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Mouse Behavioral Assays Relevant to the Symptoms of Autism*

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While the cause of autism remains unknown, the high concordance between monozygotic twins supports a strong genetic component. The importance of genetic factors in autism encourages the development of mutant mouse models, to advance our understanding of biological mechanisms underlying autistic behaviors. Mouse models of human neuropsychiatric diseases are designed to optimize (i) face validity (resemblance to the human symptoms) (ii) construct validity (similarity to the underlying causes of the disease) and (iii) predictive validity (expected responses to treatments that are effective in the human disease). There is a growing need for mouse behavioral tasks with all three types of validity, to define robust phenotypes in mouse models of autism. Ideal mouse models will incorporate analogies to the three diagnostic symptoms of autism: abnormal social interactions, deficits in communication and high levels of repetitive behaviors. Social approach is tested in an automated three chambered apparatus that offers the subject a choice between spending time with another mouse, with a novel object, or remaining in an empty familiar environment. Reciprocal social interaction is scored from videotapes of interactions between pairs of unfamiliar mice. Communication is evaluated by measuring emission and responses to vocalizations and olfactory cues. Repetitive behaviors are scored for measures of grooming, jumping, or stereotyped sniffing of one location or object. Insistence on sameness is modeled by scoring a change in habit, for example, reversal of the spatial location of a reinforcer in the Morris water maze or T-maze. Associated features of autism, for example, mouse phenotypes relevant to anxiety, seizures, sleep disturbances and sensory hypersensitivity, may be useful to include in a mouse model that meets some of the core diagnostic criteria. Applications of these assays include (i) behavioral phenotyping of transgenic and knockout mice with mutations in genes relevant to autism; (ii) characterization of inbred strains of mice; (iii) evaluation of environmental toxins; (iv) comparison of behavioral phenotypes with genetic factors, such as unusual expression patterns of genes or unusual single nucleotide polymorphisms; and (v) evaluation of proposed therapeutics for the treatment of autism.

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STRATEGIES FOR DESIGNING MOUSE MODELS OF AUTISM

Developing rodent behavioral tasks relevant to the symptoms of autism presents a unique challenge to behavioral neuroscientists. Rodent models of neuropsychiatric disorders have a long and illustrious history. Models of generalized anxiety disorder have focused on approach–avoidance conflict behaviors, including the elevated plus maze, light↔dark exploration, marble burying and Vogel thirsty-lick conflict tests (19, 35, 41, 44, 60, 62, 88, 163, 172). Cognitive deficits in Alzheimer's models are detected with learning and memory tests, including (i) spatial navigation tasks such as the Morris water maze, Barnes maze, radial maze and T-maze; (ii) working memory tasks such as object recognition and attentional set-shift, emotional memory tasks such as contextual and cued fear conditioning; (iii) olfactory memory tasks such as a social transmission of food preference; and (iv) aversive tasks such as active and passive avoidance (34, 44, 45, 82, 92, 133, 165). Parkinson's and Huntington's disease models utilize sensitive motor tasks such as balance beam walking, rotarod and footprint pattern (30, 44, 104, 112, 168). Drug abuse models use self-administration, conditioned place preference and two bottle choice tests to measure the rewarding properties of addictive drugs (27, 40, 50, 106, 161, 189, 199). Rodent tasks sensitive to antidepressant drugs include forced swim, tail suspension and stressor-induced anhedonia (19, 49, 105, 119, 120, 122, 131, 145, 158).

While a rodent model cannot replicate a human disease, fundamental symptoms can be approximated for the purposes of testing theories about the biochemical, genetic and environmental causes of the human condition. Hypotheses about genes underlying neuropsychiatric disorders are addressed by applying behaviorally relevant assays to phenotype mice with targeted gene mutations, and to compare the genetic profiles of inbred strains of mice with dissimilar behavioral phenotypes (15, 44, 46, 99, 135, 156, 175, 184, 206). Furthermore, a robust rodent model has translational value in offering preclinical surrogate markers to evaluate treatment efficacy. Rodent behavioral tasks provided useful preclinical tools for the discovery of psychopharmacological treatments for many major mental illnesses and neurological diseases (37, 47, 72, 82, 142, 174). We are engaged in a growing initiative to develop behavioral tests for mice that

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approximate the core symptoms of autism spectrum disorders in humans.

Autism is a neurodevelopmental disorder with a strong genetic basis (3, 38, 64, 97, 98, 137, 155, 169, 186, 201, 203). The diagnostic criteria for autism are aberrant reciprocal social interactions, impaired communication and stereotyped repetitive behaviors with narrow restricted interests (2, 64, 66, 100, 118, 127, 148, 154, 167, 182). The range of defining and associated features includes reduced eye contact, lack of gesturing, language delays, lack of prosody in speech, inability to interpret emotions from the facial expressions of others, reduced joint attention, impaired attentional shift, hypersensitivity to sensory stimuli, stereotyped hand flapping and upset to change in routine (9, 17, 53, 54, 61, 66, 100, 118, 182). Additional associated features in some cases include mental retardation, absence of Theory of Mind, seizures, self-injury, anxiety, sleep disturbances, gastrointestinal disturbances and larger head circumference and brain volume at young ages (2, 51, 52, 54, 66, 74, 78, 140, 153, 167, 182, 187). The importance of genetic factors in the etiology of autism is recognized in the growing literature of twin and family studies that demonstrate up to 60% concordance for autism and 90% concordance for autism spectrum disorders between monozygotic twins, as well as a male : female ratio of approximately 4:1 (13, 52, 64). Several large international and collaborative linkage analyses and association studies have identified several chromosomal regions and gene polymorphisms for autism, implicating multiple candidate genes and supporting a complex multigenic etiology (1, 4, 5, 10, 21, 97, 98, 101, 115, 159, 170, 186, 202).

Autism may be particularly difficult to model in rodents. Theory of mind, the ability to empathize and intuit the feelings and intentions of others, may be impossible to parallel in mice. Mice cannot be evaluated for speech deficits, and may not have brain regions directly comparable to those mediating human language skills relevant to autism. Luckily, *Mus musculus*, the house mouse species used in molecular and behavioral genetics research, is a social species that engages in high degrees of social interaction and social communication (73, 76). Non-verbal forms of mouse communication and interaction are amenable to quantitative analysis. Useful rodent models of autism will include behavioral features with face validity, that is, conceptual analogy, to at least one core diagnostic human symptom. Ideal mouse models will incorporate components relevant to all three diagnostic symptoms, and some of the associated symptoms. Modest goals for paralleling some of the core and associated symptoms of autism are likely to yield mouse models with considerable heuristic value for understanding genetic mechanisms and evaluating potential treatments for autism.

To date, several mouse models of autism have been proposed (3, 157, 207). One approach is targeted gene mutation for neurotransmitters and developmental genes that may regulate social behaviors. Oxytocin is a hypothalamic neuropeptide that contributes to pair-bonding and social affiliation behaviors in some species (28, 29, 204). Young, Winslow and Insel at Emory University tested a line of mice with a targeted mutation in the gene for oxytocin (95, 194, 205). Oxytocin knockout mice displayed deficits in social recognition and social memory (58, 95, 194, 204), although baseline social approach was normal (48). Oxytocin knockout mouse pups emitted fewer ultrasonic distress vocalizations when separated from their parents (195). Conversely, repeated central administration of oxytocin increased ultrasonic vocalizations in hamsters and voles (63, 108). Similarly, vasopressin, another hypothalamic neuropeptide, and its V1a receptor, facilitated social behaviors in some rodent species (110, 151). Vasopressin receptor subtype 1a knockout mice displayed reduced social recognition as measured by failure to habituate to a novel stranger (14). Vasopressin receptor subtype 1b knockout mice displayed reduced social motivation and aggression, while olfactory abilities appeared normal (191, 192). Salinger et al at the University of North Carolina at Greensboro conducted comprehensive behavioral phenotyping of Reeler mice, deficient in the *Reln* gene, reporting higher levels of social dominance in the null mutants, possibly relating to abnormal response inhibition (164). Null mutants for *dishevelled-1*, a developmental gene in the Wnt signaling pathway, showed deficits in nest building and home cage huddling, as well as subordinate behaviors in a social dominance tube test (114, 116).

A second approach is to generate defects in neurotransmitters or brain regions that are analogous to neurochemical or anatomical abnormalities seen in autism. Sulik et al at the University of North Carolina detected higher levels of serotonin in the hindbrain of the *Dhcr7* null mutant model of Smith-Lemli-Opitz syndrome (183). Hohmann at Morgan State University and coworkers investigated cortical morphology deficits following neonatal serotonin depletion (20, 86). Goldowitz et al at the University of Tennessee pursued the cerebellar abnormalities associated with autism, using heterozygous Lurcher (*Lcl/+*) mutant mice (121). Mild motor deficits were associated with minimal loss of cerebellar Purkinje cells in the heterozygotes, and significant deficits in spatial learning on the Morris water maze were detected (121). Rodier et al at the University of Rochester treated pregnant rats with a teratogenic drug, valproic acid, during the fetal developmental stage of neural tube closure, to model reports of autism that followed exposure to the teratogenic drug thalidomide (93, 162). Cranial nerves III, V, VI and XII displayed diminished motor neuron numbers, similar to that seen in the human case (162). In addition, reductions in cerebellar volume and Purkinje cell number were found to parallel those seen in cases of autism (93). Valproate-treated rats displayed faster acquisition of eye blink conditioning (173).

A third approach is based on the comorbidity of other human diseases with autism, in which a portion of the patients display autism-like symptoms. Lines of mice have been generated with targeted gene mutations relevant to Angelman syndrome (171); Smith-Lemli-Opitz syndrome (183, 185), Fragile X (33, 36, 65, 107, 144, 188), Rett syndrome (11, 132, 207) and Down syndrome (55, 83, 130, 180).

As candidate genes for autism continue to be discovered by human linkage analyses, new knockout mice are being generated to test hypotheses about the functions of these candidate genes. Since approximately 99% of human genes have ortholog counterparts in mice (178), many targeted gene mutations in mice may be forthcoming as potential models of autism-related genetic dysfunctions. Furthermore, mutations in developmental genes and synaptic proteins may offer mouse models with

construct validity to the etiology of autism. For example, mutation of neurodevelopmental genes such as *uPAR*, *Pten* and *En2* yielded mice with social and other behavioral deficits (32, 109, 113).

Advances in genetic techniques offer new tools for mouse behavioral genetics. In addition to targeted gene mutations and quantitative trait loci analyses, reverse genetics using chemical mutagenesis and DNA microarrays serve to identify genes correlated with a phenotype, for example, to discover genes mediating social behaviors (43, 135, 139). One hypothesis that our lab and others are pursuing is that a discrete number of genes regulate the natural variations in normal mouse social behaviors, and that it will be possible to discover the genes responsible for the extremes of the normal distributions in behavioral phenotypes relevant to autism, using a variety of inbred strains and mutant lines (18, 24, 43, 124, 135, 136, 139, 147).

There appears to be a growing need for a defined set of behavioral tasks relevant to the symptoms of autism, particularly in the domains of social, communication and repetitive behaviors, which can be uniformly applied across mouse models and genetic technologies (3, 43, 94). We are devoting considerable attention to understanding the clinical symptoms of autism, with the goal of designing a robust set of mouse behavioral tasks to detect autism-like traits in mutant mouse models. Our Laboratory of Behavioral Neuroscience had the good fortune to collaborate closely with Professor Joseph Piven, and colleagues in the Autism Research Centre at the Neurodevelopmental Disorders Research Centre of the University of North Carolina at Chapel Hill, who are experts in the clinical and genetic components of autism, and Dr Sheryl Moy, Director of the UNC NDRC Mouse Behavioral Phenotyping Laboratory. Their insights, along with observations shared by many other autism clinical researchers, guide our thinking and choices of appropriate behavioral tasks for mice.

MOUSE BEHAVIORAL TASKS RELEVANT TO THE CORE SYMPTOMS OF AUTISM

Sociability. Qualitative and quantitative impairments in social interaction are the first defining feature of autism (2, 118, 182). The original explication of autism (100) characterized autistic children by a dramatic lack of interest in others. Current DSM-IV criteria recognize the variable severity of deficits in reciprocal social interaction, unusual and inappropriate social approach behaviors, and the developmental changes in these symptoms across ontogeny (2, 152, 182). We reason that a critical component in a mouse model of autism is a quantitative measure of appropriate social interaction. Mice are a highly social species, displaying social investigation of an unfamiliar conspecific (an individual of the same species), communal nesting, sleeping in group huddles, aggression directed towards intruders, sexual approach and mating behavior patterns, parental care of the pups and juvenile play (16, 76, 111, 146, 157, 160). Behavioral neuroscientists use standardized scoring methods to quantitate these various types of social interactions in mice (14, 18, 25, 59, 117, 123, 157, 193, 194). Starting from this existing literature, we designed an automated apparatus to detect unusually low levels of normal mouse sociability that may be analogous to the deficits in appropriate social interaction seen in many cases of autism (48, 124, 134, 136, 138).

This simple automated social approach task measures the tendency of the subject mouse to approach another mouse and engage in social investigation. The subject is placed in a compartmentalized Plexiglas box that offers a choice of spending time with a novel conspecific vs. spending time with a novel object. Figure 1A illustrates the three-chambered automated apparatus. One side chamber contains a stranger mouse that is contained in a wire cup that permits visual, olfactory, auditory and some tactile contact. The other side cham-

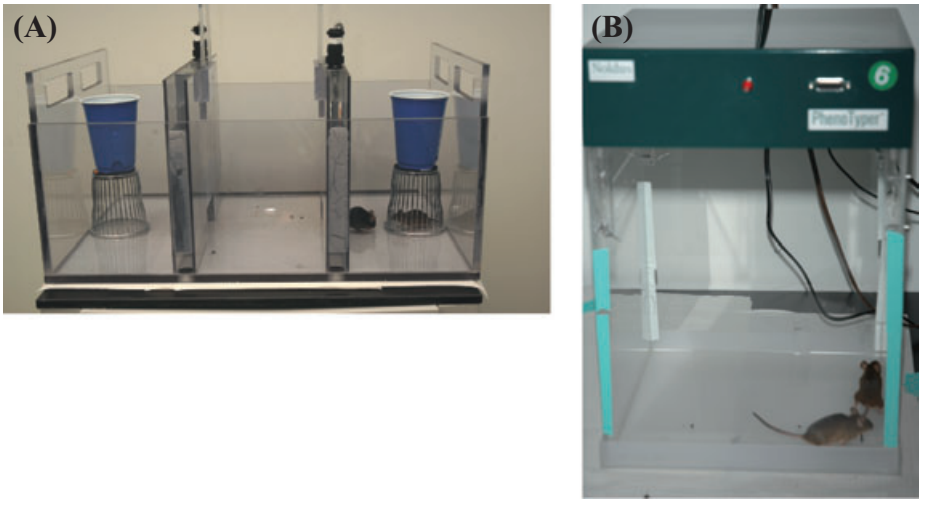

Figure 1. (**A**) Automated three-chambered apparatus for quantitating social approach behaviors in mice (48, 124, 134, 136, 138). The session starts with the subject mouse in the center chamber for a 10-minute habituation period. After habituation, the subject mouse is contained in the center chamber while a clean empty inverted wire pencil cup (novel object) is placed in one side chamber. Simultaneously, an adult male conspecific mouse that has had no previous contact with the subject (novel stranger) is placed in an inverted wire pencil cup cage in the other side chamber. Weights are placed on top of the cups, to prevent the subject mouse from climbing on top of the cups. Retractable doors between the chambers are raised to begin the 10-minute sociability test. Photocell motion detection beams across the doorways send information to a software interface that records (i) entries of the subject mouse into each chamber, and (ii) time spent in each chamber. A human observer records (i) time spent by the subject in sniffing within 1 cm of the wire cup containing the stranger mouse and (ii) time spent sniffing the novel object. Because the stranger is contained in the wire cup, social approach is initiated only by the subject. The wire cup allows visual, olfactory, auditory and some tactile contact between the subject and the stranger. This task measures sociability, the tendency of the subject mouse to spend time with a conspecific, as compared with time spent in the other two chambers. Sniffs directed towards the novel mouse as compared with sniffs directed toward the novel object confirm the social nature of the approach. Number of entries provides a control for general exploratory activity and anxiety-like behaviors. (**B**) Juvenile play apparatus for scoring reciprocal social interactions in 21-day-old mice (124). The Noldus Phenotyper 3000 Plexiglas arena is fitted with a video camera in the ceiling. Noldus Observer software is used to score individual bouts and durations of social interaction parameters, including following, pushing past, crawling over and under, nose-to-nose sniffing, anogenital sniffing and social grooming. Photographs by Janet Stephens, NIH Macrophotography, and contributed by the author.

ber contains the novel object, an inverted empty wire pencil cup which elicits a high level of exploratory behaviors in mice. The center chamber is completely empty, serving as a control for general locomotor activity. Photocell emitters embedded in the panels send infrared beams across the openings between the chambers. Photocell detectors embedded in the opposite site of the openings act as motion detectors, activated when the mouse sequentially breaks and unbreaks the series of beams. A software interface system automatically detects and records the photocell beam breaks, and counts the number of seconds spent in each compartment, over session durations chosen by the experimenter. The automated system was designed by Mr George Dold and coworkers in the NIMH/NINDS Research Services Branch in Bethesda, MD, USA. Diagrams and schematics are published (138) and available upon request.

Procedures using the three-chambered apparatus start by placing the subject mouse in the center chamber for a 10-minute habituation period, during which the center area becomes a familiar "home base." Sliding doors in the chamber divider panels are then raised, allowing the mouse to explore and habituate to all three empty chambers. The novel object, that is, the empty inverted wire pencil cup, is then placed in one side chamber, and an unfamiliar male "stranger" mouse is simultaneously placed inside another inverted wire pencil cup cage in the other side chamber. Because the wire container allows olfactory, visual, auditory and some tactile contact, the subject can detect social cues emitted by the stranger, and initiate many components of social interaction, directed towards the stranger in the wire cage. The strangers are usually adult male C57BL/6J mice that had no prior physical contact with the subject. Each stranger was previously habituated to the wire cage, so that it is generally inactive during the test session. Locating the stranger inside the wire cage ensures that all social approaches are initiated only by the subject mouse, and prevents aggressive fighting or sexual engagement. Information obtained from the photocell beam breaks across the openings between the three chambers, analyzed by the software interface, includes: (i) the movements of the subject mouse from one chamber to another; and (ii) the amount of time that the subject mouse spends in each of the three chambers. Data are collected in time bins defined by the investigator. The equipment can be programmed for various session lengths, from 5 to 30 minutes duration. To determine whether time spent in the chamber containing the stranger mouse reflects actual exploring of the stranger mouse, vs. non-social exploring of other areas of the chamber, a human observer records the time that the subject spent sniffing the wire cage containing the stranger, using two stopwatches or a computer keypad. Correlation between time spent in the chamber and time spent sniffing is significant (138), indicating that the automated parameter represents true social interaction. Many strains of mice commonly used in behavioral genetics, including C57BL/6J, DBA/2J, FVB/NJ, C3H and AKR, spent significantly more time in the chamber with the stranger mouse than in the other side chamber containing the identical but empty wire cage, or in the central start chamber (134, 136, 138). Investigatory sniffs of the chamber containing the stranger mouse were significantly higher than sniffs of the empty wire cage (136, 138). These high levels of social interaction were seen in both male and female subjects, when this sociability test was conducted at juvenile and adult ages, and when the sociability test was conducted during the light or the dark phase of the circadian cycle (124, 134, 138, 200). Repeated testing of the same subject mice at various ages yielded similar scores (134), indicating reliability of the measurements. Similar scores were obtained when the stranger was of the identical strain or a different strain, supporting the interpretation that this task measures inherent social tendencies of the subject mice (138). Analysis of 5-minute time bins across 30-minute test sessions indicated that the majority of the social interaction occurred in the first 10 minutes, supporting the use of a 10-minute test session (138). Number of entries was identical between chambers (134, 138), indicating that the subject mice explored all three chambers but preferred to spend more time interacting with the stranger mouse. Number of entries is therefore used as a control measure for general exploratory locomotor activity, to rule out false positives due to poor motor abilities and to confirm the absence of innate side preferences. Low number of entries could also reflect an anxiety-related deficit in exploration of the novel environment, prompting further testing in more specific anxiety-related tasks. Several inbred strains with low social approach, including A/J, BALB/cByJ, 129S1SvImJ and BTBR T+tf/J, have been detected to date (124, 134, 136).

Juvenile play. Our laboratory recommends that at least two corroborating tasks be conducted within a behavioral domain of interest (42, 44, 45, 89, 90). If the same direction of effects occurs in both tasks, then the interpretation of the results is very strong. For example, deficits on two different learning and memory tasks would provide stronger evidence for a fundamental cognitive deficit in a transgenic mouse than if only one task had been conducted. Alternatively, if different results are obtained in the two tasks, then the specific type of deficit could be explored in further experiments. For instance, if spatial navigation learning was normal but cued fear conditioning was impaired in a knockout, then future experiments could focus on amygdala-dependent emotional learning and memory.

Low levels of social approach are insufficient to adequately describe the complexity and variability of the social deficits in autism. More fine-grained analyses of reciprocal social interactions are available (eg, 18, 32, 59, 76, 81, 85, 88, 109, 117, 123, 146, 172, 194). Freely moving or tethered stranger mice are likely to elicit more complex approach behaviors by the subject than caged strangers, to evaluate social reciprocity. Videotracking systems and/or human observation methods that quantitate the full spectrum of social approach and reciprocal social interactions, including following, nose-to-nose contacts, nose-to-anogenital contacts, sexual approaches, aggressive intention movements, attack behaviors, escape behaviors, home cage nesting patterns, juvenile rough and tumble play, parental behaviors, etc., will enhance our understanding of the true nature of social deficits in mouse models of autism. Testing subject mice at different ages, including infant, juvenile, young adult and older adult, will address the neurodevelopmental components of autism.

Tendencies to avoid social contact can be further analyzed with a modified place preference task (147), and the social transmission of food preference task (124). Testing subject mice for complex social interactions with their parents, siblings, peers and members of the opposite sex, may provide richer detail about social interactions relevant to autism.

We have pursued juvenile play as a test for reciprocal social interactions to target the younger ages at which autism is first diagnosed. Using the Noldus Phenotyper 3000 arena (Figure 1B), video camera and Noldus Observer software, pairs of unfamiliar 21-day-old male mice were paired for 30-minute interaction sessions and scored for measures of nose-to-nose sniffing, anogenital sniffing, following, crawling over/under, social grooming, self-grooming, exploratory activity and many control measures. The BTBR T+tf/J inbred strain, which displayed low levels of adult social approach and normal scores on procedural controls, displayed low levels of juvenile nose-to-nose sniff, follow and social grooming (124). In addition, unusually high levels of self-grooming in BTBR T+tf/J were apparent, suggesting that this inbred strain incorporates social deficits and repetitive behaviors with face validity to at least two of the diagnostic criteria for autism (124).

Communication. Delayed language and poor communication skills are fundamental to the diagnosis of autism (56, 100, 118, 167). Although mice do not use language, they do display possible social communication mechanisms. Mice emit auditory signals, including ultrasonic vocalizations, and olfactory social signals, including deposition of pheromones in the environment (23, 39, 84, 102, 157). Conspecifics appear to interpret and respond to these auditory and olfactory emissions. One well-characterized method for apparent social communicative interactions in rodents is the ultrasonic vocalization emitted by pups when they are out of the nest (22, 26, 77, 84, 85, 190, 195). The parents detect this 50–80 kHz ultrasonic "distress" call, locate the pup, and retrieve it to the nest. Maternal potentiation of ultrasonic vocalizations, in which the second separation elicits more calls than the first (85), may represent a higher level of cognition and communication components. Quantitative measures of ultrasonic vocalization by pups removed from the nest, and parental retrieval of the pups, are tests that could detect deficits in communicative interactions in mice (9, 22, 157). Low levels of this type of infant vocalization may be relevant to the statements by some parents that their autistic children were very easy babies who seldom cried (66). Low levels of retrieval by the adult mice could indicate failure to respond appropriately to socially meaningful stimuli. Figure 2A illustrates an ultrasonic detector microphone that records vocalizations. In a standard test of separation-induced vocalizations, a 5- to 12-day-old mouse pup is removed from the nest and the home cage and placed in a warm soundproof chamber directly under the microphone (9, 128, 166, 190). We predict that infant mice with mutations in genes relevant to autism will show fewer ultrasonic vocalization calls, and/or less maternal quieting when placed back with their mothers. Adult mice with targeted mutations in genes relevant to autism may fail to respond to the ultrasonic vocalizations of their pups, as measured by deficits in retrieval of separated pups back into the nest.

In addition, it may be useful to record vocalizations during social play in juvenile mice and during various forms of social interaction in adult mice. Ethological observations of the behaviors associated with vocalizations may yield insights into the communicative information conveyed by different vocalizations in mice during social interactions (23, 147). Distress calls (39, 190) could conceivably measure the stress level of a juvenile or adult mouse when placed in a social milieu. Vocalizations emitted by adult mice in a social place preference conditioning task (147) and during sexual encounters (91) support the possibility that communicative vocalizations occur in the context of social situations in adult mice.

A second corroboratory approach to investigating social communication in mice is to focus on olfactory communication. Rodents deposit pheromones in their environment that appear to define territorial borders, identify members of the colony and communicate sexual receptivity (7, 79, 143). Existing olfactory tasks could conceivably be modified to address concepts such as joint attention and learning from observation of social cues. For example, olfactory habituation/dishabituation measures interest in novel olfactory stimuli and waning interest in repetitions of the same olfactory stimulus (124, 197). Social olfactory cues, such as soiled cage wipes presented on a cotton swab, are used to evaluate interest in social odors (48, 124; Figure 2B).

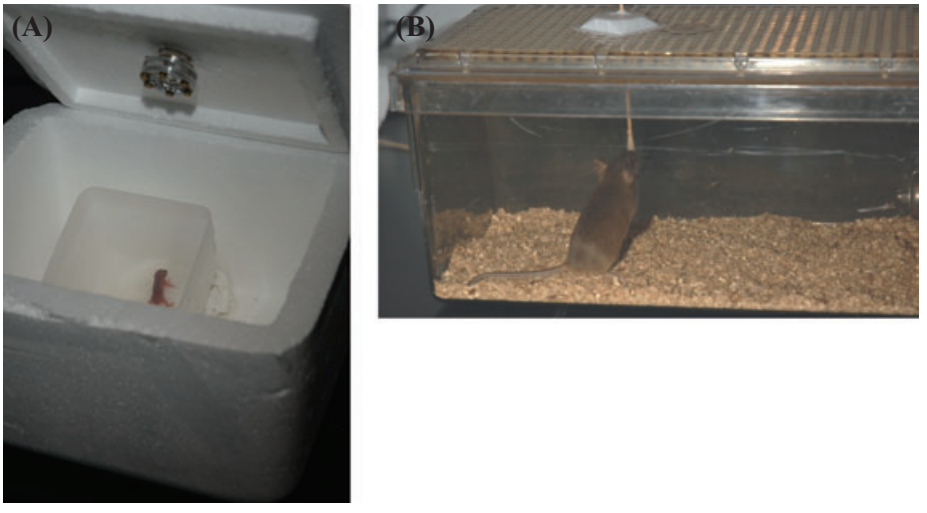

Figure 2. (**A**) An Avisoft ultrasonic vocalization detector microphone is mounted above a Styrofoam box containing a mouse pup. The equipment is used to measure ultrasonic calls emitted by mouse pups when removed from the nest at young ages such as 5 to 12 days after birth (166). The vocalizations elicit retrieval behavior by the parents, who locate the pup and return it to the nest. Software by Avisoft displays calls and calculates call parameters, frequency and duration. (**B**) Interest in social olfactory cues is quantitated as time spent sniffing a cotton swab containing mouse urine or wiped across the bottom of a soiled mouse cage. Photographs by Dr Maria Luisa Scattoni, LBN, NIMH, and Janet Stephens, NIH Macrophotography, and contributed by the author.

We predict that genetic mouse models of autism will display aberrant detection of olfactory social cues, and unusual behavioral responses to the pheromones of a conspecific. However, it is important to recognize that mouse vocal and olfactory communication do not have the same qualitative level of communication as human speech, and may derive from different brain regions. The critical component of intentionality of communication cannot be inferred from present rodent tasks. However, understanding the brain regions and genetics of mouse vocalizations could conceivably suggest new candidates to investigate in humans.

Resistance to change in routine. Autistic individuals often maintain rigid habits, similar to individuals with obsessive–compulsive disorders, and frequently show a strong insistence on sameness and upset to change in routine (66, 87). We reasoned that mice could be trained to establish a habit, and then asked to make a change in the established routine. Ability to change, resistance to change and responses to the change in routine would be analyzed. One standardized approach to developing a spatial position habit in mice is to train the subject on an appetitive task with a spatially contingent reinforcer. T-maze learning involves finding a food reward in one of two available locations at opposite ends of a T-shaped apparatus. Reversal requires the mouse to extinguish the location of the reinforcer in one arm, and learn a new location of the reinforcer in the other arm. Morris water maze learning involves locating a hidden escape platform in one quadrant location of a circular swimming pool of water. Reversal requires the mouse to extinguish the location of the hidden platform in one quadrant, and learn the new location of the hidden platform in another quadrant. Reversal learning requires different skills than the original acquisition learning. The latter serves as a control for general procedural and cognitive abilities. Ability of the subjects to switch quickly to the new location is quantitated by the number of re-training trials required to consistently choose the opposite T-maze arm to obtain the food reward, or the different escape platform location in the Morris pool. Several inbred strains of mice were able to acquire the original acquisition but failed the reversal task in either the T-maze, Morris water maze, or both (136). Failure to switch to the new position habit may be analogous to the inflexibility in routine that is characteristic of autism. In addition, it will be interesting to record any unusual behavioral responses during the change in location of the reinforcer. We can envision the expression of some form of frustration response during failures in the reversal task, such as motor stereotypies, ultrasonic vocalizations, or disrupted performance that has been reported for Fragile X mice when contingencies were changed in an operant task (129), analogous to upset to change reactions in autism.

Associated symptoms. Because autism encompasses additional symptoms with variable expression, it may be useful to include a range of additional behavioral tasks to more fully characterize a proposed genetic mouse model of autism. For example, anxiety is common in autistic individuals (57, 71, 179). Anxiety-related tasks have been well-characterized for mice. Conflict tests including the elevated plus maze, light↔dark exploration, open field emergence, probe burying, marble burying, and Vogel thirsty-lick tests are based on approach–avoidance conflicts and are sensitive to anxiolytic drugs (35, 37, 41, 60, 62, 88, 163, 172). Seizures are frequent in autistic children (8, 149). Methods for scoring seizures in mice are standard in the literature (125, 181). Spontaneous seizures, audiogenic seizures induced by loud tones or jangling keys and drug-induced seizures induced by treatment with convulsants, such as pentylenetetrazole, are well-characterized methods for assessing seizure susceptibility in mice (70, 126). Some parents report that their autistic children have disturbed sleep patterns (80, 96). Sleep patterns in mice can be evaluated by videotaping the home cages during the lights-on period, by quantitating running-wheel behavior across the circadian cycle, and by neurophysiological recording of sleep EEG patterns (176, 177). Motor stereotypies have been reported in mice (124, 180), that may be relevant to motor stereotypies in autism. Clumsiness, reported in some cases of autism (66, 69), could be tested in mice using standard motor procedures such as the balance beam, rotarod and footprint tests (31). Standardized methods to score aggressive behaviors in mice are available (123, 192). Hypersensitivity to sensory stimuli could be detected through the acoustic and tactile startle tests (68). Theory of mind could conceivably be modeled with a version of the social transmission of food preference task, in which the subject mouse bases its choice of a new flavor of food on sensory experience with olfactory cues gained during social interactions with another mouse (12, 67, 124, 196, 197). The symptom of mental retardation in some autistic patients may be detected as a learning and memory deficit in a mutant mouse model of autism. Pathological features of autism could be examined in mouse models, including head size, brain weight, ventricle shape, number of Purkinje neurons in the cerebellum and dendritic spine morphology. Biological findings from clinical studies could be examined in mouse models, including unusual serotonin levels, imaging and neurophysiological responses of the amygdala, and of cortical regions analogous to the human fusiform cortex, during social interactions. Expression of developmental genes, and the development of brain synapses and pathways, can be examined at various ages in mouse models of autism. Finally, tests of developmental milestones through the early stages of ontogeny may be useful in modeling the neurodevelopmental components of autism. Methods for scoring developmental milestone behaviors in newborn and infant mice are readily available in the literature (11, 81, 157). Table 1 presents a list of additional mouse behavioral tasks that could be further developed to model some of the associated symptoms of autism.

Control parameters. Behavioral neuroscientists are careful to control for physical problems that could produce false positives on mouse behavioral tasks. Artifacts lurk in the interpretation of behavioral results. For example, a mouse with a rhinitis infection that blocks its nasal passages, or a knockout mouse with a mutation in an olfactory gene that impairs its sense of smell, could fail social tasks that are based on detection of conspecific odors. We and many other labs routinely conduct critical control experiments to measure general health, sensory abilities and motor functions (6, 45, 75, 141, 150, 184). A battery of simple

Suggested Mouse Behavioral Tests Relevant to Autism Spectrum Disorders	
I. Core symptoms and hypothesized analogous tests for mice	C. Mental retardation
A. Inappropriate social interactions	i. Acquisition of Morris water maze tasks
i. Social approach to a stranger mouse	ii. Acquisition of T-maze tasks
ii. Reciprocal social interactions	iii. Contextual and cued fear conditioning
iii. Conditioned place preference to conspecifics	iv. Operant learning tasks
iv. Preference for social novelty	v. Attentional measures on five choice serial reaction attentional task
v. Social recognition	D. Seizures
vi. Juvenile play	i. Sensitivity to audiogenic seizures
vii. Nesting patterns in the home cage	ii. Sensitivity to drug-induced seizures
B. Impairments in social communication	E. Motor clumsiness
i. Behavioral responses to social olfactory cues from conspecifics	i. Balance beam foot slips
ii. Deposition of social olfactory pheromones	ii. Rotarod motor coordination and balance
iii. Vocalizations emitted during social interactions	iii. Footprint analysis
iv. Responses to vocalizations from conspecifics	F. Aggression
v. Parental retrieval of separated pups	i. Resident-intruder attack
vi. Ultrasonic vocalizations by separated pups	ii. Isolation-induced fighting
C. Repetitive, ritualistic, behaviors, resistance to change and restricted activities	iii. Tube test for social dominance
i. Motor stereotypies	G. Sleep disturbances
ii. Perseverative holeboard exploration	i. Circadian running wheels
iii. Extinction of a learned response in an operant chamber	ii. Videotaped observations of home cage sleep and activity patterns
iv. Reversal of a position habit in an appetitive T-maze task	H. Idiosyncratic responses to sensory stimuli
v. Reversal of a position habit in an aversive Y-maze task	i. Acoustic startle
vi. Reversal of a position habit in the Morris water maze	ii. Tactile startle
vii. Spontaneous responses to errors during reversal tasks	iii. Hot plate
II. Variable associated symptoms and hypothesized analogous tests for mice	iv. Von Frey hairs
A. Anxiety	v. Attentional neglect tape test
i. Elevated plus maze	vi. Unresponsiveness to sensory attentional cues (failure to disengage attention)
ii. Light↔dark exploration	I. Brain overgrowth
iii. Vogel conflict test	i. Brain weight, volume, size of structures and pathways
iv. Marble burying	ii. Measurements at neonatal, juvenile and adult time points
B. Theory of Mind deficits	J. Developmental progression
i. Location of buried food following observation of conspecifics	i. Developmental milestones in neonates
ii. Social transmission of food preference task	ii. Repeated testing of all relevant behaviors at juvenile and adult ages
iii. Avoidance of aggressive encounters	

Table 1. Mouse behavioral tasks with potential face validity to the diagnostic and associated symptoms of autism. Detailed descriptions of most of these mouse behavioral tasks are available in studies by Carter et al (31), Crawley (44), Crawley and Paylor (45), Geyer and Swerdlow (68), Giardina (70), Heyser (81), Holmes (88), Maxson and Canastar (123), and Turner et al (180).

observational tests for general health and physical abilities is the first step in evaluating a new line of mice with a targeted gene mutation (44). For example, olfactory tests ensure that mice are not anosmic, to avoid a false positive in social communication based on pheromones, or social learning based on food flavors (136, 191, 197). Olfactory cues with social components, for example, urine markings or home cage litter, may be both a useful control and an additional parameter in social approach tasks. Learning and memory tasks require controls for their procedural demands, for example, the hot plate test to ensure that pain detection is normal in footshock-induced fear conditioning, or the visible platform task as a control for vision and swimming abilities in the Morris water maze (103, 133, 198). Measures of physical health, home cage behaviors, neurological reflexes, vision, hearing, smell, touch, locomotion, muscle strength and others may be necessary controls for more specialized behavioral tasks to model discrete symptoms of autism.

CONCLUSIONS

Autism is a complex disease with multiple and variable symptoms. Some of these symptoms, such as the deficits in language and Theory of Mind, may be uniquely human, and therefore difficult or impossible to model in mice. However, other components may have conceptual analogies within the mouse behavioral repertoire. As a first approximation towards modeling the core symptoms of autism, we propose mouse behavioral tests that measure social approach, juvenile play, exploration of

social olfactory cues, ultrasonic vocalizations in social settings, motor stereotypies and preservation in reversal tasks. These mouse behavior tasks represent a first pass at designing practical laboratory assays with heuristic value for testing hypotheses about the causes for autism, and may serve as translational tools to evaluate proposed treatments.

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