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**REVIEWS**

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# Animal Models of Autism Spectrum Disorders and Behavioral Techniques of their Examination

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Autism spectrum disorders constitute a significant problem in modern neurology and in neuroscience in general. At present, the incidence of such disorders is increasing, reasons for their appearance remain practically unclear, and there are no sufficiently effective treatments of these pathologies. A few animal models of autistic disorders have been developed; these models reproduce one or a few key symptoms of autism (cognitive rigidity, violations of social interactions, and qualitative disorders of communication). The respective simulations are carried out using either techniques of genetic engineering (knockout rats and mice) or early (pre- or postnatal) influences of certain environmental factors. To investigate behavioral deviations in the model animals, behavioral testing methods are used. A part of them are "classic" (e.g., the open field test, Morris water maze, T-like maze, radial maze, and Skinner's chamber), while others have been designed specifically for models of autism. This review describes and analyzes the main methodical approaches in modeling of autism spectrum disorders in animals, and behavioral methods used in the studies of these models.

**KEYWORDS**: autism, autism spectrum disorders, animal models, behavioral tests.

## INTRODUCTION

Autism spectrum disorders (ASD), which include autism *per se* (Kanner's syndrome), Asperger's syndrome, childhood disintegrative disorder, Rett's syndrome, and nonspecific pervasive developmental disorder (atypical autism) [1], constitute one of the most urgent problems in the sphere of health care. These disorders are widespread in the modern world. In particular, in the US in 2011–2012, various ADSs were officially diagnosed in about 2% of schoolchildren [2]. It should be recognized that it is difficult in this case to adequately estimate which part of this statistic really reflects an increase in the incidence, and which part is related to "expansion" of the diagnostic criteria and/or earlier diagnostics; the latter aspects became possible during recent years [3]. A few decades ago, the diagnosis of "autism" was used only in severe cases, where a person was totally unable to communicate and to maintain independent functioning. At present, however, such terms as "autism spectrum disorders" and "extended autism phenotype" have appeared and begun to be used to characterize much milder autism-like symptoms [4, 5].

The so-called autistic triad includes inadequate social interactions, disorders of mutual communications (the lack of drive to share interests and to indicate interesting objects for contacting subjects), and an abnormally repetitive mode of behavior combined with limited interests [6]. Depending on the concrete type of the disorder and its severity, patients can completely be separated from the outside world, unable to realize verbal communication, and unable to adapt to society and to maintain an independent mode of life (a severe form of autism). In other cases, these subjects are rather socially active and independent despite noticeable difficulties in communication (Asperger's syndrome).

At present, there is no consensus with respect to the ASD etiology and risk factors that may cause the appearance of these disorders. There is evidence that the causes of autism are genetic, i.e. related to changes in the interaction of a number of genes and spontaneous mutations [7]. There are also indications that certain relations to prenatal effects of dangerous substances, in particular agricultural pesticides, are possible [8, 9]. It was shown that the frequency of ASD and autistic features close

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to the norm is considerably higher among brothers and sisters of autistic patients and members of their families than that within the general population [10].

About 15 to 25% of autism cases are associated with identifiable genetic disorders (with the fragile X chromosome, Rett's syndrome, neurofibromatosis, and tuberous sclerosis) and also with viral diseases, such as congenital rubella or mehaloviral pathologies [11]. It was hypothesized that the development of autism can be caused by vaccination of infants; at present, this statement, however, is not considered proven [12].

To investigate the fundamental reasons of ASD cases and to develop medication and some other methods of the control of symptoms of these disorders, animal models of autism are being developed.

## ANIMAL MODELS OF AUTISM: KEY ASPECTS

According to the generally accepted point of view, a considerable part of mental processes is peculiar only to humans. This is why the full-value induction of disorders of these processes in animals, i.e., reproduction of the full clinical pattern of autism, is hardly possible. Nonetheless, there are models in which separate characteristics of ASD, namely deterioration of social (in this case, zoosocial) interaction and a repetitive mode of behavioral manifestations, are readily reproduced.

Animal models of autism can be divided into four groups [13]:

(i) animals with certain deficiencies of neuropeptide receptors, in particular those of vasopressin (Brattleboro strain of rats and mice with a zero mutation of the subtypes of vasopressin receptors [14]), oxytocin (knockout mice with the absence of oxytocin receptors [15]), and opioids (mice with a targeted deletion of μ-opioid receptors [16]);

(ii) models with the reproduction of pathological states that increase the risk of autism in people; prenatal effects of anticonvulsants, thalidomide-induced embryopathy [17], and disorders of the mechanisms of serotonin synthesis in the course of prenatal development [18, 19] belong to the respective cases;

(iii) models with neonatal impairments of the brain zones, anomalies of which are known to be present in people with autism; the respective structures are the cerebellum, amygdalar complex, or medial prefrontal cortex [20];

(iv) genetic models of autism-associated human diseases, such as the syndrome of a fragile X-chromosome [21, 22].

However, there is a quite natural question: How adequate are the above models? In these models, only certain aspects of the etiology of the respective disorders are reproduced; at the same time, autism is a multifactorial state. In animal models, we can observe manifestations associated with "human" autistic disorders, but it is difficult to identify whether these manifestations are the reasons for these disorders, their consequences, different consequences of identical reasons, or these manifestations are interconnected by some other relations. For example, disorders in the functioning of the cerebellum correlate with the ASD occurrence, but there is no causal relation in this case [23]. In addition, a few factors inducing autism symptoms in animals are absent in humans. In particular, deletion of the V1a receptor gene proposed as one of the autism models in rodents was not found in people suffering from ASD [24].

As a rule, laboratory-bred rodents, rats or mice, are used in modeling of the autistic symptoms. At the same time, the respective experiments are carried in other animals, in particular, monkeys [25, 26] and even songbirds [27]. Laboratory rodents are quite suitable for autism models, not only because their behavior has been examined in detail but also because a number of behavioral methods and ways of the influence on the state of their nervous system have been developed. For the latter purpose, chemical agents or genetic interventions are used. Rodents, according to their nature, are social beings; this is why communication disorders in these animals can be rather easily detected [28]. As a rule, such parameters as those of reactions to pheromones secreted by other animals, contacts with known or unknown individuals of the same species, social interactions, collective nesting, ultrasound vocalization, sexual and parental behaviors, territorial marking, and aggressive behavior, are taken into account in descriptions of social interaction of the animals [29–32]. Certain standardized methods of quantitative estimation of different types of behavior of rodents (aggressive, exploratory, sexual, parental, etc.) have been described in detail [33–39].

Initially, rats were mostly used as model animals; later on, most works began to be carried out on mice. Each of these species demonstrates certain advantages and disadvantages. Mice (compared

with rats) are economically "cheaper;" they require less space, food, and other costs for their keeping. In addition, a number of genetic strains of mice with certain properties (knockout and/or mutant individuals) are at present available; among these strains, there are those with manifestations of the autistic symptoms. For example, these are strains $En2^{tm1Alj/tm1Alj}$ (other identification En2-/-) [40], BTBR [41], Shank1 [42], CNTNAP2 [43, 44], and others [45–47]). Simultaneously, examination of the function of definite brain zones is easier, under the respective conditions, in rats (because of their larger dimensions); social behavior of the rats is more clearly manifested and active.

This is why some authors propose to again concentrate attention on rats as model organisms [48]. At present, there are several lines of knockout rats that demonstrate the behavior typical of autism. For example, young *Fmr1*-knockout rats spend less time in mutual games and use fewer ultrasound signals than normal control animals [48].

Belzung et al. [13] mentioned seven models in which two key features of ASD (disorders of social interrelations and motoric stereotypicity/ cognitive rigidity) are manifested: models based on the prenatal effects of 5-methoxytryptamine (5-MT) [49] and valproic acid [50], three models of early postnatal disorders, guinea pigs of line GS [51], and rats neonatally infected by Bourne virus [52]. Eight other models were qualified in the same publication as valid ones for modeling specific symptoms of autism. The third feature of the autistic triad (disorders of social communication) is difficult to model because the specificity of the speech phenomenon typical only of humans creates definite crucial difficulties, and this aspect will be discussed below.

## BEHAVIORAL TESTS FOR EXAMINATION OF ASD MODELS

For estimation of external manifestations of disorders in animal models of autism, certain

Table 1. Crucial Symptoms of Autism, Their Possible Mechanisms, and Behavioral Methods of Their Study

| Social deficiency | Communication deficiency | Stereotyped and repetitive behavior, cognitive rigidity | Possible mechanisms | Behavioral methods of examination |
|---|---|---|---|---|
| Social interaction, social games | Olfactory labels, ultrasound vocalization (USV) produced by a female, USV induced by interaction | Motor stereotyped behavior, repeated autogrooming, restriction of research activity | Social assistance: in reactions to pain<br>Social assistance: in pseudo-sensitization to drugs<br>Joint training: learning in the social context | Open field test / a cage specialized for observation / a cage for routine keeping |
| | | Motor stereotypic behavioral events | | Test of repeated marble burying |
| Social familiarization<br>Social recognition | USV induced by interaction | | | Three-compartment testing set |
| | | Reversive learning | | Morris water maze /<br>T-like maze /<br>radial maze |
| | USV induced by isolation | | | Chamber for offspring isolation |
| Social familiarization | USC induced by interaction | | | Reproduction of USV recordings |
| | USV induced by fear | | Social assistance in learning for fear reactions. Observation-related learning for fear | Chamber for training of a fear-related conditioned reflex |
| | | | Learning by observation; operant learning | Skinner's chamber |
| | USV induced by fear | | Empathy (a rat frees another rat caught in a trap) | Movement-restricting trap |

According to M. Wöhr and M. L. Scattoni, 2013. [67].

behavioral test techniques are used (Table 1). Both universal classical tests (open field test, Morris water maze, T-like maze, radial maze, Skinner's chamber), and methods for detection of specific features of the autistic disorders are described below (a three-compartment device for estimation of social interaction, recording and reproduction of ultrasound signals in studies of social communication, etc.). Frequently, the animal's behavior is observed under conditions where these animals are routinely (familiarly) kept, immediately in the cages where they usually live. Combination and modification of certain techniques is expedient; this allows experimenters to provide more complete and detailed evaluation of the phenotypic manifestations in the respective models.

As a rule, a set of tests for studying animal ASD models includes techniques for examination of social behavior, cognitive flexibility, and anxiety [53]. The expedience of using estimation of the anxiety level in this context is, however, doubtful because changes in this index are not among specific characteristics of the autistic disorder. This is why it is expedient to concentrate attention on the former two directions of research, examination of the flexibility of cognitive processes and of changes in social interactions between animals.

## STUDIES OF DISORDERS OF SOCIAL INTERACTION

Crowley [53], when trying to estimate the level of social interactions between animals, proposed a rather simple test. A mouse or a rat is placed into a container with additional compartments at each of the two opposite sides, and the animal can freely enter these compartments. Another animal of the same species is put in one of the compartments; this animal is tied or covered with a wire cap. Another compartment is empty. According to a choice of the tested animal, to come closer to the unfamiliar individual or to move toward the empty compartment, the predisposition of the tested animal for realization of social interaction, or, *vice versa*, its indifference to such communication or even domination of the avoidance reaction can be quantitatively estimated.

A modification of this test was proposed to provide more accurate differentiation of the phenomena of social and research behavior modification [54]. A mouce covered with the wire cap is put in one of the compartments, while a similar cap, but with no animal, is put in the other compartment. In the case of autism, a significant interest to novel inanimate objects can be manifested; thus, it is logical to suppose that the animal can select the "mouse" compartment with no desire to communicate, but trying only to explore the cap in which the second mouse is positioned. When the tested animal spends the same time in two compartments or demonstrates a greater predisposition to the compartment with the empty cap, this is considered an identification feature of autistic behavior. Healthy mice significantly prefer their stay in the "social" compartment.

A device was developed that allowed experimenters to quantify the results of this test; the time spent by the tested animal in the "social" and "nonsocial" compartments was automatically measured [55]. This test is also used for evaluation of social recognition; in this case, after the first stage of testing, an unfamiliar mouse is put under the empty cap. As a rule, healthy animals in this situation prefer to communicate with the "stranger."

It was noted that precisely the test of social recognition was used in four of the five models with the development of autistic behavior resulting from a deficiency of certain neuropeptides [13]. The validity of this test remains, however, under doubt because autistic patients demonstrate difficulties with recognition of the persons rather rarely. The validity of estimation of the level of aggressiveness also looks doubtful, because manifestations of aggression can be enhanced, weakened, or remain with no changes in the case of autism.

In 2012, American scientists proposed another model version for studying social interaction in mice [56]. When exploring a transgene model of autism in mice (animals with an Ala56 allele of the gene *SLC6A4* of the serotonin receptor), they trained animals to return to the "home" cage via a plastic tube; after some time, an unfamiliar mouse was placed in this tube. In this case, transgenic mice more frequently interrupted their movement and tried to find some other way. It is, however, worth mentioning that such a reaction can serve, most probably, as a manifestation of social phobia and increased anxiety than that of autism *per se*. The main feature of the latter pathology is the rigidity of behavior, in particular, the predisposition to adhere to a well-defined route under all conditions.

Olfactory stimuli, in particular pheromones

secreted by animals, play crucial roles in the communication of rodents [57, 58]. Disorders in olfactory communication are characterized by the time intervals during which the animal sniffs aromatic traces left by an unfamiliar animal and according to the intensity of recognition and differentiation attempts with respect to the stimuli left by well-known and unfamiliar individuals.

Studies of the communication processes in which animals use sound and ultrasound auditory stimuli, chemical agents, gaze direction, facial expression, and postures are of high informative value. It should, however, be taken into account that facial expressions are relatively poor in rodents because their facial musculature is weakly developed. The respective studies are carried out by putting the experimental and "stimuli" animals in an open neutral arena, e.g., in that of open field test [59]. Testing can be carried out in animals of any age (beginning from the moment of termination of the baby milk-feeding period). When adult animals are tested, the duration of stay of the individual within the arena should be limited by a three- to five-min-long interval to prevent the development of aggressive behavior or attempts to establish sexual contacts; the above phenomena are not manifestations of the autistic phenotype [60].

## STUDIES OF THE COGNITIVE RIGIDITY AND LIMITATION OF INTERESTS

The second crucial sign of autism is cognitive rigidity. Crowley [53] proposed to evaluate this aspect using the T-maze. The animal was first taught to find a food reward in one of the branches; then, the reward was transferred to the opposite arm. The experimenters observe how quickly the animal changes its behavior and begins to look for food in a novel place. Estimation of the rate of attenuation of the conditioned reflex after withdrawal of reinforcement and measuring the time required to find the target platform in the Morris water maze after removal of this platform are other possible approaches.

The repetitive and stereotypic mode of behavior in rodents is manifested in an excessive repetition frequency or intensity of normal behavioral manifestations, such as grooming, standing upright, shaking, spinning, jumping, etc. [32, 61, 62]. These behavioral elements are observed and recorded, as a rule, in open field test or under conditions of routine behavior, directly in the cages were the animals are kept.

The "burying-stones" test (the so-called marble burying test) is an interesting technique used in studies of the stereotypic mode of behavior in ASD models (in particular, in *BTBR*-mutant mice) [63]. In this test, animals are put in the test arena covered with a fairly thick layer of bedding. Preliminarily, animals are adapted to the arena to weaken the stress reaction to the novelty of environment. Small pebbles, glass beads, or plastic beads are put on the bedding, and the experimenters watch how many of these objects the tested animal digs in the bedding. The reaction of burying the unknown objects is a typical manifestation of defensive behavior of rodents (mice and rats, in particular) [64]. Using its muzzle and forelimbs, the animal tries to cover the objects suspected to be unpleasant and/or dangerous by the ground or bending material. Under wild natural conditions, these may be insects, scorpions, or snakes; under laboratory conditions, these may be stimulating shock electrodes. Objects that are not supposed to be dangerous (bits of food, small stones, glass beads) are also readily buried. In models of autistic disorders, such a behavioral pattern acquires a repetitive, obsessive character, and this can serve as a good indicator of the recurrence of behavior, which is one of the key features of ASD in humans. Initially, burying of the stones was believed to be an anxiety correlate because the intensity of these actions was considerably weakened under the action of anxiolytics (such as diazepam) [65]. At present, however, such behavior is believed to be mostly related to the novelty of objects and reflects obsessive/compulsive manifestations. This is why at present this test is considered an acceptable one in models of autistic and obsessive/compulsive disorders [66].

The modeled restriction of interests in rodents can be manifested under conditions of the test in which the animal is placed in a chamber with many burrow-like openings in the bottom and walls. Healthy rodents intensely examine different holes, sniffing them and putting the muzzle in such "burrows." The animals manifesting autistic symptoms significantly prefer one and the same "hole" (or a few "burrows"), exploring them again and again [67].

Often when the behavior of the animal is tested in a Y-like maze, the animal is to select one of two branches, and food reinforcement is absent in both. As a rule, healthy rodents, having visited, e.g., the left arm, then go to the right one. In modeled

autism, it could be supposed that a tendency to visit one and the same arm many times should be observed. This test, however, probably characterizes the specificity of research activity and does not detect the cognitive rigidity [68].

## MODELING AND RESEARCH ON THE COMMUNICATION DEFICIENCY IN ANIMALS

We have already mentioned that modeling in animals of the third component of the "autistic triad" is rather problematic because only people use speech for communication; therefore, only in humans can we observe the respective disorders of the communication phenomena. It is known, however, that animals are able to maintain communication relationships using various systems of signals. As was found, rodents (rats, mice, hamsters, gerbils, etc.) systematically use ultrasonic auditory signals for communication [59]. These signals were detected in almost all situations of social life of the rodents, in acts of familiarization, aggression, sexual behavior, and others. Thus, it was shown [69] that ultrasonic signals produced by babies that try to find the nest help their mothers to find and return them to a safe area. Upon hearing the signal, the female left the nest and went searching. However, this female did not react to anesthetized or dead babies that did not produce such signals. Ultrasonic signals of the baby mice were used [70]. When the respective record was switched on in an empty compartment of the experimental set with the nest, most of the mothers left the latter and went to the above-mentioned compartment with full absence of baby mice, stuffed animals of the respective shape, and the corresponding smell traces. Later on, analogous studies were carried out on rats. Therefore, ultrasonic vocalization (USV) probably plays a much more important role in maternal behavior of rodents than visual and olfactory stimuli.

Significant disturbances of USV, as compared with vocalization of healthy animals, are observed in models of autistic disorders. In particular, offspring of mutant mice Shank1–/–produced fewer signals under conditions of isolation from mothers and other young animals [71]. In adult model animals, disorders of vocalizations were also obvious. Adult male mice of strains BTBRT $^{T+tf/J}$ (BTBR), when allowed to sniff urine of females, produced fewer ultrasound signals and left fewer aromatic tags than wild-type animals [72].

Disorders of vocalization began to be studied also in songbirds [27]. As is believed, such birds not only use vocalization for communications but also learn, special songs typical only of specific species during their life. It should be noted that only males are able to "sing" the respective songs, and their song is important in the competition for a female partner for reproduction. It is thought that mutation of one and the same gene, *FOXP2*, results in disorders of learning for speech in humans [73], vocalization in rodents [74, 75], and songs in songbirds [76]. It was shown that "switching off" of this gene resulting from the influence of lentivirus induced difficulties in learning songs [77]. As is known, songbirds learn a melody typical of their own species by repeating this song after an older bird (a "teacher"). Birds with disorders of the function of gene *FOXP2* reproduced the song incompletely or significantly distorted it. Such abnormal vocalization did not help the male bird in competition for a female. It cannot be ruled out that examination of the features of vocalization in birds can help to obtain certain information related to modeling of autism-like disorders.

* * *

Thus, the animal models of autism, despite a number of obvious limitations and reservations, appear to be a useful tool in studying the mechanisms of ASD and in attempts to find possible ways to alleviate their symptoms. At present, such disorders are mostly modeled on laboratory rodents, rats and mice, but other animal species, songbirds in particular, begin to be used for this purpose. As a rule, formation of deficiencies of certain neuropeptides, effects of toxic agents, or genetic mutations are used in modeling of ASD. Most models are capable of representing one or two features of the "autistic triad", but some can cover all three respective aspects.

In the studies of animal ASD models, behavioral methods, both traditional and specially developed, are used. Many techniques used are disputable and not single-valued. Nonetheless, such a direction of studies is rather important; it opens certains possibilities to obtain a significant volume of factual material necessary for successful interpretation of the mechanisms responsible for the autism phenomenon and the search for effective methods of treatment of this pathology.

This article is a review; it was not associated with any experimental studies. Therefore, the confirmation of its correspondence to the existing international ethical standards is not required.

The authors, S. V. Hrabovska and Yu. T. Salyha, confirm the absence of conflicts of any kind related to commercial or financial interests, relations with organizations or persons in any kind involved in the preparation of this material, and interrelations between the coauthors.

## REFERENCES


1. *Autism Spectrum Disorder Fact Sheet*. American Psychiatric Publishing, pp. 1-2 (2013).
2. S. J. Blumberg, M. D. Bramlett, M. D. Kogan, et al., "Changes in prevalence of parent-reported Autism Spectrum Disorder in school-aged U.S. children: 2007 to 2011–2012," *Nat. Health Stat. Rep.,* **65***,* 1-11 (2013).
3. C. J. Newschaffer, L. A. Croen, J. Daniels, et al., "The epidemiology of autism spectrum disorders", *Annu. Rev. Public Health,* **28**, 235–258 (2007).
4. J. Piven and P. Palmer, "Psychiatric disorder and the broad autism phenotype: evidence from a family study of multiple-incidence autism families," *Am. J. Psych.,* **156**, No. 4, 557-563 (1999).
5. N. Micali, S. Chakrabarti, and E. Fombonne, "The broad autism phenotype findings from an epidemiological survey," *Autism*, **8**, No. 1, 21-37 (2004).
6. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed, American Psychiatric Association, Washington, DC (1994).
7. B. S. Abrahams and D. H. Geschwind, "Advances in autism genetics: on the threshold of a new neurobiology," *Nat. Rev. Genet.,* **9**, No. 5, 341–355 (2008).
8. T. L. Arndt, C. J. Stodgell, and P. M. Rodier, "The teratology of autism," *Int. J. Dev. Neurosci.,* **23,** No. 2–3, 189–99 (2005).
9. J. F. Shelton, I. Hertz-Picciotto, and I. N. Pessah, "Tipping the balance of autism risk: Potential mechanisms linking pesticides and autism," *Environ. Health Perspect.,* 120, No. 7, 944-951 (2012).
10. T. Bourgeron, S. Jamain, and S. Granon, "Animal models of autism. Transgenic and knockout models of neuropsychiatric disorders," *Contemp. Clin. Neurosci.*, 151-174 (2006).
11. S. E. Folstein and B. Rosen-Sheidley, "Genetics of autism: complex aetiology for a heterogeneous disorder," *Nat. Rev. Genet.,* **2**, 943–955 (2001).
12. M. Rutter, "Incidence of autism spectrum disorders: changes over time and their meaning," *Acta Paediatr.,* **94**, No. 1, 2–15 (2005).
13. C. Belzung, S. Leman, P. Vourc'h, and C. Andres, "Rodent models for autism: A critical review," *Drug Discov. Today: Dis. Models,* **2**, No. 2, 93-101 (2005).
14. T. B. Van Wimersma Greidanus, "Disturbed behavior and memory of the Brattleboro rat," *Ann. N.Y. Acad. Sci.*, **394**, 655-662 (1982).
15. R. L. Pobbe, B. L. Pearson, and E. B. Defensor, "Oxytocin receptor knockout mice display deficits in the expression of autism-related behaviors," *Horm. Behav.*, **61**, No. 3, 436-444 (2012).
16. M. Wцhr, A. Moles, R. K. Schwarting, and F. R. D'Amato, "Lack of social exploratory activation in male μ-opioid receptor KO mice in response to playback of female ultrasonic vocalizations," *Soc. Neurosci.*, **6**, No. 1, 76-87 (2011).
17. M. Narita, A. Oyabu., Y. Imura, et al., "Nonexploratory movement and behavioral alterations in a thalidomide or valproic acid-induced autism model rat," *Neurosci. Res.*, **66**, No. 1, 2–6 (2010).
18. D. Kahne, A. Tudorica, A. Borella, et al., "Behavioral and magnetic resonance spectroscopic studies in the rat hyperserotonemic model of autism," *Physiol. Behav.*, **75**, No. 3, 403-410 (2002).
19. I. Lucki, "The spectrum of behaviors influenced by serotonin," *Biol. Psychiatry*, **44**, No. 3, 151-62 (1998).
20. P. T. Tsai, C. Hull, Y. X. Chu, et al., "Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice," *Nature*, **488**, 647–651 (2012).
21. M. A. Mines, C. J. Yuskaitis, M. K. King, et al., "GSK3 influences social preference and anxiety-related behaviors during social interaction in a mouse model of fragile X syndrome and autism," *PLoS ONE*, **5**, No. 3, e9706 (2010).
22. K. M. Huber, S. M. Gallagher, S. T. Warren, and M. F. Bear, "Altered synaptic plasticity in a mouse model of fragile X mental retardation," *Proc. Natl. Acad. Sci. USA*, **99**, No. 11, 7746–7750 (2002).
23. N. J. Minshew, B. Luna, and J. A. Sweeney, "Oculomotor evidence for neocortical systems but not cerebellar dysfunction in autism," *Neurology*, **52**, No. 5, 917–922 (1999).
24. T. H. Wassink, J. Piven, V. J. Vieland, et al., "Examination of AVPR1a as an autism susceptibility gene," *Mol. Psychiatry*, **9**, 968–972 (2004).
25. M. D. Bauman, J. E. Toscano, B. A. Babineau, et al., "Emergence of stereotypies in juvenile monkeys (*Macaca mulatta*) with neonatal amygdala or hippocampus lesions," *Behav. Neurosci.*, **122**, No. 5, 1005-1015 (2008).
26. L. Malkova, M. Mishkin, S. J. Suomi, and J. Bachevalier, "Long-term effects of neonatal medial temporal ablations on socioemotional behavior in monkeys (Macaca mulatta)," *Behav. Neurosci.*, **124**, No. 6, 742-760 (2010).
27. S. C. Panaitof, "A songbird animal model for dissecting the genetic bases of autism spectrum disorder," *Dis. Markers*, **33**, No. 5, 241–249 (2012).
28. J. F. Cryan and A. Holmes, "The ascent of mouse: advances in modelling human depression and anxiety," *Nat Rev Drug Discov.*, **4**, No. 9, 775-790 (2005).
29. E. Grant and J. Macintosh, "A comparison of the social postures of some common laboratory rodents," *Behaviour*, **21**, 246–259 (1963).
30. C. S. Carter, J. R. Williams, D. M. Witt, and T. R. Insel, "Oxytocin and social bonding," *Ann. N.Y. Acad. Sci.,* **652**, 204–211 (1992).
31. M. L. Terranova and G. Laviola, "Scoring of Social

Interactions and Play in Mice During Adolescence," *Curr. Protoc. Toxicol*., **13**, No. 10 (2005).
32. H. G. McFarlane, G. K. Kusek, M. Yang, et al., "Autism-like behavioral phenotypes in BTBR T+tf/J mice," *Genes Brain Behav*., **7**, No. 2, 152-163 (2008).
33. M. A. Hofer and H. N. Shair, "Ultrasonic vocalization, laryngeal braking, and thermogenesis in rat pups: a reappraisal," *Behav. Neurosci*., **107**, 354–362 (1993).
34. K. A. Miczek, S. C. Maxson, E. W. Fish, S. Faccidomo, "Aggressive behavioral phenotypes in mice," *Behav. Brain Res*., **125**, 167–181 (2001).
35. J. T. Winslow, E. F. Hearn, J. Ferguson, et al., "Infant vocalization, adult aggression, and fear behavior of an oxytocin null mutant mouse," *Horm. Behav*., **37**, 145–155 (2000).
36. C. C. Wrenn, A. P. Harris, M. C. Saavedra, and J. N. Crawley, "Social transmission of food preference in mice: methodology and application to galanin-overexpressing transgenic mice," *Behav. Neurosci.,* **117**, 21–31 (2003).
37. D. W. Wesson, M. Keller, Q. Douhard, et al., "Enhanced urinary odor discrimination in female aromatase knockout (ArKO) mice," *Horm. Behav*., **49**, 580–586 (2006).
38. J. B. Panksepp, K. A. Jochman, J. U. Kim, et al., "Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice," *PLoS One,* **2**, e351 (2007).
39. S. R. Wersinger, H. K. Caldwell, L. Martinez, et al., "Vasopressin 1a receptor knockout mice have a subtle olfactory deficit but normal aggression," *Genes Brain Behav*., **6**, 540–551 (2007).
40. J. Brielmaier, P. G. Matteson, J. L. Silverman et al., "Autism-Relevant Social Abnormalities and Cognitive Deficits in Engrailed-2 Knockout Mice," *PLoS ONE,* 7, No. 7, e40914 (2012).
41. S. S. Moy, J. J. Nadler, and N. B. Young, "Mouse Behavioral Tasks Relevant to Autism: Phenotypes of Ten Inbred Strains," *Behav. Brain Res.,* **176**, No. 1, 4–20 (2007).
42. J. L. Silverman, S. M. Turner, C. L. Barkan, et al., "Sociability and motor functions in Shank1 mutant mice," *Brain Res.,* **1380**, 120-137 (2011).
43. M. Alarcyn, B. S. Abrahams, J. L. Stone, et al., "Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene," *Am. J. Hum. Genet*., **82**, No. 1, 150-159 (2008).
44. H. C. Whalley, G. O'Connell, J. E. Sussmann, et al., "Genetic variation in CNTNAP2 alters brain function during linguistic processing in healthy individuals," *Am. J. Med. Genet. B. Neuropsychiatr. Genet*., **156**, No. 8, 941-948 (2011).
45. T. M. DeLorey, P. Sahbaie, E. Hashemi, et al., "Gabrb3 gene deficient mice exhibit impaired social and exploratory behaviors, deficits in non-selective attention and hypoplasia of cerebellar vermal lobules: a potential model of autism spectrum disorder," *Behav. Brain. Res*., **187**, 207–220 (2008).
46. B. C. Ryan, N. B. Young, S. S. Moy, and J. N. Crawley, "Olfactory cues are sufficient to elicit social approach behaviors but not social transmission of food preference in C57BL/6J mice," *Behav. Brain Res.,* **193**, 235–242 (2008).
47. K. Radyushkin, K. Hammerschmidt, S. Boretius, et al., "Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit," *Genes Brain Behav*., **8**, 416–425 (2009).
48. E. Callaway, "Rat models on the rise in autism research," *Nature News*, 23 November (2011).
49. E. C. Azmitia, A. V. Shemer, and P. M. Whitaker-Azmitia, "Dose-related effects of prenatal 5-methoxytryptamine (5-MT) on development of serotonin terminal density and behavior," *Dev. Brain Res.,* **59**, No. 1, 59-63 (1991).
50. A. G. Foley, S. Gannon, N. Rombach-Mullan, et al., "Class I histone deacetylase inhibition ameliorates social cognition and cell adhesion molecule plasticity deficits in a rodent model of autism spectrum disorder," *Neuropharmacology*, **63**, 750-760 (2012).
51. J. Caston, E. Yon, D. Mellier, et al., "An animal model of autism: behavioural studies in the GS guinea-pig," *Eur. J. Neurosci.,* **10**, No. 8, 2677-2684 (1998).
52. M. V. Pletnikov, T. H. Moran, and K. M. Carbone, "Borna disease virus infection of the neonatal rat: developmental brain injury model of autism spectrum disorders," *Front Biosci*., **7**, 593-607 (2002).
53. J. N. Crawley, *What's Wrong with My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice*, John Wiley and Sons, 329 pp. (2000).
54. M. Yang, J. L. Silverman, and J. N. Crawley, "Automated three-chambered social approach task for mice," in: *Curr. Protoc. Neurosci.*, Chapter 8: Unit 8. 26 (2011).
55. J. J. Nadler, S. S. Moy, G, Dold, et al., "Automated apparatus for quantitation of social approach behaviors in mice," *Genes Brain Behav*., **3**, No. 5, 303-14 (2004).
56. J. Veenstra-Van der Weele, C. L. Muller, H. Iwamoto, et al., "Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior," *Proc. Natl. Acad. Sci. USA*, **109**, 5469–5474 (2012).
57. J. Bakker, S. Honda, N. Harada, and J. Balthazart, "Sexual partner preference requires a functional aromatase (cyp19) gene in male mice," *Horm. Behav.,* **42**, 158–171 (2002).
58. T. H. Ahern, M. E. Modi, J. P. Burkett, and L. J. Young, "Evaluation of two automated metrics for analyzing partner preference tests," *J. Neurosci. Methods*, **182**, 180–188 (2009).
59. S. Jamain, K. Radyushkin, K. Hammerschmidt, et al., "Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism," *Proc. Natl. Acad. Sci. USA*, **105**, 1710–1715 (2008).
60. M. L. Scattoni, L. Ricceri, and J. N. Crawley, "Unusual repertoire of vocalizations in adult BTBR T + tf/J mice during three types of social encounters," *Genes Brain Behav.,* **10**, 44–56 (2011).
61. O. Pecagarikano, B. S. Abrahams, E. I. Herman, et al., "Absence of CNTNAP2 leads to epilepsy, neuronal

migration abnormalities, and core autism-related deficits," *Cell*, **147**, No. 1, 235-246 (2011).
62. J. L. Silverman, S. S. Tolu, C. L. Barkan, and J. N. Crawley, "Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP," *Neuropsychopharmacology*, **35**, No. 4, 976-989 (2010).
63. A. Thomas, A. Burant, N. Bui, et al., "Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety," *Psychopharmacology (Berlin)*, **204**, No. 2, 361-373 (2009).
64. J. P. J. Pinel and D. Treit, "Burying as a defensive response in rats," *J. Comp. Physiol. Psychol.,* **92**, No. 4, 708–712 (1978).
65. C. L. Broekkamp, H. W. Rijk, D. Joly-Gelouin, and K. L. Lloyd, "Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming in mice," *Eur. J. Pharmacol.,* **126**, No. 3, 223-229 (1986).
66. K. Njung'e and S. L. Handley, "Evaluation of marble-burying behavior as a model of anxiety," *Pharmacol. Biochem. Behav*., **38**, No. 1, 63-67 (1991).
67. M. Wöhr and M. L. Scattoni, "Behavioural methods used in rodent models of autism spectrum disorders: Current standards and new developments," *Behav. Brain Res.,* **251**, 5– 17 (2013).
68. C. Belzung, "Measuring rodent exploratory behavior," in: *Handbook of Molecular Genetic Techniques for Brain and Behavior Research,* W. E. Crusio, and R. Gerlai (eds. ), Elsevier, Amsterdam (2001), pp. 739–749.
69. H. M. Zippelius and W. M. Schleidt, "Ultraschall-Laute bei jungen Mäusen," *Naturwissenschaften*, **43**, 502 (1956).
70. G. D. Sewell, "Ultrasonic communication in rodents," *Nature*, **227**, 410 (1970).
71. M. Wöhr , F. I. Roullet, A. Y. Hung, et al., "Communication impairments in mice lacking Shank1: Reduced levels of ultrasonic vocalizations and scent marking behavior," *PLoS ONE*, **6**, No. 6, e20631 (2011).
72. M. Wöhr, F. I. Roullet, and J. N. Crawley, "Reduced scent marking and ultrasonic vocalizations in the BTBR T+tf/J mouse model of autism," *Genes Brain Behav*., **10**, No. 1, 35-43 (2011).
73. C. S. Lai, D. Gerrelli, A. P. Monaco, and S. E. Fisher, "Copp AJ. FOXP2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder," *Brain*, **126**, No. 11, 2455-2462 (2003).
74. T. E. Holy and Z. Guo, "Ultrasonic songs of male mice," *PLoS Biol*., **3**, No. 12, e386 (2005).
75. G. Arriaga, E. P. Zhou, E. D. Jarvis, "Of Mice, Birds, and Men: The Mouse Ultrasonic Song System Has Some Features Similar to Humans and Song-Learning Birds," *PLoS ONE***, 7**, No. 10, e46610 (2012).
76. M. C. Condro, S. A. White, "Distribution of language-related Cntnap2 protein in neural circuits critical for vocal learning," *J. Compar. Neurol.*, **522**, 169 (2014).
77. S. Haesler, C. Rochefort, B. Georgi, et al., "Incomplete and inaccurate vocal imitation after knockdown of FoxP2 in songbird basal ganglia nucleus Area X," *PLoS Biol,* **5**, e321 (2007).