

REVIEW

Molecular causes of sex-specific deficits in rodent models of neurodevelopmental disorders

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Abstract

Neurodevelopmental disorders (NDDs) such as intellectual disability and autism spectrum disorder consistently show a male bias in prevalence, but it remains unclear why males and females are affected with different frequency. While many behavioral studies of transgenic NDD models have focused only on males, the requirement by the National Institutes of Health to consider sex as a biological variable has promoted the comparison of male and female performance in wild-type and mutant animals. Here, we review examples of rodent models of NDDs in which sex-specific deficits were identified in molecular, physiological, and/or behavioral responses, showing sex differences in susceptibility to disruption of genes mutated in NDDs. Haploinsufficiency in genes involved in mechanisms such as synaptic function (*GABRB3* and *NRXN1*), chromatin remodeling (*CHD8*, *EMHT1*, and *ADNP*), and intracellular signaling (*CC2D1A* and *ERK1*) lead to more severe behavioral outcomes in males. However, in the absence of behavioral deficits, females can still present with cellular and electrophysiological changes that could be due to compensatory mechanisms or differential allocation of molecular and cellular functions in the two sexes. By contrasting these findings with mouse models where females are more severely affected (*MTHFR* and *AMBRA1*), we propose a framework to approach the study of sex-specific deficits possibly leading to sex bias in NDDs.

KEYWORDS

animal models, autism, neurodevelopmental disorders, sex bias, sex differences

1 | INTRODUCTION

Neurodevelopmental disorders (NDDs) comprise of a group of developmental conditions affecting cognitive, social function, and/or behavior. Among NDDs, autism spectrum disorder (ASD) and intellectual disability (ID) affect up to 2.6% of children worldwide

(Olusanya et al., 2018). The core features of ASD are social deficits and restricted/repetitive behaviors with onset during early development (American Psychiatric Association, 2013), and latest reports on ASD prevalence in the United States show an ASD diagnosis in 1 in 59 children (Baio et al., 2018). ID is reported in 1 in 100 children and is characterized by deficits in intellectual functioning and adaptive

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skills necessary for daily life (American Psychiatric Association, 2013). While these disorders are diagnosed independently, there is substantial comorbidity with around 30%–35% of children affected with ASD also being diagnosed with ID (Baio et al., 2018).

Increased prevalence of males in both ASD and ID is highly replicated, but the exact sex ratio remains unclear. Data collected by the Center for Disease Control and Prevention (Baio et al., 2018) estimates a 4:1 male:female prevalence ratio in ASD, while a meta-analysis of 54 worldwide publications by Loomes, Hull, and Mandy (2017) shows a lower sex ratio of 3.25:1. For ID, the male:female ratios range from 2:1 to 1.5:1 (Maulik, Mascarenhas, Mathers, Dua, & Saxena, 2011, 2013), consistently showing a male bias which cannot be solely explained by the prevalence of X-linked ID (Werling, 2016). Differences in the severity of the presentation of males and females with an ASD diagnosis is also often debated. Multiple studies found that autistic girls fall within the range of ID more frequently than boys, and that autistic boys have higher scores for restricted interests (Baio et al., 2018; Frazier, Georgiades, Bishop, & Hardan, 2014; Halladay et al., 2015; Loomes et al., 2017). Reports on higher intelligence quotient (IQ) autistic individuals find male:female ratios as high as 10:1 (Fombonne, 2005; Werling, 2016) and genetic studies suggested that autistic males with higher IQ may be a genetically distinct group, showing that autistic females overlap with autistic males with lower IQ (Iossifov et al., 2014). All these findings are often confounded by the differences in ascertainment and the limited number of females in some research cohorts. Autism is diagnosed later in life in girls than in boys (Begeer et al., 2013; Rutherford et al., 2016), and some reports suggest that ASD symptoms may be milder in females or masked by the occurrence of other comorbidities, such as ID, anxiety, and depression (Hartley & Sikora, 2009; Lai et al., 2011; Werling, 2016). Girls are expected by society to be more verbal and social than boys and may be more adept at hiding social deficits (Werling, 2016). Thus, it remains unclear whether there are autistic women missing from the higher IQ group due to underdiagnosis.

Different hypotheses and mechanisms for the male bias in NDDs have been suggested and are still under investigation. According to the female protective effect theory, females need a higher mutational burden as well as more deleterious copy number variants (CNVs) and single nucleotide variants (SNVs) to present an autistic presentation compared to males (Kreiser & White, 2014). Siblings of autistic girls have shown a significantly more severe presentation than siblings of autistic boys in some studies (Palmer et al., 2017; Robinson, Lichtenstein, Anckarsater, Happe, & Ronald, 2013; Werling & Geschwind, 2013a, 2013b) but not in others (Goin-Kochel, Abbacchi, Constantino, & Autism Genetic Resource Consortium, 2007; Messinger et al., 2015; Mitra et al., 2016). An alternative theory proposes that increased levels of testosterone or other steroid hormones during fetal development increase male vulnerability to ASD (Baron-Cohen, Knickmeyer, & Belmonte, 2005; Baron-Cohen et al., 2011).

Beyond genetic and endocrine effects, other factors, such as environment, innate immunology, epigenetic changes, and cell signaling, can contribute to sex bias in NDDs (for review see Ferri, Abel, &

Significance

Neurodevelopmental disorders (NDDs) such as autism spectrum disorder and intellectual disability are diagnosed more frequently in males. The molecular and cellular origins of this sex bias remain unknown. Animal models of NDDs presenting with sex-specific deficits can provide insight into sex differences in neuronal function that could underlie pathogenesis. Here we review not only male-specific, but also female-specific deficits that have been recently identified in transgenic mouse lines and summarize how these studies can guide us in understanding sex bias in NDDs.

Brodtkin, 2018). It has been proposed that synaptic plasticity plays a crucial role in NDD pathophysiology given that disruptive mutations in NDD-linked genes lead to alterations in synaptic structure, function, and plasticity (Zoghbi & Bear, 2012). It is now accepted that NDDs originate during early fetal brain development and that ASD is not caused by environmental factors in early childhood such as vaccines or parental care (Belmonte, Allen, et al., 2004; Belmonte, Cook, et al., 2004; Hviid, Hansen, Frisch, & Melbye, 2019). Mottron et al. (2015) proposed that males and females have different susceptibility to disruptions of genes involved in regulating homeostatic synaptic function in the fetal brain. While different functions such as somatosensation, motor regulation, vision, and speech are spatially distributed to specific cortical regions, multimodal association areas integrate and process different sources of information to generate a behavioral output. These circuits follow a stereotypical progression of circuit development involving synapse formation, pruning, and remodeling during fetal and postnatal life as each function is established at specific developmental time points (Tau & Peterson, 2010). Both genetic and environmental events affect this process leading to individual variability in the presentation of NDDs.

Homeostasis, the ability to return to a set state, such as a particular firing rate, is a fundamental property of neurons. Neurons are not only able to respond consistently to a stimulus, but can also display plasticity in resetting their state to a different level if this is necessary to encode information. Long-term potentiation (LTP) is a good example of stable neuronal response being increased to a new steady state, and it can be observed at the circuit, neuron, and single synapse level. This is an incredibly complex process which involves a variety of cellular functions, including synaptic transmission, intracellular signaling, gene transcription, local and nuclear protein translation and degradation, and protein trafficking (Bliss, Collingridge, Morris, & Reymann, 2018). Both homeostasis and plasticity have been found to be disrupted in NDDs (Mullins, Fishell, & Tsien, 2016; Nelson & Valakh, 2015; Ramocki & Zoghbi, 2008). Multiple studies have shown that males and females have developmentally and spatially regulated differences in gene expression and synaptic function that can differentially control how circuit function is allocated for behavior. The mechanisms underlying these differences are attributable to different patterns of brain connectivity, activation of

different brain areas, or activation of different cellular and molecular pathways to perform the same behavior (McCarthy, 2016). If homeostatic regulation of synaptic plasticity is differentially affected in males and females, similar mutations could result in sex-specific phenotypes (Mottron et al., 2015; Toro et al., 2010).

Since NDDs are highly heritable, considerable efforts have been devoted to the identification of genetic mutations leading to ASD and/or ID. Hundreds of genes have been identified as risk factors for ASD with varying levels of confidence showing great genetic heterogeneity but also informing us on the molecular mechanisms underlying NDD pathogenesis. Many of the genes now recognized as syndromic, high confidence, or strong candidates encode proteins that can be grouped within a few broad categories all affecting homeostatic processes, synaptic proteins, transcriptional and chromatin regulators, and cellular signaling proteins (Figure 1; Bayés et al., 2011; Darnell et al., 2011; De Rubeis et al., 2014; Iossifov et al., 2012, 2014; Kang et al., 2011; Pinto et al., 2014; Voineagu et al., 2011). In parallel, several mouse lines carrying mutations in NDD genes have been generated to investigate the pathological mechanism of ASD and ID. In this review, we focus on studies that have revealed a possible relationship between NDD genes and sex-specific neuronal function. The picture that emerges is still fragmentary and

often contradictory because of the use of different behavioral tests and mouse strains in different laboratories, but a pattern begins to form suggesting that sex-specific regulation of synaptic homeostasis on both the wild-type and mutant brain might be a key mechanism controlling sex-specific responses. To our knowledge, this is the first time these data have been collected in one place and we hope to provide a starting point to begin thinking about an integrated approach to elucidate sex bias in NDDs.

2 | SEX-SPECIFIC NEURONAL DEFICITS CAUSED BY SYNAPTIC GENES: GABRB3 AND NRXN1

Synaptic proteins such as neurotransmitter receptors and accessory proteins controlling synaptic plasticity and neurotransmission are often found mutated in NDDs (De Rubeis et al., 2014; Pinto et al., 2014). Sex-specific deficits in behavioral performance or circuit function caused by loss of synaptic proteins have been described in only a few cases. While the behavioral deficits identified are predominantly male-specific, there are also female-specific changes present depending on age, strain, and behavioral test (Tables 1 and 2).

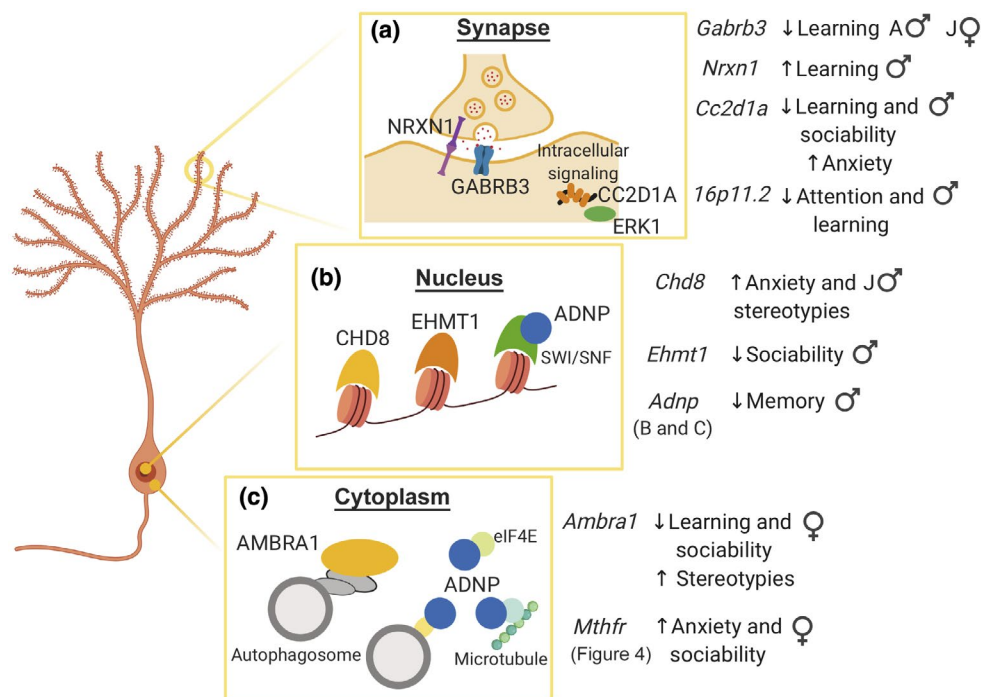


FIGURE 1 Subcellular distribution of proteins encoded by genes leading to sex-specific deficits. Molecular mechanisms are outlined in the boxes and a summary of the most severe sex-specific changes is presented on the right (A = adult; J = juvenile). (a) Proteins likely affecting the synapse directly. Gamma-aminobutyric acid A receptor $\beta 3$ (GABRB3) and Neurexin 1 (NRXN1) are essential component of the synapse where they modulate synaptic plasticity and neurotransmission. ERK1 and coiled-coil and C2 domain containing 1A (CC2D1A) are signaling proteins crucial for processing both synaptic transmission and growth factor response. (b) Chromatin regulators. Chromodomain-helicase-DNA binding protein 8 (CHD8), euchromatic histone lysine methyltransferase 1 (EHMT1) and activity dependent neuroprotector homeobox (ADNP), through the SWI/SNF complex, are chromatin regulators controlling expression of critical genes involved in brain development and synaptic function. (c) Cytoplasmic proteins. By binding to eukaryotic translation initiation factor 4E (eIF4E) and interacting with microtubule components ADNP is also involved in cytoplasmic processes. Activating molecule in beclin1-regulated autophagy (AMBRA1) promotes autophagosome formation and methylenetetrahydrofolate reductase (MTHFR) regulates folate metabolism

TABLE 1 Genes and rodent models discussed in this review

Gene	SFARI score	Function	Disease	Animal model
GABRB3	2	Synaptic transmission	Angelman syndrome	Gabrb3 (m-/p+)
NRXN1	1	Synaptic transmission	ASD, schizophrenia, ID	Nrxn1a+/- mice and rats
CHD8	1	Chromatin regulation	ASD	Chd8+/N2373k
EHMT1	1	Chromatin regulation	9q-syndrome/Kleefstra syndrome	Ehmt1+/-
ADNP	1	Chromatin regulation	ADNP syndrome, ASD, AD, FTD	Adnp+/-
16p11.2 del		Multigenic/signaling	ASD, ADHD	7qF3 (del/+)
CC2D1A	2	Intracellular signaling	ID, ASD	Cc2d1a-/-
MTHFR	3	Metabolism	Down syndrome, ASD, OCD	Mthfr+/-
AMBRA1		Autophagy		Ambra1+/-

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; FTD, frontotemporal dementia; ID, intellectual disability; OCD, obsessive-compulsive disorder; SFARI, Simons Foundation Autism Research Initiative (scores were obtained from <https://gene.sfari.org>).

2.1 | GABRB3

Female- and male-specific synaptic and behavioral changes were found in heterozygous mice for the loss of *gamma-aminobutyric acid A receptor $\beta 3$ subunit (Gabrb3)*, a component of inhibitory ionotropic GABA receptors (Figure 1a; Table 1; Mercer, Palarz, Tabatadze, Woolley, & Raman, 2016). Human GABRB3 is found in a region of chromosome 15 (15q11-q13) whose deletion is linked to different conditions and ASD depending on the maternal or paternal origin of the mutated allele. Deletions of this region are responsible for Angelman Syndrome or Prader-Willi Syndrome, if inheritance is maternal or paternal respectively (Cassidy, 1997; Cassidy, Dykens, & Williams, 2000; Cook et al., 1997). Dominant SNVs in GABRB3 cause a form of infantile epileptic encephalopathy (Epi4K Consortium et al., 2013; Epilepsy Phenome/Genome Project Epi4K Consortium, 2015; EuroEPINOMICS-RES Consortium et al., 2014; Janve, Hernandez, Verdier, Hu, & Macdonald, 2016) and several *de novo* likely gene disrupting (LGD) SNVs have been identified in ASD cohorts (De Rubeis et al., 2014; Hoischen, Krumm, & Eichler, 2014). GABRB3 is highly expressed in the cerebellum, a brain area directly associated with movement control, coordination and balance, but also involved in cognitive and social function (Buckner, 2013; Steinlin, 2007; Sullivan, 2010).

Gabrb3 heterozygous mice were developed to lack the maternal copy of the gene and model gene loss of Angelman Syndrome, *Gabrb3 (m-/p+)* (Liljelund, Handforth, Homanics, & Olsen, 2005). *Gabrb3 (m-/p+)* mice showed male-specific deficits in multiple studies: impairment in contextual learning, increased locomotion, and increased theta activity during electroencephalography (Liljelund et al., 2005), and reduced motor learning in the rotarod and tactile allodynia (De Lorey et al., 2011) (Table 2). Further analysis of neuronal activity in the cerebellar nuclei (CbN) found that male-specific motor learning is age-dependent (Mercer et al., 2016). When tested at weaning (postnatal day 22), male and female CbN neurons display different synaptic transmission properties at baseline, with wild-type females having higher firing rates and faster synaptic current decay. CbN neurons in *Gabrb3 (m-/p+)*

male mice showed higher spontaneous levels of electrical activity compared to wild-type males, which were similar to wild-type and heterozygous females. However, in weanlings, motor performance on the rotarod showed opposite sex-specific changes where *Gabrb3 (m-/p+)* female mice showed an enhanced ability at Day 1 and plateaued over the training days, indicating impaired learning behavior (Vitali & Clarke, 2004). Juvenile *Gabrb3 (m-/p+)* male mice performed equally to wild-type mice. This is opposite to what had been previously found by De Lorey et al. (2011) in adults. When Mercer et al. (2016) tested adult mice they were able to replicate the male-specific motor deficit, indicating that males become affected at a later age and females are able to compensate for earlier deficits.

It remains unclear when and how the switch in motor learning deficit in *Gabrb3 (m-/p+)* mice happens. One possible explanation for sex-specific compensation was found by measuring the activity of group I metabotropic glutamate receptors (mGluRs) at the synapses of CbN neurons in juvenile mice. mGluR1/5 receptors are less readily available for activation in juvenile male mice. In *Gabrb3 (m-/p+)* male mice mGluR1/5 receptors show higher activity which is similar to females of either genotype (Mercer et al., 2016). These findings show that maternal loss of *Gabrb3* differentially alters cerebellar physiology in a sex-specific and age-dependent fashion, leading to a sex-dependent compensatory mechanism only in weanling males that is not maintained in adulthood. Overall, these studies highlight that baseline differences in synaptic transmission can lead to sex-specific circuit deficits, but also remind us that compensatory changes may not be maintained over the long term. In addition, even when behavioral deficits are not observed, electrophysiological or molecular changes may be present, revealing that male and female brains are allocating circuit function differently.

2.2 | NRXN1

Neurexin-1 α (NRXN1 α) is a cell adhesion molecule which promotes vesicle exocytosis upon calcium channel-dependent activation at

pre-synaptic sites (Figure 1a, Table 1) (Hata, Davletov, Petrenko, Jahn, & Südhof, 1993; Missler et al., 2003). It has been shown that disruption of vesicle release leads to altered object recognition and social memory, as well as motor deficits (Glynn, 2003; Mori et al., 2002; Prado et al., 2006). *De novo* LGD mutations and heterozygous deletions in the *NRXN1α* gene have been reported in ASD and schizophrenia (Ching et al., 2010; Glessner et al., 2009; Griswold et al., 2015; Kim et al., 2008; Stefansson et al., 2014).

Multiple groups identify sex differences in behavioral performance in *Nrxn1α* heterozygous (*Nrxn1α*^{+/-}) or knock-out (*Nrxn1α*^{-/-}) mice. Laarakker, Reinders, Bruining, Ophoff, and Kas (2012) described male-specific deficits in *Nrxn1α*^{+/-} mice, where only heterozygous males exhibited enhanced exploratory behavior and faster familiarization to a new environment. Grayton, Missler, Collier, and Fernandes (2013) studied knock-out animals from the same line and found that male mice displayed increased aggression towards intruders and increased anxiety, while females showed increased social preference for a stranger mouse versus a familiar mouse in the 3-chambered test and reduced locomotion. Yet, studies in other laboratories testing the same mouse line in either knock-out (Etherton, Blaiss, Powell, & Südhof, 2009) or heterozygous mice (Dachtler et al., 2015) found no changes in locomotion and anxiety (Dachtler et al., 2015), and similar alterations in social behavior, motor learning, sensory perception and stereotypies in males and females (Table 2) (Dachtler et al., 2015; Etherton et al., 2009). This can be a common occurrence when mice are investigated behaviorally and could be due to differences in the timing of testing or handling of the animals, but also to the genetic background of the mice, a mixed C57BL/6-Sv129 background was used by Etherton et al. (2009), a pure C57BL/6J by Grayton et al. (2013) and Laarakker et al. (2012), and a pure C57BL/6N by Dachtler et al. (2015). Genetic modifiers in the background strain could contribute to changes in behavioral performance leading to conflicting results as shown by multiple studies comparing behavior in different mouse strains (Bothe, Bolivar, Vedder, & Geistfeld, 2004; Cook, Bolivar, McFadyen, & Flaherty, 2002; Moy et al., 2007; Sittig et al., 2016) and even among C57BL/6N substrains maintained by different vendors (Bryant et al., 2008).

The fact that loss of *Nrxn1α* may lead to sex-specific effects is also supported by studies on knock-out *Nrxn1α*^{-/-} Sprague Dawley rats where mutant male rats were impaired in reward learning as well as in spatial reversal learning in a novel object recognition task, while mutant female rats only showed acquisition deficits on Day 1 and performed as well as wild-type littermates in all successive trials (Esclassan, Francois, Phillips, Loomis, & Gilmour, 2015). The mechanism underlying sex-specific behavioral differences following *Nrxn1α* loss of function or haploinsufficiency remain unclear. Interestingly, similarly to *Gabrb3*, loss of *Nrxn1α* leads to variable phenotypes in males and females, and additional studies will be necessary to address whether sex-specific circuit alterations are present and depending on the genetic background.

3 | SEX-SPECIFIC NEURONAL DEFICITS CAUSED BY CHROMATIN REMODELING GENES: CHD8, EMHT1, AND ADNP

One class of genes that is often found as mutated in ASD and has shown sex-specific deficits in animal models encodes for chromatin remodeling proteins (Figure 1b). Epigenetic marks on DNA and histones are influenced by sex-hormones and other steroid hormones throughout the lifespan (McCarthy, 2016; McCarthy, Nugent, & Lenz, 2017) and it is likely that disrupting chromatin remodeling mutations in ASD and ID genes may alter sex-specific cellular functions. As in the loss of function of synaptic genes described above, deficits are mostly male-specific, but interesting female-specific deficits were present at younger ages in mice with heterozygous loss of *Adnp* (Tables 1 and 2).

3.1 | CHD8

Chromodomain-helicase-DNA binding protein 8 (CHD8) is one of the genes most frequently found to carry *de novo* LGD variants in ASD cases (De Rubeis et al., 2014; Iossifov et al., 2014; O'Roak, Vives, Fu, et al., 2012; O'Roak, Vives, Girirajan, et al., 2012). CHD8 is a transcriptional regulator which modulates expression of genes important for brain development and synaptic function by directly binding to methylated histones (Figure 1b, Table 1) (Cotney et al., 2015; Sugathan et al., 2014; Wilkinson et al., 2015). Jung et al. (2018) described male-biased behavioral deficits in *Chd8* heterozygous, *Chd8*(+/N2373k) mice, where the paralogous human truncating variant is introduced in the mouse genome. Overall, *Chd8*(+/N2373k) males, but not females, exhibited increased anxiety-like behaviors after experiencing stressful events. When male pups were separated from their mothers they displayed increased mother-seeking (ultrasonic vocalizations) and mother-attachment (time with mother) behaviors in adolescence. When adult heterozygous mice were isolated they increased self-grooming. Interestingly, wild-type female mice already displayed increased levels of all these behaviors reflecting increased susceptibility of females to separation stress, and *Chd8*(+/N2373k) males were indistinguishable from females. No cognitive or social deficits, or anxiety- and depression-like behaviors were found in either males or females.

When analyzing hippocampal activity in excitatory and inhibitory firing, wild-type males show similar excitatory firing rate, but higher inhibitory firing rate than females at baseline. In contrast, *Chd8*(+/N2373k) males showed increased excitatory firing, bursting ratio, and burst duration, while *Chd8*(+/N2373k) females increased inhibitory activity both as it related to firing rate and miniature inhibitory postsynaptic current frequency, and reduced burst duration. These changes in neuronal activity suggest a disruption in the hippocampal excitatory/inhibitory balance, a feature often altered in ASD patients as well as in ASD mouse models (Gao & Penzes, 2015; Gogolla et al., 2009; Gonçalves, Anstey, Golshani, & Portera-Cailliau, 2013; Lee, Lee, & Kim, 2017; Nelson & Valakh,

TABLE 2 Summary of behavioral deficits in animal models of NDDs in studies where both sexes were tested

Genetic model	Cognitive			Social			Motor—Stereotypies			Others			References	
	Sensory	Object Spatial	Other	Soc	Soc Mem	Nest	Other	Loc	Coord	Groom	Anx	Other		Strain
<i>Gabrb3</i> (<i>m-/-p+</i>)	↓M/F ASR ↑M/F Heat ↑M Tact	↓M Context ↓M Motor	↓M Context					↑M	↓M			↑M Seizures ↑M/F [§] Seizures	Mixed Mixed	Liljelund (2005) De Lorey (2011)
<i>Nrxn1a+/-</i>	=WT PPI		↓JF Motor	↓M/F				=WT	↓JF	=WT	=WT		Mixed	Mercer et al. (2016)
<i>Nrxn1a+/-</i>				↓M/F				↑M					B6N	Dachtler et al. (2015)
<i>Nrxn1a+/-</i>				↑M Context				↑M					B6J	Laarakker et al. (2012)
<i>Nrxn1a-/-</i>	↓M/F PPI	↑M/F Motor	↑M/F Motor	↓M/F				=WT	↑M/F	↑M/F	=WT		Mixed	Etherton et al. (2009)
<i>Nrxn1a-/-</i>	=WT	=WT		↑M/F	↑F	↓M/F	↑M Aggress	↓F	↓F	=WT	↑M		B6J	Grayton et al. (2013)
<i>Nrxn1a-/-</i> rats	↑M/F ASR	↓M	↓M Reward					↑M/F					Sprague Dawley	Esclassan et al. (2015)
<i>Chd8+/-del5</i> (<i>exon 5</i>)	=WT ASR	↓M/F	↓M/F Fear	=WT	=WT			=WT USV		=WT			B6N	Gompers (2017)
<i>Chd8+/-</i> (<i>exon 3</i>)	↑M/F Olf	=WT		↑M/F				↑M/F USV	↓pM/F	=WT			B6J	Suetterlin (2018)
<i>Chd8+/-</i> <i>N237k</i>	=WT PPI =WT Olf	=WT	=WT Motor, Context	=WT	=WT			=WT USV	↓M [#]	=WT	↑M [#]	=WT Sleep	B6J	Jung (2018)
<i>Ehmt1+/-</i>		↓M/F	=WT Motor	↓JM				↓M/F		=WT	↑M/F		B6J	Balemans et al. (2010)
<i>Adnp+/-</i>	↓F Olf	↓M		↓F	↓M/F								Mixed	Malishkevich et al. (2015), Amram et al. (2016)
	↓F Olf ↓M/F Strength	↓M			↓M/F								Mixed	Sragovich et al. (2019)
	↓JF Olf ↓JM Strength	↓JM/F		↓JF	↓JM/F		↓pF USV						Mixed	Hacohen-Kleiman et al. (2018)
<i>16p11.2 del/+</i>	=WT ABR		↓M Attention, Reward					↑M/F					Mixed	Angelakos et al. (2017) Grissom et al. (2018)
<i>Cc2d1a cKO</i>	=WT	↓M/F	↓M	↓M			↓M USV	↑M	↑M/F	↑M			B6N	Oaks et al. (2017), Zamarbide et al. (2019)

(Continues)

TABLE 2 (Continued)

Genetic model	Cognitive			Social			Motor—Stereotypies			Others			References	
	Sensory	Object Spatial	Other	Soc	Soc Mem	Nest	Other	Loc	Coord	Groom	Anx	Other		Strain
<i>Mthfr</i> ^{+/-}	=WT Olf =pWT Strength	=WT	↓pM/F Motor	↓F ^{\$}	↓F ^{\$}	↓M/F	↓F ↓pF USV	↑M ↑M/F ^{\$}	↓pM/F		↓F ^{\$}		Mixed	Blumkin et al. (2011), Levav-Rabkin et al. (2011)
<i>Ambra1</i> ^{+/-}	=WT Olf =WT ASR, PPI =WT Heat	=WT	↓F	↓F	↓F	↓M/F	↓F ↓pF USV	=WT	=WT	↑F	=WT		B6N	Dere et al. (2014)
													B6J	Mitjans (2017) Nobili et al. (2018)

Notes: ↓ and ↑ indicate respectively reduced and increased performance in the behavioral test, while =WT indicates that mutant males and female performed as well as control animals. Empty cells imply that a particular behavior has not been tested in that study.

Abbreviations: ABR, auditory brainstem response (acoustic startle); Aggress, aggressive behavior; Anx, anxiety; ASR, acoustic startle reactivity; Context, contextual memory; Coord, motor coordination; F, female; Groom, grooming; Heat, heat sensitivity; j, juvenile (PND20-30); Loc, locomotor activity; M, male; Olf, olfaction; p, pups (<PND20); PPI, pre-pulse inhibition; Soc, sociability (more time spent with conspecific); Soc mem, social memory (ability to distinguish between known and novel conspecific); Tact, tactile sensitivity; USV, ultrasonic vocalizations; \$, gene-drug interaction induced behavior; #, under stress conditions; &, pheromone-dependent behavior.

2015; Peixoto, Wang, Croney, Kozorovitskiy, & Sabatini, 2016; Sohal & Rubenstein, 2019). Moreover, transcriptome analysis in *Chd8*(+/N2373k) also showed sex-specific changes in gene expression with gene classes involved in modulation of brain development, function, and synaptic plasticity such as extracellular matrix transcripts being upregulated in females and downregulated in males (Jung et al., 2018; Kerrisk, Cingolani, & Koleske, 2014). As discussed above for *Gabrb3* mice, differential behavioral impairments in males and females correlate with sex-specific changes in circuit activity, showing that specific ASD-associated gene mutations can affect brain function in a sex-dependent manner leading to changes that can either be disruptive or compensatory. It is important to note that these sex-specific impairments are linked to increased response to stress more than core features of ASD such as social behavior and cognitive function.

Multiple additional mouse models have been developed for *Chd8* targeting different isoforms or protein domains revealing an array of phenotypes including increased social investigation of a stranger mouse, increased anxiety-like behaviors, and reduced locomotor activity (Gompers et al., 2017; Katayama et al., 2016; Platt et al., 2017; Suetterlin et al., 2018). Both sexes were investigated only by Gompers et al. (2017) and Suetterlin et al. (2018) and no sex-differences were found. Gompers et al. (2017) were the only group to identify cognitive deficits in object and fear memory, but no object memory deficits were found by Jung et al. (2018). It is important to note that Gompers et al. (2017) used a C57BL/6N background, while all other mutants were backcrossed to a C57BL/6J strain (see Table 2 for comparison). Different designs used to target the gene and other genetic or environmental modifying factors could account for the observed differences. Yet, findings in the *Chd8*(+/N2373k) mice still support that both males and females may show sex-specific synaptic deficits that can be found in the presence or absence of a sex-specific behavioral change.

3.2 | EHMT1

Euchromatic histone lysine methyltransferase 1 (EHMT1) haploinsufficiency causes chromosome 9q subtelomeric deletion syndrome (9q-syndrome), also known as Kleefstra syndrome (Figure 1c, Table 1) (Blackburn, Tischer, et al., 2017; Blackburn, Williams, et al., 2017). This disorder is characterized by ID, ASD, general developmental delay, hypotonia, and dysmorphic craniofacial features (Blackburn, Tischer, et al., 2017; Blackburn, Williams, et al., 2017; Kleefstra et al., 2005, 2006, 2009). Heterozygous SNVs in this gene have also been reported in individuals affected by ASD (de Boer et al., 2018) and schizophrenia (Chase, Gavin, Guidotti, & Sharma, 2013). EHMT1 and its homolog, EHMT2, are part of a chromatin remodeling complex that represses gene transcription after methylation of histone H3 at lysine 9 (Tachibana et al., 2005). EHMT1 is highly expressed during embryonic brain development, and heterozygous removal of *Ehmt1* in mice shows several features

of 9q-syndrome including reduced mobility and object memory, increased anxiety and grooming in both sexes, and male-specific social impairments. While *Ehmt1*^{+/-} females are indistinguishable from wild-type littermates, *Ehmt1*^{+/-} male mice show decreased juvenile playing time at 30 days of age, and delayed social preference between a familiar and stranger mouse in the social novelty portion of the 3-chambered test (Balemans et al., 2010) (Table 2). The molecular and cellular basis underlying the male-bias in social behavior of *Ehmt1*^{+/-} mice has been not investigated, but by promoting histone H3 methylation EHMT1 controls gene repression of both BDNF (Rutherford, Nelson, & Turrigiano, 1998) and Rab3b (Tsetsenis et al., 2011), which, in turn, regulate excitatory/inhibitory synaptic homeostasis. Additional information regarding the synaptic effects of mutations in this gene could decipher the possible sex-differences seen in social behavior. However, sex-differences in social play behavior have been widely studied for juvenile rats, and remain controversial in adolescent mice where they have not been fully investigated. While it is known that juvenile female rats play less than males (Auger & Olesen, 2009; Vanderschuren, Niesink, & Pee, 1997), few studies showed similar results in social play behavior for male and female mice (Dyck et al., 2009; Terranova & Laviola, 2001) or a reduced play behavior in males (Cox & Rissman, 2011).

3.3 | ADNP

De novo LGD variants in *Activity Dependent Neuroprotector Homeobox (ADNP)* were found to be strongly associated with ASD risk in multiple studies (De Rubeis et al., 2014; Hoischen et al., 2014; Iossifov et al., 2014; Stessman et al., 2017). In addition, detailed reports on the phenotypic presentation of individuals with *ADNP* mutations described an *ADNP*-syndrome characterized by facial dysmorphisms, cognitive and motor impairments, and ASD (Van Dijck et al., 2019), with girls reported to show milder ID than boys (Helsmoortel et al., 2014). In mice, *Adnp* haploinsufficiency causes different sex-specific behavioral deficits in males and females. *Adnp* heterozygous (*Adnp*^{+/-}) male mice display impairments in object recognition, while *Adnp*^{+/-} female mice have altered olfactory function and social deficits, showing less interest in the stranger mouse during the 3-chambered test (Amram et al., 2016; Hacoheh-Kleiman et al., 2018; Malishkevich et al., 2015; Sragovich, Merenlender-Wagner, & Gozes, 2017).

Interestingly, this difference in cognitive and social behavior correlated with differential *ADNP* hippocampal expression with a 2-fold increase of *ADNP* expression in wild-type males compared to females, while wild-type and female *Adnp*^{+/-} mice show a similar *ADNP* expression to the mutant males. The same sex difference has been also found in postmortem human hippocampal data, where males expressed ~25% more *ADNP* transcript than females (Amram et al., 2016; Malishkevich et al., 2015).

ADNP is part of the large ATP-dependent chromatin remodeling complex SWI/SNF (Figure 1b) which plays a critical role in brain

development and function (Gozes, 2017). In addition, *ADNP* directly interacts with the eukaryotic translation initiation factor 4E (eIF4E) modulating RNA translation (Figure 1c). Quantitative RT-PCR analysis revealed that hippocampal eIF4E mRNA was significantly increased in *Adnp*^{+/-} male mice. In addition, different neuroligins (NLGN) which are downstream effectors of eIF4E and also mutated in ASD were either increased (NLGN1 and 3) or decreased (NLGN4) in *Adnp*^{+/-} females compared to males (Malishkevich et al., 2015). Finally, *ADNP* binds to the microtubule end-binding proteins 1 and 3 (EB1 and EB3, Figure 2) affecting microtubule dynamics and dendritic spine density (Oz et al., 2014). Male *Adnp*^{+/-} mice show a significant decrease in both cortical and hippocampal spine density as well as increased immature excitatory synapses on the dendritic shaft, which is not found in females (Hacoheh-Kleiman et al., 2018). Partial *ADNP* deficiency causes hyperphosphorylation of the microtubule-associated protein tau, which is characteristic of Alzheimer disease and frontotemporal dementia-like tau pathology, resulting not only in cognitive disabilities, but also in social impairments (Garbern et al., 2010; Vulih-Shultzman et al., 2007). Tau phosphorylation is mediated via GSK3 β , which is overactivated in *Adnp*^{+/-} mice (Vulih-Shultzman et al., 2007). Genetic reduction in GSK3 β improved sociability in mice indicating that overactivation of GSK3 β and the resulting tau phosphorylation affect social behavior (Latapy, Rioux, Guitton, & Beaulieu, 2012). These downstream signaling mechanisms may be involved in the social deficits observed following *ADNP* mutations.

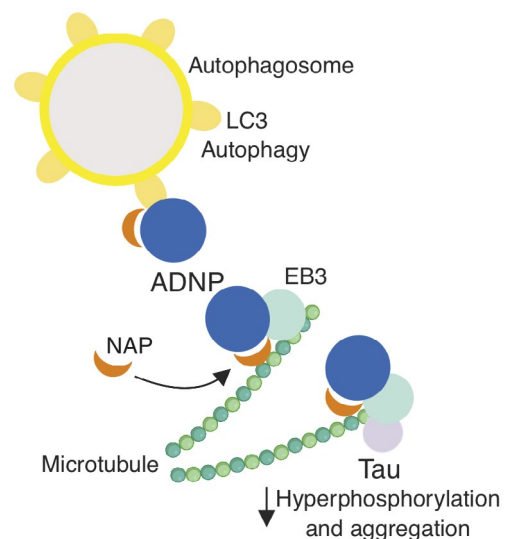


FIGURE 2 Schematic representation of activity dependent neuroprotector homeobox (*ADNP*) functions in the cytoplasm. *ADNP* binds directly to end-binding protein 3 (EB3) to modulate microtubule dynamics during synaptic plasticity and to light chain 3 (LC3) to support autophagosome formation. *ADNP* also recruits tau protein through EB3, regulating axonal transport. Treatment with NAP peptide enhances *ADNP*-microtubule interaction through EB3 binding, driving normal spine structure. NAP treatment also increase *ADNP* function in reducing tau hyperphosphorylation and deposition, and in promoting autophagy

This interaction between ADNP and EB proteins is mediated via the SIP domain in ADNP which has been investigated as a target for drug therapy. Two small peptide molecules have been widely studied: NAP, a neuroprotective ADNP-derived snippet peptide (also known as NAPVSIPQ, CP201, or davunetide) (Hacohen-Kleiman et al., 2018; Vulić-Shultzman et al., 2007) and SKIP, a four-amino-acid peptide (Amram et al., 2016). Both peptides bind to EB proteins, enhancing ADNP-EB3 interaction (Amram et al., 2016; Sragovich et al., 2017). NAP increases the association between ADNP and EB3, enhancing tau-microtubule interaction which results in a reduction of tau hyperphosphorylation and aggregation (Ivashko-Pachima, Sayas, Malishkevich, & Gozes, 2017; Oz et al., 2014). In addition, NAP increases the interaction between ADNP and microtubule-associated protein (MAP1) light chain 3 (LC3) to promote autophagosome formation and avoid the accumulation of misfolded proteins (Merenlender-Wagner et al., 2015; Figure 2). Treatment with both peptides show a potential sex-dependent therapeutic effect. Intranasal SKIP treatment does not affect male social preference for a conspecific rather than the object, but significantly reduces the time spent with the object (Amram et al., 2016). In parallel, NAP treatment can rescue abnormal dendritic spine density and spine maturation in male *Adnp*^{+/-} mice (Hacohen-Kleiman et al., 2018). As described above, NAP interacts with EB3 to promote the insertion of the microtubule at the spine, driving the plastic changes necessary for both spine formation and spine maturation during learning and memory processes (Gordon-Weeks, 2016). Moreover, both *Adnp* deficiency and NAP therapy act on mushroom spines, which form stable excitatory synapses and have been related to spatial and working memory (Mahmoud et al., 2015). Sragovich et al. (2019) also show that hippocampal VGLUT1 mRNA levels are reduced only in *Adnp*^{+/-} males but not in female mice suggesting possible broad changes in glutamatergic transmission. This decrease is restored by NAP treatment. What emerges from this analysis is that ADNP interacts with several proteins involved in the modulation of glutamatergic synapses and again suggests that disruption of sex-specific regulators of synaptic function can lead to different male and female-specific deficits.

4 | SEX-SPECIFIC NEURONAL CHANGES IN INTRACELLULAR SIGNALING: 16P11.2 CNVS AND CC2D1A

Information received by the cell via neurotransmission and transmembrane receptors is translated inside the cytoplasm by signal transduction pathways that control transcription, protein synthesis, trafficking, and ion channel function. Activity of multiple pathways that are critical for neuronal differentiation, synaptogenesis and synaptic plasticity, such as mitogen-activated protein kinases (MAPKs), protein kinase A (PKA), calmodulin kinase II and mammalian target of rapamycin (mTOR), are altered in the brain of individuals with ASD and ID (Chen, Peñagarikano, Belgard, Swarup, & Geschwind, 2015; Oron & Elliott, 2017; Wen, Alshikho, & Herbert, 2016). Here we summarize two examples of signaling disruption leading to male-specific behavioral deficits.

4.1 | 16p11.2 CNVs

CNVs can directly disrupt expression of multiple genes at once often leading to a variety of developmental phenotypes with variable presentation. Deletions and duplications in chromosomal region 16p11.2 have been described in ASD (Steinman et al., 2016) and are also observed in patients affected by Attention Deficit Hyperactivity Disorder (ADHD) (Figure 1a, Table 1) (Hanson et al., 2015; Weiss et al., 2008). Male-specific sleep disturbances were found in *del/+* mice (Angelakos et al., 2017). Altered sleep patterns and hyperactivity are two co-morbid symptoms commonly reported in NDDs, and severely affecting quality of life (Cohen, Conduit, Lockley, Rajaratnam, & Cornish, 2014; Goldman et al., 2011; Ivanenko & Johnson, 2008; Kirov & Brand, 2014). Both male and female *del/+* mice exhibited higher home-cage activity than the wild-type mice, but only *del/+* males spent significantly more time awake and significantly less time in non-rapid-eye-movement compared to the controls during polysomnography performed over a 24-hr period (Angelakos et al., 2017).

In studying reward-directed learning, Grissom et al. (2018) showed that mice with hemizygous deletion to the syntenic region to 16p11.2, 7qF3 *del/+* display male-specific deficits in outcome-action association and in motivation, paving the path for a better understanding of the underlying mechanisms of sex-difference in striatal dysfunction in NDDs. Male *del/+* mice were significantly impaired in the nine-hole nose-poke operant test, where they did not learn the association of a nose-poke action with the delivery of a positive reinforcement. Moreover, differences between mutant males and female were also described in the five-choice serial reaction time test, which measures response accuracy, impulsivity, and attention deficits with mutant males exhibiting mainly incorrect choices. These deficits were associated with male-specific increase in ERK1 phosphorylation and increase in mRNA levels of dopamine receptor 2 and adenosine receptor 2a in the striatum which are involved in the indirect pathway responsible for behavioral inhibition. ERK1 lies on the 16p11.2 chromosomal region and since its activity is involved in repression of striatal function (Mazzucchelli et al., 2002), operant learning impairments could be related to enhanced ERK1 activation. Moreover, ERK1 hyperphosphorylation is increased in both wild-type and male *del/+* mice after a sucrose reward is administered suggesting a male-specific role for ERK1 activity in the striatum.

The underlying mechanism of ERK1 hyperphosphorylation in hemideletion males remains unclear. One possible explanation was found in decreased levels of phosphatase striatum-enriched protein-tyrosine phosphatase (StEP) levels, a negative regulator of ERK (Chagniel, Bergeron, Bureau, Massicotte, & Cyr, 2014, 2016; Goebel-Goody et al., 2012; Paul et al., 2000; Figure 3). The authors found that in the striatum of *del/+* males, one isoform, StEP61, was reduced resulting in a decreased ERK1 dephosphorylation. Other upstream regulators of ERK1, such as mGluR5 expressed on both D1 and D2 medium spiny neurons were not involved in ERK1 hyperactivation and several candidates, such as, protein phosphatase-kinase intracellular signaling, and the Y-chromosome specific USP9Y protein ubiquitination/

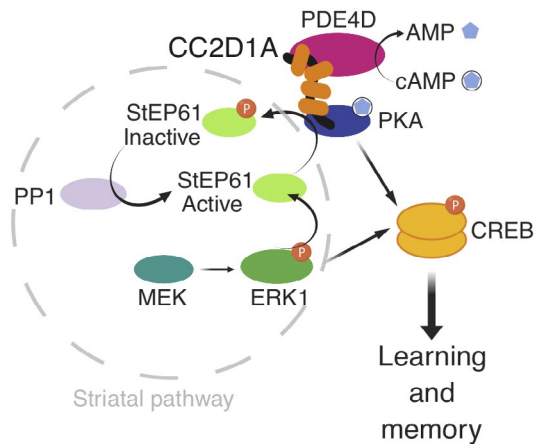


FIGURE 3 ERK1 and coiled-coil and C2 domain containing 1A (CC2D1A) signaling pathways converge in cAMP response element-binding protein (CREB) activation to enhance learning and memory processes. ERK1 activity is modulated both positively and negatively by multiple kinases and phosphatase. Here, we show ERK1 phosphorylation by MAPK/ERK kinase (MEK) and ERK1 dephosphorylation by striatum-enriched protein-tyrosine phosphatase (StEP), specifically at the striatum, as possible pathway involved in reward learning process. StEP activity is, in turn, phosphorylated by protein kinase A (PKA) and dephosphorylated by Protein phosphatase 1 (PP1). CC2D1A binds and regulates phosphodiesterase 4D (PDE4D) activity resulting in cyclic AMP (cAMP) degradation, and in PKA and CREB activation in the hippocampus

degradation factor (Lee et al., 2003) still need to be investigated. Several genes in the 16p11.2 hemideletion region, TAOK2, Sez6l2 and MVP, are associated with the regulation of neurite outgrowth and the activity of MAPK pathway, and were overexpressed in the peri-striatal and medial fiber tracts in del/+ male mice only showing a sex-specific neuroanatomical endophenotype (Kumar et al., 2018). These studies not only reveal how multiple genes affected by the 16p11.2 hemideletion could be involved in establishing a sex-specific phenotype, but also identify sex-specific anatomical changes. Changes in brain structure are often observed in autistic individuals and animal models of ASD (Courchesne et al., 2007), but do not consistently affect the same brain regions suggesting the existence of distinct groups with different alterations in connectivity (Ellegood et al., 2015; Sussman et al., 2015). Studies on large cohorts have found that brain structure changes can differ between males and females in severity and location (MRC AIMS Consortium et al., 2019; van Rooij et al., 2018). Taken together these findings support the hypothesis that interconnected disruptions in neural activity and neuroanatomy are also involved in the sex-bias in ASD.

4.2 | CC2D1A

Coiled-coil and C2 domain containing 1A (CC2D1A) is a multifunctional protein scaffold which regulates several intracellular pathways critical for neuronal function (Al-Tawashi & Gehring, 2013; Al-Tawashi, Jung, Liu, Su, & Qin, 2012; Manzini et al., 2014; Nakamura, Naito,

Tsuruo, & Fujita, 2008). Biallelic loss-of function mutations in CC2D1A cause a spectrum of ID, ASD, seizures, and aggressive behavior (Figure 1a, Table 1) (Basel-Vanagaite et al., 2006; Manzini et al., 2014; McSherry et al., 2018; Reuter et al., 2017).

Global loss of *Cc2d1a* in the mouse does not appear to grossly affect development, but causes early postnatal lethality through respiratory and swallowing deficits (Oaks et al., 2017; Zhao, Li, & Chen, 2010; Zhao, Raingo, Chen, & Kavalali, 2011). Postnatal removal of *Cc2d1a* in the forebrain, bypasses early lethality and behavioral analysis in male mice recapitulates several features of ASD and ID including cognitive and social deficits, hyperactivity, increased anxiety-like behaviors and obsessive grooming (Oaks et al., 2017; Yang, Yu, Wen, Ling, & Hsu, 2019). However, females are less severely affected showing only obsessive grooming and a deficit in object recognition, but normal spatial memory in the Morris Water Maze test, normal social function, and activity levels. CC2D1A binds phosphodiesterase 4D (PDE4D) (Al-Tawashi & Gehring, 2013) and controls its activity in degrading cyclic AMP (cAMP) which is upstream of multiple process controlling synaptic function and memory formation, including the activation of PKA (Al-Tawashi et al., 2012) and cAMP response element-binding protein (CREB), a transcription factor critical for spatial memory formation (Figure 3) (Alberini, 2009; Kandel, 2012; Ortega-Martínez, 2015; Sekeres, Neve, Frankland, & Josselyn, 2010).

Given these findings Zamarbide et al. (2019) hypothesized that CC2D1A might modulate male-specific signaling involved in learning and memory and compared signaling downstream of PDE4D in the hippocampus of male and female *Cc2d1a* conditional knock-out (cKO) mice. They found that PDE4D is hyperactive in the hippocampus of cKO males, leading to a reduction in cAMP levels and of PKA and CREB phosphorylation in male cKO mice and no difference in females. By using a selective inhibitor of PDE4D, GEBR-7b (Bruno et al., 2009, 2011), to restore wild-type levels of cAMP in the hippocampus they were able to rescue the spatial memory deficits of male cKO mice. Interestingly, GEBR-7b had no effect on female cKO performance in the Morris Water Maze test, supporting the sex-dependent role of CC2D1A in regulating molecular pathways critical for memory formation.

Similar sex-specific behavioral differences we found in a knock-out (KO) for *Cc2d1b*, the homolog for *Cc2d1a*, and in *Cc2d1a/Cc2d1b* double heterozygous animals, with more severe spatial memory deficits in *Cc2d1b* KO and *Cc2d1a/Cc2d1b* double heterozygous (+/-) males, and increased activity and anxiety only in *Cc2d1a/Cc2d1b* (+/-) males (Zamarbide, Oaks, Pond, Adelman, & Manzini, 2018). While *Cc2d1a/Cc2d1b* (+/-) males recapitulated the sex-specific phenotypes of *Cc2d1a* cKO males almost exactly, no social deficits were observed in the 3-chambered test in either males or females, suggesting that only complete loss of *Cc2d1a* may contribute to social function. No signaling information was reported to determine whether *Cc2d1a* and *Cc2d1b* both contribute to regulating the same signaling events in a sex-specific fashion.

Previous studies had shown that loss of *Cc2d1a* disrupts hippocampal synaptic plasticity and reduces spine density in both hippocampus and cortex (Oaks et al., 2017) and *Cc2d1a* had been shown

to control synaptic maturation of excitatory neurons (Zhao et al., 2011). Further studies will need to determine whether CC2D1A controls sex-specific circuit function. Conditional removal of *Cc2d1a* from the dorsal raphe showed increased anxiety and depression-like behavioral phenotypes which correlated with reduced serotonin levels and increased 5HT-1A autoreceptor in the raphe, in both males and females (Vahid-Ansari et al., 2017), suggesting that there may be regional specificity in *Cc2d1a*'s function.

PKA and MAPKs can alter synaptic transmission directly by phosphorylating a variety of ion channels to alter trafficking and activity (Mao & Wang, 2016; Woolfrey & Dell'Acqua, 2015). In parallel, downstream signaling cascades can further control neuronal plasticity by regulating local and nuclear mRNA translation and other cellular functions such as cytoskeletal remodeling and protein trafficking (Giese & Mizuno, 2013). Sex-specific synaptic signaling could be an important underlying mechanism of sex-difference in NDDs by differentially regulating electrophysiological and morphological aspects of neuronal function in males and females.

5 | LESSONS FROM FEMALE-SPECIFIC NEURONAL DEFICITS: MTHFR AND AMBRA1

Most animal models described above showed male-specific deficits that could be relevant to ASD and ID, but it remains unclear whether compensatory mechanisms may be protecting females leading to the male-bias in NDD prevalence. There are limited reports of genetic mutations that are more prevalent in girls and women with ASD. Catenin- δ (CTNND2) variants were found enriched in families with two or more severely affected females (Turner et al., 2015). No information is available on behavioral differences in *Ctndd2*^{-/-} male and female mice and no differences in synaptic activity were reported when both sexes were tested (Israely et al., 2004). Here, we will also review animal models where female-specific changes are reported in the behavioral domains relevant to ASD (Table 2). None of these genes were convincingly found to be genetic causes of ASD to date. However, these findings may be useful to understand how male- and female-specific mechanisms are established in the brain.

5.1 | MTHFR

Human *methylenetetrahydrofolate reductase* (MTHFR) is an enzyme responsible for the irreversible conversion of 5,10 methylenetetrahydrofolate into 5-methyltetrahydrofolate, a substrate necessary for homocysteine methylation to methionine, where vitamins B6 and B12 are essential cofactors (Figures 1c and 4) (Frosst et al., 1995; Goyette et al., 1994; Mitchell, Conus, & Kaput, 2014). Autosomal recessive mutations in *MTHFR* lead to a severe inborn error of folate metabolism, characterized by hyperhomocysteinemia and homocysteinuria (accumulation of homocysteine in blood and urine) leading to motor and cognitive delay, epileptic encephalopathy, brain atrophy, and death

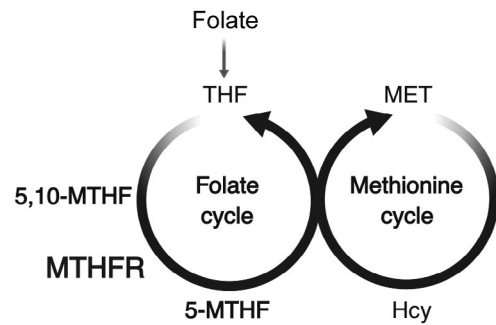


FIGURE 4 Schematic illustration of methylenetetrahydrofolate reductase (MTHFR) enzyme activity. MTHFR acts in the folate metabolic pathway. Dietary folate is converted into tetrahydrofolate (THF) that in turn is converted into 5,10 methylenetetrahydrofolate (5,10-MTHF). MTHFR irreversibly converts 5,10-MTHF into 5-methyltetrahydrofolate (5-MTHF), which is a crucial methyl donor to convert homocysteine (Hcy) into methionine (MET) and reform THF

(Goyette, Christensen, Rosenblatt, & Rozen, 1996; Goyette et al., 1994; Rozen, 1996, 1997). If diagnosed prenatally or in infancy, children can be treated with dietary supplementation of betaine to restore vitamin B metabolism and rescue brain disease (Strauss et al., 2007). The c.677C>T polymorphism in *MTHFR* has been linked to neural tube defects, heart disease, and leukemia risk (Morita et al., 1997; Skibola et al., 1999; van der Put et al., 1998). In addition, *MTHFR* has been identified as a risk gene for several neurodevelopmental and neurodegenerative diseases such as Down syndrome, depression, obsessive-compulsive disorder, Alzheimer's disease, Parkinson's disease, schizophrenia, and ASD (Jaiswal et al., 2017; Mitchell et al., 2014; Mohammad et al., 2016).

Mthfr^{-/-} mice are small and display hyperhomocysteinemia, craniofacial defects, cerebellar dyslamination, abnormal lipid deposition in the aorta, with pups often dying within two weeks from birth (Chen et al., 2001). *Mthfr*^{+/-} mice showed increased homocysteine levels, but had overall normal development exhibiting hyperactivity, anxiety, cognitive and social preference deficits (Chen et al., 2001; Levav-Rabkin, Blumkin, Galron, & Golan, 2011). Interestingly, *Mthfr*^{+/-} mice showed sex-specific responses to GABA potentiation using vigabatrin during development. Anti-epileptic drugs can interfere with homocysteine and folate metabolism during gestation when the c.677C>T polymorphism in *MTHFR* is present increasing the risk of congenital defects (Ono, Sakamoto, Mizoguchi, & Sakura, 2002). Postnatal day 4 *Mthfr*^{+/-} pups were treated with vigabatrin to block GABA degradation and tested for long-term behavioral sequelae. Female *Mthfr*^{+/-} mice showed a more marked response to treatment with reduced anxiety, hyperactivity and greater reduction in sociability than males (Blumkin, Levav-Rabkin, Melamed, Galron, & Golan, 2011; Levav-Rabkin et al., 2011), indicating that MTHFR acts together with GABAergic signaling to affect female behavior. The mechanism of such female-bias also lies on the enhancement of cortical glutamatergic signaling due to elevated levels of Reelin and fragile X mental retardation I protein which increase GluA1/GluA2 ratios at the excitatory synaptic membrane in the cortex of female *Mthfr*^{+/-} mice (Blumkin et al., 2011). Overall, these data suggest that MTHFR plays a sex-dependent role in regulating the

excitatory/inhibitory synaptic balance crucial for normal cognitive abilities and social behaviors.

5.2 | AMBRA1

Another example of female-specific deficits was found in mice with partial *activating molecule in Beclin1-regulated autophagy* (AMBRA1) deficiency (Dere et al., 2014; Mitjans et al., 2017; Nobili et al., 2018). AMBRA1 has a cytoplasmic distribution and concentrates in the endoplasmic reticulum (Sepe et al., 2014) where it interacts and positively regulates Beclin1 (coiled-coil, myosin-like BCL2-interacting protein) and the lipid kinase Vps34 (vacuolar protein sorting-associated protein 34) to promote autophagosome formation and autophagy (Fimia et al., 2007; Figures 1c and 5). Loss of *Ambra1* during mouse development causes severe neural tube defects due to a dysregulation in cell proliferation and protein turnover (Fimia et al., 2007).

In heterozygous animals from the same mutant strain comprehensive characterization of sensory, motor and anxiety behaviors found no difference between *Ambra1*^{+/-} male or female animals and wild-type (Dere et al., 2014). Female *Ambra1*^{+/-} mice displayed a significant impairment in cognitive flexibility in the Morris Water Maze test (reversal learning task) and multiple deficit in sociability tests (Dere et al., 2014). Only female pups (postnatal day 8/9) have impaired social vocalization as shown by a reduced duration and number of vocalization calls upon short separation from the mother (Dere et al., 2014). Similarly, adult *Ambra1*^{+/-} female mice do not vocalize to a female intruder mouse and spend shorter time interacting with the stranger female in two independent studies (Dere et al., 2014; Nobili et al., 2018). Though it may be difficult to define a sex-specific difference since in both cases females and males were tested with a stranger female, and social interactions with intruders for females and potential mates for males are different. In the 3-chambered

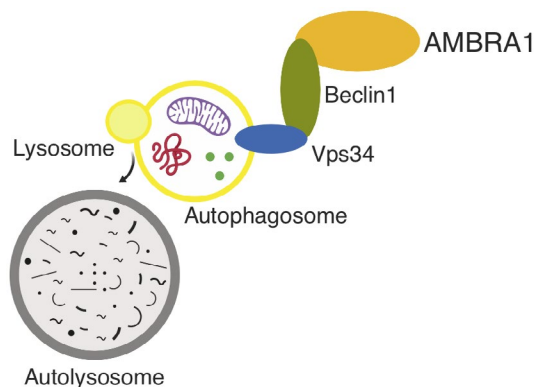


FIGURE 5 Activating molecule in Beclin1-regulated autophagy (AMBRA1) in autophagy process. AMBRA1 contribute to autophagosome formation binding Beclin1 and vacuolar protein sorting-associated protein 34 (Vps34). Autophagosome-lysosome fusion results in the autolysosome, which provide the acidic compartment where cell autophagy occurs

social test, females showed consistent deficits in social memory of a familiar animal versus a stranger mouse (Dere et al., 2014; Nobili et al., 2018), but only showed preference for a mouse versus an empty enclosure in one study (Nobili et al., 2018). In another independent study, female *Ambra1*^{+/-} mice showed no preference when given the choice to spend time in a social box containing bedding from a male versus fresh bedding (Mitjans et al., 2017). Overall, these findings show that from a young age *Ambra1*^{+/-} females show consistent sociability deficits that are not found in males. To quantify this male:female difference Dere et al. (2014) developed a severity score which includes all three major behavioral categories relevant to ASD, communication, social functions, and stereotypic/repetitive behaviors. The severity score of female *Ambra1*^{+/-} mice were higher than males indicating an ASD-like phenotype in adult *Ambra1*^{+/-} females and a milder presentation in males.

Ambra1 is expressed throughout the neuroepithelium, spinal cord, and dorsal root ganglia during the early stages of mouse development (Fimia et al., 2007) and in the forebrain, midbrain, and hindbrain, in adulthood, being specifically enriched in cholinergic neurons from the septohippocampal system, a region associated with learning and memory (Sepe et al., 2014). A greater reduction of AMBRA1 protein levels than mRNA levels was noted in female *Ambra1*^{+/-} mice and was suggested as possible molecular mechanism for the sex difference in this mouse line (Dere et al., 2014; Nobili et al., 2018). Female-specific reduction in interneuron markers GAD67 and parvalbumin (PV) and increase in PSD95 were also observed (Nobili et al., 2018). Excitatory/inhibitory synapses imbalance is a key underlying mechanism of NDD and female *Ambra1*^{+/-} mice were found to be more susceptible to seizure induction (Mitjans et al., 2017) and to have enhanced LTP (Nobili et al., 2018). In parallel, hippocampal loss of PV-positive interneurons was noted and possibly caused by increased apoptosis in the medial ganglionic eminence during mid-gestation (Nobili et al., 2018). Thus, *Ambra1* haploinsufficiency in mice result in a loss of hippocampal PV interneurons, decreases in the inhibition/excitation ratio, and altered social behaviors that are solely restricted to the female sex. Overall, this research identifies AMBRA as regulator of synaptic transmission and sociability by disrupting autophagy in a sex-specific fashion. Autophagy is a self-degradative process that modulates protein homeostasis and it has been suggested as one of the mechanisms participating in regulation of synaptogenesis and proper neural circuit organization which may underlie the pathology of ASD (Nikoletopoulou & Tavernarakis, 2018).

The role of autophagy in ASD pathogenesis has been described in two mouse models carrying mutation in the ASD-associated genes *Tsc* and *Foxp1* (Bacon & Rappold, 2012; Bourgeron, 2009; Hamdan et al., 2010; Sollis et al., 2016). *Tsc2*^{+/-} mice show mTOR hyperactivation, postnatal spine pruning defects, impaired neuronal autophagy and social behavior. mTOR inhibition by rapamycin, rescues the spine defects and the social deficits in *Tsc2*^{+/-} mice (Tang et al., 2014). Similarly, in *Foxp1* mutant mice, altered autophagy leads to abnormal cortical development with delayed radial migration and altered dendritic morphology (Li et al., 2018).

6 | CONCLUSION

The origin of sex bias in NDDs is still poorly understood, but careful analysis of males and females in mouse strains recapitulating human NDD mutations has revealed that molecular and cellular sex-specific mechanisms must be considered. The goal of this review was to summarize the data generated to date on sex-specific changes in models of NDDs. A major hindrance in defining consistent patterns in these data is the great variability in the behavioral paradigms performed or reported by different groups, often lacking corresponding electrophysiological and/or molecular findings. It remains unclear whether specific circuits or molecular pathways are prevalently affected. Cognitive deficits seem to be more often male-specific, while female-specific deficits were more frequent in sociability tests. However, these differences could also be explained by age at testing and genetic background strain and more information is needed. As a wealth of data begins to accumulate on sex-specific changes in animal models of NDDs, it will be important to compare different genetic mutations in a defined battery of tests studying both circuit function and behavior with particular attention to the genetic background and its likely role as a modifier.

Until recently, the majority of published studies on NDD mouse models were performed on male mice due to the faulty assumption that female behavior would show increased variability due to the estrous cycle and greatly hindering the detection of sex-specific deficits. It has been demonstrated by multiple groups that this is not the case in either mice or rats (Becker, Prendergast, & Liang, 2016; Dayton et al., 2016; Prendergast, Onishi, & Zucker, 2014; for review see Beery, 2018 and Shansky, 2019). We have purposefully not included a discussion of sex hormones as their role was not tested in these studies. However, the role of sex hormones is an important consideration while interpreting how sex-specific deficits are established and maintained in NDDs. Estrogens and androgens are critical in the development of the male and female anatomy, physiology, and sexual behavior. In addition to what was traditionally hypothesized as an organizational role during development, they also have continued effects throughout the lifespan. Like other steroid hormones, such as corticosteroids, sex hormones act broadly on the brain modulating neuronal plasticity and survival, and 17β -estradiol and testosterone can be produced locally in several brain regions and contribute to both neurogenesis and circuit function (for review see Frick, Tuscher, Koss, Kim, & Taxier, 2018; Hojo & Kawato, 2018; Hyer, Phillips, & Neigh, 2018). To understand the etiology of NDDs and develop therapeutic approaches, it will be beneficial to define whether sex-specific changes observed in NDD mouse models are influenced by local or gonadal sex-hormones, or whether they were established during early development through a variety of genetic, epigenetic, and/or endocrine factors.

The studies summarized here show how a genetic mutation may lead to sex-specific molecular, physiological, and/or behavioral differences, often by altering synaptic homeostasis. In addition, they

reveal that sex-specific changes in gene expression and synaptic plasticity may be present even in the sex where behavioral deficits are not observed. It is often unclear whether there are compensatory mechanisms at play, or whether convergent strategies are used by males and females to perform the same behaviors. In the *Gabrb3* and *Chd8* studies baseline differences in synaptic activity were found in wild-type mice leading to distinct alterations in the mutant animals (Jung et al., 2018; Mercer et al., 2016) and suggesting some degree of convergence. In addition, males and females may be affected at distinct developmental time-points, as observed in the *Gabrb3* (*m-p+*) mouse line, where motor performance is initially affected in juvenile females and then only in adult males (De Lorey et al., 2011; Mercer et al., 2016). Without the need of making a distinction between "female brain" versus "male brain", there are ethological and physiological regions by which the brain may need to allocate function differently in males and females using distinct circuits or molecular strategies to control behavior at different life-stages or for specific tasks. Catherine Woolley's group also introduced the concept of "latent" sex differences in synaptic function showing that the same synaptic output can be achieved via different sex-specific activation of intracellular signaling pathways (Jain, Huang, & Woolley, 2019; Tabatadze, Huang, May, Jain, & Woolley, 2015). Thus, it cannot be assumed that an identical behavioral or electrophysiological readout in males and females is achieved via identical molecular mechanisms.

Defining whether sex-specific deficits in NDDs are due to compensatory or convergent mechanisms will provide critical insight for therapy development. If the lack of behavioral deficits in one sex is due to compensation in the other, understanding how compensation is achieved, e.g., gene expression, intracellular signaling, or synaptic plasticity, may help us correct the imbalance in the more severely affected sex. If sex-specific deficits are based on convergent mechanisms where the same behavioral outcome is reached using sex-specific molecular strategies or allocating function to different brain circuits, it is possible that treatments may only be effective in males, as shown in *Adnp* and *Cc2d1a* mutant mice (Amram et al., 2016; Hacoen-Kleiman et al., 2018; Zamarbide et al., 2019). In this case, we will need to appropriately power pre-clinical and clinical studies to understand these differences and consider the need sex-specific therapies.

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CONFLICT OF INTEREST

The authors have no conflicts to declare.

AUTHOR CONTRIBUTIONS

Conceptualization, A.M. and M.C.M.; *Visualization*, A.M. and M.C.M.; *Writing - Original Draft*, A.M. and M.C.M.; *Writing - Review & Editing*, A.M. and M.C.M.; *Supervision*, M.C.M.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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