Behavioral Profiles of Mouse Models for Autism Spectrum Disorders

Elodie Ey, Claire S. Leblond, and Thomas Bourgeron

Autism spectrum disorders (ASD) are characterized by impairments in reciprocal social communication, and stereotyped verbal and nonverbal behaviors. In approximately 10–25% of the affected individuals, a genetic mutation associated with the condition can be identified. Recently, mutations altering synapse formation, cellular/synaptic growth rate and regulation of excitatory and inhibitory currents were identified in patients with intellectual disability, typical autism, Asperger syndrome or neurological syndromes associated with autistic traits. Following these genetic findings, mouse models carrying mutations similar to those identified in patients have been generated. These models offer the opportunity to investigate in vivo the physiological and behavioral consequences of the mutations. Here, we review the existing data on the phenotypes of mice carrying mutations in genes associated with ASD including *neuroligin*, *neurexin* and *Shank* mutant mice as well as the *Fmr1*, *Mecp2*, *Ube3a*, *Nf1*, *Pten* and *Tsc1*/*Tsc2* mutant mice. The diversity and complexity of the phenotype of these mouse models reflect the broad range of phenotypes observed in patients with ASD. Remarkably, results from therapeutic approaches (e.g., modulation of gene expression, administration of pharmacological and nonpharmacological substances, enriched environment) are encouraging since some behavioral alterations could be reversed even when treatment was performed on adult mice. These ongoing studies should therefore increase our understanding of the biological alterations associated with ASD as well as the development of knowledge-based treatments.

Keywords: autism spectrum disorder; mouse model; synaptic pathway; mTOR/PI3K pathway; behavior

Introduction

The diagnosis of autism is based on impairments in reciprocal social communication, and stereotyped behaviors. Beyond this unifying definition lies an extreme degree of clinical heterogeneity, ranging from profound to moderate impairments, but always with functional disability. Indeed, autism is not a single entity, but rather a complex phenotype thought to be caused by different types of defects in common pathways, producing similar behavioral phenotypes. The prevalence of autism spectrum disorders (ASD) overall is about 1/100, but closer to 1/300 for typical autism [Fernell & Gillberg, 2010]. ASD are more common in males than females with a 4:1 ratio [Abrahams & Geschwind, 2008; Freitag, 2007]. Twin and family studies have conclusively described ASD as the most ''genetic'' of neuropsychiatric disorders, with concordance rates of 82–92% in monozygotic twins vs. 1–10% in dizygotic twins; sibling recurrence risk is 6–20% [Abrahams & Geschwind, 2008; Freitag, 2007; Toro et al., 2010].

From 15 to 70% of children diagnosed as suffering from ASD have intellectual disabilities [Gillberg & Coleman, 2000], and it is now understood that, like intellectual disability, autism symptoms can be caused either by gene mutations or by chromosomal aberrations [Pinto et al., 2010]. In approximately 10–25% of the affected individuals, autism is ''syndromic,'' i.e., occurring in a child with a known genetic or environmental toxin disorder, such as fragile X, tuberous sclerosis, neurofibromatosis, valproic syndrome, or autism caused by brain herpes simplex infection [Freitag, 2007; Gillberg & Coleman, 2000].

Genes associated with ASD remain largely unknown, but two major biological pathways are emerging. Mutations in *TSC1/TSC2, NF1*, or *PTEN* activate the mTOR/PI3K pathway and lead to syndromic ASD with tuberous sclerosis, neurofibromatosis, or macrocephaly [Kelleher & Bear, 2008]. Mutations in *NLGN3/4, SHANK2, SHANK3, DLGAP2,* or *NRXN1* alter synaptic function and lead to intellectual disabilities, typical autism, or Asperger syndrome [Toro et al., 2010]. Mutations within the mTOR/PI3K pathway are associated with abnormal cellular/synaptic growth rate, whereas mutations within the NRXN—NLGN-SHANK pathway are most likely associated with abnormal synaptogenesis and imbalance between excitatory and inhibitory currents. Taken together, these data lead to the hypothesis that abnormal

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synaptic homeostasis might represent a risk factor to ASD [Bourgeron, 2009; Toro et al., 2010].

Several mouse models carrying mutations in genes associated with ASD have been generated in order to study in vivo the physiological and behavioral consequences of the mutations. Mice are mammals sufficiently close to humans to present physical and behavioral characteristics, which to some extent parallel those examined in humans. Remarkably, it was shown that traits such as decreased interest in social interaction, deficits in communication, and repetitive behavior can actually be investigated in mouse models. Indeed, wild mice are social animals, naturally living in *demes* with a single dominant male, occasionally a few subordinate ones, and several females occupying contiguous nests [but only a fraction of them reproduce; Berry, 1981; summarized in Palanza, Della Stella, Ferrari, & Parmigiani, 2005]. This social organization leads mice to use tactile, olfactory, and vocal (mostly in the ultrasonic range) communication in same-sex social interactions, male–female socio-sexual interactions, and mother–infant relationships [Brennan & Kendrick, 2006; Latham & Mason, 2004; Portfors, 2007]. Social interaction and communication have been examined in laboratory strains using different paradigms detailed in Silverman, Yang, Lord, and Crawley [2010]. However, the ethological relevance of these paradigms remains difficult to establish and further investigations in wild-derived strains and wild-living mice are necessary to design new appropriate paradigms. For instance, vocal communication has been recorded in California mice [*Peromyscus californicus*; Kalcounis-Rueppel, Metheny, & Vonhof, 2006; Kalcounis-Rueppell et al., 2010] and in wild house mice [*Mus m. musculus*; Musolf, Hoffman, & Penn, 2010], but contexts in which ultrasonic vocalizations (USVs) are uttered have not been extensively described yet.

In patients with ASD, impairments in social interactions and communication are usually combined with intellectual disability, increased anxiety, hyperactivity, abnormal circadian activity, abnormal sensory perception, and motor coordination. For these phenotypic traits, well-established behavioral paradigms can be used in animal models [reviewed in Silverman et al., 2010]. This includes tests for motor learning, stereotypic behaviors, depression, anxiety, fear conditioning, spatial learning and memory, reversal learning, home cage and nesting behavior, locomotion, seizure propensity, circadian activity, and sensory perception such as vision, olfaction, audition, and pain sensitivity. Abnormalities in locomotion and sensory perception might indeed potentially perturb cognitive performances in mouse models [see Silverman et al., 2010] and need to be controlled. All these aspects should allow us to draw a relatively exhaustive phenotype of mouse models of ASD, and to understand the effects of the mutations under study.

In the present review, we aimed at providing a tool for researchers working with mouse models of ASD by gathering data on the behavioral/physical consequences of mutations in genes associated with ASD (Fig. 1). We selected mouse models for mutations in proteins directly involved in synaptic structure, assembly, and stabilization such as the neuronal cell surface protein Neurexin1α (Nrxn1α), the neurologin family of synaptic cell adhesion proteins (Nlgn1, Nlgn2, Nlgn3, and Nlgn4), the postsynaptic scaffolding protein Shank1 and the neuronal recognition proteins involved in axonal growth and guidance of the contactin family (Cntn5 or Nb2, Cntn6 or Nb3). We also selected mice with mutations in proteins related to synaptic maturation/regulation such as the mRNA binding protein FmrP (*Fmr1* coding for FmrP), the Methyl CpG binding protein 2 (Mecp2). Mouse models with mutations in the ubiquitin ligase Ube3a regulating the turn over of synaptic proteins, the GTPase neurofibromin Nf1, the phosphatase tensin Pten influencing neuronal migration and neurite extension, as well as hamartin and tuberin encoded by *Tsc1* and *Tsc2* respectively and forming the tuberous sclerosis complex were also examined (Table I). We extended our review to all proteins within one family (i.e., Nlgn2, Shank1, Cntn5, and Cntn6; see Table I) even if these ones were not formerly associated with ASD.

Methods—Collection and Organization of the Material

The available literature was scanned using the Web of Knowledge database, with combinations of the following keywords: ''mouse,'' ''autism,'' ''behavior,'' ''vocalization,'' ''Fmr1,'' ''Mecp2,'' ''Nrxn1,'' ''Nlgn1,'' ''Nlgn2,'' ''Nlgn3,'' ''Nlgn4,'' ''Shank1,'' ''Nb2,'' ''Cntn5,'' ''Nb3,'' ''Cntn6,'' ''Ube3a,'' ''Duplication 15q11-13,'' ''Angelman syndrome,'' ''Nf1,'' ''Neurofibromatosis,'' ''Pten,'' ''Tsc1,'' ''Tsc2,'' ''Tuberous sclerosis.'' Results from the phenotypical characterization of the different mouse models were gathered in a table (see Supplementary Table I) according to the construction characteristics of the model (e.g., knock out, conditional knock out, knock in, duplication, deletion, and overexpression), the genetic background and the phenotypic traits studied (i.e., physical abilities and cognitive functions). We then summarized the phenotypic data in Figure 2 to present an overview of the different models and of the different aspects examined: physical disabilities (circadian rhythm, body weight, vision, olfaction, auditory startle, sensory-motor gating [prepulse inhibition], seizure propensity, pain sensitivity, locomotion/strength, motor learning, and stereotypes) and cognitive impairments (depression/anhedonia, anxiety, spatial learning/memory, reversal learning, fear conditioning, home cage/nesting behavior,

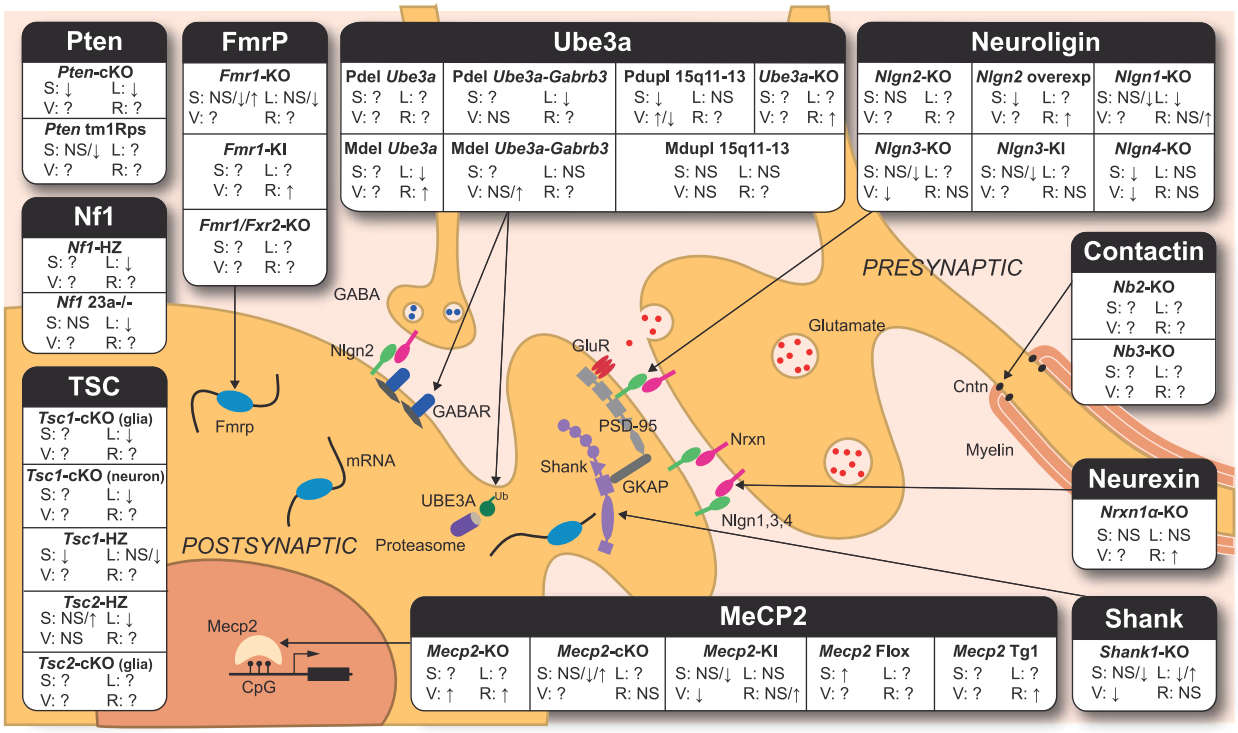

Figure 1. Location of the main proteins associated with autism spectrum disorders (ASD) and description of the behavioral deficits in the corresponding mouse models. S, social interactions; V, ultrasonic vocalizations (USVs) emission rate; L, learning; R, repetitive behaviors/stereotypes; ?, unknown; NS, nonsignificant difference; up arrow, significantly higher in mutants than in wild type animals; down arrow, significantly lower in mutants than in wild type animals.

social interactions, emission rate of USVs). For the numerous models carrying a mutation in *Fmr1*, we complemented a review published in 2006 [Bernardet & Crusio, 2006] with studies published subsequently [Dahlhaus & El-Husseini, 2010; Eadie et al., 2009; Spencer et al., 2006; Spencer, Graham, Yuva-Paylor, Nelson, Paylor, 2008; Zang et al., 2009]. For *Mecp2*, we selected studies which seemed us to be the most documented ones, and therefore we do not pretend to be exhaustive for this gene. These studies were also reviewed in De Filippis, Ricceri, and Laviola [2009] and Ricceri, de Filippis, and Laviola [2008]. We have selected models carrying mutations in *Tsc1*/*Tsc2* as previously published by Ehninger, de Vries, and Silva [2009].

In order to cluster the phenotypic categories and the mouse models, we have attributed scores to each trait studied in each model in the summary of data. When the trait was not examined in the model, score was 0. When the mutant mice did not differ significantly from wild type animals, score was 1. When the mutant mice differed significantly from wild type animals (either significantly higher and/or lower performances/scores in mutant than in wild type animals), score was 2. When differences between mutant and wild type mice were mixed (no significant difference and significantly higher and/or lower performances/scores in mutant than in wild type animals), score was 1.5. The heatmap function of the statistical software R [R Development Core Team, 2009] was used to re-order the rows (mouse models) and the columns (phenotypic traits) of the score matrix obtained according to the square distance (dissimilarity) between rows and columns. This function identifies which mouse models and which traits are the most similar through associated dendrograms for gene mutated (rows) and phenotype (columns).

Results—Behavior of ASD Mouse Models

Eight categories emerged from the clustering of phenotypic traits in the heatmap representation (Fig. 2). The first category (category A) included seizure. The second one (category B) regrouped two largely investigated cognitive functions reflecting learning (spatial learning/memory and fear conditioning). Category C was composed of body weight and two traits reflecting motor abilities (motor learning and locomotion/strength). The fourth category (category D) included the least investigated high cognitive functions (i.e., depression/anhedonia, reversal learning, and emission rate of USVs). Stereotypes represented the category E. Category F regrouped vision and olfaction with two rarely investigated traits (home cage and nesting behavior and circadian rhythms). Extensively studied high cognitive

Table I. Genes Associated With ASD in the Synaptic Pathway and the mTOR/PI3K Pathway

Gene	Location	Function	Evidence	Inheritance	Diagnosis	Reference
FMR1	Xq27.3	RNA-binding protein, synaptic translation regulation	Mutations	De novo (premutation)	ASD, FXS	Garber, Visootsak, and Warren [2008]
MECP2	Xq26	Methyl-CpG-binding protein, chromatin assembly/ remodelling, transcription regulation	CNV, mutations	De novo, inherited	ASD, RS	Amir et al. [1999]
TSC1	9q34.13	Hamartin, activation of mTOR/ PI3K pathway	CNV, mutations	De novo, inherited	ASD, TSC	Wiznitzer [2004]
TSC2	16q13.3	Tuberin, activation of mTOR/ PI3K pathway	CNV, mutations	De novo, inherited	ASD, TSC	Wiznitzer [2004]
NF1	17q11.2	Neurofibromin, regulation of tumor suppressor genes	CNV, mutations	De novo, inherited	ASD, NF	Rosser and Packer [2003]
PTEN	10q23.31	Phosphatase tensin, tumor suppressor, neuronal migration and neurite extension	CNV, mutations	De novo, inherited	ASD, CS	Butler et al. [2005]
UBE3A (Dupl 15q11-13)	15q11-13	Ubiquitin protein ligase, synaptic turn-over regulation	CNV	De novo, inherited	ASD	Glessner et al. [2009]
NRXN1	2p16.3	Synaptic CAM	CNV, mutations, SNP	De novo, inherited	ASD, SCZ, ID	Feng et al. [2006]; Kirov et al. [2008]; Szatmari et al. [2007]; Zweier et al. [2009]
NLGN1	3q26.3	Synaptic CAM	CNV, SNP	De novo, inherited	ASD	Glessner et al. [2009]
NLGN2	17p13.1	Synaptic CAM	–	–	–	–
NLGN3	Xq13.1	Synaptic CAM	Mutations	Inherited	ASD	Jamain et al. [2003]
NLGN4	Xp22.3	Synaptic CAM	CNV, mutations	De novo, inherited	ASD, ID, TS	Jamain et al. [2003]; Laumonnier et al. [2004]; Lawson-Yuen, Saldivar, Sommer, and Picker [2008]
SHANK1	19q13.3	Synaptic scaffold	–	–	–	–
SHANK2	11q13.3	Synaptic scaffold	CNV, mutations	De novo	ASD, ID	Berkel et al. [2010]; Pinto et al. [2010]
SHANK3 (del 22q13)	22q13	Synaptic scaffold	CNV, mutations	De novo, inherited	ASD, ID, SCZ	Durand et al. [2007]; Gauthier et al. [2010]; Moessner et al. [2007]
CNTN4	3p26.3	Synaptic CAM	CNV, translocations	De novo, inherited	ASD, ID	Glessner et al. [2009]; Fernandez et al. [2008]; Morrow et al. [2008]; Roohi et al. [2009]
CNTN5	11q22.1	Synaptic CAM	–	–	–	–
CNTN6	3p26.3	Synaptic CAM	–	–	–	–

CAM, cell adhesion molecule; CNV, copy number variant; SNP, single nucleotide polymorphism; ASD, autism spectrum disorder; SCZ, schizophrenia; ID, intellectual disability; FXS, fragile X syndrome; RS, Rett syndrome; TS, Tourette syndrome; TSC, tuberous sclerosis; NF, neurofibromatosis; CS, Cowden syndrome.

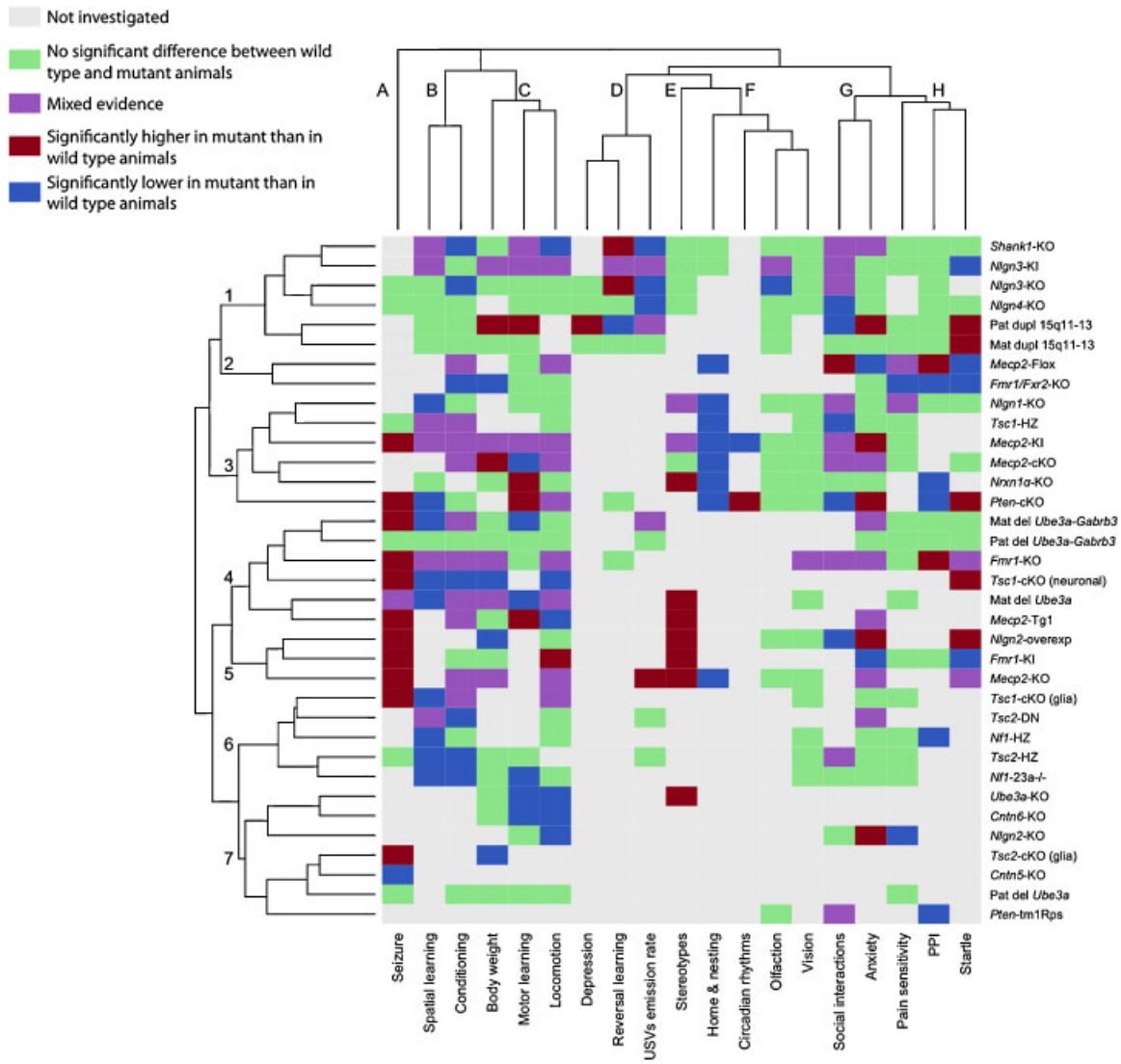

Figure 2. Heatmap representation of the clustering of mouse models for autism spectrum disorders and their phenotypic traits. The vertical dendrogram grouped mouse models according to their degree of similarity (*Nrxn1*α-KO [Etherton et al., 2009], *Nlgn1*-KO [Blundell et al., 2010], *Nlgn2*-KO [Blundell et al., 2009], *Nlgn2* overexp [brain overexpression; Hines et al., 2008], *Nlgn3*-KO [Radyushkin et al., 2009], *Nlgn3*-KI [R451C; Tabuchi et al., 2007; Chadman et al., 2008], *Nlgn4*-KO [Jamain et al., 2008], *Shank1*-KO [Hung et al., 2008; Roullet et al., 2010; Silverman et al., 2010; Roullet F., personal communication], *Cntn5*-KO [Li et al., 2003], *Cntn6*-KO [Takeda et al., 2003], Pat del *Ube3a* [paternal deletion of *Ube3a*; Jiang et al., 1998; Miura et al., 2002], Mat del *Ube3a* [maternal deletion of *Ube3a*; Heck et al., 2008; Jiang et al., 1998; Miura et al., 2002; van Woerden et al., 2007], *Ube3a*-KO [Heck et al., 2008], Pat del *Ube3a-Gabrb2* [paternal deletion of *Ube3a-Gabrb3*; Jiang et al., 2010], Mat del *Ube3a-Gabrb2* [maternal deletion of *Ube3a-Gabrb3*; Jiang et al., 2010], Pat Dupl 15q11-13 [paternal duplication of chromosome 15q11-13; Nakatani et al., 2009], Mat Dupl 15q11-13 [maternal duplication of chromosome 15q11-13; Nakatani et al., 2009], *Mecp2*-KO [deletion of exons 3 and 4; Chen et al., 2001; Guy et al., 2001; Pelka et al., 2006; Picker et al., 2006; Santos et al., 2007; Stearns et al., 2007], *Mecp2*-cKO [*Mecp2* conditional KO; Chen et al., 2001; Fyffe et al., 2008; Gemelli et al., 2006; Kerr et al., 2008], *Mecp2*-KI [truncated protein; de Filippis et al., 2010; Lawson-Yuen et al., 2007; McGill et al., 2006; Moretti et al., 2005, 2006; Shahbazian et al., 2002], $Mecp2^{Flox}$ [50% expression decrease; Samaco et al., 2008], $Mecp2^{Tg1}$ [2-fold overexpression; Collins et al., 2004], *Fmr1*-KI [I304N; Zang et al., 2009], *Fmr1*-KO [Bernardet & Crusio, 2006; Dahlhaus & El-Husseini, 2010; Eadie et al., 2009; Spencer et al., 2008], *Fmr1/Fxr2*-KO [double KO; Spencer et al., 2006], *Pten*-cKO [*Pten* conditional KO; Backman et al., 2001; Kwon et al., 2006; Ogawa et al., 2007; Zhou et al., 2009], $Pten^{tm1Rps}$ [deletion of exon 5 in *Pten*; Page et al., 2009], *Nf1*-HZ [*Nf1* heterozygous; Li et al., 2005; Silva et al., 1997], $Nf1^{23a-/-}$ [deletion of exon 23a in *Nf1*; Costa et al., 2001], *Tsc1*-cKO (glia) [conditional KO in glial cells; Erbayat-Altay et al., 2007; Uhlman et al., 2002; Zeng et al., 2007], *Tsc1*-HZ [*Tsc1* heterozygous; Goorden et al., 2007], *Tsc1*-cKO (neurons) [conditional KO in neuronal cells; Ehninger et al., 2008a,b; Meikle et al., 2007], *Tsc2*-DN [Ehninger & Silva, 2010], *Tsc2*-HZ [*Tsc2* heterozygous; Ehninger et al., 2002; Young et al., 2010], *Tsc2*-cKO (glia) [conditional KO in glial cells; Way et al., 2009]). The horizontal dendrogram grouped phenotypic traits according to their degree of similarity. Grey: trait not investigated; green: no significant difference between wild type and mutant mice; purple: mixed evidence (i.e., no significant difference and significantly higher and/or lower than wild type littermates); red: significantly higher in mutant mice than in wild type mice; blue: significantly lower in mutant mice than in wild type mice.

functions (social interactions and anxiety) composed the category G. Finally, category H included traits reflecting responses to stressful stimuli (pain sensitivity, sensory motor gaiting, and startle response).

In the mutated gene clustering, mouse models were distributed into two large groups, each composed of three and four subgroups. In the first group, the first subgroup (subgroup 1) included *Shank1*-KO, *Nlgn3*-KI, *Nlgn3*-KO, *Nlgn4*-KO, Pat dupl 15q11-13, and Mat dupl 15q11-13. These models were the most extensively studied. Mutant animals showed lower levels of social interactions and lower call rate than wild type animals. *Shank1*-KO presented increased anxiety, decreased locomotion, and remarkably, enhanced working memory, but decreased long-term memory [Hung et al., 2008; Silverman et al., 2010]. Mat dupl 15q11-13 appeared to be slightly apart from the other models in this subgroup since only a higher startle response was recorded in mutants in comparison to wild type animals. The models *Mecp2*^{Flox} and *Fmr1/Fxr2*-KO constituted the second subgroup (subgroup 2). They presented lower performances compared with wild type animals in fear conditioning and abnormal responses to stressful stimuli (categories B and H). Categories A, D, E, and F were not studied in these models. Subgroup 3 was composed of *Nlgn1*-KO, *Tsc1*-HZ, *Mecp2*-KI, *Mecp2*-cKO, *Nrxn1*α-KO, and *Pten*-cKO. These models presented locomotor impairments (category C) and generalized deficits in home cage and nesting behavior, but no impairments in vision and olfaction (category F). The investigation of high cognitive functions (categories B and G) revealed mostly lower performances in mutants than in wild type animals for spatial learning/memory, fear conditioning, and social interactions, as well as more anxiety in mutants in comparison to wild type animals. Vocal communication, depression/anhedonia, and reversal learning (category D) were not characterized in this subgroup.

The second group included four subgroups. The first one (subgroup 4) included models for Mat del *Ube3a-Gabrb3*, Pat del *Ube3a-Gabrb3*, *Fmr1*-KO, *Tsc1*-cKO (neurons), Mat del *Ube3a*, and *Mecp2*^{Tg1}. These models presented a higher seizure propensity (category A), reduced abilities in spatial learning and memory, and in fear conditioning (mostly in the context-dependent hippocampus-related task; category B), and abnormalities in body weight and locomotion (category C). Subgroup 5 was composed of the models *Nlgn2* overexpression, *Fmr1*-KI, *Mecp2*-KO. These models presented seizure propensity and stereotypes frequency higher than wild type animals (categories A and E), as well as abnormal body weight and locomotion (category C), anxiety (category G), and response to stressful stimuli (category H). Spatial learning and memory were not studied in these models, and the categories D and F were rarely investigated. *Tsc1*-cKO (glia), *Tsc2*-DN, *Nf1*-HZ, *Tsc2*-HZ, and *Nf1*$^{23a-/-}$ constituted the subgroup 6. These models presented generalized deficits in learning (spatial learning and memory and fear conditioning; category B), but impairments in anxiety (category G) and in reaction to stressful stimuli (category H) were limited. Categories A, D, E, and F were not extensively studied in this subgroup 6. The last subgroup (subgroup 7) grouped together the least studied mouse models (*Ube3a*-KO, *Cntn6*-KO, *Nlgn2*-KO, *Tsc2*-cKO (glia), *Cntn5*-KO, Pat del *Ube3a*, and *Pten*^{tm1Rps}). The first three of them presented locomotion impairments. However, this subgroup cannot be examined further given the large amount of missing data.

Discussion

Our review highlights the behavioral diversity of the mouse models of ASD. Some trends emerged despite the large amount of missing data. *Nlgn3*-KO, *Nlgn4*-KO, *Nlgn3*- R451C KI, *Shank1*-KO, paternal and maternal duplication 15q11-13 models displayed similar abnormal social and vocal behaviors despite normal to enhanced learning. *Mecp2*^{Flox} and *Fmr1/Fxr2*-KO were impaired in fear conditioning and in their reactions to stressful stimuli. *Nlgn1*-KO, *Tsc1*-HZ, *Mecp2*-KI, *Mecp2*-cKO, *Nrxn1*α-KO, and *Pten*-cKO were impaired in their home cage and nesting behavior, social behavior, and reaction to stressful stimuli. Maternal and paternal deletion of *Ube3a-Gabrb3*, *Fmr1*-KO, *Tsc1*-cKO (neurons), Mat del *Ube3a*, and *Mecp2*^{Tg1}, as well as *Nlgn2* overexpression, *Fmr1*-KI, and *Mecp2*-KO showed increased seizure propensity, deficits in spatial learning, memory and fear conditioning, abnormal locomotion, and response to stressful stimuli and more stereotypic behaviors compared with wild type animals. *Tsc1*-cKO (glia), *Tsc2*-DN, *Nf1*-HZ, *Tsc2*-HZ, *Nf1*$^{23a-/-}$ performed worse than wild type animals in spatial learning and fear conditioning, but presented no or few abnormalities in vision, anxiety, and pain sensitivity. *Ube3a*-KO, *Cntn6*-KO, *Nlgn2*-KO, *Tsc2*-cKO (glia), *Cntn5*-KO, Pat del *Ube3a*, and *Pten*^{tm1Rps} were the least investigated models.

Data on mouse models of ASD appeared to be scattered with a large amount of missing data. Given the large panel of phenotypic traits reviewed, many of them were not examined in each study. Gaps in phenotypic description might lead to bias, such as for the subgroup 7 of mouse models in Figure 2. This subgroup reflects the least investigated mouse models of ASD. In contrast, the subgroup 1 included models studied at the furthest detail levels. It was then easier to describe this subgroup in comparison to the less investigated ones. Complementing data might reshape the clustering of the different models and might lead to different clusters. There might be another type of bias. Studies highlighting only minor (or nonsignificant) impairments in a mouse model might

have more difficulties to get published than studies highlighting major deficits (especially in social interactions, learning, stereotypic behaviors, and anxiety for mouse models of ASD). These restrictions should be kept in mind when interpreting results from this review.

Given that ASD is a developmental disorder with symptoms emerging in children before three years of age, developmental studies of mouse models should provide information to better understand the emergence of symptoms in mice. Such studies were initiated for *Mecp2* mouse models [reviewed in De Filippis et al., 2009], deletion of *Ube3a-Gabrb3* [Jiang et al., 2010], and duplication 15q11-13 [Nakatani et al., 2009] and *Tsc2* heterozygous [Young, Schenk, Yang, Jan, & Jan, 2010] mouse models for pup isolation calls, but they are lacking for several models such as *Nlgn4*-KO, *Shank1*-KO, and *Fmr1* mutants. It would be of the highest importance to examine developmental aspects in mouse models of ASD to understand the emergence of the disorder. In addition, compensation for the lack of one protein could reduce phenotypic impairment in adult mice. Studying pup development might thus provide data that reflect more reliably what happens in patients carrying mutations. Studying both sexes might also be of interest, given the unbalanced sex ratio in ASD (e.g., four males for one female). Many studies concentrated only on male mice, ignoring females. The larger variability in females should not prevent investigations on them, especially for models of diseases like Rett syndrome.

The heatmap representation highlighted similarities and differences between mouse models and phenotypic traits. It grouped together high cognitive functions (categories B, D, and G), despite that only few studies have examined vocal communication and reversal learning. The cluster of anxiety with social interactions suggests the existence of a confounding factor. Very anxious animals might fear contact with unknown conspecifics as well as exploring and moving in any kind of environment, as suggested by Silverman et al. [2010]. Pain sensitivity, sensory-motor gating, and startle were included within one category. These traits are linked to the ability to respond to stressful stimuli, and belong to more basal functions. These abnormalities were observed mostly in models for syndromic ASD in subgroups 2 and 5. They are reminiscent of impairments in perception and sensitivity in some patients with ASD [Gillberg & Coleman, 2000; Rogers & Ozonoff, 2005]. Similarly, subgroups 3, 4, and 5 included mostly models which presented higher or lower anxiety than wild type animals. This characteristic is also reminiscent of high anxiety in a majority of patients with ASD [Bellini, 2004; Gillott, Furniss, & Walter, 2001]. Subgroup 1 (*Nlgn3*-KO, *Nlgn4*-KO, *Nlgn3*-KI, *Shank1*-KO, maternal and paternal duplication 15q11-13) included mice mutated in synaptic proteins, which might be directly related to synaptic plasticity. Remarkably, social interaction and vocalizations were impaired, but learning capacities were normal (*Nlgn3*-KO, *Nlgn4*-KO, Pat dupl 15q11-13) or higher (*Nlgn3*-R451C KI) compared to their wild type littermates. This impairment in social communication together with preserved or enhanced learning is reminiscent of the patients with autism with normal to elevated IQ (i.e., high-functioning autism or Asperger syndrome). In contrast, these mice showed normal to enhanced behavioral flexibility (as observed in the reversal learning paradigm, except for Pat dupl 15q11-13). This is not what it is observed in patients with ASD who usually have very little flexibility in their behavior [e.g., Green et al., 2007; but see Geurts, Corbett, & Solomon, 2009]. Remarkably, mouse models carrying mutations within the mTOR/PI3K pathway (Pten, Nf1, Tsc1, Tsc2) appear generally more impaired in spatial learning/memory and fear conditioning compared with mouse models carrying mutations within the synaptic pathway (see Supplementary Table II). This observation is consistent with a regulatory role of the mTOR/PI3K upstream of the synaptic pathway. Additionally, mice carrying mutations in proteins which have not been directly associated with ASD yet (i.e., Nlgn2, Shank1, Cntn5, and Cntn6; see Table I) are not grouped together and present similar abnormalities as mice carrying mutations in proteins directly associated with ASD. This suggests that, in these mouse models, proteins associated with ASD and proteins related to them have similar behavioral effects.

This review also highlights the diversity of the tests used to characterize the behaviors of the different models. For instance, to test anxiety, each laboratory used one or several tests among the elevated plus maze [e.g., Kulkarni & Sharma, 1991; Lister, 1987], the dark light box [e.g., Bourin & Hascoët, 2003; Hascoët, Bourin, & Nic Dhonnchadha, 2001], and the open field [e.g., Gould, Dao, & Kovacsics, 2009]. Another example is the large panel of tests used to examine social behavior: intruder-resident (adult/juvenile intruder and home cage/new environment), partition test, and three-chamber test (see Supplementary Table I). This diversity in tests most likely increases the variability of the results and therefore leads to difficulties in the interpretation. Therefore, as suggested by Crawley [2007], it should be very useful to determine a minimum set of tests (possibly among the ones described in Silverman et al. [2010]), which have to be conducted on the different mouse models. To further reduce the effects of potential confounding factors, this set of tests might include a reduced number of paradigms, but with a strict methodology shared by all laboratories. Phenotypic traits such as social interactions, vocal communication, anxiety, learning, and reversal should be examined with homogenized methods to fill the gaps indicated in this study.

In addition to standardized methods, mouse behavior should be examined with qualitative evaluation. For instance, only three studies examined the acoustic structure of USVs [Chadman et al., 2008; Jamain et al., 2008; Young et al., 2010]. No difference was observed for

Nlgn3-KO and *Nlgn4*-KO despite their lower call rate or for *Tsc2*-HZ showing no difference in call rate. However, in the *Tsc2*-HZ mouse model, the genotype of the mother greatly influenced the usage and the median frequency of pup isolation calls [Young et al., 2010]. It was shown that specific genes or genetic backgrounds could greatly influence the acoustic structure of USVs [Enard et al., 2009; Scattoni, Gandhy, Ricceri, & Crawley, 2008] Finer analyses of mouse calls might therefore reveal subtle effects of mutations in the acoustic structure of the vocalizations. Similarly, finer description of mouse behavior in same-sex free social interactions should be of interest to unravel subtle behavioral characteristics. Automation of data analyses has begun through the set up of video tracking of interacting mice. Given the progress of this technique, we might be very close to ''nose+whiskers tracking'' in mice, as a parallel to ''eye-tracking'' in humans. Indeed, olfaction and tactile perception are probably more important than vision in mouse sensory perception [Latham & Mason, 2004].

Another point highlighted in this review is the variability of the phenotypic description of apparently similar animal models. One example is the *Fmr1* mutant mice. A large number of studies have already been conducted [reviewed in Bernardet & Crusio, 2006; Dahlhaus & El-Husseini, 2010; Eadie et al., 2009; Spencer et al., 2008]. Bernardet and Crusio [2006] highlighted this variability according to the genetic background of the mice used as models (see Supplementary Table I). Different genetic backgrounds might therefore also explain the different results found by Tabuchi et al. [2007] and Chadman et al. [2008] on *Nlgn3*-KI (R451C) model and by Shahbazian et al. [2002] and Moretti, Bouwknecht, Teague, Paylor, and Zoghbi [2005] on *Mecp2*$^{308/Y}$ model. Maternal or paternal inheritance of mutation might also be taken into account, when examining heterozygous mouse models [Nakatani et al., 2009]. Environmental parameters might also play a crucial role since many of these models are sensitive to stressful stimuli or present abnormalities in anxiety levels. However, how and to what extent environmental and genetic factors interact remains largely unknown. Further studies are needed to assess the response to environmental factors in the different mouse models of ASD, and will provide useful information on the possibility to reverse phenotypic traits in each model.

Some of these studies have already been started [see reviews of Ehninger et al., 2008a; Ehninger, Li, Fox, Stryker, & Silva, 2008b; Silva & Ehninger, 2009]. They investigated the way of reversing the phenotypes of mouse models mostly for Fragile X syndrome [Paylor, Yuva-Paylor, Nelson, & Spencer, 2008; Restivo et al., 2005] and Rett syndrome [reviewed in de Filippis et al., 2009; Ricceri et al., 2008]. One possibility is to modify gene expression in the whole organism or in specific anatomical regions. For instance, using a *Mecp2* mouse model, Guy, Gan, Selfridge, Cobb, and Bird [2007] could demonstrate robust phenotypic reversal, as re-activation of Mecp2 expression leads to striking loss of advanced neurological symptoms in both immature and mature adult animals. Traits related to weight, locomotion, and lifespan were the most reversible ones [Guy et al., 2007; Ricceri et al., 2008]. Using a different approach, Paylor et al. [2008] expressed the human FmrP protein in *Fmr1*-KO mice. They reported reversal of startle reaction and sensory-motor gaiting (pre-pulse inhibition) similar to wild type animals. Another possibility to reverse symptoms is to modulate other proteins within the same pathway. A mouse with maternal deletion of *Ube3a* was crossed with a mutant without inhibitory phosphorylation of the calcium/calmodulin-dependent kinase type 2 (aCaMKII). This double mutant recovered for motor and spatial learning, fear conditioning, and seizure propensity [van Woerden et al., 2007]. An overexpression of the postsynaptic cell adhesion molecule neuroligin 1 was also set in Fmr1-KO model. It triggered recovery in locomotion, anxiety, and social interactions, but not in spatial learning and memory [Dahlhaus & El-Husseini, 2010].

Another possibility is to reverse phenotypes through the administration of pharmacological molecules (e.g., an anti-depressant drug [desipramine], an activator of glutamatergic AMPA levels [ampakines], a mTOR inhibitor [rapamycin]) or non-pharmacological (e.g., perinatal supplementation with choline) substances. Such approaches were tested in *Mecp2* models and triggered improvement in lifespan, respiratory function, and motor coordination [see Ricceri et al., 2008]. Rapamycin, a mTOR inhibitor, was injected in *Tsc1* homozygous conditional KO in neurons [Ehninger et al., 2008a,b] and in a *Pten*-cKO mouse model [Zhou et al., 2009]. The two studies reported respectively reversal of impairments in fear conditioning, spatial learning, locomotion, and brain weight [Ehninger et al., 2008a,b], and reversal of anxiety level, locomotion, social interactions, and seizure duration [Zhou et al., 2009]. Therapeutic approaches were mostly attempted in mouse models for syndromic ASD, since these ones were available a decade ago. In these models, some traits such as body weight and locomotion seemed to be most often reversible [Silverman et al., 2010]. In addition, Blundell et al. [2010] demonstrated a reversal of the increased self-grooming behavior of their mouse models *Nlgn1*-KO through administration of the NMDA receptor partial coagonist D-cycloserine. However, an attempt of reversal through administration of standard anti-epileptic drugs (phenobarbital or phenytoin) decreased seizure frequency, but did not prevent the animals' death within one month in a *Tsc1*-cKO (glia) mouse model [Erbayat-Altay, Zeng, Xu, Gutmann, & Wong, 2007].

Finally, phenotype reversal might be achieved through housing mutant mice together with wild type mice in an

enriched environment, with social (group housing), tactile (objects with various textures), olfactory (odors regularly alternated), visual (objects with different colors), auditory (e.g., bells), and locomotor (running pathways changed regularly, running wheel) stimuli. Comparisons can then be done between mutants and wild type within the enriched environment, but also between enriched and standard environment. A model for Fragile X syndrome (*Fmr1*-KO) was tested in such conditions. Enriched environment could reduce the anxiety levels and the habituation to novel objects was restored. However, hyperactivity was not reversed [Restivo et al., 2005]. In models for Rett syndrome (*Mecp2* mutants), enriched environment could restore locomotion abilities [reviewed in Ricceri et al., 2008]. More generally, Portfors [2007] reported a larger diversity in the vocal repertoire of mice in an enriched environment compared with those of mice that lived in a standard one. Therefore, environmental enrichment might be a way to restore the vocal behavior of mouse models for ASD.

These recent studies investigating therapeutic approaches represent a fascinating field of research, and the diversity of the processes as well as the preliminary results are promising. Moreover, in contrast to the homozygote mouse models usually investigated, patients with ASD are usually heterozygous for the mutations. Therefore, the characterization of heterozygous mouse models should not be neglected and therapeutic approaches aiming at over-stimulating the expression of the remaining copy could be a relevant mean to decrease the severity of autistic features in humans.

In summary, the phenotypic diversity of mouse models for ASD mirrors many aspects of the diversity of symptoms in patients. The possibility to partly reverse some behavioral abnormalities by modifying the genetic background and/or the environment suggests that additional genetic/epigenetic/environmental factors are important in the susceptibility to ASD. Future studies should concentrate on characterizing mouse models with finer analyses and paradigms to better understand the nature of the biological mechanisms underlying ASD and to provide insight for new treatments.

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References

Abrahams, B.S., & Geschwind, D.H. (2008). Advances in autism genetics: On the threshold of a new neurobiology. Nature Reviews Genetic, 9, 341–355.

Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., & Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature Genetics, 23, 185–188.

Bellini, S. (2004). Social skill deficits and anxiety in high-functioning adolescents with autism spectrum disorders. Focus on Autism and Other Developmental Disabilities, 19, 78–86.

Berkel, S., Marshall, C.R., Weiss, B., Howe, J., Roeth, R., et al. (2010). Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nature Genetics, 42, 489–491.

Bernardet, M., & Crusio, W.E. (2006). Fmr1 KO mice as a possible model of autistic features. The Scientific World Journal, 6, 1164–1176.

Berry, R.J. (1981). Population dynamics of the house mouse. Symposium of the Zoological Society of London, 47, 395–425.

Blundell, J., Tabuchi, K., Bolliger, M.F., Blaiss, C.A., Brose, N., et al. (2009). Increased anxiety-like behavior in mice lacking the inhibitory synapse cell adhesion molecule neuroligin 2. Genes, Brain and Behavior, 8, 114–126.

Blundell, J., Blaiss, C.A., Etherton, M.R., Espinosa, F., Tabuchi, K., et al. (2010). Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. The Journal of Neuroscience, 30, 2115–2129.

Bourgeron, T. (2009). A synaptic trek to autism. Current Opinion in Neurobiology, 19, 231–234.

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. European Journal of Pharmacology, 463, 55–65.

Brennan, P.A., & Kendrick, K.M. (2006). Mammalian social odours: Attraction and individual recognition. Philosophical Transactions of the Royal Society B: Biological Sciences, 361, 2061–2078.

Butler, M.G., Dasouki, M.J., Zhou, X.-P., Talebizadeh, Z., Brown, M., et al. (2005). Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. Journal of Medical Genetics, 42, 318–321.

Chadman, K.K., Gong, S., Scattoni, M.L., Boltuck, S.E., Gandhy, S.U., et al. (2008). Minimal aberrant behavioral phenotypes of neuroligin-3 R451C knockin mice. Autism Research, 1, 147–158.

Chen, R.Z., Akbarian, S., Tudor, M., & Jaenisch, R. (2001). Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. Nature Genetics, 27, 327–331.

Collins, A.L., Levenson, J.M., Vilaythong, A.P., Richman, R., Armstrong, D.L., et al. (2004). Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. Human Molecular Genetics, 13, 2679–2689.

Costa, R.M., Yang, T., Huynh, D.P., Pulst, S.M., Viskochil, D.H., et al. (2001). Learning deficits, but normal development and tumor predisposition, in mice lacking exon 23a of Nf1. Nature Genetics, 27, 399–405.

Crawley, J.N. (2007). Mouse behavioral assays relevant to the symptoms of autism. Symposium: Neurobiology of Autism. Brain Pathology, 17, 448–459.

Dahlhaus, R., & El-Husseini, A. (2010). Altered neuroligin expression is involved in social deficits in a mouse model of the fragile X syndrome. Behavioral Brain Research, 208, 96–105.

De Filippis, B., Ricceri, L., & Laviola, G. (2009). Investigating Rett syndrome through genetic mouse models: Presymptomatic,

clearly symptomatic phases, and innovative therapeutic approaches. In: Kalueff AV, Bergner CL, editors. Transgenic and mutant tools to model brain disorders, Neuromethods 44. New York: Humana Press, pp 151–178.

Durand, C.M., Betancur, C., Boeckers, T.M., Bockmann, J., Chaste, P., et al. (2007). Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nature Genetics, 39, 25–27.

Eadie, B.D., Zhang, W.N., Boehme, F., Gil-Mohapel, J., Kainer, L., et al. (2009). Fmr1 knockout mice show reduced anxiety and alterations in neurogenesis that are specific to the ventral dentate gyrus. Neurobiology of Disease, 36, 361–373.

Ehninger, D., & Silva, A.J. (2010). Increased levels of anxiety-related behaviors in a Tsc2 dominant negative transgenic mouse model of tuberous sclerosis. Behavioral Genetics. DOI 10.1007/s10519-010-9398-1.

Ehninger, D., Han, S., Shilyansky, C., Zhou, Y., Li, W., et al. (2008a). Reversal of learning deficits in a *Tsc2*$^{+/-}$ mouse model of tuberous sclerosis. Nature Medicine, 14, 843–848.

Ehninger, D., Li, W., Fox, K., Stryker, M.P., & Silva, A.J. (2008b). Reversing neurodevelopmental disorders in adults. Neuron, 60, 950–960.

Ehninger, D., de Vries, P.J., & Silva, A.J. (2009). From mTOR to cognition: Molecular and cellular mechanisms of cognitive impairments in tuberous sclerosis. Journal of Intellectual Disability Research, 53, 838–851.

Enard, W., Gehre, S., Hammerschmidt, K., Hölter, S.M., Blass, T., et al. (2009). A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. Cell, 137, 961–971.

Erbayat-Altay, E., Zeng, L.-H., Xu, L., Gutmann, D.H., & Wong, M. (2007). The natural history and treatment of epilepsy in a murine model of tuberous sclerosis. Epilepsia, 48, 1470–1476.

Etherton, M.R., Blaiss, C.A., Powell, C.M., & Südhof, T.C. (2009). Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proceedings of the National Academy of Science, 106, 17998–18003.

Feng, J., Schroer, R., Yan, J., Song, W., Yang, C., et al. (2006). High frequency of neurexin 1beta signal peptide structural variants in patients with autism. Neuroscience Letters, 409, 10–13.

Fernandez, T., Morgan, T., Davis, N., Klin, A., Morris, A., et al. (2008). Disruption of Contactin 4 (CNTN4) results in developmental delay and other features of 3p deletion syndrome. American Journal of Human Genetics, 82, 1385.

Fernell, E., & Gillberg, C. (2010). Autism spectrum disorder diagnoses in Stockholm preschoolers. Research in Developmental Disabilities, 31, 680–685.

Freitag, C.M. (2007). The genetics of autistic disorders and its clinical relevance: A review of the literature. Molecular Psychiatry, 12, 2–22.

Fyffe, S.L., Neul, J.L., Samaco, R.C., Chao, H.-T., Ben-Shachar, S., et al. (2008). Deletion of Mecp2 in Sim1-expressing neurons reveals a critical role for MeCP2 in feeding behavior, aggression, and the response to stress. Neuron, 59, 947–958.

Garber, K.B., Visootsak, J., & Warren, S.T. (2008). Fragile X syndrome. European Journal of Human Genetics, 16, 666–672.

Gauthier, J., Champagne, N., Lafrenière, R.G., Xiong, L., Spiegelman, D., et al. (2010). De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia. Proceedings of the National Academy of Sciences, 107, 7863–7868.

Gemelli, T., Berton, O., Nelson, E.D., Perrotti, L.I., Jaenisch, R., & Monteggia, L.M. (2006). Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. Biological Psychiatry, 59, 468–476.

Geurts, H.M., Corbett, B., & Solomon, M. (2009). The paradox of cognitive flexibility in autism. Trends in Cognitive Sciences, 13, 74–82.

Gillberg, C., & Coleman, M. (2000). The biology of the autistic syndromes. Cambridge: Cambridge University Press, 330p.

Gillott, A., Furniss, F., & Walter, A. (2001). Anxiety in high-functioning children with autism. Autism, 5, 277–286.

Glessner, J.T., Wang, K., Cai, G., Korvatska, O., Kim, C.E., et al. (2009). Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature, 459, 569–573.

Goorden, S.M.I., van Woerden, G.M., van der Weerd, L., Cheadle, J.P., & Elgersma, Y. (2007). Cognitive deficits in *Tsc1*$^{-/-}$ mice in the absence of cerebral lesions and seizures. Annals of Neurology, 42, 648–655.

Gould, T.D., Dao, D.T., & Kovacsics, C.E. (2009). The open field test. In: Gould TD, editor. Mood and anxiety related phenotypes in mice, Series: Neuromethods 42. New York: Humana Press, pp 1–20.

Green, V.A., Sigafoos, J., O'Reilly, M., Pituch, K.A., Didden, R., et al. (2007). Behavioral flexibility in individuals with autism: Theory, assessment, and intervention. In: Zhao LB, editor. Autism research advances. New York: Nova Science Publishers, Inc., pp 63–77.

Guy, J., Hendrich, B., Holmes, M., Martin, J.E., & Bird, A. (2001). A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nature Genetics, 27, 322–326.

Guy, J., Gan, J., Selfridge, J., Cobb, S., & Bird, A. (2007). Reversal of neurological defects in a mouse model of Rett syndrome. Science, 315, 1143–1147.

Hascoët, M., Bourin, M., & Nic Dhonnchadha, B. (2001). The mouse light-dark paradigm: A review. Program Neuro-Psychopharmacological and Biological Psychiatry, 25, 141–166.

Heck, D.H., Zhao, Y., Roy, S., LeDoux, M.S., & Reiter, L.T. (2008). Analysis of cerebellar function in *Ube3a*-deficient mice reveals novel genotype-specific behaviors. Human Molecular Genetics, 17, 2181–2189.

Hines, R.M., Wu, L.J., Hines, D.J., Steenland, H., Mansour, S., et al. (2008). Synaptic imbalance, stereotypies, and impaired social interactions in mice with altered neuroligin 2 expression. Journal of Neuroscience, 28, 6055–6067.

Hung, A.Y., Futai, K., Sala, C., Valtschanoff, J.G., Ryu, J., et al. (2008). Smaller dendritic spines, weaker synaptic transmission, but enhanced spatial learning in mice lacking Shank1. The Journal of Neuroscience, 28, 1697–1708.

Jamain, S., Quach, H., Betancur, C., Råstam, M., Colineaux, C., et al. (2003). Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nature Genetics, 34, 27–29.

Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., et al. (2008). Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. Proceedings of the National Academy of Science of the United States of America, 105, 1710–1715.

Jiang, Y.-H., Armstrong, D., Albrecht, U., Atkins, C.M., Noebels, J.L., et al. (1998). Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. Neuron, 21, 799–811.

Jiang, Y.H., Pan, Y., Zhu, L., Landa, L., Yoo, J., et al. (2010). Altered ultrasonic vocalization and impaired learning and memory in Angelman Syndrome mouse model with a large maternal deletion from *Ube3a* to *Gabrb3*. PLoS One, 5, 1–14.

Kalcounis-Rueppel, M.C., Metheny, J.D., & Vonhof, M.J. (2006). Production of ultrasonic vocalizations by *Peromyscus* mice in the wild. Frontiers in Zoology, 3, 3–14.

Kalcounis-Rueppel, M.C., Petric, R., Briggs, J.R., Carney, C., Marshall, M.M., et al. (2010). Differences in ultrasonic vocalizations between wild and laboratory California mice (*Peromyscus californicus*). PLoS ONE, 5, e9705.

Kelleher, III, R.J., & Bear, M.F. (2008). The autistic neuron: Troubled translation? Cell, 135, 401–406.

Kerr, B., Alvarez-Saavedra, M., Saez, M.A., Saona, A., & Young, J.I. (2008). Defective body-weight regulation, motor control and abnormal social interactions in Mecp2 hypomorphic mice. Human Molecular Genetics, 17, 1707–1717.

Kirov, G., Gumus, D., Chen, W., Norton, N., Georgieva, L., et al. (2008). Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. Human Molecular Genetics, 17, 458–465.

Kulkarni, S.K., & Sharma, A.C. (1991). Elevated plus-maze—A novel psychobehavioral tool to measure anxiety in rodents. Methods and Findings in Experimental and Clinical Pharmacological, 13, 573–577.

Kwon, C.-H., Luikart, B.W., Powell, C.M., Zhou, J., Matheny, S.A., et al. (2006). Pten regulates neuronal arborization and social interaction in mice. Neuron, 50, 377–388.

Latham, N., & Mason, G. (2004). From house mouse to mouse house: The behavioural biology of free-living *Mus musculus* and its implication in the laboratory. Applied Animal Behaviour Science, 86, 261–289.

Laumonnier, F., Bonnet-Brilhault, F., Gomot, M., Blanc, R., & David, A., (2004). X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. American Journal of Human Genetics, 74, 552–557.

Lawson-Yuen, A., Liu, D., Han, L., Jiang, Z.I., Tsai, G.E., et al. (2007). Ube3a mRNA and protein expression are not decreased in Mecp2R168X mutant mice. Brain Research, 1180, 1–6.

Lawson-Yuen, A., Saldivar, J.S., Sommer, S., & Picker, J. (2008). Familial deletion within NLGN4 associated with autism and Tourette syndrome. European Journal of Human Genetics, 16, 614–618.

Li, H., Takeda, Y., Niki, H., Ogawa, J., Kobayashi, S., et al. (2003). Aberrant responses to acoustic stimuli in mice deficient for neural recognition molecule NB-2. European Journal of Neuroscience, 17, 929–936.

Li, W., Cui, Y., Kushner, S.A., Brown, R.A.M., Jentsch, J.D., et al. (2005). The HMG-CoA reductase inhibitor lovastatin reverses the learning and attention deficits in a mouse model of neurofibromatosis type 1. Current Biology, 15, 1961–1967.

Lister, R.G. (1987). The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology, 92, 180–185.

Marshall, C.R., Noor, A., Vincent, J.B., Lionel, A.C., Feuk, L., et al. (2008). Structural variation of chromosomes in autism spectrum disorder. American Journal of Human Genetics, 82, 477–488.

McGill, B.E., Bundle, S.F., Yaylaoglu, M.B., Carson, J.P., Thaller, C., & Zoghbi, H.Y. (2006). Enhanced anxiety and stress-induced corticosterone release are associated with increased Crh expression in a mouse model of Rett syndrome. Proceedings of the National Academy of Science, 103, 18267–18272.

Meikle, L., Talos, D.M., Onda, H., Pollizzi, K., Rotenberg, A., et al. (2007). A mouse model of tuberous sclerosis: Neuronal loss of *Tsc1* causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival. The Journal of Neuroscience, 27, 5546–5558.

Moessner, R., Marshall, C.R., Sutcliffe, J.S., Skaug, J., Pinto, D., et al. (2007). Contribution of SHANK3 mutations to autism spectrum disorder. American Journal of Human Genetics, 81, 1289–1297.

Moretti, P., Bouwknecht, J.A., Teague, R., Paylor, R., & Zoghbi, H.Y. (2005). Abnormalities of social interactions and home-cage behavior in a mouse model of Rett syndrome. Human Molecular Genetics, 14, 205–220.

Moretti, P., Levenson, J.M., Battaglia, F., Atkinson, R., Teague, R., et al. (2006). Learning and memory and synaptic plasticity are impaired in a mouse model of Rett syndrome. The Journal of Neuroscience, 26, 319–327.

Morrow, E.M., Yoo, S.Y., Flavell, S.W., Kim, T.K., Lin, Y., et al. (2008). Identifying autism loci and genes by tracing recent shared ancestry. Science, 321, 218–223.

Musolf, K., Hoffman, F., & Penn, D.J. (2010). Ultrasonic courtship vocalizations in wild house mice, *Mus musculus musculus*. Animal Behaviour, 79, 757–764.

Nakatani, J., Tamada, K., Hatanaka, F., Ise, S., Ohta, H., et al. (2009). Abnormal behavior in a chromosome-engineered mouse model for human 15q11-13 duplication seen in autism. Cell, 137, 1235–1246.

Ogawa, S., Kwon, C.-H., Zhou, J., Koovakkattu, D., Parada, L.F., & Sinton, C.M. (2007). A seizure-prone phenotype is associated with altered free-running rhythm in *Pten* mutant mice. Brain Research, 1168, 112–123.

Page, D.T., Kuti, O.J., Prestia, C., & Sur, M. (2009). Haploinsufficiency for Pten and serotonin transporter cooperatively influences brain size and social behavior. Proceedings of the National Academy of Science of the United States of America, 106, 1989–1994.

Palanza, P., Della Stella, D., Ferrari, P.F., & Parmigiani, S. (2005). Female competition in wild house mice depends upon timing of female/male settlement and kinship between females. Animal Behaviour, 69, 1259–1271.

Paylor, R., Yuva-Paylor, L.A., Nelson, D.L., & Spencer, C.M. (2008). Reversal of sensorimotor gating abnormalities in *Fmr1* knockout mice carrying a human *FMR1* transgene. Behavioral Neuroscience, 122, 1371–1377.

Pelka, G.J., Watson, C.M., Radziewic, T., Hayward, M., Lahooti, H., et al. (2006). Mecp2 deficiency is associated with learning and cognitive deficits and altered gene activity in the hippocampal region of mice. Brain, 129, 887–898.

Picker, J.D., Yang, R., Ricceri, L., & Berger-Sweeney, J. (2006). An altered neonatal behavioral phenotype in *Mecp2* mutant mice. NeuroReport, 17, 541–544.

Pinto, D., Pagnamenta, A.T., Klei, L., Anney, R., Merico, D., et al. (2010). Functional impact of global rare copy number variation in autism spectrum disorders. Nature, 466, 368–372.

Portfors, C.V. (2007). Types and functions of ultrasonic vocalizations in laboratory rats and mice. Journal of the American Association for Laboratory Animal Science, 46, 28–34.

Radyushkin, K., Hammerschmidt, K., Boretius, S., Varoqueaux, F., El-Kordi, A., et al. (2009). Neuroligin-3-deficient mice: Model of a monogenic heritable form of autism with an olfactory deficit. Genes, Brain and Behavior, 8, 416–425.

R Development Core Team. (2009). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL http://www.R-project.org

Restivo, L., Ferrari, F., Passino, E., Sgobio, C., Bock, J., et al. (2005). Enriched environment promotes behavioral and morphological recovery in a mouse model for the fragile X syndrome. Proceedings of the National Academy of Science, 102, 11557–11562.

Ricceri, L., de Filippis, B., & Laviola, G. (2008). Mouse models of Rett syndrome: From behavioural phenotyping to preclinical evaluation of new therapeutic approaches. Behavioural Pharmacology, 19, 501–517.

Rogers, S.J., & Ozonoff, S. (2005). Annotation: What do we know about sensory dysfunction in autism? A critical review of the empirical evidence. J. Child Psychology Psychiatry, 46, 1255–1268.

Roohi, J., Montagna, C., Tegay, D.H., Palmer, L.E., DeVincent, C., et al. (2009). Disruption of contactin 4 in three subjects with autism spectrum disorder. Journal of Medical Genetics, 46, 176–182.

Rosser, T.L., & Packer, R.J. (2003). Neurocognitive dysfunctions in children with neurofibromatosis type 1. Current Neurology and Neuroscience Reports, 3, 129–136.

Roullet, F., Saxena, R., Wöhr, M., Hung, A.Y., Sheng, M., & Crawley, J.N. (2010). Scent marking as a measure of olfactory communication in Shank1null mutant mice. Poster at the IMFAR Conference, Philadelphia.

Samaco, R.C., Fryer, J.D., Ren, J., Fyffe, S., Chao, H.-T., et al. (2008). A partial loss of function allele of methyl-CpG-binding protein 2 predicts a human neurodevelopmental syndrome. Human Molecular Genetics, 17, 1718–1727.

Santos, M., Silva-Fernandes, A., Oliveira, P., Sousa, N., & Maciel, P. (2007). Evidence for abnormal early development in a mouse model of Rett syndrome. Genes, Brain and Behavior, 6, 277–286.

Scattoni, M.L., Gandhy, S.U., Ricceri, L., & Crawley, J.N. (2008). Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. PLoS ONE, 3, 1–15.

Shahbazian, M.D., Young, J.I., Yuva-Paylor, L.A., Spencer, C.M., Antalffy, B.A., et al. (2002). Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. Neuron, 35, 243–254.

Silva, A.J., & Ehninger, D. (2009). Adult reversal of cognitive phenotypes in neurodevelopmental disorders. Journal of Neurodevelopmental Disorders, 1, 150–157.

Silva, A.J., Frankland, P.W., Marowitz, Z., Friedman, E., Lazlo, G., et al. (1997). A mouse model for the learning and memory deficits associated with neurofibromatosis type I. Nature Genetics, 15, 281–284.

Silverman, J.L., Yang, M., Lord, C., & Crawley, J.N. (2010). Behavioural phenotyping assays for mouse models of autism. Nature Reviews Neuroscience, 11, 490–502.

Spencer, C.M., Serysheva, E., Yuva-Paylor, L.A., Oostra, B.A., Nelson, D.L., & Paylor, R. (2006). Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. Human Molecular Genetics, 15, 1984–1994.

Spencer, C.M., Graham, D.F., Yuva-Paylor, L.A., Nelson, D.L., & Paylor, R. (2008). Social behavior in *Fmr1* knockout mice carrying a human FMR1 transgene. Behavioral Neuroscience, 122, 710–715.

Stearns, N.A., Schaevitz, L.R., Bowling, H., Nag, N., Berger, U.V., & Berger-Sweeney, J. (2007). Behavioral and anatomical abnormalities in MeCP2 mutant mice: A model for Rett syndrome. Neuroscience, 146, 907–921.

Szatmari, P., Paterson, A.D., Zwaigenbaum, L., Roberts, W., Brian, J., et al. (2007). Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nature Genetics, 39, 319–328.

Tabuchi, K., Blundell, J., Etherton, M.R., Hammer, R.E., Liu, X., et al. (2007). A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science, 318, 71–76.

Takeda, Y., Akasaka, K., Lee, S., Kobayashi, S., Kawano, H., et al. (2003). Impaired motor coordination in mice lacking neural recognition molecule NB-3 of the contactin/F3 subgroup. Journal of Neurobiology, 56, 252–265.

Toro, R., Konyukh, M., Delorme, R., Leblond, C., Chaste, P., et al. (2010). Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. Trends in Genetic Science, 26, 363–372.

Uhlmann, E.J., Wong, M., Baldwin, R.L., Bajenaru, M.L., Onda, H., et al. (2002). Astrocyte-specific *TSC1* conditional knockout mice exhibit abnormal neuronal organization and seizure. Annals of Neurology, 52, 285–296.

van Woerden, G.M., Harris, K.D., Hojjati, M.R., Gustin, R.M., Qiu, S., et al. (2007). Rescue of neurological deficits in a mouse model for Angelman syndrome by reduction of aCaMKII inhibitory phosphorylation. Nature Neuroscience, 10, 280–282.

Wiznitzer, M. (2004). Autism and tuberous sclerosis. Journal of Child Neurology, 19, 675–679.

Ylisaukko-oja, T., Rehnström, K., Auranen, M., Vanhala, R., Alen, R., et al. (2005). Analysis of four neuroligin genes as candidates for autism. European Journal of Human Genetics, 13, 1285–1292.

Young, D.M., Schenk, A.K., Yang, S.-B., Jan, Y.N., & Jan, L.Y. (2010). Altered ultrasonic vocalizations in a tuberous sclerosis mouse model of autism. Proceedings of the National Academy of Science of the United States of America, 107, 11074–11079.

Zang, J.B., Nosyreva, E.D., Spencer, C.M., Volk, L.J., Musunuru, K., et al. (2009). A mouse model of the human fragile X syndrome I304N mutation. PLoS Genetics, 5, 1–16.

Zhou, J., Blundell, J., Ogawa, S., Kwon, C.-H., Zhang, W., et al. (2009). Pharmacological inhibition of mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific *Pten* knock-out mice. The Journal of Neuroscience, 29, 1773–1783.

Zweier, C., de Jong, E.K., Zweier, M., Orrico, A., Ousager, L.B., et al. (2009). CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in Drosophila. American Journal of Human Genetics, 85, 655–666.