Autism-Open Access
ISSN: 2165-7890

Chadman and Guariglia, Autism 2012, S1
DOI: 10.4172/2165-7890.S1-009

Review Article Open Access

The BTBR T+*tf*/J (BTBR) Mouse Model of Autism

Kathryn K. Chadman* and Sara R. Guariglia

Behavioral Pharmacology Laboratory, Department of Developmental Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA

Abstract

Mouse models of neuropsychiatric disorders are validated according to three different criteria: face validity, construct validity and predictive validity. Autism Spectrum Disorders (ASDs) are diagnosed behaviorally, therefore, mouse models of ASDs rely primarily on face validity. The three diagnostic criteria for ASDs are impairments in social interaction, communication and repetitive behavior, and/or restricted interests. The BTBR T+*tf*/J (BTBR) mice are an inbred strain used as model of ASDs. All three types of behavioral criteria have been evaluated in the BTBR mice. An advantage of using an inbred strain, such as BTBR is that, the mice are considered genetically identical and offer good controls for experimentation. The BTBR mice have demonstrated face validity for the three core behaviors that define ASDs. Low levels of social behavior, altered communication and spontaneous grooming comprise the behavioral phenotype of the BTBR mice. For construct validity, the BTBR mice have some physiological characteristics similar to humans with ASDs. Several drug and behavioral treatments for ASDs have been examined in the BTBR mice; however this area of research is still being developed. This review will offer a description of the behavior and physiology of the BTBR mice as a model for ASDs.

Introduction

A Animal models of neuropsychiatric disorders are powerful tools that resemble the corresponding human pathologies. The effectiveness of a model is evaluated by three different sets of criteria: construct validity, face validity and predictive validity. Face validity refers to a phenotype analogous to the human condition. In the case of Autism Spectrum Disorders (ASDs), face validity is determined by examining mouse behaviors relevant to the three core behavioral symptoms (impairments in social interaction, communication and repetitive behavior, and/or restricted interests), as well as the associated or co-morbid symptoms. Construct validity refers to the cause of a disease or disorder such as a genetic mutation or biological dysfunction, i.e. the model has a congruent causality to the human disease. Construct validity is not yet clearly defined for ASDs, as the clinical population is fairly heterogenous with respect to behavioral presentation, and the underlying cause has not yet been determined for the majority of cases. Theories of putative genetic mutations found in human ASDs are tested for face validity in the mouse models of ASDs. A model's predictive validity is determined by its ability to reflect a treatment response, analogous to that of the human disorder. Mouse models, such as the BTBR T+*tf*/J (BTBR) mice are invaluable tools in preclinical research for potential treatments, because they can meet the above validities.

Inbred mouse strains are an asset to research as there is little genetic variability between animals, with some exceptions (i.e. spontaneous mutations, genetic drift). Inbred strains result from sibling matings for 20 or more consecutive generations, thus eliminating genetic variability, to allow more reliable results and higher reproducibility between laboratories. Commonly used inbred mouse strains include C57BL/6J (B6), DBA/2J, FVB/NJ, C3H/HeJ, AKR/J, A/J, BALB/cByJ, 129S1/SvImJ, and BTBR. These different strains, all have unique behavioral and physiological profiles. Outbred mouse strains are genetically variable, and so may better represent the human population. Outbred strains are commonly used in toxicological studies, to better represent human response to various toxins [1].

This review outlines the recent research on ASDs, using the BTBR mouse model. This strain has become a prominent model in ASD research. The BTBR mice have a behavioral phenotype relevant to ASDs with decreased sociability, aberrant vocalizations and spontaneous grooming behavior, reminiscent of the stereotypies often observed in children with ASDs. This mouse model is now also being used for early stage pharmacological research. The main focus of this paper is on the behavioral and biological characterization of BTBR mice.

BTBR T+*tf*/J Mice

History

BTBR mice have been used for obesity research since 1997 and are the background strain for phenylalanine hydroxylase deficiency "PKU mice" [2-4]. A survey of 10 inbred strains for social behavior and reversal learning by Moy et al. [5] brought the BTBR strain to light, as a potential model for autism. Four of the ten inbred mouse strains in this study demonstrated lower levels of social behavior: A/J, BALB/cByJ, BTBR and 129S1/SvImJ. The A/J strain of mice had very low levels of exploratory behavior compared to the other mouse strains [5], which may have confounded the results of the social behavior test. The BALB/cByJ strain of mice had slightly lower levels of activity, but very high levels of anxiety-like behavior compared to the BTBR strain [5]. While anxiety is commonly found in people with ASDs, it is not required for the diagnosis. The BALB/cByJ mice have since been examined for autistic-like behaviors, and are used as a model of ASDs. For more information, see a review by Brodkin [6]. The 129S1/SvImJ mice had relatively lower levels of exploratory behavior and higher levels of anxiety-like behavior, making them a less attractive mouse strain for ASDs than the BTBR mice [5]. Following this initial examination of the BTBR mice in 2007, many other laboratories have worked to characterize the BTBR mice, behaviorally and physiologically.

***Corresponding author:** Kathryn K. Chadman, Behavioral Pharmacology Laboratory, Department of Developmental Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA, Tel: +718-494-5364; **E-mail**: kathryn.chadman@opwdd.ny.gov

Received September 10, 2012; **Accepted** November 16, 2012; **Published** November 19, 2012

Citation: Chadman KK, Guariglia SR (2012) The BTBR T+*tf*/J (BTBR) Mouse Model of Autism. Autism S1:009. doi:10.4172/2165-7890.S1-009

General phenotyping

One of the first steps in establishing an animal model for a neuropsychiatric disorder is to ensure that the mice are healthy, and able to perform behavioral tests for a phenotype with no confounding variables. This involves doing a check of the general health, neurological reflexes, sensory abilities, and ambulatory activity of the mice. For the BTBR mice, the general health was examined by Moy et al. [5], and found to be similar to nine other strains of mice. The average weight of the BTBR mice does not differ from the other strains. However, during early postnatal days, BTBR mice grow faster than B6 (C57BL/6) mice [7], and are used as a model for obesity [8]. Interestingly, data suggests that children and adolescents with ASDs are 40% more likely to be obese than neurotypical children [9]. Based on human data and BTBR data concerning autism and obesity, it seems reasonable to suspect that the two may be linked mechanistically.

A striking feature of the BTBR mice is the amount of time these mice spend grooming themselves, compared to other strains of mice. This has been likened to the stereotypical behaviors associated with ASDs, which will be discussed below. A larger percentage of the BTBR mice have coats in poor condition compared to other strains, which likely reflects the increased time that these mice spend grooming [10,11]. Other observable behaviors of the BTBR mice appear to be normal. For example, in the home cage, all of the BTBR mice show evidence of nest building and huddling together [5].

The sensory abilities of the mice have also been determined. The tactile response in the BTBR mice differs between the paws and tail. In the hot plate test, BTBR mice take significantly longer to react compared to the B6 mice [12], but in the tail flick test, the BTBR mice react as quickly as the B6 mice [12]. The lowered sensitivity to the hot plate has implications for tests that use an aversive tactile stimulus, as the cue may be less salient for the BTBR mice. Indeed, BTBR mice do not perform as well in cued and contextual fear conditioning, where a mild shock is used in training [13,14].

The olfactory system of the BTBR mice is particularly important, as mice use olfactory information in social behavior, whereas humans rely largely on visual input for social behavior. In the buried food test, the BTBR mice find the food in a similar amount of time as other strains [5]. In the olfactory habituation-dishabituation test, BTBR mice habituate to non-social odors similarly to B6 mice, however, the BTBR mice do not show that they are able to differentiate between odors from different mouse cages [14]. The sense of hearing is intact in the BTBR mice, as shown by a lack of strain differences between the BTBR and B6 mice in the startle response [12], and the Preyer reflex to an auditory stimulus [5]. The BTBR mice show normal visual ability through visual placing and are able to orient using their whiskers [5], and have no difficulties in the visual platform Morris water maze test [5]. BTBR mice have a shorter latency to fall on the accelerating rotarod, compared to many other strains of mice including B6, DBA/2J, FVB/NJ and 129S1/SvImJ mice [15], indicating somewhat impaired motor coordination. However, that does not affect the general exploratory activity of the BTBR mice in an open field, where the BTBR mice show an increased level of activity at the beginning of the test compared to B6 mice that evens out across the session [10,12,16]. This suggests that the BTBR mice may have a more intense reaction to novelty than B6 mice, but drop back to normal levels quickly. Overall, the BTBR mice have good reflexes and intact sensory modalities, and are able to be tested on behaviors relevant to ASDs.

Face Validity: Behaviors Relevant to an ASD Phenotype

The BTBR mice were initially screened for face validity for ASDs with behaviors relevant to the three core symptoms, which are impaired sociability, communication and restricted interests, and/or repetitive behaviors. To test for impairments in social behavior, the BTBR mice have been tested in a wide variety of social situations and have very consistently shown lower levels of social behavior than other strains of mice, especially the B6 mice. In all types of direct interaction tests described below, the BTBR mice consistently spend more time isolated grooming themselves, while the B6 mice are more socially interactive.

Social interaction

One common method for evaluating social behavior in mice is to pair two same sex unfamiliar mice and measure the frequency of social, investigative and other behaviors. This has been tested in both juvenile and adult BTBR mice. The juvenile BTBR mice, at P21, demonstrate fewer social behaviors such as social grooming, nose-to-nose sniffing, and push over/crawl under than pairs of B6 mice [10]. Adult BTBR mice also demonstrate significantly fewer interactive behaviors than pairs of B6 mice [17]. However, when BTBR males were paired with unfamiliar B6 males, the BTBR mice demonstrated similar levels of social behaviors as the B6 partners [17], suggesting that the strain of the partner mouse appears to affect the behavior of the BTBR mice. Another measure of social behavior in mice is the visible burrow system, a semi-natural living environment for mice that allows observation of mice in a more naturalistic environment. Consistent with the results from direct social interaction tests, the BTBR mice engage in lower levels of all interactive behaviors including approach, flight, chase/follow, allogrooming and huddling, while spending more time self-grooming and alone than the B6 mice [18]. Another test for social behavior is the social approach test, which has the advantage of minimizing the effects of the behavior of the stranger mouse in a ventilated enclosure, while allowing investigation by the subject mouse, but not direct contact. The BTBR mice do not show a preference for spending time in the chamber with the stranger mouse, or for sniffing the stranger mouse in the three chambered social approach test [5,10], consistent with the findings from direct social interaction and the visible burrow test. BTBR mice spend the least amount of time in any type of social interaction, compared with B6 mice and other strains in the resident-intruder test for aggressive behavior [19]. The 129S1/SvImJ, DBA/2J and FVB/NJ mice engage in mounting behavior, but not the B6 or BTBR mice. Mice from all of the strains will follow another mouse except the BTBR mice, indicating indifference to the other mouse [19].

There may be abnormal reward processing in ASDs, which affects the reinforcing value of social interaction. This can be examined in mouse models of ASDs using a social stimulus in the conditioned place preference test. The B6 mice demonstrate a preference for the chamber associated with the stranger mouse, while the BTBR mice do not [20].

Deficits in social communication

The second diagnostic criterion for ASDs is delayed social communication. Mice emit ultrasonic vocalizations but can also communicate using body language and olfaction. Therefore, ultrasonic vocalizations and scent markers are used to evaluate communication impairments in the mouse models of ASD. The ultrasonic vocalizations of BTBR mice have been examined in young mice, separated from dams and littermates and in adult mice during different social situations. BTBR pups call more loudly and more frequently than B6 pups, and when the calls were analyzed by pattern, BTBR pups use fewer types

of calls than B6 mice, which may indicate a more limited vocabulary [7]. Adult vocalizations and behavioral responses were assessed in three different social situations, male-male (resident-intruder), male-female, and female-female, in both BTBR and B6 mice. There was no overt fighting or mating, during any of the experimental encounters [21]. BTBR mice vocalize less and engage in less social investigation than B6 mice in all three social contexts [21]. This suggests that oral communication in social situations is different than that of the B6 mice.

Another way to examine communication in mice is the Social Transmission of Food Preference (STFP) test, where mice learn about novel foods from other mice. BTBR mice are impaired in communicating information in the STFP [10]. The BTBR demonstrators and observers spend less time sniffing each other's faces during the reunion period, and do not learn about the novel cued food, as shown by a lack of preference for the cued food compared to the B6 mice [10].

Scent marking, which is the deposition of urine, contains information that is used by mice to recognize individuals, attract mates, outline territories, and maintain family organization. Due to the information contained in the urine pheromones, it is considered a form of mouse communication. Male BTBR mice have different reactions than the B6 mice, in response to a female in estrous, by depositing fewer scent marks and emitting fewer vocalizations [22]. The difference in scent marking behavior between the BTBR and B6 is specific to female urine-elicited behavior, as there is no difference between the strains in response to male urine [23]. The male BTBR mice do react as much to the potential mating situation compared to the B6 mice, which may be analogous to a lack of reaction in human social interactions.

Repetitive behavior and restricted interests

The third diagnostic criterion for ASDs is repetitive behavior, restricted interests and insistence on sameness. Other behaviors in this category are repetitive movements, such as rocking or twirling (known as stereotypies and self-injurious behavior) [24]. Naturally occurring stereotypies or reversal learning tests, that require the mice to inhibit a specific behavior so that a new one may be acquired, are used to measure repetitive behavior in mice.

The BTBR mice spend significantly more time grooming themselves, compared to other strains of mice [10,25]. The grooming behavior is thought to represent a stereotypy because the mice groom themselves so much that fur is lost in various patterns. This repetitive behavior may be akin to some stereotyped behaviors observed in people with ASDs, including rocking or echolalia. The grooming behavior has been broken down into microstructures that include bouts, interrupted bouts, transitions, and incorrect transitions. The BTBR mice show a higher frequency and duration of every category of grooming behavior, except for paw licking [26]. The BTBR mice loose more hair during a 30 minute self-grooming session and have a higher percentage of interrupted bouts of grooming, but significantly fewer incorrect transitions [26], suggesting a rigorous pattern of repetitive, self-directed behavior.

The BTBR mice do not differ in their initial exploratory behavior in the 16 well hole-board apparatus for repetitive behavior, but do not shift preference for certain baited holes after familiarization [27]. The BTBR mice also do not prefer an appetitive olfactory stimulus after familiarization, unlike other inbred strains including B6, FVB/NJ, and BALB/cByJ [27]. Due to the extensive behavioral phenotyping of the BTBR mice, it is known that they do not have any trouble finding a buried appetitive olfactory stimulus [5], so the findings here are not due to an inability to smell the food.

Marble burying behavior measures repetitive digging behavior to model stereotypical behavior. BTBR mice bury significantly more marbles than B6 mice [28]. The repetitive behavior is so ingrained in the mice that it confounds learning in a different test. Acquisition of a wheel-running task was impaired in the BTBR mice due to the digging, such that a separate group of BTBR mice had to be trained in an apparatus free of bedding [29]. The BTBR mice were able to acquire the wheel running in the bedding free apparatus and once learned the BTBR mice do not stop spending time on the wheels, even when the wheels are jammed, suggesting cognitive inflexibility [29]. Spontaneous wheel running is one of the few behaviors where a sex difference has been found between the BTBR and B6 mice, as males had higher spontaneous wheel running activity counts, while the females showed no strain difference [30].

Reversal learning

The BTBR mice demonstrate reversal learning in some paradigms. BTBR mice acquire position-habit in the T-maze and also have high levels of reversal learning [5]. In the Morris water maze, the BTBR mice perform similarly to the B6 mice during acquisition, but there is some inconsistency in reversal learning [5,14], making it hard to interpret the reversal ability of the BTBR mice. BTBR mice demonstrate normal acquisition of the water T-maze task with strict learning criteria, but show deficits in reversal compared to B6 mice [31]. However, in a modified version of this test where the mice are tested for two days on acquisition and two days for reversal, the BTBR mice learn the reversal task, as well as B6 mice [29]. The shorter acquisition may have reduced the habit forming tendency in the BTBR mice, allowing easier reversal learning. BTBR mice perform the same as B6 mice in acquisition and reversal of a visual discrimination task, when rule switching was automatic [32]. However, when the task became more difficult as the rule switching changed to contextual information, the BTBR mice had difficulty in learning [32]. This suggests that the BTBR mice begin to show impairments, when the learning strategies become more complicated and more executive functioning is required.

Other behaviors often co-morbid with Autism Spectrum Disorders

People with ASDs are often also diagnosed with an anxiety disorder. The anxiety is separate from the core symptoms of ASDs, and has been treated with traditional anxiolytic drugs that do not affect social, communicative, or repetitive behaviors.

The elevated plus maze exploits the conflict between a mouse's natural tendency to explore a new environment versus the aversiveness of the elevated open arm of the maze. When compared to nine other strains, BTBR mice were one of the strains that spent the most time in the open arms, made a larger percentage of entries into the open arms, and a larger number of entries overall, indicating a lower level of anxiety [5,12,16,33]. There is one contradictory report showing that the BTBR mice spend less time in the open arms than the B6 mice [34]. The amount of time BTBR mice spend in the open arms can be modified by stress. Open arm time was reduced when the BTBR mice were hung by the tail for 90 seconds. This did not occur in the B6 mice [35], suggesting a greater response to stress. Neither fluoxetine nor risperidone had an effect on open arm time in the BTBR mice [33]. The elevated zero maze provides a similar conflict test for the mice, except the maze is oval shaped with different parts enclosed and open. The BTBR mice spend more time in the open arm segments than the B6 mice [10,34], supporting the idea of BTBR mice to have lower levels of anxiety compared to other strains.

Another test that explores the conflict between exploring a novel environment and avoidance of a brightly lit area is the light-dark exploration test. This test, as well as the other anxiety tests mentioned above, has potential artifacts due to nonspecific hyperactivity or sedation. These can be ruled out with the open field test (see next section). The light-dark box test exploits the conflict mice have between exploring a novel area and spending time in a safe, dark environment. The BTBR mice show the same number of transitions between the light and dark sides, the same amount of time in the dark, and the same latency to enter the dark side as B6 mice [12,16]. Overall, the BTBR mice demonstrate very little anxiety-like behavior.

Reaction to threatening stimuli, such as an anesthetized rat, is examined in the mouse defense test battery. The BTBR mice show several behaviors indicating enhanced anxiety, but also some contradictory responses to the threat stimulus. For example, BTBR mice have a reduced escape distance compared to B6 mice, but also show evidence of enhanced defensiveness [34]. The overall indication suggests that the BTBR mice have an enhanced reactivity to predator stress, compared to B6 mice. Increased reactivity has also been observed in the BTBR mice, as higher levels of anxiety follow restraint stress [35].

The BTBR mice do not show a depressive phenotype. Depression is not one of the core symptoms of ASDs. Depression-like behavior is evaluated with suspension test as drugs that treat depression in humans reduce immobility in this test. BTBR mice spend significantly less time immobile than B6 mice, and are more responsive to the effects of the selective serotonin reuptake inhibitor citalopram [12,36]. The forced swim test also has predictive validity for the effects of antidepressants in mice. BTBR mice also spend significantly less time immobile than B6 mice in this test [12,30], which confirms a less depressive phenotype for the BTBR mice compared to B6 mice.

Pavlovian fear conditioning is a well-developed behavioral paradigm to assess learning and memory. BTBR mice exhibit significantly less freezing behavior compared to B6 mice, in both cued and contextual fear tests [13,14].

Construct Validity: Biology of the BTBR Mice

Construct validity is commonly used to create an animal model of a disorder, often through genetic manipulation. The BTBR strain of mice is inbred and was discovered as a model for ASDs through behavioral similarities. Construct validity is being determined for the BTBR mice, by examining the neurophysiology of these mice and looking for similarities to ASDs.

Neurophysiology

There is mounting evidence that humans with ASDs have a corpus callosum that is smaller or malformed [37-39]. BTBR mice lack a corpus callosum and have a severely reduced hippocampal commissure, compared to other mouse strains [40]. The reduced hippocampal commissure is also found in about 30% of the mice from another strain, BALB/cByJ, which also has low sociability, but high anxiety-like behavior. Crosses between the BTBR and BALB/cByJ mice have demonstrated that the absent corpus callosum and reduced hippocampal commissure have X-linked dominant inheritance [41]. When BTBR mice are crossed with BALB/cJ, 129S1/SvImJ and I/LnJ mice, the offspring are less affected, when compared to the parent strains. The mixed offspring tend to harbor abnormalities similar to their dams, further confirming the X-linked inheritance of commissural abnormalities [42].

Likely related to the observed commissural abnormalities in the BTBR mice, there is a marked reduction in white matter in the midline of the forebrain. Small, ectopic white matter bundles that are <200 μm in diameter have been found in the cingulate cortex, adjacent to the midline. These ectopic structures are possibly remnants of callosal fibers that were unable to cross the midline [43]. Reductions in white matter, in both the frontal lobes and in tracts involved in speech and language, have been found in children with ASDs [44,45].

The inability of callosal axons to cross the midline may be the result of a marked decrease in heparan sulfates secreted by fractones. In BTBR mice, there are fewer fractones as compared to B6 mice [46-48]. Fractones are specialized structures in the ependymal-subventricular zone interface that produce heparin sulfates, which are polysaccharides in the extracellular matrix, near the cell surface. Heparin sulfates regulate a wide variety of biological activities, including axonal guidance and the development of the corpus callosum [49,50]. Peripherally, plasma sulfates are reduced in BTBR mice compared to B6 or CD-1 mice [51]. Decreased concentrations of plasma sulfates are found in children with ASDs, compared to neurotypical controls [52,53].

Gliosis, which is the proliferation of astrocytes in response to neuronal damage is not found in BTBR mice, but is typically found in persons with ASDs. This dissimilarity may result from variation in the environment, and/or inflammatory factors. However, in BTBR mice, glial fibers are increased in the cingulum and the alveus, which are white matter regions in the forebrain. Astrocyte fibers are misoriented in regions of the brain that normally receive callosal innervation in the BTBR mice [43]. This suggests that callosal agenesis likely contributes to the glial fiber misorientation [43].

Additional glial abnormalities are found in polydendrocytes, which are a specific glial cell population that participates in neuronal migration, neurogenesis and myelin repair. Polydendrocytes are increased in the Anterior Cingulate Cortex (ACC) in the BTBR forebrain, but not the striatum [43]. Currently, there is limited information regarding polydendrocytes in persons with ASDs. Further investigation of this cell population may be important in understanding the mechanism underlying white mater abnormalities in ASDs, especially since the increase in polydendrocytes is spatially distributed in the ACC.

Besides agenesis of the corpus callosum and white matter abnormalities, there are a wide variety of other neuroanatomical differences between BTBR and B6 mice. These differences include lateral displacement of the hippocampus, the lateral septum and the striatum, elongated falx cerebri, collapsed lateral ventricles, and a shrunken choroid plexus in the anterior portions of the lateral ventricles [47,54]. It is likely that many of these neuroanatomical abnormalities result from callosal agenesis [40,43].

There are no gross neuroanatomical changes in the cerebellum, brainstem, or in the prefrontal cortex, nor are there any differences in total brain weight between BTBR and B6 mice [43]. These findings highlight that there are differences in gross pathology in the BTBR model and ASDs.

Neuronal biology

Cellular neuroanatomical differences in BTBR mice include a reduced number of granule cells in the dentate gyrus and a thinning of the hillus, compared to B6 mice [43]. Decreased adult hippocampal neurogenesis in the dentate gyrus of the hippocampus, the subventricular zone, amygdale, or in the piriform and entorhinal cortices, are also noted in BTBR mice [43]. Mossy fiber pathway synapses appear normal.

This suggests spatial heterogeneity in protein expression. In persons with ASDs, hippocampal abnormalities are inconsistent. Some studies suggest that there are differences in hippocampal volume and shape, as well as cellular density, whereas other studies have not revealed these same differences [41,55-59].

Neuroendocrinology

There are neuroendocrine differences in BTBR mice, compared to other mouse strains that are relevant to ASDs. BTBR mice have elevated plasma corticosterone compared to B6 mice, which is peculiar when considering their low anxiety phenotype. Corticosterone, a glucocorticoid steroid hormone, is indicative of stress. It promotes storage of fat, prevents mobilization of lipids from visceral adipose reserves, and regulates insulin. Elevated corticosterone may contribute to the mild insulin resistance and obesity found in BTBR mice [8]. Quite possibly, corticosterone feedback to the hypothalamus is impaired in the BTBR mice and to central receptors which modulate anxiety, thus contributing to low anxiety. There are some reports of persons with ASDs with hypothalamic-pituitary-adrenal dysregulation, although it seems that adrenocorticotrophic hormone is elevated and cortisol level is diminished [60,61].

Additionally, BTBR mice have elevated plasma progesterone and 5α-pregnan-3α-ol-20-one compared to B6 mice, and elevated levels of 5α-pregnan-3α-ol-20-one in the hypothalamus compared to B6 and 129S1/SvImJ mice [62]. In male rodents, reduction of 5α-pregnan-3α-ol-20-one formation can enhance aggression and decrease cognitive function [62]. No studies have been done which examine the level of 5α-pregnan-3α-ol-20-one in persons with ASDs.

Oxytocin is involved in the regulation of sociability, depression, anxiety, and in other physiological events (i.e. parturition). Abnormalities in either the oxytocin peptide or receptor have been found in persons with ASDs [63-65]. Treatment with oxytocin has been found to alleviate repetitive behaviors and increase social retention in persons with ASDs [66,67]. BTBR mice were found to have elevated oxytocin peptide in the Paraventricular Nucleus (PVN) of the hypothalamus [12], which is counterintuitive, since BTBR mice show deficits in sociability. However, elevated oxytocin peptide in the PVN does not necessarily reflect the amount of oxytocin that is available for signaling. Impairments in exocytotic mechanisms can cause the observed increase of oxytocin in the PVN [68].

Neurotransmission

Serotonin neurotransmission varies between BTBR and B6 strains. There is 20-30% fewer serotonin transporters in the BTBR brain, particularly in the hippocampus [69]. However, hippocampal 5-HT (1A) and 5-HT (2A) receptor assays were similar between the two strains, indicating that there are no major differences in the number of receptors [69]. Though receptor binding was comparable, 8-OH-DPAT-stimulated a 28% increase in GTPγS binding in the BTBR hippocampal CA1 region, indicating elevated 5-HT(1A) capacity to activate G-proteins [70]. This suggests that reduced serotonin transporter expression or elevated 5-HT (1A) receptor capacity to activate G-proteins may account for some of the behavioral phenotypes that are found in BTBR mice. In people with ASDs, some SERT gene variants confer enhanced serotonin uptake [71-73]. Interestingly, animals carrying one of the gain-of-function SERT mutations, SERT Ala56, displayed enhanced 5-HT receptor sensitivity, and hyperserotonemia, as well as alterations in a number of ASD-relevant behaviors [74]. It is possible that, like the Ala56 model, BTBR mice may harbor gain-of-function SERT as compared to B6 mice, which may account for the decreased SERT receptor number, as well as the enhanced 5-HT binding that was observed.

Cholinergic fibers and cholinergic neurons in the forebrain are not altered, nor are there changes in dendritic spines in any region of the forebrain in the BTBR mice, as compared to the B6 mice [43]. Furthermore, there are no profound differences in the number of inhibitory or excitatory synapses [43]. A lack of irregular cholinergic innervation, spine density and GABAergic synapses suggest that these particular phenotypes do not underlie the ASD relevant pathology in the BTBR mice. However, it has been shown that there are abnormalities in the cholinergic system of persons with ASDs [75]. Since the data that suggests cholinergic involvement is obtained from adults with ASDs, the abnormalities found may result from compensation for other developmental programs gone awry [75-77]. Therefore, investigation of cholinergic innervation at a later time point in the BTBR mice, may reveal a similar phenotype.

Neuroimmunology

There are immunological differences between the BTBR and B6 mice. The BTBR mice have more serum IgG and IgE, IgG anti-brain antibodies, as well as more IgG and IgE in the brain [78]. Furthermore, there is elevated expression of cytokines in the brain of BTBR mice compared to B6 mice, and an increased proportion of MHC class II-expressing microglia [78]. Supporting these findings are the increased numbers of CD40 hi/I-A hi B cells and IgG-secreting B cells [78]. BTBR mice produce 2-3 times more antibodies to KLH upon immunization, and are significantly more susceptible to listeriosis than B6 mice [78]. Abnormal immune responses are common in ASDs. Many children with ASDs have food allergies, gastrointestinal allergies, and are highly susceptible to viral infection [79]. Interestingly, newborns and children with ASDs were found to have a reduced level of immunoglobulins, as well as reduced cytokines of various types [79-83]. Even though there is a peripheral reduction in immune response, there is a higher density of activated microglia in the brain of persons with ASDs [84]. This suggests that chronic neuroinflammation in the BTBR mice is involved in their aberrant behaviors, which may further relate this mouse model to humans with ASDs.

Predictive Validity: Potential Treatments for ASD Symptoms

Drugs targeting various neurotransmitter systems have been tested to determine their effect on direct social interaction behavior, as well as other autism relevant behaviors in the BTBR mice.

It is possible to increase social preference in BTBR mice using behavioral interventions, such as weaning the BTBR mice with same sex B6 mice for 20 days [85]. However, fostering BTBR mice to B6 dams does not increase social preference [25]. This suggests that either the timing of the social intervention, pre or post weaning, is important for social development or that siblings provide more social "training" than dams. Pharmacological interventions have been shown to influence social preference as well, with mixed results. For example, GRN-529, a negative allosteric modulator of the metabotropic glutamate receptor, increases the number of nose-to-nose sniffing bouts and the time spent in social contact by the BTBR mice, while reducing the self-grooming and digging time [86]. GRN-529 also increases both measures of social preference, chamber time and sniffing the stranger mouse, in the BTBR mice [86]. Fluoxetine, a Selective Serotonin Reuptake Inhibitor antidepressant (SSRI), and diazepam, a benzodiazepine

anxiolytic, both increase social preference, measured by chamber time and sniffing the stranger mouse, in the BTBR mice [33,34]. CX 1837 and CX 1739, positive modulators of AMPA receptors that enhance excitatory neurotransmission, increase sniffing behavior towards a stranger mouse in the three chambered test, but do not increase the time spent in the chamber with the stranger mouse [86]. A high dose of acetaminophen, a non-steroidal anti-inflammatory drug, also increases the time spent in the chamber with the stranger mouse in the BTBR mice, but WIN 55,212-2, a cannabinoid agonist does not [70]. Therefore, both behavioral interventions and drugs from a wide variety of neurotransmitter systems, glutamate, serotonin and cannabinoid, all of them increase social behavior. Conversely, diazepam has other actions as well, decreasing crawl under social behavior and behaviors associated with anxiety, rears and jump escape behavior in the BTBR mice [17].

There have been fewer investigations targeted to ameliorating the other behavioral phenotypes of ASDs in BTBR mice. The repetitive grooming behavior in the BTBR mice is decreased, following administration of GRN-529 [86], or methyl-6-phenylethynyl-pyridine (MPEP), a metabotropic glutamate antagonist [87]. However, CX-1837 and CX-546, positive modulators of AMPA receptors, do not affect grooming behavior in BTBR mice [86]. Another measure of repetitive behavior, marble burying, is diminished in the BTBR mice with risperidone (an atypical antipsychotic), but not acetaminophen, WIN 55,212-2 (a cannabinoid agonist), buspirone (SSRI) or fluoxetine [20,70].

Conclusions

The BTBR inbred strain displays construct validity, face validity and predictive validity for ASDs. Subsequently, these mice may be used in many different ways to elucidate the etiology of the symptoms of ASDs, as well as potential treatments. It is now possible to examine how environmental influences over different periods of development either enhance or reduce the ASD phenotype. Conversely, treatments at various times during development may be examined for prevention of the symptoms of ASDs. The BTBR mouse model has become a powerful tool in the search of a better understanding of ASDs.

References

1. Festing MFW (2010) Inbred Strains Should Replace Outbred Stocks in Toxicology, Safety Testing, and Drug Development. Toxicol Pathol 38: 681-690.
2. Cerreto M, Mehdawy B, Ombrone D, Nisticò R, Ruoppolo M, et al. (2012) Reversal of metabolic and neurological symptoms of phenylketonuric mice treated with a PAH containing helper-dependent adenoviral vector. Curr Gene Ther 12: 48-56.
3. Liang L, Gu X, Lu L, Li D, Zhang X (2011) Phenylketonuria-related synaptic changes in a BTBR-Pah(enu2) mouse model. Neuroreport 22: 617-622.
4. Zagreda L, Goodman J, Druin DP, McDonald D, Diamond A (1999) Cognitive deficits in a genetic mouse model of the most common biochemical cause of human mental retardation. J Neurosci 19: 6175-6182.
5. Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, et al. (2007) Mouse behavioral tasks relevant to autism: Phenotypes of 10 inbred strains. Behav Brain Res 176: 4-20.
6. Brodkin ES (2007) BALB/c mice: low sociability and other phenotypes that may be relevant to autism. Behav Brain Res 176: 53-65.
7. Scattoni ML, Gandhy SU, Ricceri L, Crawley JN (2008) Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. PLoS One 3: e3067.
8. Flowers JB, Oler AT, Nadler ST, Choi Y, Schueler KL, et al. (2007) Abdominal obesity in BTBR male mice is associated with peripheral but not hepatic insulin resistance. Am J Physiol Endocrinol Metab 292: E936-E945.
9. Curtin C, Anderson SE, Must A, Bandini L (2010) The prevalence of obesity in children with autism: a secondary data analysis using nationally representative data from the National Survey of Children's Health. BMC Pediatr 10: 11.
10. McFarlane HG, Kusek GK, Yang M, Phoenix JL, Bolivar VJ, et al. (2008) Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes Brain Behav 7: 152-163.
11. Yang M, Scattoni ML, Zhodzishsky V, Chen T, Caldwell H, et al. (2007) Social approach behaviors are similar on conventional versus reverse lighting cycles, and in replications across cohorts, in BTBR T+ tf/J, C57BL/6J, and vasopressin receptor 1B mutant mice. Front Behav Neurosci 1: 1.
12. Silverman JL, Yang M, Turner SM, Katz AM, Bell DB, et al. (2010) Low stress reactivity and neuroendocrine factors in the BTBR T+tf/J mouse model of autism. Neuroscience 171: 1197-1208.
13. MacPherson P, McGaffigan R, Wahlsten D, Nguyen PV (2008) Impaired fear memory, altered object memory and modified hippocampal synaptic plasticity in split-brain mice. Brain Res 1210: 179-188.
14. Yang M, Abrams DN, Zhang JY, Weber MD, Katz AM, et al. (2012) Low sociability in BTBR T+tf/J mice is independent of partner strain. Physiol Behav.
15. Nadler JJ, Zou F, Huang H, Moy SS, Lauder J, et al. (2006) Large-scale gene expression differences across brain regions and inbred strains correlate with a behavioral phenotype. Genetics 174: 1229-1236.
16. Yang M, Clarke AM, Crawley JN (2009) Postnatal lesion evidence against a primary role for the corpus callosum in mouse sociability. Eur J Neurosci 29: 1663-1677.
17. Defensor EB, Pearson BL, Pobbe RL, Bolivar VJ, Blanchard DC, et al. (2011) A novel social proximity test suggests patterns of social avoidance and gaze aversion-like behavior in BTBR T+ tf/J mice. Behav Brain Res 217: 302-308.
18. Pobbe RL, Pearson BL, Defensor EB, Bolivar VJ, Blanchard DC, et al. (2010) Expression of social behaviors of C57BL/6J versus BTBR inbred mouse strains in the visible burrow system. Behav Brain Res 214: 443-449.
19. Bolivar VJ, Walters SR, Phoenix JL (2007) Assessing autism-like behavior in mice: Variations in social interactions among inbred strains. Behav Brain Res 176: 21-26.
20. Pearson BL, Bettis JK, Meyza KZ, Yamamoto LY, Blanchard DC, et al. (2012) Absence of social conditioned place preference in BTBR T+tf/J mice: Relevance for social motivation testing in rodent models of autism. Behav Brain Res 233: 99-104.
21. Scattoni ML, Ricceri L, Crawley JN (2011) Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. Genes Brain Behav 10: 44-56.
22. Wöhr M, Roullet FI, Crawley JN (2011) Reduced scent marking and ultrasonic vocalizations in the BTBR T+tf/J mouse model of autism. Genes Brain Behav 10: 35-43.
23. Roullet FI, Wöhr M, Crawley JN (2011) Female urine-induced male mice ultrasonic vocalizations, but not scent-marking, is modulated by social experience. Behav Brain Res 216: 19-28.
24. Muehlmann AM, Lewis MH (2012) Abnormal repetitive behaviours: shared phenomenology and pathophysiology. J Intellect Disabil Res 56: 427-440.
25. Yang M, Zhodzishsky V, Crawley JN (2007) Social Deficits in BTBR T+tf/J Mice are Unchanged by Cross-Fostering with C57BL/6J Mothers. Int J Dev Neurosci 25: 515-521.
26. Pearson BL, Pobbe RL, Defensor EB, Oasay L, Bolivar VJ, et al. (2011) Motor and cognitive stereotypies in the BTBR T+tf/J mouse model of autism. Genes Brain Behav 10: 228-235.
27. Moy SS, Nadler JJ, Poe MD, Nonneman RJ, Young NB, et al. (2008) Development of a mouse test for repetitive, restricted behaviors: relevance to autism. Behav Brain Res 188: 178-194.
28. Amodeo DA, Jones JH, Sweeney JA, Ragozzino ME (2012) Differences in BTBR T+ tf/J and C57BL/6J mice on probabilistic reversal learning and stereotyped behaviors. Behav Brain Res 227: 64-72.
29. Karvat G, Kimchi T (2012) Systematic autistic-like behavioral phenotyping of 4 mouse strains using a novel wheel-running assay. Behav Brain Res 233: 405-414.
30. Onaivi ES, Benno R, Halpern T, Mehanovic M, Schanz N, et al. (2011)

Consequences of cannabinoid and monoaminergic system disruption in a mouse model of autism spectrum disorders. Curr Neuropharmacol 9: 209-214.

31. Guariglia S, Braccolino J, Buenaventura D, Stapley N, Begum U, et al. (2011) Water-t-maze: A test useful for examining perseverance behaviors in mice.
32. Rutz HL, Rothblat LA (2012) Intact and impaired executive abilities in the BTBR mouse model of autism. Behav Brain Res 234: 33-37.
33. Chadman KK (2011) Fluoxetine but not risperidone increases sociability in the BTBR mouse model of autism. Pharmacol Biochem Behav 97: 586-594.
34. Pobbe RL, Defensor EB, Pearson BL, Bolivar VJ, Blanchard DC, et al. (2011) General and social anxiety in the BTBR T+ tf/J mouse strain. Behav Brain Res 216: 446-451.
35. Benno R, Smirnova Y, Vera S, Liggett A, Schanz N (2009) Exaggerated responses to stress in the BTBR T+tf/J mouse: An unusual behavioral phenotype. Behav Brain Res 197: 462-465.
36. Crowley JJ, Blendy JA, Lucki I (2005) Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. Psychopharmacology (Berl) 183: 257-264.
37. Casanova MF, El-Baz A, Elnakib A, Switala AE, Williams EL, et al. (2011) Quantitative analysis of the shape of the corpus callosum in patients with autism and comparison individuals. Autism 15: 223-238.
38. El-Baz A, Elnakib A, Casanova MF, Gimel'farb G, Switala AE, et al. (2011) Accurate automated detection of autism related corpus callosum abnormalities. J Med Syst 35: 929-939.
39. Hong S, Ke X, Tang T, Hang Y, Chu K, et al. (2011) Detecting abnormalities of corpus callosum connectivity in autism using magnetic resonance imaging and diffusion tensor tractography. Psychiatry Res 194: 333-339.
40. Wahlsten D, Metten P, Crabbe JC (2003) Survey of 21 inbred mouse strains in two laboratories reveals that BTBR T/+ tf/tf has severely reduced hippocampal commissure and absent corpus callosum. Brain Res 971: 47-54.
41. Kemper TL, Bauman ML (1993) The contribution of neuropathologic studies to the understanding of autism. Neurol Clin 11: 175-187.
42. Bohlen MO, Bailoo JD, Jordan RL, Wahlsten D (2012) Hippocampal commissure defects in crosses of four inbred mouse strains with absent corpus callosum. Genes Brain Behav 11: 757-766.
43. Stephenson DT, O'Neill SM, Narayan S, Tiwari A, Arnold E, et al. (2011) Histopathologic characterization of the BTBR mouse model of autistic-like behavior reveals selective changes in neurodevelopmental proteins and adult hippocampal neurogenesis. Mol Autism 2: 7.
44. Radua J, Via E, Catani M, Mataix-Cols D (2011) Voxel-based meta-analysis of regional white-matter volume differences in autism spectrum disorder versus healthy controls. Psychol Med 41: 1539-1550.
45. Voineskos AN, Lett TA, Lerch JP, Tiwari AK, Ameis SH, et al. (2011) Neurexin-1 and frontal lobe white matter: an overlapping intermediate phenotype for schizophrenia and autism spectrum disorders. PLoS One 6: e20982.
46. Blanchard DC, Defensor EB, Meyza KZ, Pobbe RL, Pearson BL, et al. (2012) BTBR T+tf/J mice: autism-relevant behaviors and reduced fractone-associated heparan sulfate. Neurosci Biobehav Rev 36: 285-296.
47. Mercier F, Kwon YC, Douet V (2012) Hippocampus/amygdala alterations, loss of heparan sulfates, fractones and ventricle wall reduction in adult BTBR T+ tf/J mice, animal model for autism. Neurosci Lett 506: 208-213.
48. Meyza KZ, Blanchard DC, Pearson BL, Pobbe RLH, Blanchard RJ (2012) Fractone-associated N-sulfated heparan sulfate shows reduced quantity in BTBR T+tf/J mice: a strong model of autism. Behav Brain Res 228: 247-253.
49. Conway CD, Howe KM, Nettleton NK, Price DJ, Mason JO, et al. (2011) Heparan sulfate sugar modifications mediate the functions of slits and other factors needed for mouse forebrain commissure development. J Neurosci 31: 1955-1970.
50. Inatani M, Irie F, Plump AS, Tessier-Lavigne M, Yamaguchi Y (2003) Mammalian brain morphogenesis and midline axon guidance require heparan sulfate. Science 302: 1044-1046.
51. Corley MJ, Meyza KZ, Blanchard DC, Blanchard RJ (2012) Reduced sulfate plasma concentrations in the BTBR T+tf/J mouse model of autism. Physiol Behav.
52. Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, et al. (2011) Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. Nutr Metab (Lond) 8: 34.
53. Alberti A, Pirrone P, Elia M, Waring RH, Romano C (1999) Sulphation deficit in "low-functioning" autistic children: a pilot study. Biol Psychiatry 46: 420-424.
54. Mercier F, Cho Kwon Y, Kodama R (2011) Meningeal/vascular alterations and loss of extracellular matrix in the neurogenic zone of adult BTBR T+ tf/J mice, animal model for autism. Neurosci Lett 498: 173-178.
55. Amaral DG, Schumann CM, Nordahl CW (2008) Neuroanatomy of autism. Trends Neurosci 31: 137-145.
56. Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, et al. (2004) The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci 24: 6392-6401.
57. Dager SR, Wang L, Friedman SD, Shaw DW, Constantino JN, et al. (2007) Shape mapping of the hippocampus in young children with autism spectrum disorder. AJNR Am J Neuroradiol 28: 672-677.
58. Nicolson R, DeVito TJ, Vidal CN, Sui Y, Hayashi KM, et al. (2006) Detection and mapping of hippocampal abnormalities in autism. Psychiatry Res 148: 11-21.
59. Bailey A, Luthert P, Dean A, Harding B, Janota I, et al. (1998) A clinicopathological study of autism. Brain 121: 889-905.
60. Hamza RT, Hewedi DH, Ismail MA (2010) Basal and adrenocorticotropic hormone stimulated plasma cortisol levels among Egyptian autistic children: relation to disease severity. Ital J Pediatr 36: 71.
61. Curin JM, Terzić J, Petković ZB, Zekan L, Terzić IM, et al. (2003) Lower cortisol and higher ACTH levels in individuals with autism. J Autism Dev Disord 33: 443-448.
62. Frye CA, Llaneza DC (2010) Corticosteroid and neurosteroid dysregulation in an animal model of autism, BTBR mice. Physiol Behav 100: 264-267.
63. Wu S, Jia M, Ruan Y, Liu J, Guo Y, et al. (2005) Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. Biol Psychiatry 58: 74-77.
64. Jacob S, Brune CW, Carter CS, Leventhal BL, Lord C, et al. (2007) Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. Neurosci Lett 417: 6-9.
65. Liu X, Kawamura Y, Shimada T, Otowa T, Koishi S, et al. (2010) Association of the oxytocin receptor (OXTR) gene polymorphisms with autism spectrum disorder (ASD) in the Japanese population. J Hum Genet 55: 137-141.
66. Hollander E, Novotny S, Hanratty M, Yaffe R, DeCaria CM, et al. (2003) Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. Neuropsychopharmacology 28: 193-198.
67. Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, et al. (2007) Oxytocin increases retention of social cognition in autism. Biol Psychiatry 61: 498-503.
68. Zhang G, Bai H, Zhang H, Dean C, Wu Q, et al. (2011) Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. Neuron 69: 523-535.
69. Gould GG, Hensler JG, Burke TF, Benno RH, Onaivi ES, et al. (2011) Density and function of central serotonin (5-HT) transporters, 5-HT1A and 5-HT2A receptors, and effects of their targeting on BTBR T+tf/J mouse social behavior. J Neurochem 116: 291-303.
70. Gould GG, Seillier A, Weiss G, Giuffrida A, Burke TF, et al. (2012) Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice. Prog Neuropsychopharmacol Biol Psychiatry 38: 260-269.
71. Sutcliffe JS, Delahanty RJ, Prasad HC, McCauley JL, Han Q, et al. (2005) Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. Am J Hum Genet 77: 265-279.
72. Prasad HC, Steiner JA, Sutcliffe JS, Blakely RD (2009) Enhanced activity of human serotonin transporter variants associated with autism. Philos Trans R Soc Lond B Biol Sci 364: 163-173.
73. Prasad HC, Zhu CB, McCauley JL, Samuvel DJ, Ramamoorthy S, et al. (2005) Human serotonin transporter variants display altered sensitivity to protein kinase G and p38 mitogen-activated protein kinase. Proc Natl Acad Sci U S A 102: 11545-11550.

74. Veenstra-VanderWeele J, Muller CL, Iwamoto H, Sauer JE, Owens WA, et al. (2012) Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior. Proc Natl Acad Sci U S A 109: 5469-5474.

75. Deutsch SI, Urbano MR, Neumann SA, Burket JA, Katz E (2010) Cholinergic abnormalities in autism: is there a rationale for selective nicotinic agonist interventions? Clin Neuropharmacol 33: 114-120.

76. Perry EK, Lee ML, Martin-Ruiz CM, Court JA, Volsen SG, et al. (2001) Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. Am J Psychiatry 158: 1058-1066.

77. Martin-Ruiz CM, Lee M, Perry RH, Baumann M, Court JA, et al. (2004) Molecular analysis of nicotinic receptor expression in autism. Brain Res Mol Brain Res 123: 81-90.

78. Heo Y, Zhang Y, Gao D, Miller VM, Lawrence DA (2011) Aberrant immune responses in a mouse with behavioral disorders. PLoS One 6: e20912.

79. Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, et al. (2008) Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. Autism Res 1: 275-283.

80. Grether JK, Croen LA, Anderson MC, Nelson KB, Yolken RH (2010) Neonatally measured immunoglobulins and risk of autism. Autism Res 3: 323-332.

81. Abdallah MW, Larsen N, Mortensen EL, Atladóttir HÓ, Nørgaard-Pedersen B, et al. (2012) Neonatal levels of cytokines and risk of autism spectrum disorders: An exploratory register-based historic birth cohort study utilizing the Danish Newborn Screening Biobank. J Neuroimmunol 252: 75-82.

82. Jyonouchi H, Geng L, Cushing-Ruby A, Quraishi H (2008) Impact of innate immunity in a subset of children with autism spectrum disorders: a case control study. J Neuroinflammation 5: 52.

83. Manzardo AM, Henkhaus R, Dhillon S, Butler MG (2012) Plasma cytokine levels in children with autistic disorder and unrelated siblings. Int J Dev Neurosci 30: 121-127.

84. Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, et al. (2010) Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry 68: 368-376.

85. Yang M, Perry K, Weber MD, Katz AM, Crawley JN (2011) Social peers rescue autism-relevant sociability deficits in adolescent mice. Autism Res 4: 17-27.

86. Silverman JL, Smith DG, Rizzo SJ, Karras MN, Turner SM, et al. (2012) Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. Sci Transl Med 4: 131.

87. Silverman JL, Tolu SS, Barkan CL, Crawley JN (2010) Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. Neuropsychopharmacology 35: 976-989.

This article was originally published in a special issue, **Animal Models in Autism** handled by Editor(s). Dr. Craig M. Powell, The University of Texas Southwestern Medical Center Dallas, USA