impaired sociability, reduced corticostriatal excitatory transmission, longer dendritic spines, and lower density of dendritic spines, as compared with wild-type controls.<sup>81</sup> These divergent outcomes of mutations at differing sites within the same gene provide a unique opportunity to

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understand the binding partners and their downstream signaling actions that determine the severity of symptoms in humans. For example, deficits in mGluR5 signaling have been reported after *Shank3* knockdown in neuronal cultures.<sup>115</sup> Augmentation of mGluR5 activity could be beneficial in cases of autism with *SHANK3* mutations, and in individuals with Phelan-McDermid syndrome, an intellectual disability syndrome in which the *SHANK3* mutation is central to the 22q13 chromosomal deletion.<sup>108</sup>

## Contactin associated protein 2 (Cntnap2)

Contactin associated protein 2 (*Cntnap2*), a member of the neurexin superfamily, plays a role in neuron-glia interactions and neuronal migration during early brain maturation. Mutations in *CNTNAP2* are associated with autism in a small number of individuals, particularly with language disabilities.<sup>107,116</sup> *Cntnap2* knockout mice were generated to understand the actions of this protein on brain development and autism-relevant behaviors.<sup>40</sup> Seizures were detected in 9 out of 10 null mutants. Social behaviors were impaired on the 3-chambered task, during reciprocal interactions, and in home cage nesting. Repetitive self-grooming was elevated. Resistance to change was seen in the Morris water maze, in which the initial learning was normal but the *Cntnap2* knockouts failed the reversal test when the escape platform location was changed. Less spontaneous alternation in a T-maze was seen in the null mutants, concomitant with moderate hyperactivity. Reduced number of GABAergic interneurons and impaired migration of cortical projection neurons in this line of *Cntnap2* mice underlie their seizures and some of their behavioral abnormalities. The Geschwind team proceeded to test risperidone, the antipsychotic approved by the US Food and Drug Administration for the treatment of irritability in autism. At 0.2 mg/kg IP daily for 7 days, a dose and regimen which did not affect locomotion in the wildtype controls, risperidone reduced the hyperactivity and repetitive self-grooming in *Cntnap2* null mutant mice.<sup>40</sup> Social behaviors were unaffected by the treatment with risperidone, which is an atypical antipsychotic.

Single gene mutations, chromosomal deletions, and duplications cause a variety of neurodevelopmental disorders, including Fragile X, Rett, Angelman, Prader-Willi, Smith-Lemli-Opitz, Timothy, Williams, and Phelan-McDermid syndromes, and tuberous sclerosis.<sup>97,108</sup> A

surprisingly large number of these de novo mutations code for signaling proteins that mediate the biochemical events downstream to postsynaptic neurotransmitter receptors. Interactome network analyses revealed convergences in genes that mediate transcriptional and splicing mechanisms that may be dysregulated in autism spectrum disorders.<sup>117</sup> Mutant mouse models of many of these syndromes have been generated.<sup>43,44,114,118-122</sup> While clinically distinct disorders caused by known single gene mutations suggest straightforward targets, as compared with complex disorders such as cases of autism in which the genetic substrates are unknown, increasing knowledge about the actions of downstream signaling proteins could identify pharmacological interventions which target key mechanistic sites in convergent biochemical cascades. Mice with homologous mutations are being employed as translational tools to evaluate convergent downstream target mechanisms, and to screen compounds that yield useful interventions at those sites.

## Tuberous sclerosis

Tuberous sclerosis, caused by a mutation in the *Tsc1* or *Tsc2* gene, is characterized by benign tubers in the cerebral cortex, seizures, a high incidence of intellectual impairment, and frequent comorbidity with autism.<sup>123,124</sup> *Tsc1* and *Tsc2*, which dimerize, are downstream targets of the PI3K/Akt postsynaptic signaling pathway elements that bind to mTORC2, and regulate mTORC1 at a further downstream site.<sup>125</sup> mTOR, the mammalian target of rapamycin, is a serine/threonine kinase which regulates many facets of brain development and cytoskeletal organization.<sup>126</sup> Deletion of *Tsc1* in knockout mice, hippocampal slices, or cortical cultures resulted in enlarged brains, large dysmorphic astrocytes, decreased myelination, reductions in  $\gamma$ -aminobutyric acid (GABA)-ergic interneurons in the cerebral cortex, and loss of mGluR-dependent long-term depression.<sup>126,127</sup> Mice with mutations in *Tsc2* display neuronal hypertrophy, reduced long-term potentiation in hippocampal slices, impaired hippocampally mediated fear conditioning, and impaired water maze learning.<sup>128</sup> Treatment with the mTOR inhibitor rapamycin for 5 days reversed the fear conditioning deficit and improved water maze learning, along with reducing brain weight and increasing survival.<sup>128</sup>

This early demonstration of a pharmacological rescue of phenotypes in a mouse model of a neurodevelopmental



disorder sparked optimism for treating disorders caused by perturbations in signal transduction.<sup>129</sup> In a separate mutant line, 4 weeks of treatment with rapamycin reduced the macrocephaly and increased the low social interaction in mice with a mutation in *Pten*, an upstream regulator of mTOR that is implicated in cancers, seizures, and autism.<sup>38</sup> Rapalogs, analogs of mTOR, are in clinical trials for cancers.<sup>130</sup> Rapalogs and compounds targeting PI3K and Akt<sup>131</sup> present possibilities for therapeutic interventions in neurodevelopmental disorders with underlying mechanisms in the mTOR signaling pathway.

### Fragile X syndrome

Fragile X syndrome is the most frequent genetic cause of intellectual disabilities. Constriction at the end of the X chromosome, termed a fragile site, is associated with a dramatic expansion of CGG triplet repeats, which transcriptionally silence the *FMR1* gene.<sup>132,133</sup> Fragile X mental retardation protein (FMRP) is highly expressed in the brain, where it negatively regulates the synthesis of a large number of downstream proteins.<sup>134</sup> Mice with a mutation in *Fmr1* display impairments in long-term potentiation, unusual social behaviors, and some unusual cognitive and anxiety-related behaviors.<sup>135-139</sup> One functional consequence of the *FMR1* mutation is upregulation of mGluR5 receptors.<sup>140</sup> Bear and colleagues discovered that crossing *mGluR5* knockout mice with *Fmr1* knockout mice rescued the impaired long-term depression, elevated the dendritic spine densities in the hippocampus, and attenuated seizures.<sup>141</sup> Negative allosteric modulators of the mGluR5 receptor were therefore postulated as potential treatments for Fragile X Syndrome. Clinical trials are in progress to test this hypothesis.<sup>142</sup> Approximately 30% of individuals with Fragile X syndrome meet the diagnostic criteria for autism.<sup>143</sup> Considering this high comorbidity, we reasoned that a treatment effective in Fragile X Syndrome

might act through a pathway convergent with other risk genes for autism.<sup>144,145</sup> We discovered that the prototypic mGluR5 antagonist 6-methyl-2-(phenylethynyl) pyridine (MPEP), and GRN-529, a more selective negative allosteric modulators of the mGluR5 receptor, reduced the high levels of repetitive self-grooming in BTBR mice,<sup>79,146</sup> an inbred strain that displays robust social deficits, low vocalizations in social settings, and high repetitive self-grooming and digging.<sup>42,58,60,65,78,79,147,148</sup> In addition, GRN-529 reduced the high levels of stereotyped jumping that characterize another inbred strain, C58/J.<sup>80,146,149</sup> Further, MPEP reduced marble burying in *Fmr1* knockout mice, reduced stereotypies in Swiss-Webster mice, and reduced repetitive self-grooming and marble burying in mice pretreated prenatally with valproic acid.<sup>14,150,151</sup> These reports lend credence to the notion that interventions acting through mGluR5 receptors could confer specific benefits for treating repetitive behaviors, a major component of the third diagnostic symptom of autism.

### Conclusions

Promising early findings of therapeutic rescues in mouse models have energized the rational search for pharmacological treatments of autism spectrum disorder. While the optimal developmental period for pharmacological intervention remains to be determined, adults with autism will likely be recruited for the first clinical trials,<sup>152</sup> since the risks of adverse drug reactions are predicted to be greater in children. Challenges will include discovering the critical window during development and/or adulthood at which interventions are useful, dosages, and treatment regimens which minimize toxicity. We have taken the first step in a long journey. □

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# Clinical research

## Modelos animales traslacionales de autismo y trastornos del neurodesarrollo

El autismo es un trastorno del neurodesarrollo cuyo diagnóstico se basa en tres criterios conductuales: interacciones sociales recíprocas inusuales, déficit en la comunicación y conductas repetitivas estereotipadas con intereses disminuidos. Los trastornos del espectro autista están asociados con un gran número de mutaciones monogénicas de novo y deleciones cromosómicas. En base a la gran evidencia genética, se han generado ratones con mutaciones específicas en genes homólogos como herramientas de investigación traslacional. Hay modelos de autismo en el ratón que han revelado resultados conductuales y biológicos que corresponden a mutaciones en genes de riesgo en genes de riesgo. Este campo ahora está preparado para emplear los fenotipos más potentes en los modelos de ratón más reproducibles para la evaluación preclínica de nuevas terapéuticas.

## Modèles animaux translationnels des troubles autistiques et neurodéveloppementaux

L'autisme est un trouble du neurodéveloppement dont le diagnostic se fonde sur 3 critères comportementaux : des interactions sociales réciproques inhabituelles, des déficits de communication et des comportements répétitifs stéréotypés accompagnés d'intérêts restreints. Les troubles autistiques sont associés à de nombreuses mutations monogéniques de novo et à des délétions chromosomiques. Sur la base des arguments génétiques solides, des souris aux mutations ciblées sur des gènes homologues ont été élevées comme outil de recherche translationnelle. Les modèles murins d'autisme ont présenté des mutations biologiques et comportementales correspondant aux mutations des gènes à risque. Ce domaine de recherche est maintenant prêt à employer les phénotypes les plus fiables des modèles murins les plus reproductibles pour le dépistage préclinique de nouveaux traitements.

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