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Designing Mouse Behavioral Tasks Relevant to Autistic-Like Behaviors

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The importance of genetic factors in autism has prompted 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 (1) face validity, i.e., resemblance to the human symptoms; (2) construct validity, i.e., similarity to the underlying causes of the disease; and (3) predictive validity, i.e., 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 for modeling the symptoms of autism. We are in the process of designing a set of tasks with face validity for the defining features of autism: deficits in appropriate reciprocal social interactions, deficits in verbal social communication, and high levels of ritualistic repetitive behaviors. Social approach is tested in an automated three-chambered apparatus that offers the subject a choice between a familiar environment, a novel environment, and a novel environment containing a stranger mouse. Preference for social novelty is tested in the same apparatus, with a choice between the start chamber, the chamber containing a familiar mouse, and the chamber containing a stranger mouse. Social communication is evaluated by measuring the ultrasonic distress vocalizations emitted by infant mouse pups and the parental response of retrieving the pup to the nest. Resistance to change in ritualistic repetitive behaviors is modeled by forcing a change in habit, including reversal of the spatial location of a reinforcer in a T-maze task and in the Morris water maze. Mouse behavioral tasks that may model additional features of autism are discussed, including tasks relevant to anxiety, seizures, sleep disturbances, and sensory hypersensitivity. Applications of these tests include (1) behavioral phenotyping of transgenic and knockout mice with mutations in genes relevant to autism, (2) characterization of mutant mice derived from random chemical mutagenesis, (3) DNA microarray analyses of genes in inbred strains of mice that differ in social interaction, social communication and resistance to change in habit, and (4) evaluation of proposed therapeutics for the treatment of autism.

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Key Words: autism; mice; social interaction; ultrasonic vocalizaitons; olfactory communication; reversal learning; social approach; anxiety

HOW WOULD YOU MODEL AUTISM IN MICE?

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, and Vogel thirsty-lick conflict tests [Crawley, 1985; Rodgers, 1997; Crawley, 2000; File, 2001; Holmes, 2001; Clement et al., 2002; Finn et al., 2003]. Memory deficits in Alzheimer's models are detected with learning and memory tests, including spatial navigation tasks such as the Morris water maze, Barnes maze, radial maze, and T-maze; emotional memory tasks such as contextual and cued fear conditioning; olfactory memory tasks such a social transmission of food preference; and aversive tasks such as active and passive avoidance [Sarter, 1987; Crawley and Paylor, 1997; Crawley, 2000; Morris, 2001; Hsiao Ashe, 2001; Higgins and Jacobsen, 2003]. Parkinson's and Huntington's disease models utilize sensitive motor tasks such as balance beam walking and footprint pattern [Carter et al., 1999; Crawley, 2000; Kitamura, 2000; Sedelis et al., 2001]. Drug abuse models employ self-administration, conditioned place preference, and two bottle choice tests to measure the rewarding properties of addictive drugs [Caine et al., 1999; Crawley, 2000; Wehner et al., 2001; Crabbe, 2002; Rocha et al., 2002; Cunningham et al., 2003; Yamada et al., 2003]. Rodent tasks sensitive to antidepressant drugs include forced swim, tail suspension, and stressor-induced anhedonia [Markou and Koob, 1991; Moreau, 1997; Crawley, 2000; Cryan et al., 2002; Ripoll et al., 2003; Overstreet et al., 2003; Konkle et al., 2003; Matthews and Robbins, 2003].

While a rodent model cannot replicate a human disease, fundamental symptoms can be approximated for the purposes of testing theories about the biochemical and genetic causes of the human condition. Hypotheses about genes underlying neuropsychiatric disorders are addressed by applying these behavioral procedures to phenotype mice with targeted gene mutations and to compare the genetic profiles of inbred strains of mice with dissimilar behavioral phenotypes [Crawley et al., 1997; Jones et al., 1999; Crawley, 2000; Pongrac et al., 2002; Wahlsten et al., 2003; Tabakoff et al., 2003; Biola et al., 2003; Zirlinger and Anderson, 2003]. Further, the rodent model has translational value in offering preclinical surrogate markers to evaluate treatment efficacy. Rodent behavioral tasks provided useful preclin-

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ical tools for the discovery of psychopharmacological treatments for many major mental illnesses and neurological diseases [Contarino et al., 1999; Gingrich and Hen, 2001; Nestler et al., 2002; Crawley et al., 2002; Higgins and Jacobsen, 2003]. We are engaged in a new effort to develop behavioral tests for mice that approximate some of the core symptoms of autism spectrum disorder in humans.

Autism is a neurodevelopmental disorder with a strong genetic basis [Wassink and Piven, 2000; Folstein and Rosen-Sheidley, 2001; Cook, 2001; Andres, 2002; Shastry, 2003; Yonan et al., 2003; Jamain et al., 2003a,b; Muhle et al., 2004; Wassink, 2004]. The diagnostic criteria for autism spectrum disorders, including Asperger syndrome, continue to be refined, a difficult task given the complexity of the syndrome and the variability in symptoms and severity. The fundamental symptoms of autism are aberrant reciprocal social interactions, poor communication skills, and ritualistic repetitive behaviors [Kanner, 1943; Schloper and Mesibov, 1987; Frith, 1991; American Psychiatric Association, 1994; Piven et al., 1997; Lord et al., 2000; Folstein and Rosen-Sheidley, 2001; Mesibov et al., 2001; Paul, 2003; Volkmar and Pauls, 2003]. The range of defining symptoms includes lack of meaningful language, lack of gesturing, diminished facial expression of emotion, inability to interpret emotions from the facial expressions of others, reduced social orienting, reduced joint attention, hypersensitivity to sensory stimuli, hand flapping, and stereotyped movements [Kanner, 1943; Frith, 1991; Filipek et al., 1999; Lord et al., 2000; Bodfish et al., 2000; Baranek, 2002; Deuel, 2002; Volkmar and Pauls, 2003; Dawson et al., 2004]. Additional commonly associated characteristics include large head circumference, mental retardation, seizures, self-injury, anxiety, sleep disturbances, minimal eye contact, upset to change in routine, narrow range of interests, and absence of Theory of Mind [Schloper and Mesibov, 1987; Frith, 1991; American Psychiatric Association, 1994; Happé et al., 1996; Deuel, 2002; Dawson et al., 2002; Volkmar and Pauls, 2003; Glasson et al., 2004; Cure Autism Now, 2004; National Alliance for Autism Research, 2004]. 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 between monozygotic twins, 90% heritability, and a male:female ratio of approximately 4:1 [Folstein and Rosen-Sheidley, 2001; Dawson et al., 2002; Bespalova and Buxbaum, 2003]. Several large international and collaborative linkage analyses of autism pedigrees have identified several regions of significant linkage harboring multiple candidate genes, supporting a complex multigenic cause of autism [Barrett et al., 1999; Risch et al., 1999; Wassink and Piven, 2000; Bradford et al., 2001; Liu et al.,

Table 1. Symptoms of Autism Spectrum Disorders and a Sampling of Mouse Behavioral Tasks Proposed as Relevant to these Symptoms

I. Core symptoms and hypothesized analogous tests for mice
- A. Inappropriate social interactions
  - i. Social approach to a stranger mouse
  - ii. Conditioned place preference to conspecifics
  - iii. Preference for social novelty
  - iv. Social recognition
  - v. Juvenile play
  - vi. Social interactions under dim lighting
  - vii. Nesting patterns in the home cage
- B. Impairments in social communication
  - i. Behavioral responses to social olfactory cues
  - ii. Vocalizations and responses to vocalizations during social interactions
  - iii. Parental retrieval of separated pups
  - iv. Ultrasonic vocalizations by separated pups
- C. Repetitive, ritualistic, behaviors, resistance to change, and restricted activities
  - i. Reversal of a position habit in an appetitive T-maze task
  - ii. Reversal of a position habit in the Morris water maze
  - iii. Extinction of a learned association
  - iv. Novel object exploration

II. Variable symptoms and hypothesized analogous tests for mice
- A. Anxiety
  - i. Elevated plus maze
  - ii. Light–dark exploration
  - iii. Vogel conflict test
- B. Theory of Mind deficits
  - i. Location of buried food following observation of conspecifics
  - ii. Social transmission of food preference task
  - iii. Avoidance of aggressive encounters
- C. Mental retardation
  - i. Acquisition of Morris water maze tasks
  - ii. Acquisition of T-maze tasks
  - iii. Contextual and cued fear conditioning
  - iv. Operant learning tasks
  - v. Attentional measures on five-choice serial reaction attentional task
- D. Seizures
  - i. Sensitivity to audiogenic seizures
  - ii. Sensitivity to drug-induced seizures
- E. Clumsiness
  - i. Balance beam foot slips
  - ii. Rotarod motor coordination and balance
  - iii. Footprint analysis
- F. Stereotypies
  - i. Videotaped observations of home cage behaviors
  - ii. Observations during stressful situations such as habit reversal
  - iii. Sensitivity to motor stereotypies induced by dopaminergic agonists
- G. Aggression
  - i. Isolation-induced fighting
  - ii. Resident-intruder attack
  - iii. Tube test for social dominance
- H. Sleep disturbances
  - i. Circadian running wheels
  - ii. Videotaped observations of home cage sleep and activity patterns
- I. Idiosyncratic responses to sensory stimuli
  - i. Acoustic startle
  - ii. Tactile startle
  - iii. Hot plate
  - iv. Von Frey hairs
- J. Brain overgrowth
  - i. Brain weight, volume, size of structures
  - ii. Measurements at neonatal, juvenile, and adult time points
- K. Developmental progression
  - i. Repeated testing of all relevant behaviors at juvenile and adult ages
  - ii. Developmental milestone tests in neonates

2001; Shao et al., 2002; Alarcon et al., 2002; Keller and Persico, 2003; Jamain et al., 2003a,b; Yilisaukko-oja et al., 2004; Bacchelli et al., 2003].

Autism may be particularly difficult to model in rodents. Theory of mind, the ability to intuit the feelings and intentions of others, will be difficult to parallel in mice. Mice cannot be evaluated for speech deficits and may not have brain regions comparable to those mediating human language skills relevant to autism. Luckily, *Mus musculus,* the house mouse species used in molecular genetics research, is a social species that engages in high degrees of social interaction and social communication [Grant and MacIntosh, 1963; Gheusi et al., 1994]. Nonverbal forms of mouse social communication and interaction are amenable to quantitative analysis. Useful rodent models of autism will include behavioral features with conceptual analogy to at least one core human symptom. The best mouse models will also incorporate some of the variable symptoms. Modest goals for paralleling some of the core and variable 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 [Andres, 2002; Zoghbi, 2003]. 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 [Carter et al., 1992; Young, 2001; Carter, 2003]. Larry Young, Jim Winslow, and Tom Insel at Emory University tested a line of mice with a targeted mutation in the gene for oxytocin [Insel et al., 1999; Winslow and Insel, 2002; Young et al., 2002]. Oxytocin knockout mice displayed deficits in social interaction and social recognition [Insel et al., 1999; Young, 2001; Winslow and Insel, 2002]. Oxytocin knockout mouse pups emitted fewer ultrasonic distress vocalizations when separated from their parents [Winslow et al., 2000]. Conversely, repeated central administration of oxytocin increased ultrasonic vocalizations in hamsters and voles [Floody et al., 1998; Kramer et al., 2003]. Similarly, vasopressin, another hypothalamic neuropeptide, and its V1a receptor facilitated social behaviors in some rodent species [Pitkow et al., 2001; Landgraf et al., 2003]. Vasopressin receptor knockout mice displayed reduced social recognition as measured by failure to habituate to a novel stranger [Bielsky et al., 2004]. Walter Salinger and coworkers 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 [Salinger et al. 2003]. 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 [Lijam et al., 1997; Long et al., 2004].

A second approach addresses a human disease 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 [Sinkkonen et al., 2003]; Smith-Lemli-Opitz syndrome [Wassif et al., 2001; Waage-Baudet et al., 2003], fragile X [Comery et al., 1997; Chen and Toth, 2001; Nielsen et al., 2002; Watase and Zoghbi, 2003; Kooy, 2003; Frankland et al., 2004], Rett syndrome [Berger-Sweeney, 2003; Zoghbi, 2003] and Down syndrome [Dierssen et al., 2001; Turner et al., 2001; Moran et al., 2002; Hill et al., 2003]. Social behaviors have not yet been extensively analyzed in these mutant mouse models. As candidate genes for autism continue to be discovered by human linkage analyses, it seems likely that new knockout mice will be generated to test hypotheses about the functions of these candidate genes. Since approximately 99% of human genes have ortholog counterparts in mice [Tecott, 2003], many targeted gene mutations in mice may be forthcoming as potential models of autism-related genetic dysfunctions.

A third approach is to generate defects in neurotransmitters or brain regions that are analogous to neurochemical or anatomical abnormalities seen in autism. Kathy Sulik and coworkers 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 [Waage- Baudet et al., 2003]. Patricia Rodier and coworkers 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 [Rodier et al., 1996; Ingram et al., 2000]. Cranial nerves III, V, VI, and XII displayed diminished motor neuron numbers, similar to that seen in the human case [Rodier et al., 1996]. In addition, reductions in cerebellar volume and Purkinje cell number were found to parallel those seen in cases of autism [Ingram et al., 2000]. No reports of behavioral testing of these valproate-treated rats have appeared to date. Dan Goldowitz and coworkers at the University of Tennessee pursued the cerebellar abnormalities associated with autism, using heterozygous Lurcher (*Lcl/+*) mutant mice [Martin et al., 2003]. 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; however, social tests were not conducted in this study [Martin et al., 2003].

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 to identify genes correlated to a phenotype may emerge as advantageous approaches for discovering genes mediating social behaviors. Our hypothesis is that a discrete number of genes regulate the expression of the wide range of normal mouse social behaviors and that it will be possible to discover the genes responsible for the extremes in behavioral phenotypes, using a variety of inbred strains and mutant lines.

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 ritualistic repetitive behaviors, that can be uniformly applied across mouse models and genetic technologies [Insel, 2001; Andres, 2002]. As mentioned in the report of a meeting at The Jackson Laboratory [Insel, 2001], we are devoting considerable attention to understanding the clinical symptoms of autism, with the goal of designing mouse behavioral tasks to detect autism-like traits in mutant mouse models. Our Laboratory of Behavioral Neuroscience has the good fortune to collaborate closely with experts in the clinical and genetic components of autism at the Neurodevelopmental Disorders Research Center of the University of North Carolina at Chapel Hill. Their generosity in sharing observations and insights into the fundamental defining features of autism, and the variable traits associated with these core symptoms, guide our thinking about analogous behaviors in mice. Over the past two years, our new Mouse Behavioral Phenotyping Laboratory at the Uni-

versity of North Carolina Neurodevelopmental Disorders Research Center began designing, testing, validating, and automating a set of mouse behavioral tasks that focuses on the three defining symptoms of autism [Moy et al., 2004; Nadler et al., 2004]. This article presents the rationales underlying the first set of tasks that we have designed and describes the methods and results obtained to date.

CORE SYMPTOMS

Sociability

Qualitative and quantitative impairments in social interaction are the identifying feature of autism [American Psychiatric Association, 1994; Lord et al., 2000; Volkmar and Pauls, 2003]. The original explication of autism [Kanner, 1943] characterizes 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 during ontogeny for these symptoms [American Psychiatric Association, 1994; Piven, 2001; Volkmar and Pauls, 2003]. We reasoned that a critical component in a mouse model of autism was 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 toward intruders, sexual approach and mating behavior patterns, parental care of the pups, and juvenile play [Grant and MacIntosh, 1963; Panksepp et al., 1984; Laviola and Terranova, 1998; Rissman et al., 1999; Blanchard et al., 2003]. Behavioral neuroscientists employ standardized scoring methods to quantitate these various types of social interactions in mice [File, 1997; Winslow and Insel, 2002; Winslow, 2003; Lonstein and Fleming, 2003; Maxson and Canastar, 2003; Brodkin et al., 2004]. 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 [Moy et al., 2004; Nadler et al., 2004].

The first 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 near a conspecific or in adjacent chambers that do not contain another mouse. Figure 1A illustrates the three-chambered automated apparatus. One side chamber contains a stranger mouse that is contained in a wire cage that permits visual, olfactory, auditory, and some tactile contact. The other side chamber contains only the empty wire cage, serving as a control for exploring a novel object in a novel environment. 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 on 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 times 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. Diagrams and schematics are published [Nadler et al., 2004] and are available upon request.

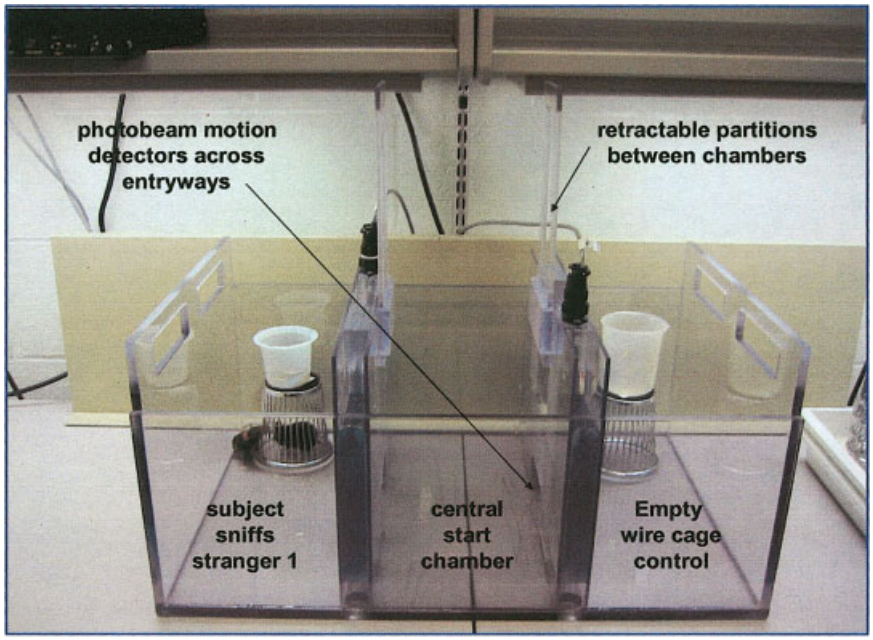

Fig. 1. Automated three-chambered apparatus for quantitating social approach behaviors in mice. The session starts with the subject mouse in the center chamber for a 10-min habituation period. An adult male conspecific mouse that has had no previous contact with the subject is placed in a wire cage in one of the side chambers. A clean empty wire cage is placed in the other side chamber. Retractable doors between the chambers are raised to begin the 10-min sociability test. Photocell motion detection beams across the doorways send information to a software interface that records (a) entries of the subject mouse into each chamber and (b) time spent in each chamber. A human observer records (a) time spent by the subject in sniffing the wire cage containing the stranger mouse and (b) time spent sniffing the empty wire cage. Because the stranger is contained in the wire cage, social approach is initiated only by the subject. The wire cage allows visual, olfactory, auditory, and some tactile contact between the subject and the stranger. This task measures sociability, the preference of the subject mouse to spend time with a conspecific, compared to time spent in the other two chambers. Sniffs directed toward the conspecific compared to sniffs directed toward the empty wire cage confirm the social nature of the approach. Number of entries provides a control for general exploratory activity and anxiety-like behaviors. (Adapted by Elizabeth Koenig from Nadler et al., 2000). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Our first experiments with the three-chambered apparatus began by placing a juvenile male subject mouse in the center chamber for a 10-min habituation period, during which the center area becomes a familiar "home base." Divider panels separate the center chamber from two identical side chambers. Sliding doors in the divider panels are then raised, allowing the mouse to explore all three chambers. An unfamiliar male "stranger" mouse is quietly sitting in a wire cage in one of the side chambers. Because the wire cage 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 toward the stranger in the wire cage. The strangers are adult male C57BL/6J mice

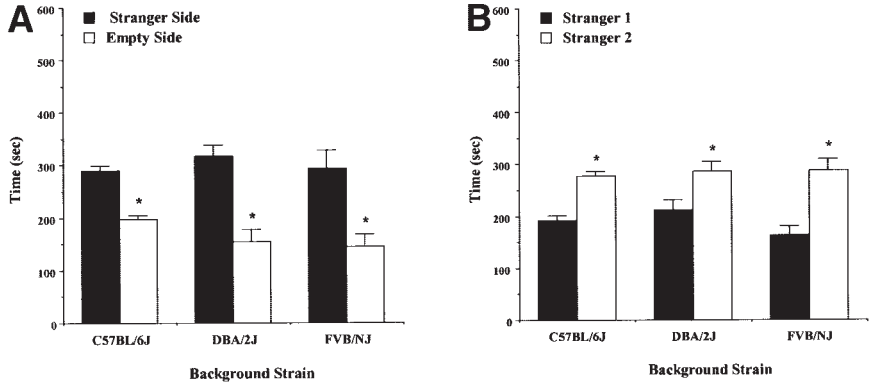

Fig. 2. Three inbred strains of mice commonly used in behavioral genetics showed (A) significant sociability and (B) preference for social novelty. Time spent in the chamber containing the stranger mouse was greater than time spent in the chamber containing the empty wire cage for juvenile male C57BL/6J, DBA/2J, and FVB/NJ. $N = 20$ per strain, data are expressed as mean + standard error of the mean, $*p < .05$; adapted from Moy et al., 2004. Time spent in the chamber containing a new stranger 2 mouse was greater than time spent in the chamber containing the now-familiar stranger 1 mouse.

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. Information obtained from the photocell beam breaks across the openings between the three chambers, analyzed by the software interface, includes (1) the movements of the subject mouse from one chamber to another and (2) 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 min duration. To determine whether time spent in the chamber containing the stranger mouse reflects actual exploration of the stranger mouse versus nonsocial exploration of other areas of the chamber, a human observer recorded the time that the subject spent sniffing the wire cage containing the stranger.

Representative data are shown in Figure 1B. Three strains of mice commonly used in behavioral genetics, C57BL/6J, DBA/2J, and FVB/NJ, all 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 [Moy et al., 2004; Nadler et al., 2004]. Investigatory sniffs of the chamber containing the stranger mouse were significantly higher than sniffs of the empty wire cage [Nadler et al., 2004]. These high levels of social interaction were seen in both male and female subjects, and when this sociability test was conducted at juvenile and adult ages [Moy et al., 2004; Nadler et al., 2004]. In all three strains, significant correlations were found between time spent sniffing and time spent in the chamber, confirming the social nature of the time spent in the chamber containing the stranger [Nadler et al., 2004]. Repeated testing of the same subject mice yielded similar scores [Moy et al., 2004], 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 [Nadler et al., 2004]. Analysis of 5-min time bins across 30-min test sessions indicated that the majority of the social interaction occurred in the first 10 min, supporting the use of a 10-min test session [Nadler et al., 2004]. Number of entries was identical between chambers [Moy et al., 2004; Nadler et al., 2004], indicating that the subject mice explored all three chambers but preferred to spend more time interacting with the stranger mouse. Number of entries appears to be a good control measure for general exploratory locomotor activity, to rule out false positives due to poor motor abilities. 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. One inbred strain with low social approach, A/J, has been detected to date [Moy et al., 2004].

Preference for social novelty

Our laboratory recommends that two corroborating tasks be conducted within a behavioral domain of interest [Crawley and Paylor, 1997; Crawley, 2000, 2003; Holmes et al., 2003a,b]. 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.

The automated three chambered apparatus can be used in many ways to corroborate a social approach deficit and to address additional hypotheses about social behaviors [Nadler et al., 2004]. For example, social deficits in autism may appear as inappropriate or indiscriminate approaches to strangers, rather than an overall lack of social approach [Loveland et al., 2001]. Normal mice usually habituate quickly to the presence of one new conspecific and then move on to approach and investigate another new conspecific [Ferguson et al., 2000; Moy et al., 2004]. We theorize that a lack of normal preference for social novelty in mice could be analogous to the preference of autistic individuals to remain with familiar individuals or to indiscriminately approach strangers. Using the three-chambered apparatus, preference for social novelty is quantitated by habituating the subject mouse to one stranger mouse, then providing access to a second, newer stranger, and calculating relative approaches to the habituated versus the novel stranger. Since the majority of the sociability scores appeared during the first 10 min spent with access to stranger 1 [Nadler et al., 2004], a 10-min habituation period may be sufficient for stranger 1 to become familiar. We predict that a mouse model of autism will show equal or less investigation of a novel stranger 2 as compared to investigation of the habituated, now-familiar stranger 1. Preference for social novelty is illustrated in Figure 2B. C57BL/6J, DBA/2J, and FVB/NJ, the three standard inbred strains commonly used in behavioral genetics research, displayed the expected preference for the novel

stranger 2 compared to the habituated stranger 1.

Sociability and preference for social novelty appear to be simple tasks that are easily measured. The short session lengths and the automation of the parameters will permit high-throughput screening and avoid observer fatigue. Uniform methods for automation will ensure that results are consistent across investigators and laboratories. High interrater reliability between students, technicians, and senior investigators in scoring the sniffing behaviors was obtained in our studies [Moy et al., 2004; Nadler et al., 2004], suggesting that scoring of sniffs, the nonautomated additional parameter, is readily mastered. Repeatability of the results within a strain indicates that the social scores represent a trait rather than a state for inbred strains of mice. From these preliminary data in inbred strains, it appears that these tasks will be sufficiently sensitive to detect low tendencies to initiate social approach, or aberrant types of social approach, in mice with targeted gene mutations relevant to autism.

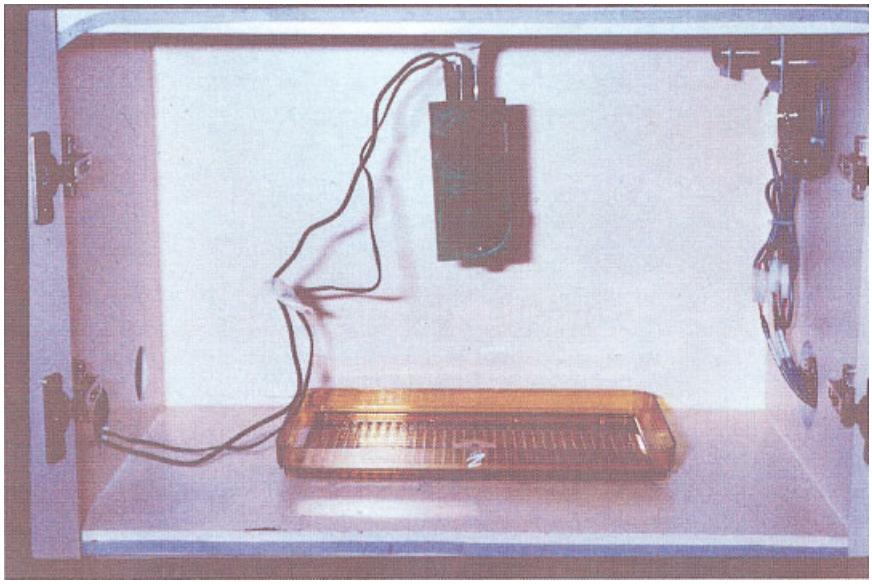

Fig. 3. The ultrasonic vocalization detector microphone is mounted above a warming tray, where a mouse pup is placed. The task measures ultrasonic calls emitted by mouse pups when removed from the nest at young ages, such as 7 days after birth. The vocalizations elicit retrieval behavior by the parents, who locate the pup and return it to the nest. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Further analyses of social tendencies

Low levels of social approach are insufficient to adequately describe the complexity and variability of the social deficits in autism. It will be useful to conduct more fine-grained analyses of social interactions, using established methods [e.g., Grant and MacIntosh, 1963; Panksepp et al., 1984; File, 1997; Hofer et al., 2001; Winslow and Insel, 2002; Holmes, 2001; Heyser, 2003; Lonstein and Fleming, 2003; Maxson and Canastar, 2003] and developing new tests. Tethered stranger mice are likely to elicit more complex approach behaviors by the subject. Freely moving stranger mice will allow better evaluation of reciprocal social interactions. Videotracking systems and/or human observers that quantitate the full spectrum of social approach behaviors, including following, nose-to- nose contacts, nose-to-anogenital contacts, sexual approaches, aggressive intention movements, attack behaviors, escape behaviors, home cage nesting behaviors, 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, could address the neurodevelopmental components of autism. Tendencies to avoid social contact could be further analyzed with a standard place preference task and the social transmission of food preference task. 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.

Social Communication

Poor social communication is fundamental to autism spectrum disorders [Kanner, 1943; Schloper and Mesibov, 1987; Lord et al., 2000; Doussard-Roosevelt et al., 2003]. Although mice do not use language, they do display strong social communication mechanisms. Mice emit auditory signals, including ultrasonic vocalizations, and olfactory social signals, including deposition of pheromones in the environment [Hofer, 1996; Keverne, 2002; Covington and Miczek, 2003; Branchi et al., 2004]. 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 that appears to be reflexively emitted by pups when they are out of the nest [Hofer, 1996; Branchi et al., 1998; Brunner et al., 1999; Winslow et al., 2000; Hofer et al., 2001; Hahn and Schanz, 2002]. The parents detect this 50–80 kHz ultrasonic "distress" call, locate the pup, and retrieve it to the nest. Our thinking is that 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. 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 [Frith, 1991]. Low levels of retrieval by the adult mice could indicate failure to respond appropriately to socially meaningful stimuli. Figure 3 illustrates the ultrasonic detector microphone that records vocalizations. In a standard test of separation vocalizations, a 7-day-old mouse pup is removed from the nest in the home cage and placed in a warm holding cage under the microphone. 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 gene mutations 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 [Branchi et al., 2004]. Distress calls [Weller et al., 2003; Covington and Miczek, 2003] could conceivably measure

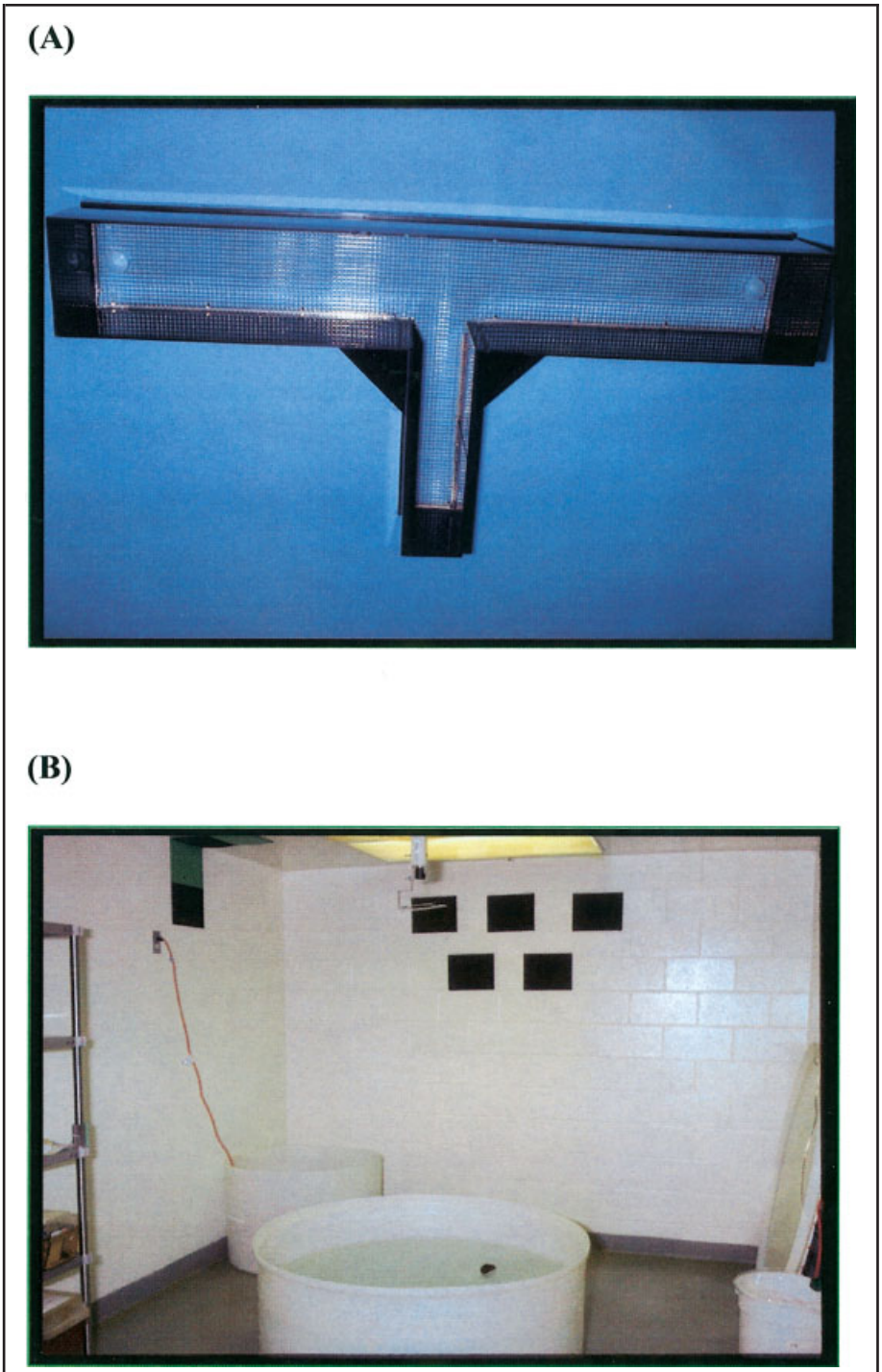

Fig. 4. (A) T-maze and (B) Morris water maze for evaluating resistance to change in routine. Adult subject mice are trained to choose one arm of the T-maze to obtain a sucrose food reward. After reaching the criterion of 8 correct responses out of 10 trials per day, on 3 consecutive days, the location of the sucrose food pellet is switched to the opposite arm of the T-maze. Similarly, adult subject mice are trained to locate a hidden platform in a large pool of deep water. After reaching the criterion of 15 s or less to reach the hidden platform, the platform location is changed to a different quadrant of the pool. These tasks measure reversal learning, to evaluate the ability of the subject mice to change an established habit. Any unusual reactions to the new location of the reinforcer are recorded. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the stress level of a juvenile or an adult mouse when placed in a social milieu.

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 [Harrington, 1976; Bakker, 2003; Nevison et al., 2003]. 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. 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.

Repetitive Ritualistic Behaviors

Resistance to change in routine

Autistic individuals often maintain rigid habits, similar to individuals with obsessive–compulsive disorders, and frequently show a strong upset to change in routine [Frith, 1991; Hollander et al., 2003]. We reasoned that mice could be trained to establish a habit and then be asked to make a change in the routine. Ability to change, resistance to change, and responses to the change in routine would be analyzed. One standardized approach to forming 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 (Fig. 4A). Morris water maze learning involves locating a hidden escape platform in one quadrant location of a circular swimming pool of water (Fig. 4B). These two cognitive tasks are generally corroborative for spatial learning and memory but require different sets of sensory and motor skills. We propose a reversal task, in which mice are well- trained to locate the food reward at one fixed end of the T-maze. As a second corroboratory reversal task, mice would be well-trained to locate the hidden platform at one fixed quadrant of the Morris water maze. The location of the reinforcer is then changed. For the T-maze, the food is placed into the opposite arm. For the Morris water maze, the hidden platform is placed in a different quadrant of the pool. Ability of the subjects to switch quickly to the new location would be 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. Failure to develop 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, such as motor stereotypies or ultrasonic vocalizations, during failures in the reversal task. These hypothesized reactions could be analogous to the upset reactions to change seen in many autistic individuals.

Additional Symptoms

Because autism includes 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 [Tsai, 1999; Edelson et al., 1999; Gillot et al., 2001]. Anxiety-related tasks have been well-characterized for mice. Conflict tests including the elevated plus maze, light–dark exploration, open field emergence, probe burying, and Vogel thirsty-lick tests are based on approach–avoidance conflicts and are sensitive to anxiolytic drugs [Crawley, 1985; Rodgers, 1997; Contarino et al., 1999; File, 2001; Holmes, 2001; Clement et al., 2002; Finn et al., 2003]. Seizures are frequent in autistic children [Ballaban-Gil and Tuchman, 2000; Pellock, 2004]. Methods for scoring seizures in mice are standard in the literature [Meisler et al., 2001; Upton and Stratton, 2003]. 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 [Meldrum, 1997; Giardina, 2000]. Some parents report that their autistic children have disturbed sleep patterns [Harvey and Kennedy, 2002; Ivanenko et al., 2004]. 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, or by neurophysiological recording of sleep EEG patterns [Tafti and Franken, 2002; Taheri and Mignot, 2002]. Motor stereotypies have been reported in mice [Turner et al., 2001], which may be relevant to motor stereotypies in autism. Clumsiness, reported in some cases of autism [Frith, 1991; Ghaziuddin and Butler, 1998], could be tested in mice using standard motor procedures such as the balance beam, rotarod, and footprint tests [Carter et al., 2001]. Standardized methods to score aggressive behaviors in mice are available [Maxson and Canastar, 2003]. Hypersensitivity to sensory stimuli could be detected through the acoustic and tactile startle tests [Geyer and Swerdlow, 1998]. Theory of mind could conceivably be modeled with the social transmission of food preference task, in which the subject mouse chooses a new flavor of food based on social interactions with another mouse that has eaten that novel flavored food [Galef, 1992; Berger-Sweeney et al., 2000; Wrenn et al., 2003]. 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, and cerebellar irregularities. Biological findings from clinical studies could be examined in mouse models, including unusual serotonin levels, imaging and neurophysiological responses of the amygdala, and cortical regions analogous to the human fusiform cortex, during social interactions. Expression of developmental genes and the development of brain 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 behaviors in young mice are readily available in the literature (Heyser, 2003; Berger-Sweeney, 2003). Ontogenic measures of brain weight in a mutant line or inbred strain that models some of the behavioral symptoms of autism could address the biological mechanisms underlying the age-specific overgrowth of some brain regions in autistic children (Piven et al., 1992; Courchesne et al., 2003).

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 mouse behavioral tasks. 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 (Crawley and Paylor, 1997; Nelson and Young, 1998; Gold, 1999; Picciotto and Wickman, 1998; Crawley, 2000; Wahlsten et al., 2003). A battery of simple observational tests for general health and physical abilities is the first step in evaluating a new line of mice with a targeted gene mutation. 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 (Wrenn et al., 2003). Olfactory cues with social components, e.g., pheromones markings or home cage litter, may be both useful controls and additional parameters in social approach tasks. Learning and memory tasks require controls for their procedural demands, e.g., 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 (Kinney et al., 2002; Wrenn et al., 2004). 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.

CONCLUSION

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 in the mouse behavioral repertoire. As a first approximation toward modeling several of the core symptoms of autism, we propose mouse tasks that measure sociability, vocalizations, and change in routines. These mouse behavior tasks represent a first pass at designing practical laboratory assays with heuristic value for testing hypotheses about the cause and treatment of autism.

The suggestions offered in this article are preliminary. We expect to learn from early results using inbred strains of mice and targeted gene mutations and then refine the procedures and expand the scope of mouse behavioral tasks relevant to autism. Feedback from clinical researchers in the autism community is anticipated and encouraged. It may be interesting to discuss the design of analogous tests for mice and humans, e.g., a suite of three rooms to test social tendencies in autistic subjects, analogous to the three- chambered apparatus to test sociability in mice. Development of useful mouse behavioral tasks relevant to autism will be an iterative process by many lab-

oratories over the course of several years. Our goal is to optimize a set of mouse tasks that can be applied to investigations of transgenics and knockouts, inbred and recombinant inbred strains, quantitative trait loci, DNA microarrays, and chemical mutagenesis phenotyping. A small set of simple, perhaps automated, behavioral tasks may provide a high- throughput approach to discover a particularly interesting line of mice. More in-depth behavioral tasks would then expand on the initial behavioral phenotype. Ideally, experiments with these behavioral tasks will discover genes in mice that mediate social, communication, and repetitive behaviors. Identification of these genes in mice may offer suggestions toward the identification of homologous human genes within the chromosomal loci that have been linked to autism.

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