Modeling autism-relevant behavioral phenotypes in rats and mice: Do 'autistic' rodents exist?

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Autism spectrum disorders (ASD) are among the most severe developmental psychiatric disorders known today, characterized by impairments in communication and social interaction and stereotyped behaviors. However, no specific treatments for ASD are as yet available. By enabling selective genetic, neural, and pharmacological manipulations, animal studies are essential in ASD research. They make it possible to dissect the role of genetic and environmental factors in the pathogenesis of the disease, circumventing the many confounding variables present in human studies. Furthermore, they make it possible to unravel the relationships between altered brain function in ASD and behavior, and are essential to test new pharmacological options and their side-effects. Here, we first discuss the concepts of construct, face, and predictive validity in rodent models of ASD. Then, we discuss how ASD-relevant behavioral phenotypes can be mimicked in rodents. Finally, we provide examples of environmental and genetic rodent models widely used and validated in ASD research. We conclude that, although no animal model can capture, at once, all the molecular, cellular, and behavioral features of ASD, a useful approach is to focus on specific autism-relevant behavioral features to study their neural underpinnings. This approach has greatly contributed to our understanding of this disease, and is useful in identifying new therapeutic targets. *Behavioural Pharmacology* **26:522–540**

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Introduction

Autism spectrum disorders (ASD) are among the most severe psychiatric disorders in childhood in terms of prevalence, outcome, impact on families, and cost to society (Fombonne, 2003; Elsabbagh *et al.*, 2012; Buescher *et al.*, 2014). In recent years, worldwide epidemiological studies have consistently reported an increased prevalence of ASD compared with that 30 years ago. Thus, as a result of changes in diagnostic criteria, the adoption of the much broader concept of 'ASD' rather than simply 'autism', the recognition of the disease among normally intelligent individuals, and improved social services, the current prevalence estimate for ASD is about 1% in the general population (Ghosh *et al.*, 2013; Lai *et al.*, 2014a, 2014b).

In the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5, American Psychiatric Association, 2013), the traditional triad of ASD symptom domains (i.e. social, communication, and atypical/repetitive behaviors) has been reduced to two domains by combining social and communication symptoms into one single diagnostic criterion: criterion A: social/communicative deficits; criterion B includes restricted, repetitive patterns of behaviors, interests, or activities (American Psychiatric Association, 2013). These symptoms are present since early childhood (criterion C) and limit or impair everyday life (criterion D; American Psychiatric Association, 2013).

The term 'spectrum' to define this disorder has been adopted because its manifestations vary greatly depending on the severity of the core symptoms and diversity of comorbid features. For instance, ASD patients may present anxiety, hyperactivity, impulsivity, as well as seizures or gastrointestinal disorders (Lai *et al.*, 2014a, 2014b). Although there is considerable variation in the extent to which these associated symptoms manifest in overt behavior, a core and disabling feature of ASD is impaired social behavior and social communication (American Psychiatric Association, 2013). Parents are usually the first to notice symptoms of social dysfunction and impaired social communication in their children (Young *et al.*, 2003). As early as infancy, babies with ASD may demonstrate the following symptoms: (i) lack of or weak response to their parents' voice; (ii) not using their voice to attract attention to themselves, express emotions, or establish contact; and (iii) lack of attempts at nonverbal communication. Furthermore, they may be unresponsive to social stimuli, or may focus intently on one item, refusing to interact with others for long periods of time. As the children get older, withdrawal from social

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interactions, indifference to social activities, and deficits in social communication become more evident, and several components of the social repertoire and language appear highly impoverished (Jones *et al.*, 2014; Lai *et al.*, 2014a, 2014b; Zachor and Curatolo, 2014).

No specific and effective drug treatment is currently available for ASD (Mohiuddin and Ghaziuddin, 2013; Lai *et al.*, 2014a, 2014b). Antidepressant drugs are often prescribed to ameliorate symptoms of anxiety, depression, or compulsive and aggressive behaviors. Antipsychotic medications are used to treat stereotypies, irritability, and self-injuring behaviors. Seizures can be treated with one or more anticonvulsant drugs. Stimulant drugs are sometimes used effectively to decrease impulsivity and hyperactivity (Mohiuddin and Ghaziuddin, 2013; Zachor and Curatolo, 2014). It is noteworthy that none of these drugs selectively ameliorate the altered social behavior and social communication displayed by ASD patients. Thus, there is an urgent need for new treatments that can ameliorate the symptomatology of the disease, particularly in the core social domain.

Given the diverse set of symptoms displayed by ASD patients, it is not surprising that multiple etiological components are thought to be involved in the pathogenesis of the disease, including genetic and environmental factors (Jeste and Geschwind, 2014). Genetic factors clearly play an important role, as the concordance rate for ASD ranges from ~ 2–6% in dizygotic twins to 60–95% in monozygotic twins (Abrahams and Geschwind, 2008). Accordingly, single-gene mutations, polymorphisms, and copy number variations have been associated with ASD. At the same time, the evidence that the concordance is less than 100% in monozygotic twins prompted investigation of environmental and epigenetic factors as causes of ASD (Chaste and Leboyer, 2012). Several prenatal and perinatal risk factors have been identified, including exposure to intrauterine infections, maternal treatment with drugs such as valproic acid (VPA) and thalidomide, or exposure to toxicants such as organophosphate insecticides or heavy metals (Chaste and Leboyer, 2012).

Researchers working in the field now face the challenge of understanding the impact of the genetic and environmental alterations possibly involved in the disease, and their potential interactions. In this context, animal models mimicking the specific symptoms of the disease are excellent translational research tools: first, they enable dissection of the role of genetic and environmental perturbations in the pathogenesis of ASD, thus providing a better understanding of the biological mechanisms underlying the core features of the disease; second, they make it possible to unravel the relationships between altered brain function in ASD and behavior; finally, they are essential to validate new therapeutic targets and the ability of potential drug candidates to reverse and/or prevent the core and associated symptoms.

The aim of this review was to outline the importance of rodent models in ASD research. First, we discuss whether and how the concepts of construct, face, and predictive validity can be applied to rodent models of ASD. Then, we discuss how behavioral phenotypes relevant to ASD can be mimicked in rodents, particularly at early developmental ages. Finally, we provide several examples of environmental and genetic rodent models widely used and validated in ASD research.

The concept of validity in rodent models of autism spectrum disorders

Validity is a key criterion used when assessing the utility of animal models. As originally postulated by McKinney and Bunney (1969), an ideal animal model should mimic a certain disease in its etiology, biochemistry, symptomatology, and treatment. The McKinney and Bunney (1969) criteria laid the foundation for the concept of face, construct, and predictive validity of animal models (Willner, 1984; Blanchard *et al.*, 2013). In animal models of neuropsychiatric disorders, face validity refers to the similarities between the symptoms displayed by the patients and the behaviors observed in the animal model of the disease. Construct validity refers to the theoretical rationale of the model – that is, similarity between the neurobiological mechanisms underlying the behavior in the model and those underlying the behavior in the pathological state that is being modeled. The criterion of predictive validity is related to the ability of the model to predict interventions that will be successful (or unsuccessful) in the clinical setting (Willner, 1984), including sensitivity of the proposed model to pharmacological manipulations affecting the disease in humans, either in a positive or in a negative direction (Blanchard *et al.*, 2013). A valid animal model should also be reliable – that is, the results obtained through the model should be the same between one time and another within the same laboratory and between one laboratory and another (Willner, 1997; van der Staay *et al.*, 2009).

Obviously, given the multifactorial etiology of ASD, the lack of effective and specific treatments, and the great phenotypic variation within each core symptom domain, there is no animal model that can capture, at once, all the molecular, cellular, and behavioral features of ASD. Rather, as ASD is defined by specific behavioral characteristics, a useful approach in ASD research is to focus on animal behaviors that are relevant to the core diagnostic symptoms of the disease (Crawley, 2012), in order to study both the underlying neural mechanisms and the potential pharmacological targets to mitigate the phenotype. Thus, a valid animal model would show behaviors relevant to each category of the diagnostic symptoms of ASD. An added value of the model would be its ability to mimic not only the core but also some of the associated symptoms of the disease. In the following paragraphs, we will address how it is possible to mimic ASD-relevant

behaviors in laboratory animals by describing some behavioral tests commonly used to assess altered behavioral phenotypes relevant to ASD in rodent models.

Measuring autism-relevant behavioral phenotypes in the laboratory setting

Diagnostic and Statistical Manual of Mental Disorders, 5th edition criterion A: deficits in reciprocal social communication and social interaction

Social communication

Social communication skills include verbal and nonverbal behaviors used in reciprocal social interaction. Autistic children may show different social communication deficits, depending upon their intellectual and social development (Wetherby *et al.*, 2007). Some children may be unable to speak, whereas others may have rich vocabularies and be able to talk about specific subjects in great detail. The majority of ASD patients, however, have difficulties in using language effectively, especially when talking to other people or understanding what others say to them. They also may have difficulty in nonverbal communication, such as through hand gestures, eye contact, and facial expressions, and may be unable to understand body language.

One of the most widely used means to study social communication in rodents is the analysis of ultrasonic vocalization (USV) emission in social settings. Like many other vertebrates, rodents emit USVs in different social situations across their entire lifespan: for instance, to communicate distress due to separation from mother and siblings or following a predator attack, to communicate pleasure in the anticipation of and during mating or playful interactions, or to communicate food location to other group members (Cuomo *et al.*, 1988; D'Amato, 1991; Panksepp and Burgdorf, 2000; Panksepp *et al.*, 2007; Portfors, 2007; Scattoni *et al.*, 2009, 2011; Lahvis *et al.*, 2011). The transmission of different types of information through USVs depends on the specific frequency and temporal properties of the acoustic signals (Portfors, 2007): in rats, low-frequency (around 22 kHz) USVs have been associated with negative social experiences (e.g. exposure to predator odor, intermale fighting), whereas high-frequency (around 50 kHz) USVs have been detected in social contexts involving potential reward (e.g. sexual approach, play fighting; Burgdorf *et al.*, 2011). Although rodent USVs are generally inaudible to humans, they can be studied in laboratory settings using specific equipment.

As communicative deficits in ASD appear as early as in infancy, a very useful tool in rodent models of ASD is the analysis of the USVs emitted by rodent pups in response to separation from the mother and the nest. When separated from their mother and siblings, rats and mice emit USVs with frequencies between 30 and 90 kHz (Zippelius and Schleidt, 1956). These USVs play an essential communicative role in mother–offspring interaction and are crucial for pup survival (Hofer and Shair, 1991). They are, indeed, a potent stimulus for maternal retrieval and elicit care-giving behaviors in the dam (Insel *et al.*, 1986; Cuomo *et al.*, 1987; Branchi *et al.*, 2001; Scattoni *et al.*, 2009; Trezza *et al.*, 2011). The idea that USV emission in pups serves a communicative function originated from early findings showing that dams selectively retrieve vocalizing pups, but not anesthetized or killed pups (Zippelius and Schleidt, 1956). Since this first study, many experiments have confirmed that maternal retrieval of pups can be induced by solely presenting pup USVs (for a review see Ehret, 2005). This early vocalization response is observed in most mammalian species, including humans, when infants separated from their familiar surroundings cry to elicit maternal care. As rodent pups vocalize immediately after birth, isolation-induced USVs can be detected from the first days of life, when other behaviors are still immature (Portfors, 2007). This has translational relevance, as the first communicative deficits found in autistic patients can be observed during the first months of life (Dawson and Bernier, 2013).

The most widely used and simplest protocol for the study of isolation-induced USVs in infant rodents is to separate the pup from its mother and littermates and place it alone in a soundproof arena for a few minutes. The USVs emitted by the pup are detected by an ultrasonic microphone fixed above the arena, connected to an ultrasound detector. Although USVs can be counted manually by the experimenter by listening to the audible output through earphones, nowadays specific software is available to analyze each spectrogram for the number and shape of the USVs emitted. As low temperatures also elicit USV emission, the testing room is maintained around 25°C and the body temperature of each pup is controlled before and after the test.

Several genetic and environmental rodent models of ASD (described in more detail below) show changes in pup isolation-induced USV emission (Jamain *et al.*, 2008; Scattoni *et al.*, 2008; Gandal *et al.*, 2010; Malkova *et al.*, 2012). These changes include either increases or decreases in call rate or in the unusual patterns of vocalizations. For instance, BTBR mouse pups separated from their mothers and siblings at postnatal days 2–12 emitted significantly more calls of longer duration than control mice; from a qualitative point of view, BTBR mice also showed a narrower USV repertoire than control pups (Scattoni *et al.*, 2008). Conversely, lower rates of USVs were emitted by mouse pups lacking the neuroligin-4 gene (Jamain *et al.*, 2008) and by rat pups prenatally exposed to either VPA (Gandal *et al.*, 2010) or maternal infection (Malkova *et al.*, 2012). It has been suggested that USV emission in rodent pups may be related to human infant crying (Elsner *et al.*, 1990) and that any particular change in the acoustic features of the neonatal cry may be an indicator of long-term

neurobehavioral alterations caused by adverse prenatal and postnatal events (Lester 1987). In this perspective, the findings showing either increases or decreases in call rate and unusual patterns of vocalizations in preclinical models of ASD are reminiscent of the altered and heterogeneous vocalization profile displayed by autistic infants, with some autistic infants crying for long periods of time (Johnson, 2008), whereas others producing lower rates of sounds (Paul *et al.*, 2011).

Limitations of the analysis of pup isolation-induced USVs are that these calls may also depend on the propensity of the pup to show anxiety-like behaviors, and that anxiolytic drugs reduce, whereas anxiogenic compounds increase the call rate. However, as the pups get older, USV emission can be detected in other social contexts, such as during juvenile social play behavior, during adult social interaction, or in response to female odors (for reviews, see Wöhr and Schwarting, 2013 and Portfors, 2007). Therefore, measuring USV emission in these social contexts at older ages may provide further useful information about the altered social behavior and communication in rodent models of ASD.

Social play and sociability

One of the most characteristic social behaviors that is altered in ASD is social play behavior (Jordan, 2003; Young *et al.*, 2003). Social play behavior is the first form of nonmother-directed social behavior displayed by most developing mammals. The experience of social play is crucial for neural growth and proper development, both in children and in young mammals: it helps to develop communicative skills, to acquire cognitive and social competence, and it contributes to the development of behavioral and mental flexibility (Vanderschuren and Trezza, 2014). Social play has a key role in the identification and diagnosis of ASD. In ASD, play patterns are characterized not only by deficient cognitive complexity, but also by a typical asocial dimension. Children with ASD are able to engage in certain forms of play. However, these are passive, stereotyped, and rigid, with no adjustment of play patterns to involve others and with fewer opportunities to join and share play scenarios. As a result, the play of children with ASD is less likely to engage the interest of other children. The lack of socialized patterns of play and the failure to engage in play with peers lead, in turn, to further social isolation (Jordan, 2003). As the opportunity to engage in social play is crucial for the acquisition of proper social and cognitive skills, the lack of social play in children with ASD likely has deleterious effects on their development, leading to long-lasting deficits in self-awareness, social competence, problem-solving, and behavioral flexibility. It has been shown that, similar to that in autistic children, social play is impaired in rat models of ASD (Schneider and Przewlocki, 2005; Chomiak *et al.*, 2010).

Social play behavior can be easily assessed in laboratory animals. In particular, the laboratory rat is an ideal species to study this behavior (Vanderschuren and Trezza, 2014). Whereas other muroid species, like mice, often engage in solitary or rudimentary forms of play, social play is the most commonly occurring form of play displayed by rats. Social play in rats peaks between weaning and sexual maturation: in this time window, adolescent rats spend a significant amount of time and energy engaging in social play with peers. The social play of young rats can be easily and objectively quantified by measuring the frequency and duration of specific behaviors (Trezza *et al.*, 2010). A bout of social play behavior in adolescent rats typically starts with one rat soliciting ('pouncing' on) another animal, by attempting to nose or rub the nape of its neck. The animal that is pounced upon can respond in different ways: if the animal fully rotates to its dorsal surface, 'pinning' is the result – that is, one animal lying with its dorsal surface on the floor with the other animal standing over it. From this position, the supine animal can easily initiate another play bout, by trying to gain access to the other animal's neck. If the animal that is pounced upon responds by evading, the soliciting rat may start to chase it, thus making another attempt to launch a play bout (Panksepp and Beatty, 1980; Pellis and Pellis, 1987; Trezza *et al.*, 2010). Pinning, pouncing, and chasing are the behavioral acts occurring during social play that can easily be recognized and quantified. Furthermore, analysis of these behaviors both per pair of animals and separately for each individual animal of a test pair makes it possible to determine play responsiveness – that is, the probability of an animal rotating to the supine position (resulting in it being pinned) in response to play solicitation (pouncing) by the test partner. Other components of the social repertoire of adolescent rats, such as social investigation and social grooming, are considered social behaviors not primarily related to play, and can also be easily assessed. It is also possible to correlate the performance of social behaviors, both related and unrelated to play, with the emission of USVs. Indeed, it has been reported that rats emit more 50 kHz USVs while playing together than while alone (Knutson *et al.* 1998), although other studies have failed to correlate the display of social play behavior in adolescent rats with the emission of 50 kHz USVs (Willey and Spear, 2012; Manduca *et al.*, 2014a, 2014b).

As the social play behavior of young mice involves only a small subset of the complex playful activities displayed by rats (Pellis and Pasztor, 1999; Whishaw *et al.*, 2001), other behavioral tests are usually used to assess sociability in mouse models of ASD. A commonly used behavioral paradigm is the three-chamber test, which provides a simple measure of general sociability (Nadler *et al.*, 2004). The apparatus usually consists of a Plexiglas three-chamber box with openings between the lateral chambers and the central compartment. The two lateral

chambers are identical, except for the fact that, during the testing session, one chamber contains a stimulus animal (same age and sex but unfamiliar to the experimental subject) inside a cage, whereas the other chamber contains an identical but empty cage. After habituation to the empty apparatus, the experimental subject is placed in the central compartment and can choose to spend time either in the chamber that contains the cage containing the unfamiliar stimulus animal, or in the chamber that contains the empty cage. The time spent sniffing the stimulus animal or the empty cage, the time spent in each chamber, and the number of entries into each chamber are measured. A second session may follow, in which a new stimulus animal is placed into the cage that had been empty during the first session, thus allowing the experimental animal to choose to spend time either in the chamber containing the same stimulus animal encountered in session 1, or in the chamber containing the new stimulus (Kaidanovich-Beilin *et al.*, 2011). During the first session, 'sociability' is defined as propensity to spend time in the chamber containing the stimulus animal, as compared with time spent alone in the identical but empty opposite chamber. During the second session, 'preference for social novelty' is defined as propensity to spend time with a new stimulus rather than with the same stimulus encountered in the first session (Moy *et al.*, 2004). Usually, rodents spend more time with a peer rather than alone, and with an unfamiliar rather than a familiar animal. Conversely, rodents showing social dysfunctions tend to make fewer entries into and spend less time in the chamber with the stimulus compared with control animals in the first session, and they spend less time with the novel compared with the familiar stimulus during the second session (Moy *et al.*, 2004, 2013; Yang *et al.*, 2007; McFarlane *et al.*, 2008; Silverman *et al.*, 2010; Kerr *et al.*, 2013; Baronio *et al.*, 2015).

In adult rodents, reciprocal social interactions can also be studied by assessing the behaviors of pairs of animals placed together in a neutral arena and optimizing the experimental conditions according to the specific aims of the experiment (File, 1980). Altered social interaction has been observed in several rodent models of ASD (Goorden *et al.*, 2007; Jamain *et al.*, 2008; Markram *et al.*, 2008; McFarlane *et al.*, 2008; Kaidanovich-Beilin *et al.*, 2011; Banerjee *et al.*, 2014).

Diagnostic and Statistical Manual of Mental Disorders, 5th edition criterion B: repetitive and stereotyped behaviors

The second domain of predominant symptoms displayed by autistic patients includes stereotypies and repetitive behaviors. These behaviors are highly heterogeneous in presentation and are characterized by repetition, rigidity, and invariance, as well as a tendency to be inappropriate in nature (Turner, 1999). Common examples include hand flapping, body rocking, toe walking, and obsession with the alignment of objects (Cunningham and Schreibman, 2008).

As in humans, rodents also show, under certain circumstances, particular behaviors that can be cataloged as stereotyped behaviors. Common motor stereotypies displayed by rodents are high levels of vertical jumping, circling, digging, rearing, and excessive self-grooming (Hart *et al.*, 2009; Patterson, 2009). To assess stereotypies, the animal is typically placed in a Plexiglas arena and, after a period of habituation, the time and frequency of stereotyped behaviors are scored (McFarlane *et al.*, 2008).

The marble burying test is commonly used to detect repetitive digging behavior. This test is based on defensive burying, which is part of a repertoire of defensive reactions that are naturally displayed by rodents (De Boer and Koolhaas, 2003). In this test, the animal is placed in a Plexiglas cage containing clean bedding and some marbles spaced evenly on the surface of the bedding. After 10–20 min, the animal is removed from the cage and the number of marbles buried with bedding up to two-thirds of their depth is counted (Deacon, 2006). Marble burying behavior is highly repetitive and perseverative and therefore is a very useful tool to quantitatively evaluate repetitive/perseverative responses in rodents. The occurrence of perseverative behaviors can also be assessed in T-maze and water maze reversal learning tasks, which enable analysis of the inability of rats and mice to change a learned pattern of behavior when the testing conditions demand it. The T-maze is a choice task that has been conceived on the basis of the innate tendency of animals to explore their environment and obtain natural rewards, like food, in one of two available locations at opposite ends of a T-shaped apparatus. The water maze task is a test of spatial learning for rodents that involves locating a submerged escape platform in one quadrant of a circular swimming pool. In these tasks, the difficulty in learning a new location of either food in the T-maze or the hidden platform in the water maze after the initial learning of a first location can be used as a measure of perseverative behavior, which may be reminiscent of the rigid patterns of behaviors displayed by autistic patients.

Several rodent models of ASD show stereotypies and repetitive behaviors, such as self-grooming and marble burying, as well as perseverative behaviors (Schneider and Przewlocki, 2005; Schneider *et al.*, 2006; McFarlane *et al.*, 2008; Mehta *et al.*, 2011Spencer *et al.*, 2011; Sungur *et al.*, 2014Baronio *et al.*, 2015).

Associated symptoms

The most frequent associated symptoms displayed by autistic patients include anxiety, epileptic seizures and cognitive impairments (American Psychiatric Association, 2013). Several established behavioral tests are available to assess these symptoms in rodents. The elevated plus

maze test is probably the most widely used test to detect an anxious phenotype in rats and mice. This test is based on an innate conflict in rodents: the natural fear for open spaces and the innate interest in the exploration of new environments (Pellow and File, 1986). The open-field test, in which the behavior of the animal in a large empty arena is scored for a variable period of time, is often used to assess, at the same time, the occurrence of anxiety-like and stereotyped behaviors.

Various aspects of learning and memory can be evaluated in rodents using behavioral tests designed to assess specific cognitive competencies, such as associative learning, nonspatial or spatial learning, short-term and long-term memory, attention, and emotional memory. A useful experimental tool to evaluate cognitive processing at a very early developmental age is the homing behavior test, which exploits the strong tendency of immature rodent pups to maintain body contact with the dam and siblings by discriminating the nest odor from a neutral odor. The experimental procedure for the homing test includes brief isolation of the pup before testing. Next, the pup is placed for a few minutes in a cage with two parts of the floor covered with clean sawdust and one part with sawdust from its own nest. The latency to reach the familiar bedding and the total time spent by the pup in the familiar bedding are scored. An increased susceptibility to audiogenic or chemically induced seizures can also be easily assessed in both rats and mice (Kandratavicius *et al.*, 2014).

Rodent models based on genetic factors

The BTBR mouse strain

The BTBR T+ tf/J mouse strain is an inbred mouse strain that displays behavioral deficits with face validity to the core symptoms observed in ASD patients. First, BTBR mice show several communication deficits: when separated from their mother and siblings, BTBR mouse pups show an unusual pattern of USVs and vocalize more and longer compared with C57BL/6J pups (Scattoni *et al.*, 2008). This behavioral profile may be reminiscent of the atypical vocalizations and more intense and longer crying seen in some autistic infants (Johnson, 2008). Interestingly, a recent study showed that BTBR mouse pups display a calming response and emit fewer isolation-induced USVs when tested under soiled-bedding conditions with home cage bedding material, similar to control pups. However, unlike that in control animals, the presence of odors from mothers and littermates has no effect on the altered acoustic call features displayed by BTBR mouse pups. This indicates that BTBR mice are able to detect environmental changes, but have a limited ability to adjust to them (Wöhr, 2015). The communicative and social deficits displayed by BTBR animals persist through development. When juvenile, BTBR mice show low levels of home cage social behaviors (Babineau *et al.*, 2013), impaired social behavior (McFarlane *et al.*, 2008), the absence of social conditioned place preference (Pearson *et al.*, 2012), and reduced social investigation, associated with a decrease in USV emission (Scattoni *et al.*, 2013). At adulthood, they show reduced social approach (McFarlane *et al.*, 2008), impaired social transmission of food preference (McFarlane *et al.*, 2008), altered sociability (Jasien *et al.*, 2014), lower scent marking, and minimal USV responses to female urine (Wöhr *et al.*, 2011a). It has recently been shown that, compared with C57BL/6J animals, adult BTBR mice display reduced social motivation in a social motivation operant task. These deficits in motivation, however, seem to be not specific to social reward, as BTBR mice also make significantly fewer lever presses for a food reward (Martin *et al.*, 2014). Furthermore, both juvenile and adult BTBR mice display high levels of self-grooming behavior (McFarlane *et al.*, 2008; Brodkin *et al.*, 2014) and protracted, elevated levels of perseverative behavior, manifested as persistence in interacting with a wheel even when it is jammed (Karvat and Kimchi, 2012). The behavioral deficits displayed by BTBR mice seem to be specific to the core ASD domains, as olfactory abilities and locomotion are unaffected (McFarlane *et al.*, 2008; Jasien *et al.*, 2014). BTBR mice also show some ASD-associated symptoms, such as cognitive impairments in the water maze reversal task (Silverman *et al.*, 2010; Guariglia and Chadman, 2013) but not in the novel object discrimination task (Jasien *et al.*, 2014), and deficits in contextual and cued fear conditioning (Stapley *et al.*, 2013). Furthermore, when tested in a computer-automated touch-screen apparatus, BTBR mice perform normally on a visual discrimination reversal task in which rule switching is relatively automatic, but are severely impaired in a task-switch paradigm that requires the active use of contextual information to switch between rules in a flexible manner (Rutz and Rothblat, 2012). Conversely, BTBR mice do not show marked anxiety-like behaviors when exposed to a novel environment or relevant changes in epileptogenic activity (Pobbe *et al.*, 2011; Chadman and Guariglia, 2012; Ruskin *et al.*, 2013).

Despite the fact that the genetic alterations underlying the behavioral deficits displayed by this inbred mouse strain are still unclear, a polymorphism in the *KMO* gene has been reported (McFarlane *et al.*, 2008). This gene encodes kynurenine 3-hydroxylase, an enzyme that regulates the synthesis of kynurenic acid, a glutamate and nicotinic receptor antagonist that may play a role in neuroprotection, dendritic spine formation, and dopamine release (Hilmas *et al.*, 2001; Alkondon *et al.*, 2004; Yu *et al.*, 2004; Sapko *et al.*, 2006; Wu *et al.*, 2007), the levels of which are abnormal in several neuropsychiatric diseases (Schwarcz *et al.*, 2001; Sapko *et al.*, 2006). From an anatomical point of view, BTBR mice show some specific neuroanatomical features, such as the absence of the corpus callosum and a reduced hippocampal commissure (Wahlsten *et al.*, 2003; Yang *et al.*, 2009).

Concerning the predictive validity of this rodent model of ASD, certain pharmacological manipulations can correct some behavioral abnormalities displayed by BTBR mice. For instance, it has been shown that mGluR5 antagonism can decrease repetitive behaviors, improve learning and memory in the Morris water maze task, correct long-term object location deficits, and increase social responses of BTBR mice (Silverman *et al.*, 2012; Seese *et al.*, 2014; Yang *et al.*, 2015). Furthermore, it has recently been shown that the selective $GABA_B$ receptor agonist R-baclofen reverses social approach deficits, repetitive self-grooming, and high marble burying scores of BTBR mice (Silverman *et al.*, 2015). In addition, treatment with low, nonsedating/nonanxiolytic doses of benzodiazepines can improve the deficits in social interaction, repetitive behavior, and spatial learning displayed by BTBR mice, through positive allosteric modulation at the α2,3-subunits of the $GABA_A$ receptor (Han *et al.*, 2014). Risperidone, an atypical antipsychotic, approved 'off-label' to treat irritability in ASD, can improve reversal learning in BTBR mice (Amodeo *et al.*, 2014). Interestingly, dietary and behavioral interventions can also ameliorate certain behavioral abnormalities displayed by BTBR mice. A recent study has shown that tryptophan supplementation can improve sociability, but not repetitive self-grooming of BTBR mice, indicating specificity of this supplementation to the social domain (Zhang *et al.*, 2015). It has also been shown that perinatal choline supplementation or a ketogenic diet can correct both the increased repetitive behavior and the impaired social interactions displayed by BTBR mice (Ruskin *et al.*, 2013; Langley *et al.*, 2015). Concerning behavioral manipulations, social peer enrichment has been proven to ameliorate social interactions in BTBR mice reared as juveniles with the highly social C57BL/6J mice (Yang *et al.*, 2011), whereas environmental enrichment is effective in decreasing the repetitive behaviors displayed by BTBR mice (Reynolds *et al.*, 2013).

Mutation in the fragile X mental retardation 1 (*FMR1*) gene

Fragile X syndrome (FXS) is a leading inherited cause of intellectual disability and the most common monogenic cause of autism, with ~30% of boys with FXS meeting the full diagnostic criteria for ASD (although only a small percentage of autistic children have FXS; O'Donnell and Warren, 2002). At the behavioral level, the most common symptoms displayed by FXS patients are impaired cognition, social phobia, altered social behaviors, anxiety, hyperactivity, and repetitive behaviors (Bardoni *et al.*, 2006; Tranfaglia, 2011). At the morphological level, FXS patients may show facial dysmorphology and macroorchidism (Zarnescu *et al.*, 2005; McLennan *et al.*, 2011).

FXS is caused by expansion of an unstable CGG repeat in the *FMR1* gene, triggering hypermethylation and partial or complete gene silencing that results in partial or complete lack of the gene product, the fragile X mental retardation protein (FMRP; Verkerk *et al.*, 1991; O'Donnell and Warren, 2002). FMRP is an mRNA-binding protein that is ubiquitously expressed in the cytoplasm of neurons throughout postnatal life (O'Donnell and Warren, 2002; Till *et al.*, 2012), which plays a crucial role in brain development, synaptogenesis, and synaptic pruning (Verkerk *et al.*, 1991; Greenough *et al.*, 2001). Loss of FMRP has been related to uncontrolled activity of group I metabotropic glutamate receptors (mainly mGlu5; Bear *et al.*, 2004), reduced GABAergic transmission (D'Hulst and Kooy, 2007), and dysregulation of the mammalian target of the rapamycin (mTOR) signaling cascade (Sharma *et al.*, 2010), all of which appear to play an important role in the pathology of FXS.

The most widely used model of FXS in preclinical research is the *FMR1*-deficient [*fmr1* knockout (KO)] mouse model, which reproduces many of the symptoms of FXS in humans. *Fmr1* KO mice show quantitative and qualitative differences in pup isolation-induced USV emission, marked susceptibility to audiogenic seizures, social anxiety, reduced social interaction, hyperactivity, repetitive behaviors, and cognitive deficits, such as impairments in spatial and reversal learning (Mineur *et al.*, 2002; Spencer *et al.*, 2005, 2011; Mineur *et al.*, 2006; Connor *et al.*, 2011; Lai *et al.*, 2014a, 2014b). At the neurochemical level, *fmr1* KO mice show uncontrolled activity of metabotropic glutamate receptors, such as altered mGluR5-dependent endocannabinoid activity (Maccarrone *et al.*, 2010) and endocannabinoid-mediated long-term depression at excitatory synapses of the forebrain (Bear *et al.*, 2004; Jung *et al.*, 2012; Busquets-Garcia *et al.*, 2013). Furthermore, they show elevated cortical spine densities, similar to those observed in ASD and FXS (Grossman *et al.*, 2010).

In line with the idea that mGluR5-dependent dysregulation of mRNA translation at synapses is a core pathogenetic mechanism in FXS, pharmacological antagonism or genetic reduction of mGluR5 has been shown to rescue several features of the FXS phenotype in this animal model (Michalon *et al.*, 2012; Michalon *et al.*, 2014). However, clinical trials with mGluR5 antagonists have recently been discontinued because of a lack of significant improvement in abnormal behaviors compared with placebo in both adults and adolescents (Braat and Kooy, 2014; De Esch *et al.*, 2014). Alternative pharmacological targets currently under investigation include the serotonergic and endocannabinoid systems. It has been shown that drugs targeting 5-HT7 serotonin and cannabinoid receptors can correct certain behavioral and neurochemical alterations displayed by *fmr1* KO mice (Costa *et al.*, 2012; Jung *et al.*, 2012; Busquets-Garcia *et al.*, 2013).

Mutations in neuroligin-3 (*NLGN*-3) and neuroligin-4 (*NLGN*-4) genes

Neuroligins (NLGNs) are postsynaptic neuronal cell adhesion molecules that are essential for proper maturation and function of both excitatory glutamatergic and inhibitory GABAergic synapses in the mammalian brain, acting in concert with their presynaptic and intracellular binding partners, the β-neurexins and SHANK3, respectively (Graf *et al.*, 2004; Prange *et al.*, 2004). Several mutations in neuroligin-3 (*NLGN*-3) and neuroligin-4 (*NLGN-4*) genes have been associated with ASD and mental retardation (Jamain *et al.*, 2003; Laumonnier *et al.*, 2004). In particular, one missense mutation each in *NLGN*-3 and *NLGN-4* and two nonsense mutations in *NLGN*-4 have been identified in autistic patients (Betancur *et al.*, 2009). The mutations found in these genes seem to alter the ability of NLGNs to guide maturation of synapses during the development of neural circuits and to shift the balance between glutamatergic and GABAergic synapses (Chih *et al.*, 2004; Chubykin *et al.*, 2007). Three different nonsense mutations in *SHANK*3, the intracellular binding partner for NLGNs, have also been found in patients with ASD (Durand *et al.*, 2007).

Preclinical studies with *Nlgn-3* and *Nlgn-4*-mutant mice support a role of NLGNs in synaptic function and social behavior, although the available findings are sometimes controversial. In particular, Radyushkin *et al.* (2009) showed that, although *Nlgn-3* KO male mice do not show deficits in the social interaction test, they show reduced USV emission upon contact with a female in estrous and altered social novelty preference in the three-chamber test, potentially due to an olfactory deficiency found in these animals. *Nlgn-3* KO mice also show a decrease in the freezing response in the contextual fear conditioning task, but normal behavior in the Morris water maze, and no prepulse inhibition, seizure propensity, and sucrose preference (Radyushkin *et al.*, 2009).

Tabuchi *et al.* (2007) reported that *Nlgn-3* knockin (KI) mice, which have a point mutation in the endogenous mouse gene that is identical to the mutation observed in a subset of ASD patients, display increased inhibitory synaptic transmission associated with impaired social behavior and enhanced spatial learning abilities. In contrast, a study by Chadman *et al.* (2008) did not find relevant autistic-like behaviors in these mice, except for a deficit in communication, identified by a reduction in USV emission when the pups were isolated from the nest on postnatal day 8. Controversial results have also been obtained with *Nlgn-4* KO mice. Jamain *et al.* (2008) reported that *Nlgn-4* KO mice display reduced social interactions and USV emission at adulthood upon contact with a female, although they show normal sensory ability, locomotor and exploratory activity, anxiety behavior, learning, and memory. Social interaction and ultrasonic communication deficits have also been found in female *Nlgn-4* KO mice (El-Kordi *et al.*, 2013; Ju *et al.*, 2014). From these studies, it appears that *Nlgn-4* KO mice display two of the three classic core autistic symptoms: impaired social interaction and altered USV communication, but do not show repetitive behavior or some of the associated symptoms, such as increased anxiety or deficits in learning and memory (Jamain *et al.*, 2008; El-Kordi *et al.*, 2013; Ju *et al.*, 2014). These results are consistent with clinical findings in patients carrying the *NLGN-4* mutation, who also do not show these symptoms (Patterson, 2011). Conversely, another study conducted in later generations of the same line of *Nlgn-4* KO mice found no differences between mutant and wild-type mice in pup isolation-induced USV emission or between adults during reciprocal social interactions. Anxiety-like behaviors, self-grooming, rotarod and open-field exploration also did not differ across genotypes (Ey *et al.*, 2012). These discrepant preclinical findings highlight the important roles of environmental, generational, and/or procedural factors in the behavioral characterization of rodent models of ASD.

Mutations in the *SH3* and multiple ankyrin repeat domains (*SHANK*) genes

The *SHANK* genes encode a family of synaptic scaffolding proteins located postsynaptically on excitatory synapses. These proteins exist in three major isoforms (Shank1, Shank2, and Shank3). All of them are particularly expressed in the hippocampus and the cortex, Shank 1 and 2 are almost absent in the striatum, whereas Shank 3 is highly expressed in this region (Sheng and Kim, 2000). Several mutations of the *SHANK* genes have been found in autistic patients (Berkel *et al.*, 2010; Sato *et al.* 2012; Leblond *et al.*, 2014). In particular, rare (0.04%) *SHANK1* deletions have been found in autistic patients with a normal intelligence quotient (Sato *et al.* 2012; Leblond *et al.*, 2014), mutations in *SHANK2* have been reported in ASD patients with mild intellectual disability (Berkel *et al.*, 2010; Leblond *et al.*, 2014), whereas mutations in *SHANK3* have been found in 0.69% of ASD patients, and may be associated with moderate to profound intellectual disability (Leblond *et al.*, 2014).

On this basis, in the recent years, mice mutant for the three *SHANK* genes have been produced. The first *Shank*-mutant mouse model described was the *Shank1*-deficient mouse model (Hung *et al.*, 2008). *Shank1*-KO mice are similar to their wild-type controls on standardized measures of general health, neurological reflexes, and sensory skills, and show no gross abnormalities in the size or histological structure of the brain (Hung *et al.*, 2008; Silverman *et al.*, 2011). However, they show deficits in social communication at different stages of life. In particular, compared with wild-type animals, *Shank1*-KO mouse pups emit fewer USVs when isolated from their mother and siblings (Wöhr *et al.*, 2011b). At adulthood, both *Shank1*-KO and wild-type male mice emit a similar

amount of USVs when exposed to female urine. However, whereas control males change their calling pattern depending on their previous exposure to a female, *Shank1*-KO males are unaffected by prior female experience. In addition, *Shank1*-KO mice show altered scent marking behavior, depositing fewer urine traces in proximity to the female urine spot compared with control mice (Wöhr *et al.*, 2011b). Despite these relevant alterations found in the social communication domain, *Shank1*-KO mice show no changes in reciprocal social interactions, compared with wild-type animals, during both adolescence and adulthood (Silverman *et al.*, 2011). Interestingly, the study by Silverman *et al.* (2011) reported no significant differences between control and *Shank*1-mutant mice in the cumulative time spent self-grooming, although this finding may be related to the unusually high levels of self-grooming behavior observed in control animals in this study. In line with this possibility, a more recent study showed that adult but not juvenile *Shank*1-mutant mice display higher levels of self-grooming behavior, particularly in a social context, with no changes in digging behavior and with reduced marble burying (Sungur *et al.*, 2014). *Shank1*-KO mice also display hypoactivity and motor coordination deficits in the open-field and rotarod tests, together with increased anxiety-related behaviors, impaired contextual fear memory, and enhanced spatial learning, but impaired long-term retention of spatial memory (Hung *et al.*, 2008; Silverman *et al.*, 2011). Neurochemically, *Shank1*-KO mice show altered postsynaptic density protein composition and smaller hippocampal dendritic spines and synapses, despite normal hippocampal long-term potentiation, long-term depression, and late-phase long-term potentiation (Hung *et al.*, 2008).

Concerning rodent models of *Shank2* mutations, two main mouse models have been produced, in which either exon 7 (*Shank2*^{e7}-mutant mice) or both exons 6 and 7 (*Shank2*$^{e6-7}$-mutant mice) were targeted for deletions (Schmeisser *et al.*, 2012; Won *et al.*, 2012). These two mouse models show similar core autistic-like features: impaired social interaction in the three-chamber apparatus, altered USV emission in both infancy and adulthood, and increased self-grooming and repetitive jumping (Schmeisser *et al.*, 2012; Won *et al.*, 2012; Ey *et al.*, 2013; Yoo *et al.*, 2014). As for the ASD-associated symptoms, both types of *Shank2*-mutant mice exhibit increased anxiety-like behavior, whereas only *Shank2*$^{e6-7}$-mutants show deficits in working memory, compared with wild-type mice, when tested in the Morris water maze task (Won *et al.*, 2012). Despite the similar behavioral phenotypes, certain neuroanatomical features differ between *Shank2*^{e7}-mutant and *Shank2*$^{e6-7}$-mutant animals. Unlike *Shank2*$^{e6-7}$-mutant mice, *Shank2*^{e7}-mutant mice show a small reduction in spine numbers at CA1 dendritic spines (Schmeisser and Boeckers, 2012). Furthermore, *Shank2*^{e7}-mutant mice show an increase, and *Shank2*$^{e6-7}$-mutant mice a decrease, in NMDA receptor-mediated synaptic function (Schmeisser and Boeckers, 2012).

Different lines of *Shank3*-mutant mice have been generated, with specific mutations involving exons 4–9 (Bozdagi *et al.*, 2010; Wang *et al.*, 2011; Yang *et al.*, 2012), 4–7 (Peça *et al.*, 2011), or 13–16 (Peça *et al.*, 2011). Compared with wild-type animals, *Shank*3-mutant mice show abnormal social behavior (Bozdagi *et al.*, 2010; Peça *et al.*, 2011; Wang *et al.*, 2011) and altered USV emission during social interactions (Bozdagi *et al.*, 2010; Wang *et al.*, 2011), together with stereotyped and repetitive behaviors (Peça *et al.*, 2011; Wang *et al.*, 2011). It should be noted, however, that a strong autistic-like phenotype is not always observed in all the *Shank3*-mutant models that have been generated. For instance, the study by Yang *et al.* (2012) on *Shank*3-mutant mice with mutations involving exons 4–9 found only mild social impairments in juveniles during reciprocal interactions, normal adult sociability in the three-chamber task, and reduced USVs only in some cohorts of animals. These findings show that the severity of autistic-like symptoms displayed by *Shank*3-mutant mice can be variable, and may depend on the specific targeted domain within the *Shank3* gene sequence. *Shank3*-mutant mice also show some cognitive deficits, such as impairments in reversal learning, object recognition memory, and social transmission of food preference (Wang *et al.*, 2011; Yang *et al.*, 2012). These behavioral changes are associated with several neurochemical alterations, such as reduced corticostriatal excitatory transmission, longer dendritic spines, and a lower density of dendritic spines, as compared with wild-type controls (Bozdagi *et al.*, 2010; Peça *et al.*, 2011; Yang *et al.*, 2012).

Mutations in the tuberous sclerosis (*TSC*) 1 and 2 genes

Tuberous sclerosis (TSC) is a genetic disease with high comorbidity with ASD: approximately half of the patients with TSC meet the criteria for ASD (Bolton *et al.*, 2002; Lewis *et al.*, 2004) and 1–4% of ASD cases are attributable to TSC (Caglayan, 2010). TSC is an autosomal dominant neurocutaneous disorder caused by heterozygous mutations in the *TSC1* or *TSC2* genes. These two genes encode for two proteins, TSC1 and TSC2, which dimerize, forming a complex that negatively regulates the mTOR pathway. mTOR is a serine/threonine kinase that regulates the synthesis and translation of proteins involved in cell growth and proliferation, including neuronal proteins that play key roles in synaptic plasticity (Jaworski and Sheng, 2006). Thus, mutations in either *TSC1* or *TSC2* result in uncontrolled cellular proliferation.

At the morphological level, TSC is characterized by growth of benign tumors in the brain and other vital organs (Roach *et al.*, 1998). The brain abnormalities include subependymal nodules, subependymal giant cell astrocytomas, and cortical tubers (DiMario, 2004). At the behavioral level, several features displayed by TSC

patients fall into the autistic domain, such as impairments in communication, stereotypies, and repetitive language patterns (Levy *et al.*, 2009). Other symptoms may include seizures, intellectual disability, and developmental delay (Roach *et al.*, 1998). MRI and PET studies correlated cerebellar pathology in TSC patients with increased ASD symptomatology (Eluvathingal *et al.*, 2006). Accordingly, animal models of TSC show several autistic-like symptoms. In particular, $Tsc2^{+/-}$ mouse pups show compromised communication with the mothers, indicated by altered emission of isolation-induced USVs (Young *et al.*, 2010). Furthermore, they show impaired water maze learning and aberrations in synaptic plasticity associated with long-term potentiation in hippocampal slices (Ehninger *et al.*, 2008), together with abnormal axon guidance in the visual system (Nie *et al.*, 2010). However, these mice show normal motor skills, anxiety, exploratory activity, and social approach behavior (Ehninger *et al.*, 2008). Impaired learning and memory associated with reduced social approach and nesting behavior have been found in $Tsc1^{+/-}$ mice (Goorden *et al.*, 2007). Astrocyte-specific conditional Tsc1 KO mice show significant increases in astrocyte numbers throughout the brain by 3 weeks of age and abnormal neuronal organization in the hippocampus between 3 and 5 weeks, associated with epileptic seizures (Uhlmann *et al.*, 2002). Tsai *et al.* (2012) found that both heterozygous and homozygous loss of *TSC1* in cerebellar Purkinje cells induce autistic-like behaviors in mice, including abnormal social interaction, repetitive behavior, and altered USVs, in addition to decreased excitability of Purkinje cells. Interestingly, both the behavioral and neural alterations displayed by these mice were prevented by treatment with the mTOR inhibitor rapamycin (Tsai *et al.*, 2012).

Rodent models of autism spectrum disorders based on environmental factors

Most of the rodent models of ASD based on environmental factors are supported by strong epidemiological data (Newschaffer *et al.*, 2007; Gardener *et al.*, 2009; Veiby *et al.*, 2013). Indeed, in addition to the role of genetic factors in ASD (Kim *et al.*, 2014), there is extensive literature to show correlations between non-heritable factors and the disease. In particular, several environmental factors have been correlated with ASD (Arndt *et al.*, 2005), such as maternal infection (Chess, 1971) or maternal exposure to ethanol (Nanson, 1992), thalidomide (Stromland *et al.*, 1994), VPA (Moore *et al.*, 2000), and misoprostol (Bandim *et al.*, 2003). These findings support the multifactorial theory of the etiology of ASD: according to this theory, a crosstalk between genetic susceptibility and exposure to environmental factors is at the basis of the disease (Kim *et al.*, 2014; Tordjman *et al.*, 2014). Several studies have suggested that most of the major anatomical anomalies observed in autistic patients, including cerebellar and brainstem impairments, together with atypical neuropeptide profiles at birth, are induced by neuropathological processes *in utero* (Newschaffer *et al.*, 2003; Copp and Greene, 2010). It is generally assumed that the effects of environmental factors on offspring development are strongly related to the gestational stages at which the exposure occurs, with the first trimester of pregnancy being the most susceptible period (Copp and Greene, 2010). In particular, several birth defects and central nervous system abnormalities in humans are related to insults that occur around the time of formation of the neural tube, which may result not only in neural tube defects, but also in neuropsychiatric disorders, including ASD (Rodier *et al.*, 1996; Arndt *et al.*, 2005). The neural tube is the embryo's precursor of the central nervous system, the formation of which starts on the 17th day of gestation and ends after 25–26 days, whereas the closure of the vertebral arches is completed at 11 weeks of gestation (Copp and Greene, 2010).

The most common rodent models of ASD based on environmental factors that are known to induce the disease in humans use prenatal exposure to either pharmaceutical agents or infections at the time of neural tube closure. Below, we discuss two models that are widely used.

Prenatal exposure to valproic acid

VPA is an anticonvulsant and mood-stabilizing drug, primarily prescribed to treat epilepsy and bipolar disorder (Johannessen and Johannessen, 2003). Despite the high prescription rates and its clinical efficacy, the drug has well-known teratogenic effects. Indeed, its use during pregnancy is related to the onset of several minor and major malformations in the offspring. In particular, a higher incidence of neural tube defects, cleft lip and palate, cardiovascular abnormalities, genitourinary defects, endocrinological disorders, limb defects, developmental delay, and ASD has been reported in children prenatally exposed to VPA (Kozma, 2001; Jentink *et al.*, 2010). The widespread occurrence of physical malformations induced by the drug observed in the 1980s indicated a correlation of these outcomes with the use of VPA during pregnancy (Kozma, 2001). Later, an association between VPA exposure during pregnancy and behavioral alterations in the offspring was hypothesized when Christianson *et al.* (1994) reported a study involving four children exposed to VPA *in utero*, among whom three showed developmental delay and one showed autism. Further epidemiological studies confirmed that some behavioral alterations found in children exposed to VPA during pregnancy had several aspects in common with the symptoms displayed by autistic patients (Williams and Hersh, 1997; Williams *et al.*, 2001). In particular, the behavioral phenotype observed in these children was characterized by impaired communication, reduced sociability, and stereotyped and repetitive behaviors and

interests, providing evidence that prenatal VPA exposure is a risk factor for ASD.

On the basis of these epidemiological findings, prenatal exposure to VPA in rodents has been validated and standardized in many laboratories and, at present, is widely used in preclinical research on ASD. One of the first studies that demonstrated that prenatal VPA exposure reproduced some of the symptoms of ASD in laboratory animals was performed by Rodier *et al.* (1996). On the basis of clinical studies showing that thalidomide exposure, probably on days 20–24 after conception, induced lesions of the motor cranial nerve nuclei and autistic-like symptoms in children, they hypothesized that the disease originates from an injury at the time of closure of the neural tube, which occurs around those gestational days (Stromland *et al.*, 1994). To test this possibility, they predicted that rats exposed to such a teratogen during motor neuron production would show brain abnormalities comparable to those found in the brains of autistic patients – that is, a reduced number of motor neurons while the rest of the central nervous system develops normally enough to produce a brain of grossly normal morphology. As thalidomide does not have the same teratogenic effect in rodents as it does in primates (Schumacher *et al.*, 1968), they exposed rat embryos to VPA, as this anticonvulsant drug was known to induce in rodents many of the same neural abnormalities seen after human exposure (Binkerd *et al.*, 1988; Collins *et al.*, 1991; Ehlers *et al.*, 1992), which are similar to those observed in humans exposed to thalidomide. In rats, the neural tube closes on day 11 of gestation, and within the following day, neuron production for the motor nucleus of trigeminal, the trochlear, abducens, and hypoglossal nuclei is completed. Hence, pregnant rats were exposed to 350 mg/kg of VPA on gestational day 11.5, 12, or 12.5. Postmortem brain analysis revealed that animals exposed to VPA during gestation showed a decrease in the number of motor nuclei, comparable to the damage found in autistic patients, with no overt signs of other physical malformation (Rodier *et al.*, 1996). Altogether, these findings indicate that the abnormalities in at least some cases of ASD include an injury to cranial nerve motor neurons, initiated by an insult to the developing neural tube (Rodier *et al.*, 1996). Since this first study, prenatal exposure to VPA has become the most widely used environmentally triggered model of ASD, in both rats (Rodier *et al.*, 1996; Markram *et al.*, 2008) and mice (Wagner *et al.*, 2006; Gandal *et al.*, 2010). Despite the well-known outcomes of VPA exposure during pregnancy, the underlying mechanism is still unclear. Classically, the mechanism of action of this drug includes an increase in GABAergic activity, a reduction in excitatory neurotransmission, and modification of monoamines, as well as modulation of voltage-gated sodium and calcium channels (Johannessen and Johannessen, 2003). However, these main mechanisms of action do not seem to be correlated to the brain alterations related to ASD in the offspring (Jessberger *et al.*, 2007). Currently, the most widely accepted theory suggests that VPA could act as a histone deacetylase inhibitor at the time of closure of the neural tube (Jessberger *et al.*, 2007; Szyf, 2009). The drug is indeed a nonselective inhibitor of histone deacetylases, enzymes implicated in the regulation of gene transcription and phenotype differentiation (Szyf, 2009). In particular, the modulation of chromatin structure controlled by histone deacetylases is important for the regulation of transcription and differentiation of neural stem cells. Therefore, following VPA exposure, the functions that involve these cells could be compromised.

Concerning the validity of this rodent model of ASD, VPA-exposed animals exhibit most of the behavioral and anatomical abnormalities observed in autistic patients. At the behavioral level, they show impairments in social interaction, repetitive and stereotyped behaviors, increased anxiety, alteration in fear memory, and compromised emission of pup isolation-induced USVs. In particular VPA-exposed animals show impaired social behavior when tested in the social play behavior (Schneider and Przewlocki, 2005; Chomiak *et al.*, 2010), three-chamber (Kim *et al.*, 2011; Kerr *et al.*, 2013; Baronio *et al.*, 2015), resident-intruder (Felix-Ortiz and Febo, 2012), and social interaction tests (Schneider and Przewlocki, 2005; Markram *et al.*, 2008; Felix-Ortiz and Febo, 2012). Furthermore, several studies have shown alterations in the number of USVs emitted by pups prenatally exposed to VPA when isolated from their nests (Schneider and Przewlocki, 2005; Dufour-Rainfray *et al.*, 2010; Gandal *et al.*, 2010). Together with impairments in social behavior and communication, repetitive and stereotyped behaviors have also been found in VPA-exposed animals, such as increased digging behavior and repetitive self-grooming (Mehta *et al.*, 2011; Kim *et al.*, 2014; Baronio *et al.*, 2015). These findings show that this animal model of ASD shows the characteristic symptoms essential for the diagnosis of the disease, together with associated symptoms, such as increased anxiety and cognitive deficits (Schneider and Przewlocki, 2005; Dufour-Rainfray *et al.*, 2010; Kim *et al.*, 2011; Mychasiuk *et al.*, 2012; Roullet *et al.*, 2013).

The behavioral abnormalities displayed by animals exposed to VPA during pregnancy are often accompanied by neural impairments. In particular, the offspring prenatally exposed to VPA show cranial nerve abnormalities (Rodier *et al.*, 1996; Tashiro *et al.*, 2011), a rearrangement of the dendritic morphology in several limbic and cortical regions (Snow *et al.*, 2008; Bringas *et al.*, 2013), hyperreactivity of pyramidal neurons after electrical stimulation and increased synaptic plasticity in the amygdala (Markram *et al.*, 2008), decreased excitability with a reduction in putative synaptic contacts in pyramidal neurons (Rinaldi *et al.*, 2008), and a reduced number of

Purkinje cells in the posterior lobes of the cerebellum (Ingram *et al.*, 2000).

Altogether, these findings suggest that the VPA rodent model of ASD has face and construct validity. As for predictive validity, some studies tested the ability of 'off-label' drugs commonly prescribed to autistic patients to ameliorate the behavioral and brain abnormalities displayed by VPA-exposed animals. For instance, Choi *et al.* (2014) found that the attention deficit hyperactivity disorder medication, atomoxetine, mitigated the hyperlocomotion displayed by VPA-exposed rats, whereas methylphenidate was ineffective. As atomoxetine is a selective noradrenaline transporter inhibitor, whereas methylphenidate inhibits both the noradrenaline and the dopamine transporters, these results suggest that abnormalities in the noradrenaline transporter may underlie the hyperactive phenotype in VPA-exposed animals.

Kim *et al.* (2014) reported that the acetylcholinesterase inhibitor, donepezil, improved sociability and prevented repetitive behavior and hyperactivity in VPA-treated mouse offspring, a result that is in line with the dysregulation of the brain cholinergic system frequently reported in ASD patients (Lam *et al.*, 2006). Another recent study reported that the histamine receptor 3 antagonist, ciproxifan, attenuated the social deficits and stereotypic behaviors displayed by VPA-exposed mice (Baronio *et al.*, 2015).

Interestingly, it has been shown that preweaning and postweaning environmental enrichment reversed almost all the behavioral alterations observed in male rats prenatally exposed to VPA (Schneider *et al.*, 2006). This finding is in line with clinical studies showing that environmental enrichment is effective in ameliorating some of the symptoms displayed by autistic children (Woo and Leon, 2013). Altogether, these findings indicate that the VPA animal model of ASD shows a certain degree of predictive validity, because of the positive effects that some 'off-label' drugs, potential new drug candidates, or environmental manipulations known to ameliorate the disease in humans have in correcting the autistic-like behavioral and brain abnormalities displayed by VPA-exposed animals.

Maternal infections

As for prenatal exposure to VPA, preclinical models of ASD based on maternal infections are supported by clinical findings. Epidemiological studies have indeed demonstrated a correlation between maternal infections during pregnancy and ASD (Patterson, 2009; Atladottir *et al.*, 2010). The exposure to some specific pathogens seems to be a risk factor, such as congenital rubella (Chess, 1971), cytomegalovirus (Yamashita *et al.*, 2003), bacteria (Atladottir *et al.*, 2010), and influenza virus (Deykin and MacMahon, 1979). The effect of viral infections seems to be more prominent in the first trimester of pregnancy, whereas bacterial infections seem to be more pathogenic in the second trimester (Atladottir *et al.*, 2010). The mechanism by which infections may lead to ASD is not yet completely understood. It may involve direct infection of the central nervous system, infection elsewhere in the body acting as a trigger for brain disease, alteration of the immune response of the mother or offspring, or a combination of these factors (Libbey *et al.*, 2005). The activation of the maternal immune system after exposure to these microbiological agents during pregnancy induces release of cytokines that cross the placenta and alter fetal brain development. Thus, increases in maternal interleukin-6 (IL-6) alter many parameters that influence fetal growth, such as nutrient transfer, anoxia, and vascular permeability (Desai *et al.*, 2002; Kendall and Peebles, 2005). Furthermore, IL-6 can act directly on progenitor cells to regulate fetal neurogenesis and gliogenesis (Deverman and Patterson, 2009). IL-1, IL-2, and IL-6 also influence the release of monoamines in the hippocampus and other brain regions (Libbey *et al.*, 2005).

In line with epidemiological studies, preclinical work has shown that maternal infection in laboratory animals induces autistic-like symptoms in the offspring. Rodent models of ASD induced by maternal infection are based on maternal respiratory infection with influenza virus or maternal immune activation (MIA) induced by polyinosine:cytosine, poly(I:C), a synthetic double-stranded RNA evoking an antiviral-like immune reaction, or by lipopolysaccharide (LPS), which evokes an antibacterial-like immune reaction (Patterson, 2011).

The consequences of maternal infections are highly dependent on the stage of fetal development at the time of the infection. Again, exposure around the time of closure of the neural tube seems to be related to the onset of autistic-like behaviors in the offspring. For instance, injection of poly I:C on gestational day 9.5 or 12.5 caused impairments in social interaction and anxiety behaviors in the offspring (Shi *et al.*, 2003; Smith *et al.*, 2007; Hsiao and Patterson, 2011), together with altered cerebellar development (Shi *et al.*, 2009). Furthermore, injection of poly I:C on gestational days 10.5, 12.5, and 14.5 induced qualitative and quantitative differences in pup isolation-induced USV emission, together with decreased sociability and repetitive/stereotyped behavior in both marble burying and self-grooming tests at adulthood (Malkova *et al.*, 2012). Interestingly, clozapine injection at postnatal days 34–47 prevented the behavioral abnormalities displayed by the adult offspring prenatally exposed to poly I:C (Piontkewitz *et al.*, 2009).

LPS exposure during gestation altered USV emission (Kirsten *et al.*, 2012) and impaired nest-seeking behavior and associative learning in the exposed pups, associated with a significant decrease in the expression of *5HT1A*

Table 1 Overview of the rodent models of ASD that show behavioral abnormalities relevant to the core symptoms of ASD, as listed in DSM-5

Rodent models	Behavioral tests
BTBR mice	
DSM-5 criterion A	Home cage social behavior (Babineau *et al*., 2013) Juvenile social behavior (McFarlane *et al*., 2008) Social conditioned place preference (Pearson *et al*., 2012) Resident intruder (Scattoni *et al*., 2013) Three-chamber test (McFarlane *et al*., 2008; Silverman *et al*., 2012; Yang *et al*., 2012; Ruskin *et al*., 2013; Han *et al*., 2014; Jasien *et al*., 2014; Langley *et al*., 2015; Silverman *et al*., 2015; Zhang *et al*., 2015) Social interaction (McFarlane *et al*., 2008; Silverman *et al*., 2012; Han *et al*., 2014) Social motivation operant task (Martin *et al*., 2014) Social transmission of food preference (McFarlane *et al*., 2008; Ruskin *et al*., 2013) Pup isolation-induced USVs (Scattoni *et al*., 2008; Wöhr, 2015) USV recording during resident-intruder test (Scattoni *et al*., 2013) Scent marking (Wöhr *et al*., 2011a) USV response to female urine (Wöhr *et al*., 2011a)
DSM-5 criterion B	Self-grooming behavior (McFarlane *et al*., 2008; Silverman *et al*., 2012; Yang *et al*., 2012; Ruskin *et al*., 2013; Brodkin *et al*., 2014; Reynolds *et al*., 2013; Silverman *et al*., 2015) Jammed running wheel test (Karvat and Kimchi, 2012) Marble burying test (Langley *et al*., 2015; Silverman *et al*., 2015; Zhang *et al*., 2015) Stereotyped circling behavior (Han *et al*., 2014) Repetitive novel object contact (Reynolds *et al*., 2013)
Fmr1 mutant mice	
DSM-5 criterion A	Resident-intruder (Mineur *et al*., 2006) Social interaction (Spencer *et al*., 2005) Pup isolation-induced USVs (Lai *et al*., 2014a)
DSM-5 criterion B	Marble burying test (Spencer *et al*., 2011)
Nlgn-3 mutant mice	
DSM-5 criterion A	Social interaction (Tabuchi *et al*., 2007) Three-chamber test (Tabuchi *et al*., 2007; Radyushkin *et al*., 2009) Pup isolation-induced USVs (Chadman *et al*., 2008) USV response to female exposure (Radyushkin *et al*., 2009)
Nlgn-4 mutant mice	
DSM-5 criterion A	Social interaction (Jamain *et al*., 2008; El-Kordi *et al*., 2013) Pup isolation-induced USVs (Ju *et al*., 2014) USV response to female exposure (Jamain *et al*., 2008) USV recording during social encounter (El-Kordi *et al*., 2013)
Shank1-mutant mice	
DSM-5 criterion A	Pup isolation-induced USVs (Wöhr *et al*., 2011b) Scent marking (Wöhr *et al*., 2011b) USV response to female exposure (Wöhr *et al*., 2011b)
DSM-5 criterion B	Self-grooming and marble burying test (Sungur *et al*., 2014)
Shank2-mutant mice	
DSM-5 criterion A	Three-chamber test (Schmeisser *et al*., 2012; Won *et al*. 2012) USV response to female exposure (Schmeisser *et al*., 2012; Won *et al*., 2012; Ey *et al*., 2013) Pup isolation-induced USVs (Ey *et al*., 2013) USV recording during resident-intruder test (Ey *et al*., 2013)
DSM-5 criterion B	Self-grooming and digging behaviors (Schmeisser *et al*., 2012; Won *et al*., 2012)
Shank3-mutant mice	
DSM-5 criterion A	Juvenile social behavior (Yang *et al*., 2012) Social interaction (Bozdagi *et al*., 2010; Wang *et al*., 2011) Three-chamber test (Peça *et al*., 2011) Social transmission of food preference (Wang *et al*., 2011) USV recording during social encounter (Bozdagi *et al*., 2010; Wang *et al*., 2011; Yang *et al*., 2012)
DSM-5 criterion B	Hole-board test (Wang *et al*., 2011) Repetitive novel object contact (Wang *et al*., 2011)
Tsc1 mutant mice	
DSM-5 criterion A	Social interaction (Goorden *et al*., 2007) Three-chamber test (Tsai *et al*., 2012) Pup isolation-induced USVs (Tsai *et al*., 2012)
DSM-5 criterion B:	Self-grooming behavior (Tsai *et al*., 2012)
Tsc2-mutant mice	
DSM-5 criterion A	Pup isolation-induced USVs (Young *et al*., 2010)

Table 1 (*continued*)

Rodent models	Behavioral tests
VPA prenatal exposure	
DSM-5 criterion A	Social play (Schneider and Przewlocki, 2005; Schneider *et al.*, 2006; Chomiak *et al.*, 2010) Three-chamber test (Dufour-Rainfray *et al.*, 2010; Kim *et al.*, 2011; Kerr *et al.*, 2013; Baronio *et al.*, 2015) Resident intruder (Felix-Ortiz and Febo, 2012; Banerjee *et al.*, 2014) Social interaction (Schneider and Przewlocki, 2005; Schneider *et al.*, 2006; Markram *et al.*, 2008; Felix-Ortiz and Febo, 2012) Pup isolation-induced USVs (Schneider and Przewlocki, 2005; Dufour-Rainfray *et al.*, 2010; Gandal *et al.*, 2010)
DSM-5 criterion B	Self-grooming and digging behaviors (Mehta *et al.*, 2011; Kim *et al.*, 2014) Repetitive/stereotypic-like behaviors (Schneider and Przewlocki, 2005; Schneider *et al.*, 2006) Marble burying test (Baronio *et al.*, 2015)
Maternal immune activation	
DSM-5 criterion A	Social interaction (Shi *et al.*, 2003; Smith *et al.*, 2007; Malkova *et al.*, 2012) Pup isolation-induced USVs (Malkova *et al.*, 2012)
DSM-5 criterion B	Marble burying test (Malkova *et al.*, 2012) Self-grooming behavior (Malkova *et al.*, 2012)
LPS prenatal exposure	
DSM-5 criterion A	Pup isolation-induced USVs (Baharnoori *et al.*, 2012; Kirsten *et al.*, 2012)
DSM-5 criterion B	Repetitive/stereotypic-like behaviors (Kirsten *et al.*, 2012)

ASD, autism spectrum disorder; DSM-5, *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed.; LPS, lipopolysaccharide; USV, ultrasonic vocalization; VPA, valproic acid.

and *5HT1B* genes in the frontal cortex (Baharnoori *et al.*, 2012). During adulthood, LPS-exposed animals showed deficits in prepulse inhibition (Borrell *et al.*, 2002), increased anxiety, learning impairments, repetitive behaviors, and increased amphetamine-induced locomotion (Bakos *et al.*, 2004; Fortier *et al.*, 2004; Kirsten *et al.*, 2012). Furthermore, at the neuroanatomical level, maternal LPS administration increased cell density and compromised development of dendritic arbors in the hippocampus (Ito *et al.*, 2010). In line with the inflammatory dysregulation found in the brain of autistic patients, astrogliosis and altered microglial immunostaining has been found in the offspring of LPS-treated mothers (Jonakait, 2007; Patterson, 2009).

Conclusion

A revised diagnosis of ASD has been proposed by the DSM-5 released in May 2013, on the basis of the current state of knowledge about autism and the clinical experience in the years since DSM-4 was published in 1994. In DSM-5, the four separate subdiagnoses indicated by DSM-4 (autistic disorder, Asperger's disorder, pervasive developmental disorder not otherwise specified, and disintegrative disorder; American Psychiatric Association, 1994) have been unified under the umbrella of ASD, which refers to a range of closely related disorders with a shared core of symptoms: deficits in social communication and social interaction, and restricted and repetitive behaviors (American Psychiatric Association, 2013).

ASD has a multifactorial etiology, being caused by a complex interplay of genetic and environmental factors. Furthermore, there is a wide degree of phenotypic variation in the severity of the core and associated symptoms displayed by ASD patients. As a result, it is difficult, if not impossible, to reproduce in one single laboratory animal all the molecular, cellular, and behavioral features of ASD. Nevertheless, behaviors relevant to the core and associated symptoms of ASD can be easily and effectively assessed in laboratory animals, and several rodent models are available that show specific autism-relevant behavioral features, whose neural underpinnings resemble those found in autistic patients. The genetic and environmental animal models of ASD discussed here display most or all of the core behavioral features of autism, together with some associated symptoms (Table 1). Interestingly, the altered behavioral patterns found in these animal models are already apparent at an early developmental age, as evinced for instance by the atypical USV emission by rodent pups isolated from the nest or by the altered social play behavior displayed by these animals when juvenile. Over the last few years, these models have contributed greatly to our understanding of ASD. However, much remains still to be done in ASD research. Continuous refinement of the behavioral assays relevant to ASD, parallel studies at different levels of investigation, ranging from the behavioral to the molecular level, and combined preclinical and clinical approaches should be encouraged to further clarify gene–environment interactions in ASD, to identify potential neurobiological mechanisms, and to find new pharmacological targets able to ameliorate the symptomatology of the disease, both as a whole and in the core communicative and social domains.

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Conflicts of interest

There are no conflicts of interest.

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