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The identification of a novel electrophysiological endophenotype in the Valproic Acid rat model of autism unveils new disease pathways for the development of specific pharmacological treatments

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SUMMARY

INTRODUCTION

1. Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a highly heritable and heterogeneous neurodevelopmental disorder characterized by a deficit in social behavior, nonverbal interaction, sensory anomalies, repetitive behavior, and variable levels of intellectual disability, often associated with comorbid features such as hyperactivity and attention disorders (such as attention-deficit/ hyperactivity disorder (ADHD)), anxiety, depression, and epilepsy (Lord et al. 2020). The prevalence of ASD is about 1% ~ 2%, placing this disorder as one of the most common pervasive developmental disorders raising strong public concern. ASD affects males 2~3 times more than females suggesting a possible female-specific protective effect against ASD. Interestingly, prevalence has been increasing over the past 50 years as a result of changes in Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria, and increase in risk factors (Park et al. 2016). Autism was first reported by Kanner in 1943 with a clinical description of 11 children showing "extreme aloneness from the very beginning of life, not responding to anything that comes to them from the outside world." He described the behavioral combination of autism, obsessiveness, stereotypy, and echolalia as "childhood schizophrenia". However, ASD was not classified as a separate developmental disorder with a specific organic origin until 1980s. In the early 1980s, studies demonstrated the strong inheritance (~90%) of ASD and its association with other genetic syndromes, providing compelling evidence for a genetic component in the aetiology of ASD and fuelling the conceptualization of autism as a distinct neurodevelopmental disorder. In recent years, some researchers suggest that ASD is the result of functional and structural changes during Central Nervous System (CNS) development in the pre- and post-natal period, which appear to be at the basis of the complex behavior and cognitive features of this disorder.

1.1. Clinical characteristics and diagnosis of ASD

Autism is typically diagnosed within the third year of life. Although substantial heterogeneity exists between and within individuals across development, a set of core diagnostic features of autism including deficit in social interaction, verbal communication, restricted and repetitive or sensory behaviors can be reliably identified. According to DSM-5, for a diagnosis of ASD, a child must display persistent deficits in each of three areas of social communication and interaction plus at least two of four types of restricted, repetitive behaviors ("must" criteria A, B, C, D) (Diagnostic and Statistical Manual of Mental Disorders [DSM-5]):

A. Persistent deficits in social communication and social interaction across multiple contexts, not explainable through a generalized delay in development and manifested by all 3 of the following points:

1. Deficits in social-emotional reciprocity, ranging from abnormal social approach and failure of normal back-and-forth conversation to reduced sharing of interests, emotions, affect, or failure to initiate or respond to social interactions.
2. Deficits in nonverbal communicative behaviors used for social interaction, ranging from poorly integrated verbal and nonverbal communication to abnormalities in eye contact and body language or deficits in understanding and use of gestures, to a total lack of facial expressions and nonverbal communication.
3. Deficits in developing, maintaining, and understand relationships, ranging from difficulties to adjust their behavior to suit various social contexts. to difficulties in sharing imaginative play or in making friends, to the lack of interest in peers.

C. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history:

1. Stereotyped or repetitive motor behavior, use of objects, or speech (e.g., simple motor stereotypes, lining up toys or flipping objects, echolalia, idiosyncratic phrases).
2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior (e.g., extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take the same route or eat the same food every day).
3. Highly restricted, fixated interests that are abnormal in intensity or focus (e.g., strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interests);
4. Hyper- or hyporeactivity to sensory input or unusual interest in sensory aspects of the environment (e.g. apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement).

D. Symptoms must be present in the early developmental period, although they may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life.

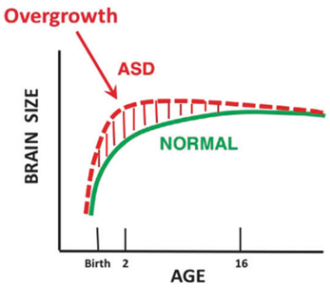
E. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning. In association with these core features, autistic children often manifest non-specific symptoms including aggression, self-injury, sleep disorders, seizure or epilepsy, gastrointestinal complaint, depression, and learning disability.

1.2. Neurochemical and neuropathological features of ASD

Studies of neurological syndromes with autistic-like symptoms indicate that ASD is the result of functional and structural changes during CNS development in the pre- and post-natal periods. Neuroanatomical studies have reported macrocephaly and defect in brain cytoarchitecture accompanied by impaired neuronal connectivity. This suggests that the formation of functional neuronal circuits plays an important role in the genesis of ASD (Gilbert and Man 2017).

1.2.1 Neuroanatomical alterations: macrocephaly in ASD

Evidence of anatomical and morphological abnormalities in ASD patients have been primarily obtained using magnetic resonance imaging (MRI) starting from the second year of life. MRI finds an overall brain overgrowth, termed macrocephaly, in ~20% of autistic children. Abnormal acceleration is observed during early brain development (Fig.1) and affects several brain regions including the frontal lobe, parietal-temporal lobe, cerebellum, subcortical limbic structures (in particular the amygdala), and is due by increased white matter, accompanied by reduced gray matter, attributed to over-connectivity (Amaral, Schumann, and Nordahl 2008). Anatomical alterations observed strongly correlated with the key features of ASD such as social and communication deficits and motor impairment (Fig.1).



Social impairment	Communication deficits	Repetitive behaviors
OFC – Orbitofrontal cortex ACC – Anterior cingulate cortex FG – Fusiform gyrus STS – Superior temporal sulcus A – Amygdala mirror neuron regions IFG – Inferior frontal gyrus PPC – Posterior parietal cortex	IFG- Inferior frontal gyrus (Broca's area) STS – Superior temporal sulcus SMA – Supplementary motor area BG – Basal ganglia SN – Substantia nigra Th – Thalamus PN – Pontine nuclei cerebellum	OFC – Orbitofrontal cortex ACC – Anterior cingulate cortex BG – Basal ganglia Th – Thalamus

Figure 1: Illustration of early brain overgrowth and the correlation between anatomical changes and ASD. Brain overgrowth in the first years of life is a common feature of ASD probably due to prenatal cell cycle dysregulation that causes an overabundance of cortical neurons that could lead to disrupted neural network development and function (left). Brain areas implicated in the mediation core ASD-behaviors: social behavior, language and communication, and repetitive and stereotyped behaviors. Adapted from (Amaral et al. 2008; Courchesne et al. 2019).

The cerebellum is a key brain area for motor coordination and control. It is also involved in other cognitive functions including attention and language as well as emotional control, such as regulating fear and pleasure responses (Schmahmann and Caplan 2006; Wolf, Rapoport, and Schweizer 2009). The cerebellum anatomy is strongly compromised in ASD as MRI studies have reported

hypoplasia of the vermis and hemispheres (Amaral et al. 2008). Anatomical changes were accompanied by evidence of a reduction in the number of cerebellar Purkinje cells in post-mortem studies. Certain genetic animal models demonstrate the correlation between specific gene mutations, associates with ASD in human, and cerebellar dysfunctions. For instance, selective deletion of tuberous sclerosis 1 (Tsc1) and neuroligin-3 (Nlgn3) genes induces a deficit in motor coordination, reduces Purkinje cells excitability, and impairs cerebellar connectivity (Baudouin et al. 2012; Tsai et al. 2012).

The amygdala is a cluster of nuclei located deeply and medially within the temporal lobes and anteriorly to the hippocampal formation. It is considered a major component of the limbic system and a critical hub in the cortico-striato-thalamo-cortical circuit. This brain region is particularly involved in the modulation of memory, decision-making, and emotional response, including fear, anxiety, and aggression. Given its role in the social-emotional component and fear processing, it is no surprise that some evidence for the amygdala deficit in patients with ASD has been suggested. The amygdala in children with autism appears to undergo an abnormal developmental process that includes a period of precocious enlargement that persists through late childhood (Amaral et al. 2008; Park et al. 2016; Won, Mah, and Kim 2013).

The frontal lobe is another target area involved in ASD. MRI studies show abnormal enlargement in the frontal area probably due to increased cortical surface area or thickness and increased volume of frontal axonal tracts (Courchesne et al. 2019). Individuals with frontal lobe deficit demonstrate autism-like dysfunction in higher-order cognitive, language, social, and emotional processing. In particular, the prefrontal cortex is especially linked with ASD as it is involved in social cognition and understanding self and others a variety of 'higher' cognitive functions (Wood and Grafman 2003), higher-order cognitive functions that are severely compromised in ASD as will be described in "*The cognitive theories of ASD*".

However, although major neuroanatomical defects are found in autistic brains, a direct causal link between such malformations and behavioral symptoms remains to be established.

1.2.2 Extracellular factors: growth and neurotrophic factors in ASD

It has been found that both growth and neurotrophic factors are associated with ASD. Clinical studies in Italian and American families have shown that the levels of MET (a transmembrane receptor for a hepatocyte growth factor HGF with tyrosine kinase activity) mRNA and corresponding protein were decreased in the cerebral cortex of ASD patients (Campbell et al. 2007). By activating MET signaling, HGF acts as a neurotrophic factor and influences neurite outgrowth and dendritic morphology (Fig.2), suggesting that changes in MET activity cause abnormal neuronal structures typical of ASD pathology (Gutierrez 2004; Sun, Funakoshi, and Nakamura 2002). Another growth factor linked to ASD is WNT2 (Wingless-type MMTV integration site family, member 2), a member of the canonical Wnt pathway involved in several developmental processes including embryonic development, cellular differentiation, cell-polarity generation, neuronal migration, axon guidance,

and dendrite branching (Fig.2). The WNT2 gene is located at the ASD susceptibility chromosomal locus 7q31(Vincen et al. 2000; Warburton et al. 2000) and animal models of WNT2 mutation reproduce autistic-like behavior. As WNT2, also the Brain-derived neurotrophic factor (BDNF), calcium-dependent secretion activator 2 (CADPS2, that interacts with BDNF to regulate exocytosis), and reelin (RELN), genes are located in an autism susceptibility locus. BDNF is crucial for neurogenesis, axodendritic growth, neuronal/synaptic differentiation (Fig.2). Reelin is a large secreted extracellular matrix glycoprotein that helps regulate processes of neuronal migration and positioning in the developing brain by controlling cell-cell interactions (Fig.2). In the adult brain, RELN modulates synaptic plasticity by enhancing the induction and maintenance of long-term potentiation (LTP). It also stimulates dendrite and dendritic spine development and regulates the continuing migration of neuroblasts generated in adult neurogenesis (Won et al. 2013). Given their role in CNS development and organization is not surprising that any mutation in these genes is implicated in the genesis of various neurodevelopmental disorders, including ASD.

1.2.3 Transcription factors

Transcription factors have been involved in syndromic forms of ASD. By regulating gene expression, transcription factors have a significant influence on different neuronal and cellular functions. MeCP2 (X-linked gene methyl CpG binding protein 2) is one of the best characterized ASD-related transcription factors. MeCP2 is a methyl-CpG binding domain (MBD) protein that represses gene transcription when DNA is methylated (Fig.2) (Bienvenu and Chelly 2006). Among its downstream targets, BDNF and CDKL5, previously reported as involved in ASD. Moreover, MeCP2 mutations cause Rett syndrome, a neurodevelopmental disorder with autistic features (Amir et al. 1999; Bienvenu and Chelly 2006). Engrailed-2 is a homeodomain transcription factor encoded by the *EN2* gene in humans. It is a member of the engrailed gene family important for embryological development and segmental polarity to brain development and axon guidance (Fig.2) (Brunet et al. 2005; Joyner 1996). *EN2* gene is located in the autism susceptibility locus and single nucleotide polymorphisms (SNPs) of *EN2* gene. In particular, rs1861972 and rs1861973 were found in 518 ASD families demonstrating a strong correlation with the disease (Benayed et al. 2005; Gharani et al. 2004). Since Engrailed-2 is expressed in response to WNT2-Dvl1 signaling activation, it appears that the WNT2- Dvl1-Engrailed-2 pathway, given its role in regulation in neuronal migration and axonal guidance, may contribute to the neuroanatomical abnormalities of ASD.

1.2.4 Synaptic alterations in ASD

Synapses are highly specialized structures essential to neuronal functioning at the basis of fast neuron-neuron communication and plastic remodeling of neuronal networks following experience. Synaptic dysfunctions, associated with functional mutations in pre- or postsynaptic proteins, are considered as a major component in the pathophysiology of ASD.

- ***Presynaptic proteins linked to ASD***: Neurexins (NRXN) are presynaptic cell adhesion proteins, that connect at the synapse. They are encoded by three different genes: NRXN1, NRXN2, and NRXN3 (Fig.2). The extracellular domain interacts with Neuroligins (NLGNs), their counterparts on the postsynaptic membrane. NRXNs are linked to ASD because multiple mutations or copy number variations having been identified in ASD diagnoses (Bremer et al. 2011). NRXN1α KO mice show functional synaptic aberrations such as reduced excitatory synaptic strength, decreased input-output relationship of evoked postsynaptic potentials, and frequency of miniature excitatory postsynaptic currents (mEPSC). Furthermore, these mice have been found to have an increase in grooming behavior (Etherton et al. 2009). The Synapsin protein family (SYN1, 2, 3) have long been implicated in the regulation of neurotransmitter release at synapses and neurite outgrowth. Indeed, they control the neurons' number of synaptic vesicles available for release at the active site (Evergren, Benfenati, and Shupliakov 2007). Synapsin KO mice display behavioral deficits in social novelty test and avoidance behavior in social approach as well as epileptic activity, all of which are typical in ASD (Greco et al. 2013; Ketzef and Gitler 2014). Mutations in SYN1 have been identified in a family with epilepsy and autistic phenotypes, suggesting its link as a causative factor for ASD. Within these mutations, the nonsense mutation, Q555X, is associated with a reduction in CaMKII and MAPK/ERK activity, which influence synaptic vesicle trafficking and neurite growth, while missense mutations in A550T and T567A impaired presynaptic synapsin localization (Fassio et al. 2011). Additionally, SYN2 KO mice are characterized by autistic-like signs as impairments in social recognition tests and an increase in repetitive self-grooming (Greco et al. 2013).
- ***Postsynaptic proteins linked to ASD:*** Neuroligins (NLGN) are cell adhesion proteins located postsynaptically that mediate synapse formation through physical interaction with their presynaptic partners, NRXNs (Fig.2). NLGN family includes five genes (NLGN1, 2, 3, 4, 4Y). NLGNs have distinct expression patterns and a role in synaptic transmission. Nlgns-1, -3, and -4 are localized to excitatory synapses, while Nlgn2 is found at inhibitory synapses (Missler et al. 2003). Because of the nature of their functions, mutations in gene encoding, NRXNs and NLGN could be considered as in the pathogenesis of cognitive diseases. (Südhof 2008). SH3 and multiple ankyrin repeat domains 3 (Shank3), also known as proline-rich synapse-associated protein 2 (ProSAP2) is a scaffolding protein located postsynaptically in excitatory synapses, involved in synaptic development and function (Fig.2). The SHANK family is composed of three members, Shank1, Shank2, and Shank3. Mutations in the member of SHANK family genes have been associated with syndromic and idiopathic ASD (Sala et al. 2015), as well as with typical ASD-behavioral such as repetitive routines, social impairment, and anxiety. At the morphological level, loss of Shank3 in vitro results in a decrease in both the length and density of spines. On the contrary, over-expression of Shank3 results in more mature and larger spines (Betancur, Sakurai, and Buxbaum 2009). The deletion of Shank3 is strongly linked to Phelan-McDermid syndrome, a severe neurodevelopmental disorder characterized by developmental

delay, impaired language, ASD, and intellectual disability (Phelan 2008a). Transgenic mice carrying different mutations or deletions were key to better understanding the role of SHANK in ASD. Transgenic mice reproduce human characteristic alterations, in particular social impairments and repetitive grooming (Bozdagi et al. 2010; Peça et al. 2011; Wang et al. 2011). SHANK3 interacts with postsynaptic density protein 95 (PSD-95), a protein that in humans is encoded by the discs large homolog 4 (*DLG4*) gene (Fig.2). PSD-95 is an important postsynaptic scaffolding protein that localizes to excitatory synapses. PSD-95 is a membrane-associated guanylate kinase (MAGUK) member that, along with PSD-93, is recruited into the same NMDA receptor and potassium channel clusters. It is almost exclusively located in the postsynaptic density of neurons as well as to spine heads in excitatory synapses (Hunt, Schenker, and Kennedy 1996), and is involved in anchoring synaptic proteins. Its direct and indirect binding partners include neuroligin, NMDA receptors, AMPA receptors, and potassium channels (Sheng and Sala 2001). It plays an important role in synaptic plasticity and the stabilization of synaptic changes during LTP (Meyer, Bonhoeffer, and Scheuss 2014). PSD-95 KO mice show not only multiple ASD-like behavioral abnormalities but also display defects in dendritic spine morphology (Feyder et al. 2010). Gephyrin is a postsynaptic scaffolding protein, that in humans is encoded by the *GPHN* gene, found in inhibitory synapses where it stabilizes inhibitory neurotransmitter receptors, including GABAA, and glycine receptors. It is composed of three N terminal G domain, C terminal E domain, and a large unstructured linker domain that connects the two. Mice KO mice for this gene display a decrease in GABAA and glycine receptor clustering (Kneussel et al. 1999). Rare exonic deletions within the gephyrin (GPHN) gene have been reported in ASD diagnosed patients (Lionel et al. 2013).

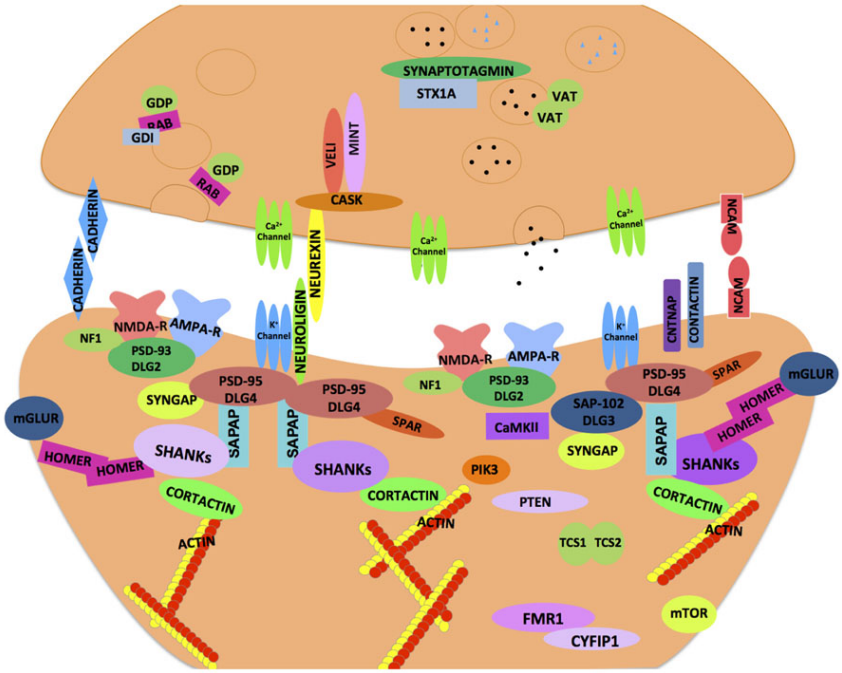


Figure 2: Synaptic proteins associated with ASD. A schematic illustration of an ensemble of pre- and post-synaptic proteins genes linked with ASD. Adapted from (Kanjhan et al. 2005).

1.3. The cognitive theories on the genesis of ASD

Although the precise causes of ASD are still unknown, some theories have been proposed in the attempt to explain the cognitive and biological basis of the core deficit observed in autism. The prevailing hypothesis of autism's origin are the following:

- ***The theory of mind (ToM)***. Proposed for the first time by Simon Baron-Cohen and Uta Frith in the early '80s, with this theory the authors proposed a novel paradigm to explain social impairment in children diagnosed as autistic. In their article, "*Does the autistic child have a theory of mind*?", the authors explain how the inability of ASD children to represent the mental state of themselves and others, prevents them to understand or predict the others' behavior and intentions. As a consequence of this lack of a theory of mind of the other, ASD children are impaired in their understanding of mental states (Baron-Cohen, Leslie, and Frith 1985).
- ***Executive function theory***. According to this theory, there seems to be a relationship between prefrontal deficit and autism as a consequence of executive function impairment in autistic subjects. Due to the similarity of this pattern to patients with frontal lobe damage the "*executive function theory"* hypothesizes that some developmental disorders cause disturbances in neuronal circuits of the frontal lobe or hippocampus or yet the basal ganglia (Upton and Corcoran 1995). Autistic patients would not be able to relate to the organization and perception of inter-human experience as a structured set of goal-directed behaviors.
- ***Central coherence theory***. "Central coherence" defines the human ability to derive overall meaning from details. The Central coherence process is based on understanding meaning. Uta Frith, in 1989, hypothesized that the social deficit observed in ASD patients was a result of specific impairment in the ability to integrate information at different levels. ASD is characterized by a "*weak central coherence";* in fact, autistic children have a peak of performance in activities that do not require integration but, on the contrary, segmentation of information. Following this theory, ASD brains would have a cognitive modality that favors segmentation due to the limitation or impossibility of more complex information processing (Frith 1996; Frith and Happé 1994).

2. PREVALENCE OF ASD

Epidemiological studies conducted over the past 50 years demonstrate that the prevalence of ASD appears to be increasing globally. There are many possible explanations for this apparent increase, which include improved awareness, expansion of diagnostic criteria, better diagnostic tools, and improved reporting. During the first epidemiological study, conducted in the UK in 1966, the prevalence rate of autism was 4.5 in 10,000 children. In subsequent years prevalence increased to 19 in 10,000 American children during 1992 and rose steeply to 1 in 150 in 2002 (Newschaffer et al. 2007), 1 in 110 in 2006 (Autism et al., 2009), 1 in 132 in 2010 until to get to 1 in 54 in 2020(see also data from the US Centers for Disease Control and Prevention [CDC]). Epidemiological studies have suggested that ASD is more common in males than in females, with reported ratios of 4:1 in the 2010 Global Burden of Disease study. In Italy, data for ASD prevalence are available from the regions of Emilia-Romagna and Piedmont. The most recent findings indicate a total prevalence in the population up to 18 years of 2.3/1000 in Emilia-Romagna during 2011 and 2.9/1000 in Piedmont during 2010, with estimates rising to 2, respectively. 8/1000 and 4.2/1000 in primary school age (6-10 years).

The currently estimated global prevalence of ASD, based on consistent reports of ASD prevalence by multiple sources in different populations, is ∼1%, placing this disorder as one of the most common pervasive developmental disorder raising public concern (Lotter 1966; Maenner et al. 2020; Won et al. 2013).

In conclusion, epidemiology is a fundamental discipline for the study of ASD, as it allows to estimate the prevalence and incidence of these disorders, to describe the variations in their distribution in time and space, and to investigate their etiopathogenesis, as well as the role of individual genetic or environmental risk factors and their possible interactions.

3. DRUG THERAPY IN ASD

Despite early diagnosis and intervention, to date, there is no specific therapy to completely reverse the core symptoms of ASD. Indeed, in current practice, there is no specific treatment for ASD as the state-of-the-art therapy involves primarily an educational approach (behavior analysis, speech therapy, sensory integration therapy, auditory therapy) to improve learning, communication, adaptive and social functioning, and reduction in inappropriate behaviors such as aggression or hyperactivity. Currently, ASD treatment included pharmacological and non-pharmacological therapy such as food supplementation, detoxification, dietary intervention, treatment of gastrointestinal disturbances, treatment of chronic inflammation in the brain and intestine, and immunologic treatments. ASD cannot be cured completely with medications, however many pharmacologic agents may be effective in treating single behavioral symptoms that are interfering with daily life and that may be causing impairment or distress (Kumar et al. 2012). Only two medicines have been approved by the Food and Drug Administration (FDA), including risperidone (Risperdal®) and aripiprazole (Abilify®) specifically used for the treatment of additional symptoms such as irritability and motor deficit. Unfortunately, to date, no pharmacological treatment for the social deficit has been found, although some improvements have been demonstrated with the intranasal administration of oxytocin (Won et al. 2013). Clinical and preclinical ASD researches have identified different pathobiological targets to develop new drugs for the treatment of ASD children. Table 1 summarized the various drugs under developmental stages. These drugs act on different targets to cure the behavioral as well as neurological symptoms of ASD.

Drugs	Action	Developmental s
KM 391	Acts on serotonin uptake	Preclinical sta
Docosahexaenoic acid	Omega-3-fatty acids	Clinical trials
Fluconazole	Antifungal action	Clinical trials
Methylphenidate	Psychostimulant	Clinical trials
Oxcarbazepine	Anticonvulsant and mood stabilizing drug	Clinical trials
Arbaclofen (STX209)	Specific GABA receptor agonist	Phase II
Buspirone	Psychoactive agent	Phase II
Citalopram	Selective serotonin reuptake inhibitor	Phase II
CX516	Cognition/memory enhancement	Phase II
Dimercaptosuccinic acid	Chelating agent	Phase II
Divalproex sodium	Anticonvulsant	Phase II
Donepezil HCl	Acetylcholinesterase inhibitor	Phase II
Lenalidomide	Anti-inflammatory	Phase II
Mecamylamine	Nicotinic antagonists	Phase II
Memantine	NMDA blocker	Phase II
Methylcobalamin	Vitamin	Phase II
N-Acetylcysteine	Antioxidant	Phase II
Olanzapine	Decrease disruptive behaviors in autism	Phase II
Oralgam	Human immunoglobulin	Phase II
Oxytocin	Hormone	Phase II
Pioglitazone	Antihyperglycemic	Phase II

R-baclofen	Specific GABA receptor agonist	Phase II
Riluzole	Sodium channel blocker	Phase II
Ziprasidone	Atypical antipsychotic	Phase II
Fluoxetine	Selective serotonin reuptake inhibitor	Phase III
Fluvoxamine	σ-1 and selective serotonin reuptake inhibitor	Phase III
Galantamine	Cholinesterase inhibitor	Phase III
Naltrexone	Opioid receptor antagonist	Phase III
Paliperidone ER	Atypical antipsychotic	Phase III
RG1068	Synthetic human secretin	Phase III
Bumetanide	Diuretic action	Phase III
D-cycloserine	NMDA modulator	Phase III
Saproterin dihydrochloride	An enzymatic cofactor	Phase III
Sertraline	Selective serotonin reuptake inhibitor	Phase III
Valproate	Anticonvulsant	Phase III
Divalproex sodium ER	Anticonvulsant	Phase IV
Levetiracetam	Anticonvulsant	Phase IV
Minocycline	Antibiotic	Phase IV
Risperidone	Atypical antipsychotic drug	Phase IV
Aripiprazole	Antipsychotic drug	Phase IV
Atomoxetine (Strattera)	Selective norepinephrine transporter inhibitor	Phase IV

Table 1: Different drugs for ASD treatment. Potential preclinical drugs with the corresponding development stage. Adapted from (Kumar et al. 2012).

4. ETIOLOGY OF ASD

In spite of the numerous studies that have been undertaken to elucidate the pathogenic mechanisms underlying ASD, the exact cause remains still unknown. ASD etiology is very complex and heterogeneous and currently, it is broadly considered to be a multifactorial disorder resulting from genetic and non-genetic risk factors and their interaction.

- ***Genetic risk of factors in ASD:*** Twin and family studies consistently demonstrate that autism has a particularly large genetic contribution, with estimated inheritance ranging from ~40% to 90%. Besides, one analysis demonstrated that ASD is among the most inheritable common medical conditions. To support this, the risk of developing ASD is 50 times greater in families with even just one ASD member, with a recurrence rate of 5%~8% (Szatmari et al. 1998). Genome-wide linkage studies suggested linkages on chromosomes 2q, 22q, 7q, 15q, and 16p as the location of susceptibility genes (Wassink et al. 2004). Genetic causes, including gene defects and chromosomal anomalies, have been implicated in the disruption of neural connections, brain growth, and synaptic/dendritic morphology. HOXA1 is a critical gene that, within its functions, is involved in the development of the central nervous system. HOXA1 polymorphism has been found to induce an increased head growth rate in ASD children (Muscarella et al. 2007). Within several candidate genes, both tuberous sclerosis 1 (TSC1, also known as hamartin) and fragile X mental retardation 1 (FMR1) genes are strongly associated with ASD. They lead respectively to tuberous sclerosis and Fragile X syndrome (FXS), both characterized by ASD and intellectual disability (ID). ASD is estimated to affect 40–60% (Jeste et al. 2008; Richards et al. 2015) of patients with TSC. The cognitive and behavioral profiles of children with TSC and idiopathic ASD are very similar, suggesting shared abnormalities circuit-level (Bruining et al. 2014; Jeste et al. 2016). The main function of the TSC complex is to integrate multiple upstream signaling pathways and inhibit the activity of RHEB (Ras homolog enriched in brain) and mTORC1. The link between the TSC complex and mTORC1 was demonstrated in TSC-deficient animals, in which neurobehavioral phenotype was attributed to mTORC1 hyperactivation. Indeed, abnormal social behavior and cognitive impairment were ameliorated after the administration of rapamycin, a mTORC1 inhibitor (Ehninger et al. 2008). ASD prevalence in FXS is approximately 30% (Hagerman, Hoem, and Hagerman 2010), and like TSC is associated with ID, epilepsy, and ASD. FXS is usually caused by a triplet repeat expansion within fragile X mental retardation protein (FMRP), an mRNA-binding protein involved in translational regulation (Darnell and Klann 2013). Animal models carrying this mutation showed enhanced protein synthesis-dependent, metabotropic glutamate receptor (mGluR)-mediated long term depression (LTD) and dendritic spine elongation, together with cognitive deficits, impaired in peer interactions and socio-emotional reciprocity (Bernardet and Crusio 2006). Moreover, as previously described for TSC, also in FXS there is a link with mTORC1.

Increased phosphorylation of S6K1 (a hallmark of mTOR activation indicating hyperactivation of mTORC1) was found in the hippocampus of FMR1 KO mice. In line with this, evidence of mTORC1 activation was also found in fibroblasts, lymphocytes, and post-mortem brain tissue of human individuals with FXS (Kumari et al. 2014; Sharma et al. 2010). Phosphatase and tensin homolog (PTEN) gene is a negative intracellular regulator of phosphatidylinositol-3,4,5-trisphosphate (PIP3) levels in cells and a tumor suppressor that negatively regulates the Akt/PKB (protein kinase B) signaling pathway. Originally associated with cancer (Cowden syndrome), PTEN gene mutations have also been associated with brain morphological changes observed in ASD (Goffin et al. 2001). PTEN mutations can be detected in 7%-17% of individuals with ASD (Varga et al. 2009). In mouse models, PTEN-deficient enhances levels of PIP3 that lead to activation of the Akt/PKB and mTOR pathway. Since mTOR signaling is involved in multiple cellular functions, including cell growth and proliferation, PTEN-deficient animals display neuronal over-growth, brain enlargement, epilepsy, and premature death (Garcia-Junco-Clemente and Golshani 2014; Kwon et al. 2006a). Pharmacological inhibition of mTORC1 ameliorates the core features of PTEN-deficient animals, suggesting that mTORC1 hyperactivation is responsible for this phenotype (Xiong et al. 2012; Zhou et al. 2009). Angelman syndrome (AS) is characterized by refractory epilepsy, significant motor and language impairment, and ASD. Despite the difficulty in formal testing due to profound ID, more than 50% of patients with AS meet the criteria for ASD (Wink et al. 2015). AS is commonly caused by a deletion in the 15q11–13 region but can be caused by mutation of ubiquitin-protein ligase E3A (UBE3A), which is also in this locus and thought to be the main driver of symptoms. UBE3A gene encodes a ubiquitin ligase involved in protein degradation. mTORC1 activity was found to increase in the cerebellum and hippocampus of mice with a UBE3A deletion. Indeed inhibition of mTORC1 with rapamycin ameliorates motor and learning phenotypes in Ube3A mice (Sun et al. 2015, 2016). Interestingly, contrary to mTORC1, mTORC2 signaling was reduced and its activation was able to rescue LTP in acute slices, suggesting that modulation of mTORC1/2 might play an important role (Sun et al. 2016). These data demonstrate that some of the neurological phenotypes in mutant animals are due to hyperactive mTORC1 signaling. The mechanism by which UBE3A modulates mTORC1 remains still unclear. Phelan-McDermid syndrome (PMS) is a neurodevelopmental disorder characterized by neonatal hypotonia, global developmental delay, normal to accelerated growth, absent to severely delayed speech, and minor dysmorphic features (Phelan 2008b) caused by chromosome 22q13 deletion. There is evidence that, in PMS, autistic behavioral features are linked to the deletion of SHANK3. However, loss of SHANK3 is thought to be responsible for the neurodevelopmental symptom of PMS. causing loss of SHANK3 locus. SHANK3 deletion

is strongly associated with PMS responsible for the neurodevelopmental symptoms (neurodevelopmental delay, impaired language, ASD, and ID) (Phelan 2008a). As previously mentioned, SHANK3 encodes a structural protein present in the postsynaptic density identified like postsynaptic proteins linked to syndromic and idiopathic ASD (Sala et al. 2015). Approximately 80% of PMS patients have ASD phenotype (Soorya et al. 2013). SHANK3-deficient rodent neurons displayed a reduction in phosphorylation of AKT and mTORC1 levels, which was replicated in iPSC-derived (induced Pluripotent Stem Cells) neurons from patients with PMS. This was caused by the accumulation of cdc-like kinase 2 (CLK2 a negative regulator of AKT,), due to the loss of the SHANK3 gene. Consistently, CLK2 inhibitor ameliorated social deficit observed in SHANK3 deficient mice (Bidinosti et al. 2016). Currently, a specific biological marker for ASD diagnosis has not been identified, yet. Indeed, ASD should be considered a multi factors disease in which a gene×environment interaction has to be taken into consideration.

- ***Environmental risk of factors in ASD:*** it is currently accepted that, in addition to genetic causes, exposure to specific environmental factors might contribute to the development of ASD. Environmental risk factors include prenatal, perinatal, and postnatal factors. Among prenatal factors, exposure to teratogens and anticonvulsants drugs such as thalidomide and valproic acid (VPA), certain viral infections (congenital rubella syndrome), and diet contribute to ASD susceptibility. Instead, low birth weight, abnormally short gestation length, and birth asphyxia are considered perinatal factors. Finally, autoimmune disease, viral infection, hypoxia, mercury toxicity are classified as postnatal factors (Kolevzon, Gross, and Reichenberg 2007). To date ASD diagnosis is made before 2 years of life, suggesting a window of developmental vulnerability that must occur during the prenatal and/or early postnatal period of development. Figure 3 shows the critical prenatal window for ASD that has been described to date that identifies the first trimester of pregnancy as a critical period for some categories of environmental agents (Ploeger et al. 2010). Exposure to pharmacological agents during pregnancy represents a high risk for ASD; thalidomide and VPA are among the best examples to date of drugs linked to ASD.

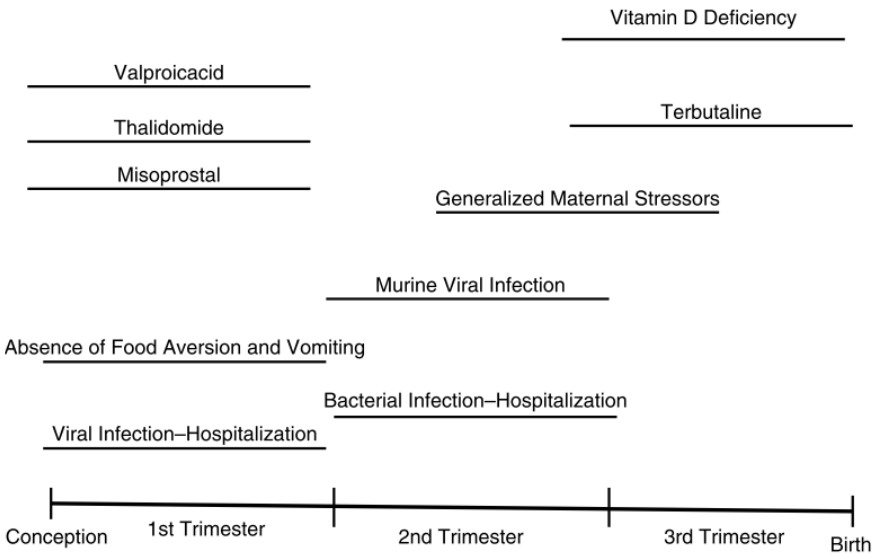


Figure 3: Critical prenatal windows for risk of ASD: The know and putative environmental risk of factors contributing to ASD during the first trimester of pregnancy. Adapter from (Dietert, Dietert, and DeWitt 2011).

Thalidomide is a known human teratogen famous for causing birth defects when used as an antiemetic in pregnancy in 1950/1960. Indeed, clinical studies reported increased incidences of autism in children following prenatal exposure to thalidomide (Strömland et al. 1994).

Together thalidomide also VPA is known for its teratogen effect if administrated during pregnancy in particular within the first trimester. It is an anticonvulsant commonly used in the treatment of epilepsy and also bipolar disorder due to its mood-modifying ability. When taken during pregnancy, it can result in children having a well-defined syndrome of abnormalities affecting several systems known as the valproate syndrome (Ornoy 2009a). In children from women who have taken this medication during pregnancy, ASD is increased several-fold (Bromley et al. 2008). Indeed, when taken during pregnancy, VPA not only increases the risk for various congenital malformations (Kini et al. 2006; Kozma 2001), but also induces core autistic symptoms in the offspring, i.e., impaired communication, reduced sociability, and stereotyped behaviors (Bescoby-Chambers, Forster, and Bates 2001). Based on these findings, prenatal VPA exposure in rodents has been accepted as a drug-induced preclinical model of ASD (Nicolini and Fahnestock 2018; Roullet, Lai, and Foster 2013; Tartaglione et al. 2019).

5. VALPROIC ACID

The 2-propylvaleric acid (also called 2-pro-pylpentanoic acid or n-dipropylacetic acid) is commonly referred to as valproic acid (VPA). It is a branched short-chain fatty acid derived from naturally occurring valeric acid (naturally produced by valerian, Valeriana officinalis) first synthesized in 1882, originally used as a solvent for organic compounds. Only in 1963, the pharmacological activity of VPA was shown to prevent pentylenetetrazol-induced convulsions in an animal model of seizures (Meunier et al. 1963). VPA is used primarily as an anti-epileptic drug (AED), either in mono or polytherapy. Importantly, AED drugs are teratogenic as their use by the pregnant mother has been associated with an increased risk of major congenital abnormalities in the embryo and fetus (Kultima et al. 2004; Ornoy 2003). These anomalies are anatomical and/or functional and may also have neurological, behavioral, and cognitive effects (Kaneko et al. 1999). However, there is a variation in the rate and degree of teratogenic potential of the different antiepileptic drugs. There seems to be sufficient data to imply that VPA is a highly teratogenic drug (Kaneko et al. 1999; Kini et al. 2006). The first reports in which has been demonstrated the teratogenicity of VPA in humans were published in the early 1980s (Dalens, Raynaud, and Gaulme 1980); French children who mothers treated with VPA during the first trimester of pregnancy displayed lumbosacral neural tube defect (Nau 1994).VPA is also used in migraine prevention (but not to treat the active symptoms), bipolar, mood, anxiety, and psychiatric disorders (Diederich et al. 2010). Recent work has explored its use as an adjuvant agent in cancer, HIV therapy, and neurodegenerative disease because of its action as a histone deacetylase (HDAC) inhibitor (Terbach and Williams 2009).

5.1. Pharmacokinetics

VPA is highly protein-bound (87–95%) resulting in slow clearance (6–20 ml/h/kg) (Leppik and Birnbaum 2010). It is metabolized through two principal ways: glucuronidation, β oxidation in the mitochondria, or cytochrome P450 (CYP)-mediated oxidation, considered as a minor way. The principal metabolite in urine is Valproate glucuronide (Ghodke-Puranik et al. 2013). VPA is a fatty acid and can be metabolized through endogenous pathways in the mitochondria. It has been observed that some of the mitochondrial metabolites of VPA generated by this pathway are hepatotoxic.

As previously introduced within AEDs, VPA has major teratogenic potential if taken during pregnancy (Kaneko et al. 1999; Kini et al. 2006). A basic principle for the action of any teratogenic agent is its ability to cross the placenta. VPA is known to cross the human placenta, and the clearance of VPA is increased during pregnancy (Albani et al. 1984; Kondo et al. 1987). Valproic acid levels in cord serum are often higher than in the mother and may be up to 5 times higher than the levels in maternal serum. This increased concentration in the fetus has been attributed to a better binding in the fetal compartment than in the maternal (Johannessen 1992). Moreover, VPA is excreted into human milk in low concentrations (G.E. et al. 1984), and it has been estimated that infants may ingest about 5% of the weight-adjusted maternal daily dose (Albani et al. 1984).

5.2. Pharmacodynamics

VPA exhibits its PD effects in different ways: it acts on I) γ aminobutyric acid (GABA) levels in the brain, II) blocks voltage-gated ion channels, and finally III) acts as an HDAC inhibitor. A reduced inhibitory tone in the CNS, due to a reduction in GABAergic transmission, can lead to a seizure, making the GABA receptor a target for antiepileptic drugs. Indeed, VPA increases the synthesis of the neurotransmitter GABA and promotes its transmission by inhibiting the metabolic enzyme 4-aminobutyrate aminotransferase (ABAT). VPA targets include also voltage-gated sodium, potassium, and calcium channels. Indeed, the antiepileptic action results also from the blocking of these channels with a consequent reduction in neuronal firing (Johannessen 2003). Interestingly, for a stable and persistent mood-stabilizing effect, VPA needs about 10 days, suggesting that its mechanism of action involves also delayed effects such as changes at the transcriptional level. Recently, VPA was demonstrated to be an inhibitor of HDAC1 as well as other HDACs (Göttlicher et al. 2001; Phiel et al. 2001), which potentially increases the expression of genes involved in apoptosis and antitumor action. HDAC inhibitor-induced activation/overexpression of specific deacetylases in tumor cells can increase the effectiveness of antineoplastic therapies by promoting the selective killing of tumor cells (Bradbury et al. 2005). HDAC inhibition induces expression dependent genes Wnt (Götze et al. 2014) a family of glycoproteins that playing an important role in embryogenesis and homeostasis of adult tissues.

5.3. Effect of VPA during pregnancy

VPA is also in a variety of neurological and psychiatric conditions including bipolar disorder, migraine, and schizophrenia. It was first licensed for use in Europe starting from the mid-1970s. But starting from February 2018, the Pharmacovigilance Committee of the European Medicines Agency strongly suggested to not use VPA during pregnancy unless all other available AEDs had failed (Dalens et al. 1980). Successively in April of the same year, the Pharmacovigilance Committee recommended that women using VPA should use effective contraception and agreed that VPA use during pregnancy should be restricted to women in which the treatment with others antiepileptic drugs is ineffective. For the past forty years, since VPA use began in Europe, many women have taken this drug during pregnancy for its better effectiveness than other AEDs (Marson et al. 2007). From the early 1980s reports began to be published suggesting that VPA exposure during pregnancy was associated with an increased risk for congenital malformations and poorer developmental trajectories (Clayton-Smith and Donnai 1995; Robert and Guibaud 1982). But it was only when results from prospective cohort studies of children born to mothers taking VPA and data from pregnancy registers became available (Wyszynski et al. 2005), that the serious teratogenic effects of VPA were appropriately acknowledged. Thus, doctors in the EU are advised not to prescribe VPA for epilepsy or bipolar disorder to pregnant women, or women of childbearing age, unless other treatments are ineffective or not tolerated. At present, the percentage of risk for congenital malformation after VPA exposure during pregnancy is about 11% (Meador et al. 2008; Weston et al.

2016), but it is strictly correlated with dose, indeed the risk rises to 24% when the dose is over 1500 mg daily (Meador et al. 2008). In line with this scenario, in March 2016, the French Inspection Générale des Affaires Sociales reported at least 450 cases of malformations in children born between 2006 and 2014 from mothers exposed to VPA during pregnancy (IGAS 2016). The most recent consensus guidelines by the American Academy of Neurology, the American College of Obstetricians and Gynecologists, and the International League Against Epilepsy might be summarized as follows: 1) optimize treatment before conception, 2) use monotherapy if possible, 3) choose the most effective AED for seizure type and syndrome, 4) use the lowest effective dose, 5) supplement with folate, 6) treat the child with vitamin K at birth and possibly the mother late in pregnancy for AEDs that interfere with vitamin K.

The risks of neurodevelopment impairment, as a consequence of VPA prenatal exposure (VPE), appears diverse and can influence specific domains. A recent evidence-based review of prospective cohort studies of major congenital malformations (MCMs) after exposure AEDs concluded that first-trimester VPA exposure is highly likely to increase the risk for MCMs compared with the use of either no AED or a few other single AEDs (Wendy S. Biggs 2009). Congenital malformations are more likely in children exposed in utero to antiepileptic drugs (AEDs) and are increased with higher AED dosages, higher AED serum levels, or polytherapy (Ornoy 2009b). On the 3rd of December 2009, the US Federal Drug Administration released a drug safety alert for health care professional indicating that female patients should be warned about the possible consequences of valproate treatment in offspring such as heart malformations, orofacial defects, urologic defects, skeletal abnormalities, and neural tube defects (e.g. spina bifida) (Hill et al. 2010).

In addition to congenital malformation, in utero AED exposure caused also behavioral/cognitive defects. Developmental delay, including reduced cognitive function, attention deficit disorder, and learning difficulties have been repeatedly described among the offspring of VPA-treated mothers. Recently, it was shown that children exposed to VPA in utero showed reduced verbal and non-verbal cognitive outcomes (Meador et al. 2011). There was a significant negative correlation between VPA dose (200–3000 mg/day) and language scores in children aged 6 to 8 years, and a greater deficit in children exposed to VPA in comparison to other AEDs. Finally, children exposed to VPA had significantly lower scores than controls on Verbal Comprehension and Working Memory assessed by the Wechsler Intelligence Scale for Children in a dose-dependent manner, specifically, children exposed to doses ≥800 mg/day showed a greater risk of intellectual impairment (C. Nadebaum et al. 2011a; 2011b). Clinical studies also show that VPA exposure during the first trimester of pregnancy is associated with a higher incidence of ASD in the offspring. A possible association between in utero VPA exposure and ASD was first observed by Christianson et al. (Christianson, Chester, and Kromberg 1994) who described 4 children exposed in utero to VPA that demonstrated developmental delay and, one of them, also ASD. Later, Williams and Hesh (Gail Williams and Hersh 1997) described an additional child with the typical facial features of VPA embryopathy who also developed the typical findings of ASD-symptoms, and several years later additional 5 children

(Bescoby-Chambers et al. 2001). Clinical findings demonstrated that VPA monotherapy during pregnancy leads to a seven-fold greater risk for the offspring to develop ASD or its core features such as language impairment, reduced attention, social impairment, and restricted interests (Bromley et al. 2008). Among studied children, 8.9% had ASD or Asperger's syndrome caused by prenatal exposure to VPA monotherapy (Rasalam et al. 2005). The mechanism by which exposure to VPA during pregnancy causes ASD-like behavior is not entirely known, but some hypotheses have been proposed.

VPA potently inhibits histone deacetylase (HDAC), a negative regulator of gene expression in multiple settings. Importantly, the teratogenicity of VPA in vertebrate embryos is mimicked by the HDAC inhibitor trichostatin A, whereas non-teratogenic analogs of VPA do not inhibit HDAC. These findings lead us to propose that HDAC is an important target of VPA in the pathogenesis of birth defects (Phiel et al. 2001). HDACs can be separated into two classes: HDACI and HDACII. The first is expressed in neural stem cells/progenitors, but as they exit the cell cycle HDACI expression is lost but maintained in glia throughout the cell cycle. On the contrary, HDACII is expressed in post-mitotic neuroblasts and neurons, and up-regulated as they commit to a neuronal lineage but not in fully differentiated glia (MacDonald and Roskams 2008). Transient hyperacetylation of H3 and H4 histones induced by VPA during a critical window of embryonic mouse brain might play a key role in autism pathogenesis (Kataoka et al. 2013; Moldrich et al. 2013). Of note, exposure to valpromide, a VPA analog lacking HDAC inhibitory activity, neither affects behavior nor induces a transient increase in the levels of acetylated histones (Kataoka et al. 2013). Moreover, VPA treatment changes the expression of various genes (Barrett 2017; Cipriani et al. 2018; J.-W. Kim et al. 2014a; 2014b; Konopko, Densmore, and Krueger 2017; Stodgell et al. 2006; Wiltse 2005) including the Hox genes that are critical to the early patterning of the embryo and the methyl-CpG-binding protein 2 (MECP2) gene, implicated in CNS maturation. More in detail, Hoxa1 is important in the development of the brainstem, as higher or lower Hoxa1 protein is teratogenic (Stodgell et al. 2006). MeCP2 is the gene (located on X chromosome) responsible for Rett syndrome, an X-linked neurodevelopmental disorder, which shows commonalities with ASD-like behavioral phenotype (Amir et al. 1999).

Furthermore VPA, through HDAC inhibition, regulates the cellular response to DNA double-strand break (DSB) formation by affecting checkpoint activation, DSB repair, and stability of crucial enzymes involved in the resection of DNA ends (Shubassi et al. 2012). When taken during pregnancy VPA-induces DNA damage, activation of apoptotic mechanisms, leaving an insufficient number of neuroepithelial cells to undergo neural tube closure and resulting in neural tube defects (Tung and Winn 2011), that is a major consequence of VPE (Hill et al. 2010). This evidence supports the hypothesis that HDAC inhibition during a critical window of embryonic brain development contributes to autism pathogenesis.

However, it does not preclude that other mechanisms of action could be involved in ASD-like behavior resulting from exposure to this drug during brain development. Indeed VPA exposure could result in elevated GABA levels and signaling, as VPA inhibits GABA transaminases, enzymes involved in GABA degradation while enhancing the activity of glutamic acid decarboxylase, a GABA synthesizing enzyme (Johannessen 2003). GABA plays a critical role in the maturation of neural networks in the developing brain (Baltz, de Lima, and Voigt 2010; Ben-Ari et al. 2007; Sernagor et al. 2010). Thus, changes in GABA levels due to exposure to VPA during early development could disrupt the developing neuronal circuitry and result in ASD behavioral phenotypes. Moreover, VPA, by indirectly inhibiting glycogen synthase kinase 3 beta (GSK3β), impacts axonal remodeling in developing neurons (Chen et al. 1999; Hall et al. 2002; Phiel et al. 2001), promoting Wnt signaling (Hall et al. 2002). The latter regulates the differentiation of cortical intermediate progenitors into neurons (Munji et al. 2011) and the patterning of cerebral cortex (Chenn 2008). Given its crucial functions in the differentiation of neural precursor populations and the regional specification of the cortex, abnormal activation of the Wnt pathway induced by in utero VPA exposure could disrupt spatio-temporal windows of embryonic brain development that are essential for the establishment of normal neuronal networks.

Also, the mTOR pathway has been suggested as a potential mechanism in the development of ASD both in humans and animal models (Sahin and Sur 2015). Indeed, mTOR dysregulation was found highly associated with several genetic forms associated with ASD. Moreover, recent advances in preclinical research have demonstrated that VPE leads to an up-regulation of mTOR signaling that results in ASD phenotype (Kotajima-Murakami et al. 2019; Kuo and Liu 2018; Qin, Dai, and Yin 2016; Sato 2016; Winden, Ebrahimi-Fakhari, and Sahin 2018; Xing et al. 2019). Since mTOR governs various processes implicated in CNS development, its implications in neurodevelopmental disease will be extensively discussed in the next chapter.

6. ANIMAL MODEL OF AUTISM INDUCED BY PRENATAL EXPOSURE TO VPA

Considering clinical evidence that VPE causes ASD symptoms in the offspring, VPE in rodents has been validated as a drug-induced preclinical model of ASD (Nicolini and Fahnestock 2018; Roullet et al. 2013; Tartaglione et al. 2019). The VPA model was created by Rodier, who exposed rat embryos to a single dose of 350 mg/kg VPA (Rodier et al. 1996a). Since then, prenatally VPA-exposed rodents have been used extensively to investigate the molecular pathways linked to ASD behaviors as well as to identify new targets for ASD drug therapy. Thus, prenatal VPA exposure in animals has been proposed as a model of ASD that has both constructs (similarity in underlying causes) and face (resemblance to human symptoms) validity. Indeed, VPA rodents show marked behavioral impairments resembling the core and secondary signs of ASD (Melancia et al. 2018; Miyazaki, Narita, and Narita 2005a; Narita et al. 2002a; Rodier et al. 1996a; Schneider and Przewłocki 2005; Servadio et al. 2016a).

Embryonic alterations induced by VPE: VPA exposure in the embryo causes short- and long-term changes in the trajectory of neurodevelopment. Both in vitro and in vivo studies have provided key details into the ways that VPA might influence neurodevelopment. For example, in an *in vitro* model, the exposure of neural crest cells to VPA resulted in a loss of N-cadherin expression, which is necessary for neural crest cells to migrate from the midline individually (Fuller et al. 2002) suggesting that VPA-related interference with neural migration at key times in embryonic development could contribute to neural tube defects. Moreover, rats prenatally exposed to VPA *in vivo* on embryonic day 12 (E12) showed upregulation of anti-apoptotic factors and reduced levels of apoptotic factors in whole brain lysates (Fuller et al. 2002). Since programmed cell death is an important component of healthy brain development, VPA exposure may interfere with normal embryonically regulated apoptotic programs leading to select tissue overgrowth that could contribute to deficits observed in VPA-exposed offspring. Since VPA is an HDAC inhibitor, epigenetic regulation has been proposed as a key mechanism by which VPA influences neurodevelopment in ASD (Kataoka et al. 2013; Moldrich et al. 2013). Recently, Soham and his collaborators have demonstrated that VPA specifically affects only early neurodevelopment through HDAC inhibition, causing a transcriptional downregulation of genes essential for dendritic morphogenesis and synapse maturation during early neurodevelopment (Soham Chanda et al. 2019). Overall, these data provide a window into how VPA influences the developing nervous system in utero that could underline the typical postnatal alterations.

Behavioral alteration in the VPA-rodent model: since impairment in social interaction is a key feature of ASD, a valid animal model is expected to exhibit deficits in this behavioral domain. Rats prenatally exposed to VPA show a wide range of deficits in the social domain. For both animals and humans, social play is crucial for neural growth and proper development: it helps to develop communicative skills, to acquire cognitive and social competence, and it contributes to the development of behavioral and mental flexibility (Vanderschuren, Achterberg, and Trezza 2016; Vanderschuren and Trezza 2014). At infancy, they show deficits in social communication and social

discrimination (Cartocci et al. 2018; Dufour-Rainfray et al. 2010; Melancia et al. 2018; Schneider and Przewłocki 2005; Servadio et al. 2016a). At adolescence, VPA-exposed rats display atypical patterns of social play behavior, that is the most characteristic and rewarding social activity displayed by young mammals: indeed, compared to control animals, rats prenatally exposed to VPA respond to play solicitation mainly by partial rotation and evasion, rather than reciprocating the playful interaction (Melancia et al. 2018; Servadio et al. 2016a). The social deficits displayed by VPA-exposed rats are long-lasting since they also persist in adulthood (K. C. Kim, Lee, et al. 2014a; Kim et al. 2011; Markram et al. 2008; Melancia et al. 2018; Schneider et al. 2008; Schneider, Turczak, and Przewłocki 2006; Schneider and Przewłocki 2005; Servadio et al. 2016a, 2018). Interestingly, the social interaction deficit observed in rats prenatally exposed to VPA was ameliorated after *in vivo* treatment with rapamycin (Kotajima-Murakami et al. 2019), suggesting that a perturbation in mTOR signaling could be linked with the behavioral phenotype observed in VPA-exposed rats.

Additionally to the social impairment, VPA animals displayed communication deficits measured with ultrasonic vocalizations (USVs). Indeed, when separated from the dam and siblings, mice and rats prenatally exposed to VPA show a reduction in the number of calls (Servadio et al. 2016a).

Finally, according to the DSM-5, the second core feature of ASD includes repetitive/stereotypic behaviors. Several studies reported these behaviors in VPA animals in term of self-grooming (Gandal et al. 2010; Mehta, Gandal, and Siegel 2011; Moldrich et al. 2013), digging (J.-W. Kim et al. 2014; Mehta et al. 2011; Moldrich et al. 2013), and dipping (Melancia et al. 2018) behaviors.

Neuronal network activity in VPA animal model: the disruption in the excitatory and inhibitory (E/I) balance has been proposed among the possible mechanisms involved in ASD etiology and symptoms. E/I imbalance is associated with normal development and functioning of the brain. It represents a key process for optimal tuning of the circuits to respond to salient inputs (Canitano and Pallagrosi 2017; Shew et al. 2011). Male rats prenatally exposed to VPA present E/I imbalance, similar to ASD patients (K. C. Kim, Choi, et al. 2014; K. C. Kim, Lee, et al. 2014b; Kim et al. 2013). Particularly, VPA rat brains were characterized by the increased neuronal number and increased protein markers such as PSD95, α-Ca (2+)/calmodulin-dependent protein kinase II (α-CaMKII), N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid (AMPA) receptors (Gandal et al. 2010; Go et al. 2012; K. C. Kim, Lee, et al. 2014b; Kim et al. 2013; Mehta et al. 2011; Nagode et al. 2017). Moreover, VPA-exposed rats displayed hyper-connected micro-circuits at the cellular level in different brain areas implicated in ASD, such as the prefrontal cortex and the somatosensory cortex, which was reflected in enhanced expression and function of the NMDA receptor protein (Rinaldi 2008; Rinaldi et al. 2007; Rinaldi, Silberberg, and Markram 2008; Silva et al. 2009).

7. THE MAMMALIAN TARGET OF RAPAMYCIN (mTOR)

mTOR is a serine/threonine-protein kinase (predicted molecular weight 280 kD) in the PI3K-related kinase (PIKK) family, that is encoded by the *MTOR* gene, localized on chromosome 1p36 (Baretić and Williams 2014). mTOR can combine with protein binding partners to form two distinct protein complexes known as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Harris and Lawrence 2003).

- mTORC1: figure 4 shows that it is defined by its three core components: mTOR, RAPTOR (regulatory protein associated with mTOR), and mLST8 (mammalian lethal with Sec13 protein 8, also known as GβL). RAPTOR recruits the mTORC1 substrate across the binding of the TOR signaling (TOS) motif. Instead, mLST8 is associated with the catalytic domain of mTORC1. In addition to mTOR, RAPTOR, and mLST8 mTORC1 present inhibitory subunits PRAS40 (proline-rich Akt substrate of 40 kDa) and DEPTOR (DEP domain-containing mTOR interacting protein). mTORC1 is regulated both directly and indirectly through the TSC1/TSC2 complex by adenosine monophosphate (AMP)-activated kinase (AMPK) and AKT. Signaling through the PI3K pathway by growth factors such as insulin and insulin-like growth factor 1 is an important regulator of AKT activity (Baretić and Williams 2014). Finally, mTORC1 regulates p70 S6K (S6K) and eukaryotic translation initiation factor 4E binding proteins 1 and 2 (4EBP1/2), both of which facilitate mRNA translation (Gingras et al. 1999; Magnuson, Ekim, and Fingar 2012). Selective inhibition of mTORC1 is caused by rapamycin through its binding of FK506 binding protein 12 (FKBP12). The complex also regulates autophagy through inhibition of unc51-like autophagy activating kinase 1 (ULK1) and autophagy-related 13 (ATG13) (Baretić and Williams 2014; Harris and Lawrence 2003).
- mTORC2: mTORC2 contains DEPTOR, MLST8, telomere maintenance 2 (TEL2), and TELO interacting protein 1 (TTI1) but differs, from mTORC1 with the presence of a rapamycin-insensitive companion to mTOR (RICTOR), mammalian stress-activated protein kinase 1 (MSIN1), and protein observed with rictor (PROTOR) (Fig.4). It can be activated by growth factors that induce PI3K such as insulin, and it interacts with the TSC1/TSC2 complex, which is necessary for its activation. mTORC2 activity is also necessary for the full activation of AKT (Saxton and Sabatini 2017).

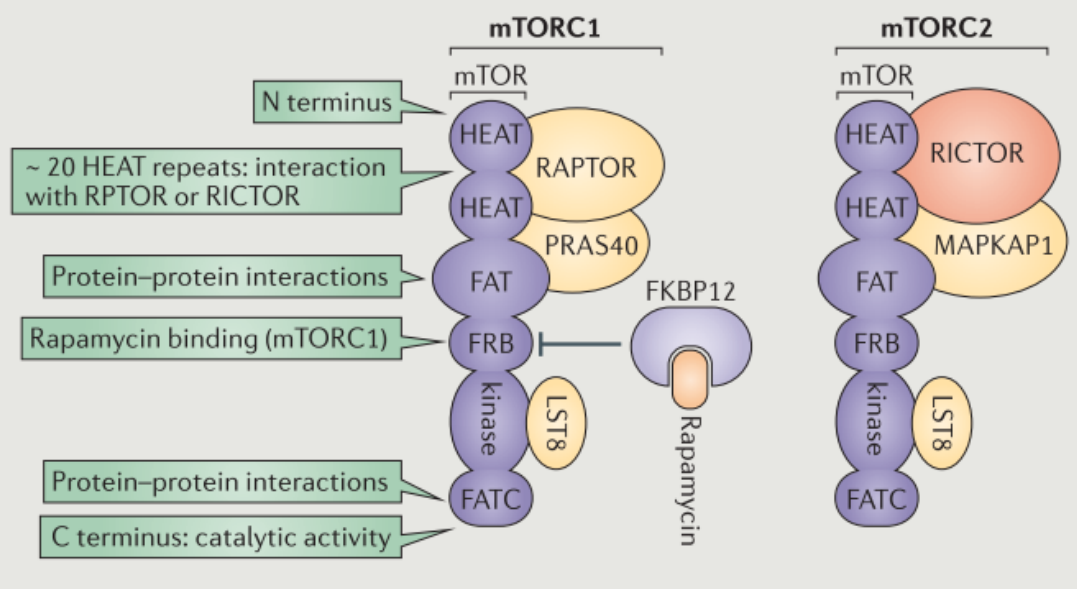


Figure 4: mTORC molecular and protein organization. Adapter from (Crino 2016)

7.1. The mTOR signaling network

In the brain, mTORC1 and mTORC2 regulate different cellular processes across many cell lineages, such as neurons, astrocytes, and neuroglial progenitor cells. It is involved in distinct functions such as neuronal excitability, memory formation, and learning. mTORC1 has multiple subcellular localization including cytoplasm, endoplasmic reticulum, and nucleus. mTORC1 assumes an active signaling conformation when it is tethered to the lysosomal membrane. Whereas mTORC2 is required for cytoskeletal integrity and cell migration. To date, the majority of neurological disorders associated with mTOR signaling have been linked to mTORC1. For this reason, for the remainder of this thesis, I will focus on mTORC1 ("mTOR" henceforth).

7.1.1. Upstream of mTORC1

mTOR integrates the inputs from upstream regulatory proteins that transduce information from growth factors, insulin, ATP, nutrient stores (especially amino acids), oxygen, and energy cellular levels (Fig.5). making mTOR a cellular sensor of cell growth and survival. The most important protein involved in the regulation of mTOR activity is the TSC, a heterotrimeric complex composed of TSC1, TSC2, and Tre2-Bub2-Cdc16 (TBC) 1 domain family, member 7 (TBC1D7), and functions as a GTPase activating protein (GAP) for the small GTPase Rheb. Since only the GTP-bound form of Rheb can directly bind and activate mTOR. Therefore, as a Rheb GAP, TSC suppresses mTOR by converting Rheb from an active GTP-bound form to an inactive GDP-bound state. The growth factor regulates mTOR activity mainly through activation of two classical upstream pathways of mTOR: PI3K/AKT/mTORC1 and Ras/Raf/MEK/ERK/mTORC1 signaling pathways. Oxygen level affects mTOR activity in many ways. During hypoxia state activation of TSC through transcriptional regulation of regulated in DNA damage and development 1 (REDD1) inhibit mTORC1 signal transduction. Besides, under mild hypoxic conditions, the decrease of ATP level activated AMPK, also promoted TSC activation and inhibited mTORC1 signal transduction.

Furthermore, among amino acids, leucine and arginine, can lead to mTORC1 activation. Recently, there are two different ways in which amino acids regulate mTORC1: one is that leucine and arginine depend on Rag GTPase to activate mTOR, the other is glutamine to activate mTOR independently of the Rag GTPases (Hay and Sonenberg 2004).

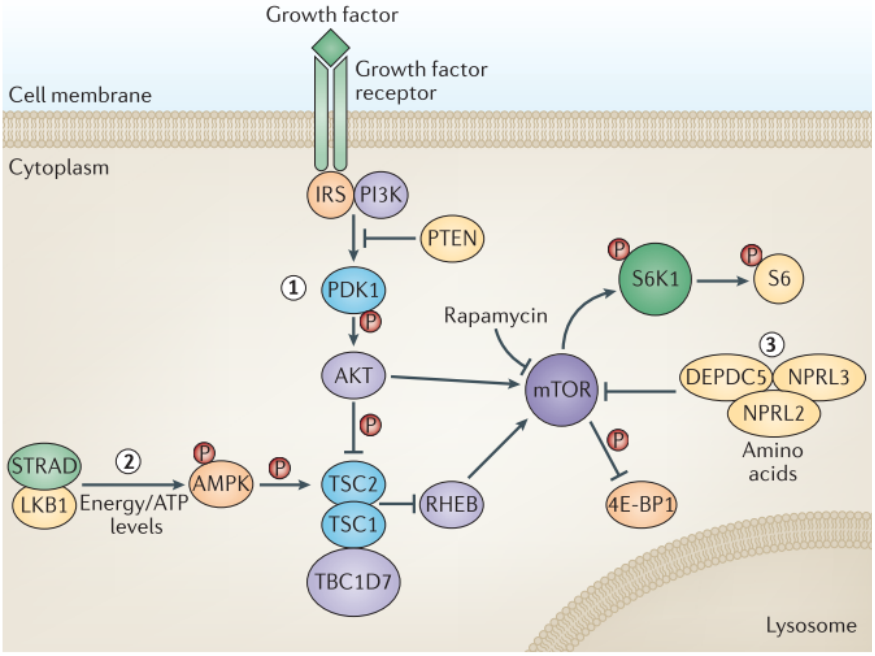


Figure 5: The mTOR Signalling Network. mTOR activation depends on three principal signals: the growth factor pathway (1) characterize by PI3K activation that leads to inhibition of TSC2, phosphorylation of protein S6 kinase beta-1 (S6K1), or eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). Low cellular energy activates a cellular pathway that results in mTOR inhibition (2). Finally, changes in amino acid levels lead to GATOR1 complex6 (DEP domain-containing protein 5, DEPDC5; nitrogen permease regulator proteins NPRL2 and NPRL3) modulation (3). Adapted from (Crino 2016).

7.1.2. Downstream of mTORC1

During the maturation phase, the production of proteins, lipids, and nucleotides increases, while catabolic process pathways, such as autophagy, decrease (Fig.6). mTOR regulates these processes and controls the anabolism/catabolism balance in response to environmental conditions. It integrates a variety of stimulation and signaling networks to promote anabolism. For example, it can promote protein synthesis by directly phosphorylating two key proteins: ribosomal protein S6 kinase 1 (p70S6K1) and the eukaryotic translation initiation factor 4E binding protein (4EBP) (Saxton and Sabatini 2017). Moreover, it can promote novel lipid synthesis through the sterol response element-binding protein (SREBP) transcription factor to promote membrane formation during cell growth (Porstmann et al. 2008). Furthermore, mTOR also promotes the synthesis of nucleotides required for DNA replication and ribosome synthesis in cell growth and proliferation (Ben-Sahra et al. 2013; Saxton and Sabatini 2017). In addition to promoting the above anabolic processes, mTORC1 also negatively regulates the catabolic process-autophagy, thus indirectly promoting cell growth (Fonseca and Proud 2009).

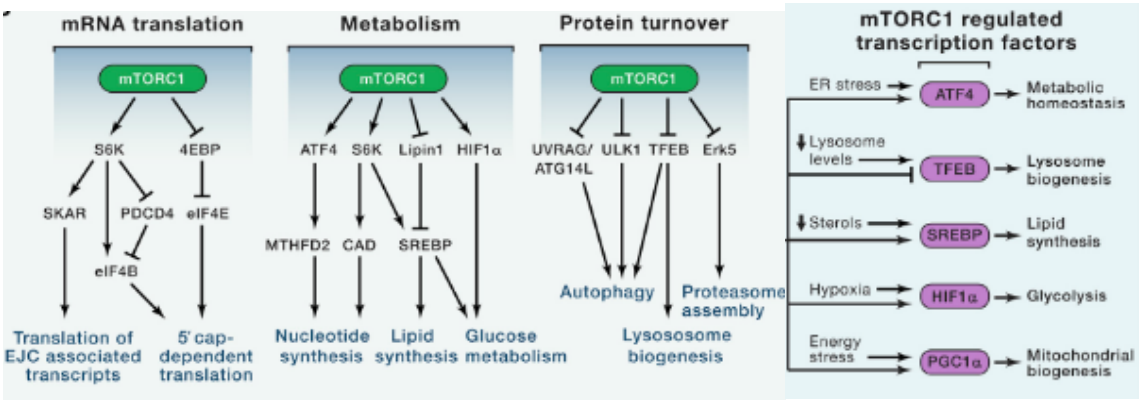


Figure 6:Downstream signaling pathways of mTOR. Activation of mTOR modulates different targets molecules, resulting in downstream processes such as mRNA translation, metabolism, protein turnover, and transcription. Adapted from (Saxton and Sabatini 2017).

7.2. Role of mTOR in the brain

In the CNS, mTOR pathway regulates several cellular processes required for correct neural development and circuits formation. During the embryonic and neurogenesis period, it regulates neural stem cells (NSCs) differentiation as well as neural progenitors migration. Moreover, mTOR influences also axon maturation and neuronal communication through the regulation of synaptic strength (Jaworski and Sheng 2006).

Abnormal mTOR signaling leads to a host of neurodevelopmental, psychiatric, and neurodegenerative disorders, including ASD, schizophrenia, and Rett syndrome (Winden et al. 2018). As previously described in chapter 2, many neurodevelopmental diseases are associated with disruptions in known members of the mTOR pathway, including TSC1, TSC2, and PTEN (Fig.7) (Sato 2016). The first evidence about the importance of mTOR in development has been obtained in the "flat-top" mutant mouse in which a mutation in the mTOR gene leads to a defect in telencephalon formation and premature death during mid-gestation (Hentges et al. 2001), suggesting a central role of mTOR in normal development and viability (Murakami et al. 2004). Based on these findings, using specific knockout animal models for members of the mTOR pathway, it was possible to dissect the role of this complex pathway in brain development. The loss in mTOR negative regulators TSC1/2 and PTEN leads to an increase in mTOR signaling causing profound changes in neuronal architecture and differentiation (Fig.7) (Kwon et al. 2006b; Tavazoie et al. 2005). In particular, loss of TSC1/ 2 function causes impairment in cellular maturation, axon development, and reduction in dendritic spine numbers (Choi et al. 2008; Tavazoie et al. 2005). In addition to cellular changes, mTOR has a prominent role also in synaptic plasticity, learning, and memory processes, by regulating new protein synthesis (Kandel 2001). Tang et al. showed that rapamycin impaired protein synthesis-dependent late phase of long-term potentiation (L-LTP) in rat hippocampal slices. The same authors further found that mTOR, translation initiation components (eIF4E), and mTOR substrates (4E-BP), co-localized with post-synaptic markers, suggesting that mTOR inhibition with rapamycin targeted synaptic translation machinery (Shao and Schuman 2002). mTOR is also required for mGluR

dependent-LTD (Huber, Roder, and Bear 2001), another known form of protein synthesis-dependent synaptic plasticity, which is found altered in the FXS mouse model (Huber et al. 2002).

Activation of mGluRs leads to increased ERK-MAPK and PI3K-mTOR signaling and activation of protein synthesis near synapses. Therefore mTOR inhibition and block of protein synthesis result in loss of mGluR-dependent LTD, confirming the involvement of mTOR in LTD regulation (Auerbach, Osterweil, and Bear 2011; Banko et al. 2006; Hou and Klann 2004; Huber et al. 2002). In keeping with the neurophysiological evidence linking mTOR signaling to synaptic plasticity, this pathway has important roles in cognitive behaviors. Indeed, Tsc2+/- mice, characterized by enhanced L-LTP and reduced LTD, displayed impairment in hippocampus-dependent learning paradigms. This defect has been reverted whit rapamycin administration (Ehninger et al. 2008). Similarly to behavioral alteration found in Tsc2+/- mice also, S6K1 and 4EBP2-null mice displayed abnormal fear conditioning and defective hippocampus-based learning (Antion et al. 2008; Banko et al. 2007). Since S6K1 and 4EBP2 loss are expected to have at least partially opposing net downstream effects these findings that any disruption in mTOR downstream signaling can affect cognition. suggesting the strong link between mTOR signaling, synaptic plasticity impairment, and altered cognitive function.

Figure 7 shows as mTOR pathway dysregulation cause a series of the cellular, circuit, and neurophysiological effects underlying many pathological conditions including ASD. Indeed, several genetic disorders that directly affect mTOR signaling are associated with ASD. Moreover, animal models with mutations in mTOR family members display impairment in motor and social domain that represents the core features of ASD (Ehninger et al. 2012; Goorden et al. 2007; Kwon et al. 2006b; Waltereit et al. 2011). Interestingly, rapamycin ameliorates the behavioral phenotype in the same animal models, (Sato et al. 2012). These studies have illuminated translational regulation pathways as crucial mediators of higher-order cognitive function and suggest that manipulation of these pathways may be pharmacological targets for treating ASD.

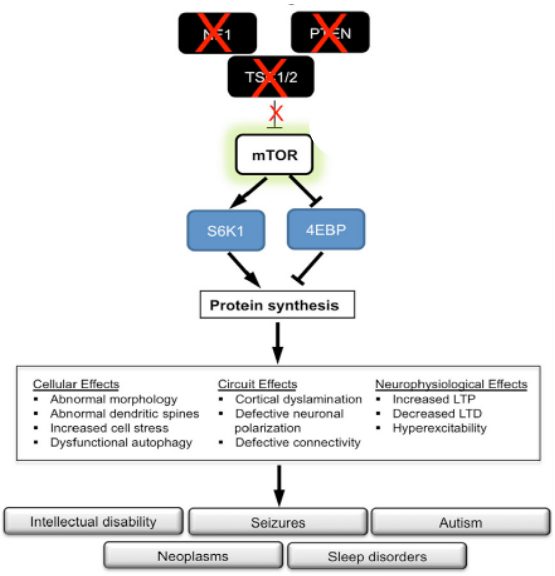


Figure 7: The mTOR Pathway in Neurodevelopmental Diseases. In Neurodevelopmental disease, the loss of NF-1, TSC1/2, or PTEN, may stimulate mTOR-dependent protein synthesis, which leads to a host of cellular, structural, and physiological responses that culminate in clinical symptoms. Adapted from (Lipton and Sahin 2014).

8. POTASSIUM CHANNELS

Potassium (K^+) channels represent the oldest membrane channel family in evolution, highly conserved across eukaryotic cells, bacteria, and yeasts (Hille 1978). They selective conduct K^+ ions down their electrochemical gradient (Lim and Dudev 2016). At rest, the K^+ extracellular concentration is about less compared to the other ions, and for this when K^+ channels are open, a positive K^+ charge moves from the cytoplasm to the extracellular compartment, resulting in a polarization of the membrane potential.

In neurons, these channels are master regulators of electrical properties. Indeed, K^+ channels influence neuronal excitability through the ability to stabilize the resting membrane potential (Hille 1978). Moreover, they regulate action potential (AP) frequency and waveform, and the strength of synaptic contacts between neurons (Bean 2007). Given their role in the control of neuronal activity in the CNS, mutations of these channels are strongly implicated in several neurological disorders associated with alterations in excitability, such as epilepsy and episodic ataxia (Browne et al. 1994; Shieh et al. 2000).

Functional K^+ channels are tetrameric structures composed of two or four α-subunits organized around a central ion-conducting pore. Each K^+ channel α-subunits includes a variable number of trans membrane segments(TMS) connected through intra- and extra-cellular loops in which amino (N)- and carboxy (C) terminal are located in the cytoplasmatic compartment. From the amino-acid sequences of α-subunits, these channels can be divided into three families: 1) the six transmembrane (6TM) domain channels which are mainly gated by voltage. They can also respond to both Ca^{2+} and voltage or by Ca^{2+} alone; 2) the two transmembrane (2TM) known as inward-rectifier K^+ channels (Kir) and finally 3) the four transmembrane (4TM) or two-pore domain (K2P) also called leak K^+ channel(s) as shown in Figure 8.

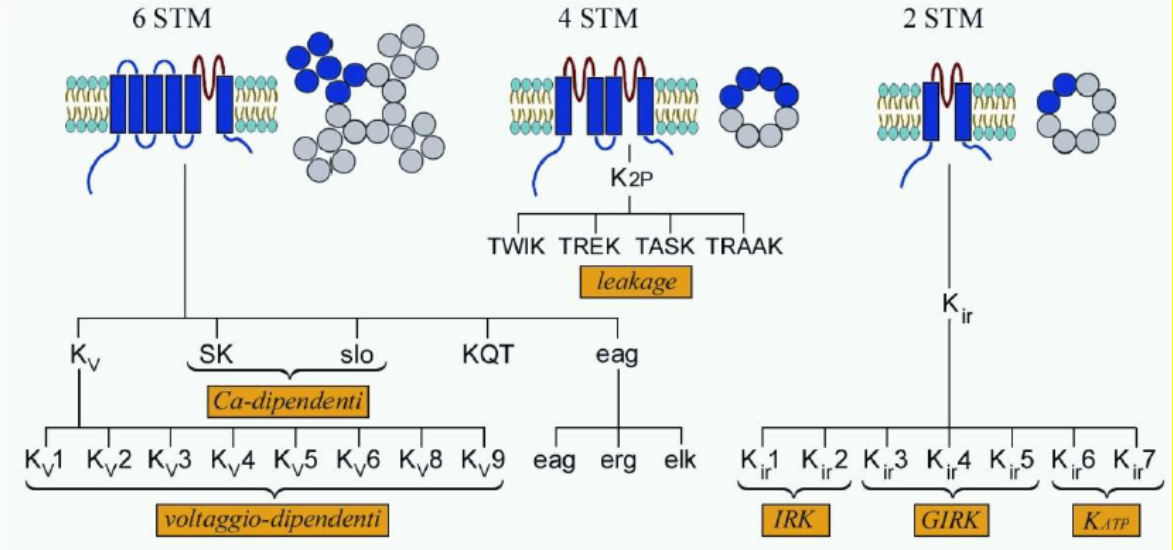


Figure 8: Potassium channels classification. Schematic representation of K^+ channels families structure Adapted from (Benarroch 2009).

In the last decade, many studies have been suggested the importance of the K^+ channels in ASD-related disease (Cristina D'adamo et al. 2011; Luca et al. 2015; Sun et al. 2018). Among K^+ channels, in this thesis, I will discuss only Kir channels as possible ion channels involved in the ASD phenotype.

8.1. Inward-rectifier K^+ channels (Kir)

The 2TMS channels family is also known as Inward-rectifier K^+ channels (Kir). It is involved in several biological processes in both nervous and non-nervous tissues. In the latter, Kir channels regulate heart rate, vascular tone, insulin release, and salt flow across epithelia, whereas in CNS Kir channels are strongly implicated in the regulation of neuronal excitability (Shieh et al. 2000).
In mammals, KCNJx genes encode Kir channels, that can be divided into seven subfamilies (Kir1.x to Kir7.x), based on their strength of rectification and their responses to cellular signals(Fig.9A). These subfamilies include 1) classical Kir channels constitutively active and strong inward rectification (Kir2.x), 2) G protein-gated Kir channels gated by G-protein coupled 3 receptors (Kir3.x), 3) ATP-sensitive K^+ channels regulated by ATP (Kir6.x) which combine with sulphonylurea receptors (SUR), and 4) K+ -transport channels (Kir1.x, Kir4.x, Kir5.x, and Kir7.x). The common molecular structure is composed of two transmembrane (TM) segments called M1 and M2, a pore loop (P), and amino (N)- and carboxy (C)-terminal cytoplasmic domains (Hibino et al. 2010) (Fig.9B).

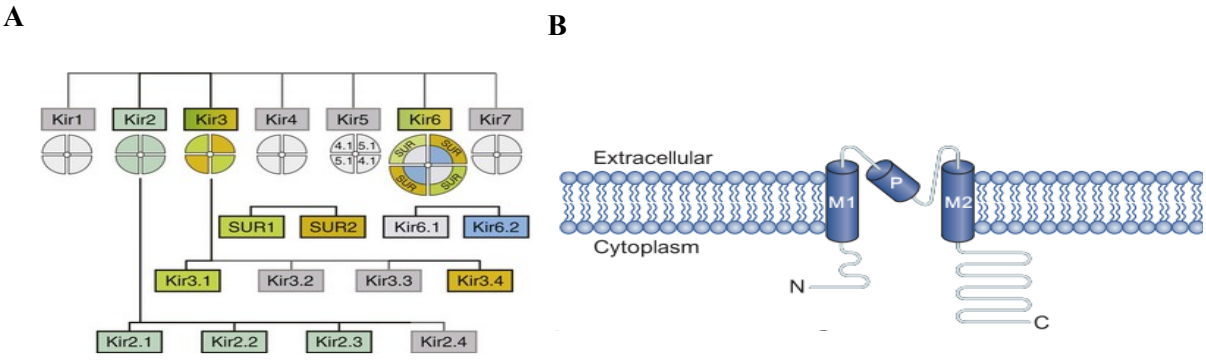


Figure 9: Overall organization of Inward-rectifier K^+ channels. A) Kir channels families can be divided into seven subgroups including the strong inward-rectifier K^+ channels (Kir2), the G-protein-activated inward-rectifier K^+ channels (Kir3), and the ATP-sensitive K^+ channels (Kir6), which combine with sulphonylurea receptors (SUR). B) Schematic of a Kir channel subunit indicating the cytoplasmic N- and C-terminus domains, two transmembrane-spanning domains (M1 and M2), and the pore-forming loop with selectivity filter (P) (Bichet, Haass, and Jan 2003).

Unlike canonical voltage-gated potassium (Kv) channels, Kir channels do not display a voltage-sensing domain. Their voltage dependence is determined by their characteristic ability to mediate the inward flow of K^+ ions at hyperpolarizing membrane voltages more readily than the outward flow of K^+ at depolarizing voltages. Importantly, a small outward current exists when the membrane potential (Em) is equal to the equilibrium of potassium (Ek), but the rectification of current occurs when Em > Ek through Kir blockage by polyamines (e.g. putrescine, spermine, and

spermidine) and/or magnesium (Mg^{2+}). This rectification allows for greater inward currents at hyperpolarized potentials than outward currents at depolarized potentials. Although relatively simple biophysical properties when compared to voltage-gated ion channels, the inward flux of K^+ ions at hyperpolarized potentials regulates various physiological processes ranging from maintaining the resting membrane potential, neuronal excitability, and muscle contraction (Hibino et al. 2010).
Finally, the activity of Kir channels can be modulated by several cytoplasmic factors, including Mg^{2+} ions, polyamines, nucleotides, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), lipids, and many intracellular proteins that can interact directly with the channel and modulate its function (Hibino et al. 2010).

8.2. Pharmacology of Kir channels

Biophysical characterization of Kir channels was possible by using inorganic and organic blockers of canonical K^+ channels. Unlike Kv channels, which are blocked by 4-aminopyridine (4-AP) and tetraethylammonium (TEA) salts but insensitive to and Ba^{2+}, Kir channels show opposite pharmacological profile. Indeed, they are sensitive to Ba^{2+} and Cs^+ but not to 4-AP, while showing sensitivity to TEA at millimolar concentration. Moreover, also toxins show several blocking activities on Kir channels (Doupnik 2017; Jin and Lu 1998). Among these, tertiapin is the most potent toxins that specific block Kir1.1 and Kir3.1/3.4 channels, with a little effect on other types of Kir channel (Imredy, Chen, and MacKinnon 1998; Jin and Lu 1998; Kanjhan et al. 2005). Kir blocker site is localized in the conduction pore formed by the linker between TM1 and TM2 segments (Felix et al. 2006; Jin et al. 1999; Reimann, Proks, and Ashcroft 2001).

8.3. Kir channels in neurodevelopmental disease

Given their role is not surprising that any alteration in Kir channels activities could lead to abnormal functioning of brain electrical circuits. Indeed, several neurological disorders including neuropsychiatric, neurodegenerative, and neurodevelopmental diseases have been attributed to ion channel mutations (Gargus 2009; Imbrici, Camerino, and Tricarico 2013; Kullmann 2010). Remarkably, it has recently demonstrated the causative role of Kir channels in the etiopathology of ASD (Cristina D'adamo et al. 2011; Ebert and Greenberg 2013; Luca et al. 2015) in both human and animal models. Recently, a genetic analysis of ASD children resulted in the identification of twelve tag single nucleotide polymorphisms (SNPs) from the KCNJ2 and KCNJ10 genes strengthening the notion that their intrinsic dysfunction may play a central role in ASD pathogenesis (Sun et al. 2018). Sun and his collaborators have further found that Kir2.1 and Kir4.1 expression was lower in VPA rats than in control (Sun et al. 2018), indicating that these channels play an important role in ASD susceptibility. KCNJ2 gene encodes for Kir2.1 channels that in the brain is predominantly expressed in the hippocampus, caudate, putamen, nucleus accumbens, and to lower levels in habenula and amygdala (Kanjhan et al. 2005), where it contributes to control

neuronal excitability. Importantly, these channels are expressed in the brain areas involved in cognition, mood, learning, memory, and reward-related behavior, demonstrating the putative role of these channels in the development of key features of ASD. For instance, FSX, loss of fragile X mental retardation protein resulted in potassium channel dysregulation and cognitive dysfunction as well as autistic behavioral symptoms (Lu et al. 2016). In Mecp2-null mice, an animal model of Rett syndrome, overexpression of Kir channels was associated with abnormal neuromodulation and autism-like behaviors (Zhang et al. 2010). Thus, aberrant Kir channel expression and function may lead to an imbalance of K+ homeostasis in the brain and defective brain development, which could underlie neuropsychiatric disorders.

9. VENTRAL STRIATUM (NUCLEUS ACCUMBENS (NAc)): anatomical and cellular organization

The ventral striatum (VS), commonly known as Nucleus Accumbens (NAc), is a circular structure located anterior to the posterior border of the anterior commissure (AC) and lies parallel to the midline. It is composed of two distinct areas: a central core (NAcc), localized closer to the AC, surrounded by an outer shell (NAcSh). NAcc is considered a part of VS, while NAcSh an extension of the amygdala (Heimer and Alheid 1991). NAc is anatomically indistinguishable from the dorsal striatum. Indeed, neither cytoarchitectonic nor histochemistry distinctions mark a clear boundary between the two areas. Thus, the best way to define the VS is by its afferent projections from cortical and subcortical areas (Salgado and Kaplitt 2015). Unlike the dorsomedial (DMS) and lateral striatum (DML), NAc receives I) glutamatergic inputs from the prefrontal cortex (in particular from prelimbic and infralimbic cortex), basolateral amygdala, hippocampus and thalamic nuclei, II) histaminergic projections from the tuberomammillary nucleus, and finally, III) dopaminergic inputs from the ventral tegmental area (VTA) (Salgado and Kaplitt 2015; Tremblay, Worbe, and Hollerman 2009) (Fig.10)

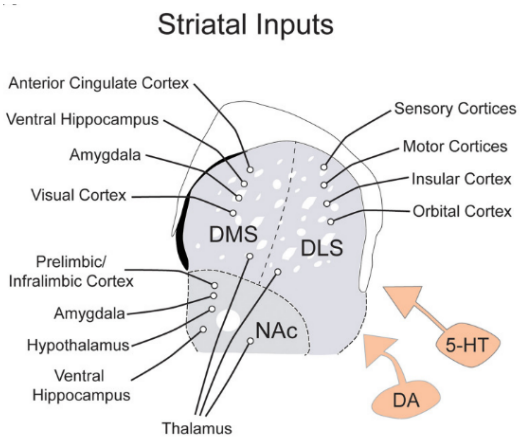


Figure 10: Schematic representation of striatal inputs. Diagram of glutamatergic, serotoninergic, and dopaminergic inputs to distinct striatal sub-regions. Dorsomedial striatum (DMS), dorsolateral striatum (DLS), and nucleus accumbens (NAc). Adapted from (Fuccillo 2016).

Striatal neurons can be divided into two principal classes based on their morphology: (a) spiny projection neurons and (b) aspiny interneurons including unique classes of GABAergic interneurons such as fast-spiking (FS) and low-threshold spiking (LTS) GABAergic interneurons, and tonically active, aspiny cholinergic neurons (TAN) (Kawaguchi et al. 1995) (Fig.11 left). Spiny projection neurons, also called medium spiny neurons (MSNs), are characterized by several dendritic spines (Kawaguchi et al. 1995). In the entire striatum, MSNs represent the most abundant neuronal type (more than 95%). They can be further classified based on the expression of D1 or D2 dopamine (DA) receptors expression. D1R-expressing MSNs are part of the direct pathway while D2R-expressing MSNs are part of the indirect pathway of basal ganglia (Kawaguchi et al. 1995; Salgado and Kaplitt 2015). These pathways function in opposition to one another in reward-based learning, with the direct pathway mediating reinforcement and the indirect pathway mediating

punishment and aversion (Bromberg-Martin, Matsumoto, and Hikosaka 2010; Frank, Seeberger, and O'Reilly 2004; Kravitz and Kreitzer 2012). Since the NAc is a major target area of the ML dopaminergic system, the overall impact of NAc output on behavior depends on the relative activity of D1- vs. D2-expressing MSNs. Indeed, proper activation of direct and indirect ML pathways underlies proper motor learning as well as the acquisition of reward-related behaviors (Kawaguchi et al. 1995; Shin, Kim, and Jung 2018).

Electrophysiological properties of NAc-MSNs: MSNs are characterized by a hyperpolarized resting membrane potential (Vr), low input resistance (IR), and many potassium conductances that shape their characteristic firing patterns (Nisenbaum and Wilson 1995). In MSNs both Vr and IR are strictly controlled by Kir channels, particularly by Kir 2.1 that are the most abundant subtype expressed in the somatodendritic compartment of NAc-MSNs (Prüss et al. 2003). Indeed at rest, Kir channels are responsible for their distinctive negative resting potential and low IR. Membrane depolarization inactivates Kir channels and activates both fast- (Kv4.2) and slow- inactivating (Kv1.2) A-type potassium currents, as well as a persistent potassium conductance (Kv7), which together yield a slow depolarization and delay to the initial spike (James Surmeier, Bargas, and Kitai 1989; Nisenbaum et al. 1996; Shen et al. 2004; Surmeier et al. 1991; Tkatch, Baranauskas, and Surmeier 2000). Depolarization and spiking also yield calcium influx, which can activate both small- and large-conductance calcium-activated potassium channels (Bargas et al. 1999) and limit MSN firing rates (Fig.11 right).

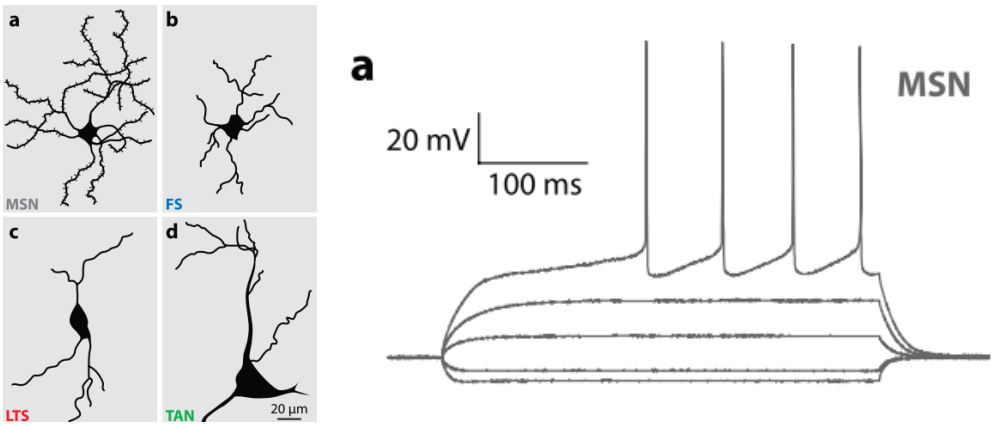


Figure 11: Striatal neurons. Schematic representations of (a) a striatal medium spiny neuron (MSN), (b) a fast-spiking interneuron (FS), (c) a low-threshold spiking interneuron (LTS), and (d) a cholinergic tonically active neuron (TAN) (left). Firing properties of medium spiny neurons (right). Adapted from (Kreitzer 2009).

The intrinsic membrane properties of these neurons are crucial for the correct response to synaptic inputs because MSNs fluctuates between two stable states, the "downstate", characterized by a hyperpolarized RMP (~-80 mV) in which MSNs are in a quiescent, and "upstate," characterized by a depolarized RMP (~-55 mV), which facilitates action potential firing (Bargas et al. 1999). The stability of MSNs at rest in downstate is due to high levels of Kir that limit membrane depolarization in response to excitatory synaptic inputs. However, if sufficient numbers of inputs

become active, MSNs can be depolarized enough to block Kirs, shifting MSNs into the up responsive state (Bargas et al. 1999).

9.1. The mesolimbic (ML) reward pathway

Thanks to dopaminergic projection from the VTA, the NAc is part of the mesolimbic (ML) pathway, also known as the *reward pathway*, that is the principal brain pathway involved in reward-related behavior as well as motivation and emotional processes, motor behaviors, and addiction (Dafny and Rosenfeld 2017; Floresco 2015; Haghparast et al. 2017). In this scenario, dopamine has a crucial role in the reward system, because is the principal neurotransmitter realizes in NAc, as well as in other brain regions, involved in pleasure-related cognition (Berridge and Kringelbach 2015; Berridge, Robinson, and Aldridge 2009). Importantly, since NAc-MSNs are considered the output of the ML system, it plays a central role to assign a positive value to an emotional response, counterbalanced by a negative emotional response pathway mediated by the amygdala (Wager et al. 2008). Based on its important role in reward and motivation, ML pathway dysfunction could lead to altered reward representation reducing motivation for rewarding experiences. Moreover, when this alteration is restricted to the social domain the ability to assign a positive value to social experiences could be lost. Since reduced social interaction is a key feature of ASD, this feature could be linked to ASD brains might fail to register social experiences as rewarding, further reducing the motivation to seek social interactions and develop social abilities. To support this, functional MRI studies in both children and adults with ASD, demonstrate the reduction in ML reward pathway accompanied by a strong impairment in social measured by the ADI-R social interaction subscale (Supekar et al. 2018). Furthermore, in ASD subjects, dopamine imbalances manifested as either hyperactivity or hypoactivity of midbrain dopaminergic pathways have been detected, highlighting the heterogeneity of the disease (Pavăl 2017). In agreement with the clinical data, also animal models characterized by ASD like phenotype display impairment in the social domain. Among these, rodents exposed to VPA during pregnancy show marked behavioral impairments resembling the core and secondary signs of ASD (Melancia et al. 2018; Miyazaki, Narita, and Narita 2005b; Narita et al. 2002b; Rodier et al. 1996b; Schneider and Przewłocki 2005; Servadio et al. 2016b). The social dysfunctions displayed by VPA-exposed rats may be caused by either their inability to properly understand and respond to social signals by the social partner or by a failure of their reward system to assign a positive value to the social experience, suggesting the possible involvement of ML pathway and more specifically of the NAc. Moreover, it has been demonstrated that striatal MSNs show enriched expression of genes associated with ASD (Chang et al. 2015) and that ASD-associated mutations affect specific MSN subtypes (Portmann et al. 2014; Rothwell 2016; Rothwell et al. 2014).

Taken together, it seems likely that the profound deficits in the social approach and rigid behavioral patterns that typify ASD may stem in part from specific abnormalities in striatal-based reward processing.

10. AIM OF THESIS

Reduced social interaction is a key feature of ASD. Since VPA is known to mimic ASD in rodents, this Ph.D. project aimed to investigate whether the social impairment displayed by VPA-exposed rats could be caused by a dysfunction in the mesolimbic (ML) reward system. To achieve this goal, *in vivo* (1) and *in vitro* (2) experiments were pursued.

(1) The effect of VPE on the social domain was initially studied performing behavioral characterization in rats.

(2a) NAc is considered the output of the ML system. Given its important role in rewarding forms of social interaction, we addressed the role of the NAc in the social impairment displayed by VPA-exposed rats by performing electrophysiological experiments in this brain area.

(2b) Recently, it has been demonstrated that rapamycin ameliorates the social deficit displayed in animal models showing ASD-like phenotype (Kotajima-Murakami et al. 2019; Sato et al. 2012). Thus, we investigated the neuroprotective efficacy of rapamycin on NAc-MSNs alterations. Hence, we performed electrophysiological experiments during acute rapamycin application to evaluate its effects on NAc-MSNs properties in VPA-exposed rats.

11. METHOD

11.1 Animals

All procedures required for ex vivo experiments were conducted in compliance with the Council Directive of the European Community (2010/63/EU), Decreto Legislativo Italiano 26 (13/03/2014) and approved by the Animal Care Committee of the Department of Neurofarba, University of Florence (authorization number: 31-2019-PR). Female Wistar rats were obtained from Charles River (Arbresle, France) and housed singly in a temperature-controlled (24 °C) animal colony under a 12 h light/dark cycle with food and water available ad libitum. Female rats were mated overnight. The morning when spermatozoa were found was designated as gestational day 1. The valproic acid sodium salt was dissolved in 0.9% saline and administered at a dose of 500 mg/kg that has been shown to induce autistic-like behavior in the offspring (Nicolini and Fahnestock 2018). On gestational day 12.5, females received a single intraperitoneal injection of either VPA or saline (SAL). On postnatal day (PND) 21, the pups were weaned and housed in groups of three. The experiments were carried out on the male offspring during adolescence (PNDs 30–40) and adulthood (PNDs 90–95). One male pup per litter from different litters per treatment group was used in each experiment. For the flow cytometric experiments, we used brain samples from the VPA- and SAL-exposed rats tested in the social play behavior, the three-chamber, and the social discrimination tests. Other cohorts of VPA- and SAL-exposed rats were used for the electrophysiology experiments.

11.2. Behavioral test

11.2.1. Social Play Test

The test was performed in a sound-attenuated chamber under dim light conditions. At PNDs 35–40, rats were individually habituated to the test cage for 10 min on the 2 days before testing. On the test day, the animals were isolated for 3 h before testing. The test consisted of placing each experimental rat together with an untreated animal for 15 min in the testing chamber. In rats, social play behavior starts with one rat soliciting (‘‘pouncing’’) another animal, by attempting to nose or rub the nape of its neck. The animal that is pounced upon can respond in different ways: if the animal fully rotates to its dorsal surface, ‘‘pinning’’ is the result (one animal lying with its dorsal surface on the floor with the other animal standing over it), which is considered the most characteristic posture of social play behavior in rats. The following parameters were scored for each animal of a pair using the Observer 3.0 software (Noldus, The Netherlands): (1) number of pinning events; (2) number of pouncing events; (3) evasion (the animal that is pounced upon does not prolong the playful interaction but rather runs away); and (4) play responsiveness (the percentage of response to play solicitation, as the probability of an animal of being pinned in response to pouncing by the stimulus partner). Time spent in social exploration (the total amount of time spent in non-playful forms of social interaction, like sniffing any part of the body of the test partner, including the anogenital area, or grooming any part of the partner body).

11.2.2. Three-Chambers Test

The apparatus was a rectangular three-chamber box, with two lateral chambers (30 × 35 × 35 cm; l × w × h) connected to a central chamber (15 × 35 × 35 cm; l × w × h). Each lateral chamber contained a small Plexiglas cylindrical cage. At PND 90, each experimental rat was individually allowed to explore the three-chamber apparatus for 10 min and then confined in the central compartment. An unfamiliar stimulus animal was confined in a cage located in one chamber of the apparatus, while the cage in the other chamber was left empty. Both doors to the side chambers were then opened, allowing the experimental animal to explore the apparatus for 10 min. The discrimination index was scored using the Observer 3.0 software (Noldus, The Netherlands) and was calculated as the difference in time spent sniffing the stimulus animal and the time spent exploring the empty chamber, expressed as the percentage ratio of the total time spent exploring both the stimulus animal and the empty chamber.

11.2.3. Social Discrimination Test

Animals were isolated for 7 days before testing. The test consisted of a learning trial and a retrieval trial, which were separated by a 30 min intertrial interval. During the learning trial, a juvenile (PND 30) unfamiliar rat was introduced into the home cage of the experimental rat for 5 min. The time spent by the experimental rat investigating (sniffing, allogrooming and following) the juvenile was measured. Thirty-minutes after, the juvenile used in the learning trial was returned to the same adult's cage together with a novel juvenile. The time spent by the adult exploring the novel and the familiar juveniles was monitored for 5 min. The discrimination index was calculated as the difference in time exploring the novel and the familiar animal, expressed as the percentage ratio of the total time spent exploring both animals.

11.3. Brain Samples Collection

Rats were rapidly decapitated, and their brains were removed and cut into coronal slices on a cold plate. The PFC, DS, NAc and hippocampus (HIPP) were dissected by hand under microscopic control within 2 min.

11.3.1. Flow Cytometric Experiments

Brain samples for each brain area were transferred with PBS into 1.5 ml tube. After centrifugation, the pellet was digested with Trypsin (0.1%) for 30 min at 37◦C on slight agitation. Cell suspensions were filtered with CellTrics (100 μm) and washed with 5 ml of PBS. A little sample of suspension was analyzed for forward and side scatter parameter for neuron population quality control (Cruz et al. 2013). Each sample was fixed with PFA (1%), permeabilized with ice-cold ethanol (70%) and incubated for at least 2 h at −20◦C. Next, cells were centrifuged, rehydrated with 1 ml PBS/BSA (1%)/Triton (0.1%), aliquoted in 96 well tissue culture plates with conical bottom. Samples were incubated for 1 h RT with primary antibody anti-D1 (1:100 diluted, Novusbio AB81296) and D2 dopamine receptors (1:100 diluted, Santa Cruz SC-7523). The cells were washed

and incubated with secondary antibody anti-rabbit and anti-goat Alexa 488 conjugated. All samples were counterstained with propidium iodide/RNase buffer for nuclei identification (with G0 cell cycle phase DNA content) and for singlet/doublets discrimination. Mean fluorescence intensity of 20,000 useful cellular events for each sample, was calculated.

11.4. Striatal slice preparation

1-month old male Wistar rats were anesthetized with isoflurane and decapitated. The brain was quickly removed and glued to the bottom of a vibroslicer slicing chamber (Leica VT 1000S, Leica Microsystem, Wetzlar, Germany). Coronal brain slices (350 μm) containing the NAc were cut in a sucrose-based solution composed of (in mM): 87 NaCl, 75 sucrose, 25 glucose, 4 KCl, 2.1 MgCl2, 0.5 CaCl2, 18 NaHCO3, and 1.25 NaH2PO4. During slicing, the solution was kept cold and infused with a 95% O2 + 5% CO2 gas mixture. Immediately after cutting, slices were stored for 1 h at 32◦C in a low calcium artificial cerebrospinal fluid (low Ca2+ aCSF) that contained in mM: 130 NaCl, 11 Glucose, 2.5 KCl, 2.4 MgCl2, 1.2 CaCl2, 23 NaHCO3, 1.2 NaH2PO4, and was equilibrated with 95%O2/5%CO2 and then at room temperature until the time of recording. During recordings, a single slice was kept in a flow chamber positioned under the microscope objective and continuously perfused with aCSF (same as low Ca2+ aCSF with the following exception: 2.4 mM CaCl2 and 1.2mM MgCl2).

11.5. The patch-clamp technique

The patch-clamp technique, invented by Erwin Neher and Bert Sakmann in 1976, consists of sealing the tip of a pipette filled with a physiological solution to the membrane of a cell, maintaining it at a specific voltage to measure currents flowing through ion channels. Alternatively, the experimenter can control the current injected into the cell through the recording electrode, measuring its potential. There are five different configurations of patch-clamping: “whole-cell”, “cell-attached”, “inside-out”, “outside-out” and “perforated”. Among them the less invasive is the cell-attached configuration, where the patch electrode is sealed to the membrane (reaching an electrical resistance in the order of a GΩ), leaving the cell intact and allowing the recording of currents through single ion channels. The whole-cell configuration is obtained with the rupture of the seal, allowing the pipette to become continuous with the membrane. If alter the formation of gigaseal the pipette is quickly withdrawn from the cell, the piece of membrane inside of it will be exposed to the extracellular media (inside-out configuration). Pulling the membrane from both sides of a whole-cell configured patch in order to let the external faces being in contact with the external solution will result in the outside-out configuration. During whole-cell recordings an irreversible washout of diffusible intracellular constitutes (e.g. ATP, phosphorylating molecules, etc) into the relatively larger volume of the pipette through dialysis can occur. As a consequence, properties and functions of ion channels can be impaired, leading to a decrease of ionic currents over time. The permeabilized-patch whole-cell configuration overcomes this problem by accessing

the intracellular space not by seal rupture but forming pores thanks to a pipette solution containing antibiotics. In this way, the dialysis is much slower and the intracellular environment is preserved for long recordings.

11.6. Electrophysiology

Whole-cell patch-clamp recordings

Patch pipettes were pulled from thin-walled borosilicate capillaries (Harvard Apparatus, London, UK) with a vertical puller (Narishige PP830, Narishige International Limited, London, UK) and back-filled with one of following intracellular solutions containing (in mM): (i) K+ methanesulfonate (120), KCl (15), HEPES (10), EGTA (0.1), $MgCl_2$ (2), Na_2Phosphocreatine (5), Na_2GTP (0.3) and MgATP (2); (ii) KCl (140), $MgCl_2$ (1.6), MgATP (2.5), NaGTP (0.5), EGTA (2), HEPES (10) resulting in a bath resistance of 3–5 MΩ or (iii) K+ gluconate (145), NaCl (3), $MgCl_2$ (1), EGTA (1), $CaCl_2$ (0.3), Na^+ATP (2), Na^+GTP (0.3), cAMP (0.2), HEPES (10) (pH 7.25-7.3, osmolality, 280-300 mOsm) resulting in a bath resistance of 4-6 MΩ. Unless otherwise specified, all drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA) and bath-applied. Access resistance was monitored during voltage-clamp recordings with 100-ms, −10- mV steps, throughout the experiment. Recordings undergoing a drift in access resistance ≥ 10% were discarded. No whole-cell compensations were used. Signals were sampled at 10 kHz and low-pass filtered at 3 kHz with an Axon Multiclamp 700B (Molecular Devices, Sunnyvale, CA, USA). Recordings were made in the medial ventral NAc core close to the anterior commissure (Salgado and Kaplitt 2015) where MSNs were identified by their morphological and electrophysiological properties (Cepeda et al. 2008). Current-voltage (I-V) curves were made by a series of hyperpolarizing to depolarizing current steps with amplitude ranging from −50 to +550 pA with 50 pA increments immediately after breaking into the cell. Inwardly rectifying potassium currents (IKir) were obtained by imposing 500 ms-voltage steps with amplitude ranging from −150 to −60 mV with 10 mV increments. Potassium currents were recorded in the presence of tetrodotoxin (TTX, 1 μM), a blocker of voltage-dependent sodium channels. IKir was isolated as the 1 mM CsCl-sensitive component. IKir current density was obtained by normalizing current amplitudes for membrane capacitance (derived from the area underlying current peaks elicited by −10 mV steps) and expressed as pA/pF. Excitatory synaptic activity during recordings of spontaneous EPSCs (sEPSCs) was isolated by using the $GABA_A$ receptor blocker gabazine. On the contrary, inhibitory activity during recordings of spontaneous IPSCs (sIPSCs) was isolated by using the APV and NBXQ. These latter blocks NMDA and AMPA receptors, respectively. Finally, in the experiments involving recordings of miniature excitatory postsynaptic currents (mEPSCs) the external solution was supplemented with TTX. A -10 mV hyperpolarizing pulse was applied before recordings of both sEPSCs and sIPSCs to evaluate the access resistance.

During recordings rapamycin (dissolved in DMSO 100%, Sigma-Aldrich) was added to gabazine or

TTX in a final concentration of 200 nM. After a 10 min perfusion in rapamycin, the neurons were recorded up to 25 min. Control experiments were performed by adding only the same amount of solvent (i.e., DMSO)

11.7. Reagents

Unless otherwise specified, reagents were purchased from Sigma-Aldrich (Saint-Louis, MO, USA). The sodium salt valproic acid (NaVPA; Sigma-Aldrich) was dissolved in 0.9% saline to obtain a concentration of 250 mg/ml and administered at a dose 500 mg/kg at gestational day 12.5 to induce autistic-like behavioral changes in the offspring. IKir was isolated with potassium channels blocker CsCl (1mM; Sigma-Aldrich). Voltage-dependent sodium channels (NaV) were blocked with TTX (1 µM; Tocris Bioscience, Bristol, UK). AMPA, NMDA, KAINATE receptors were blocked with respectively, NBQX, D-APV, CNQX (10 µM, 50 µM, 20 µM; Tocris bioscience, Bristol, UK). GABAA receptors were blocked with gabazine (SR95531, 10 µM; Tocris). Rapamycin was used for mTOR inhibition (200 nM, Sigma-Aldrich). Drugs were added at the final concentration to the recording aCSF media.

11.8. Data analysis

Whole-cell patch-clamp recordings were analyzed using Clampfit 10.6 (Molecular Devices, Sunnyvale, CA, USA). Passive membrane properties were measured from current transient elicited by 10 mV hyperpolarizing pulses from the holding potential of −70 mV in voltage-clamp. Membrane capacitance (C_m) was measured from the capacitive charge (the area underlying current transients), and cell input resistance (Ri) was calculated by dividing the steady-state voltage response by the current pulse amplitude (Rinput =$\Delta V/\Delta I$). Resting membrane potential (RMP) was measured immediately after whole-cell formation during the current-clamp protocol. The input-output curve was made by measuring the number of action potentials elicited by depolarizing current steps of increasing amplitude. IKir was defined by subtracting the ionic currents obtained at hyperpolarized membrane potentials in the presence and the absence of CsCl; IKir current density was obtained by normalizing current amplitudes for membrane capacitance (derived from the area underlying current peaks elicited by −10 mV steps) and expressed as pA/pF. The AP threshold was determined from the first AP evoked in a current-clamp protocol and was defined as the voltage at the AP inflection point of the rising phase of AP. Spontaneous and miniature EPSCs/IPSCs amplitude and frequency were analyzed with Axograph X using a double exponential template: f(t)=exp(−t/rise)+exp(−t/decay), rise=0.4 ms. The threshold of amplitude detection was set at -7 pA.

11.9. Statistical Analysis

Pooled data are presented as mean ± standard error of the mean (SEM) of n cells obtained from N animals. Statistical analysis of data was performed with Prism 7.0 (GraphPad Software) using tests indicated in the main text after outlier subtraction. Significance at the $p < 0.05$, 0.01, 0.001 and

0.0001 level is indicated, when achieved, with *, **, and ****, respectively, in figures. Graphs and representative traces were generated with Prism. Example traces represent typical observations.

12. RESULTS

12.1. VPA prenatal exposure reduces social play behavior

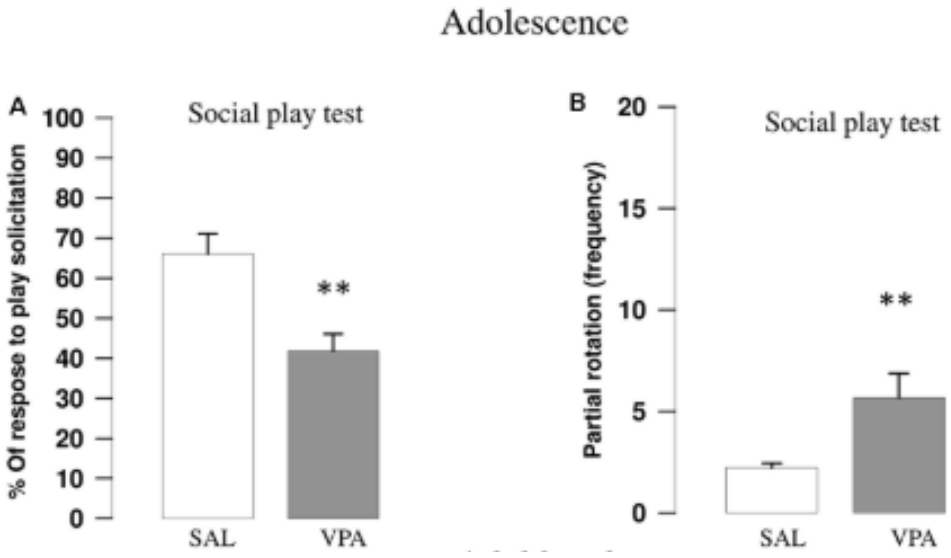
Social play has a strong emotional component, its most characteristic feature being its high reward value. Therefore, we performed behavioral tests to evaluate the effects of VPA prenatal exposure on social skills. We found that VPE reduced play responsiveness in VPA-exposed rats compared with SAL-exposed animals ($t = 3.67$, $p = 0.002$, $df = 16$; Fig. 12A). Indeed, while no differences were found between SAL- and VPA-exposed animals in the number of pinning ($t = 0.81$, p = n.s., $df = 16$; data not shown) and pouncing behaviors ($t = -0.86$, p = n.s., $df = 16$; data not shown), VPA-exposed rats displayed a higher frequency of partial rotations ($t = -2.81$, $p = 0.013$, $df = 16$; Fig. 12B) compared to SAL-exposed animals. No differences in the total time spent in general social exploration were found between SAL- and VPA-exposed animals ($t = 1.43$, p = n.s., $df = 16$, data not shown).

12.2. Prenatal exposure to VPA reduces social interaction

VPA-exposed rats showed decreased sociability in the three-chamber test, as they spent less time sniffing the stimulus animal compared to SAL-exposed rats, resulting in a lower discrimination index ($t = 2.27$, $p = 0.039$, $df = 14$; Fig. 12C).

12.3. Prenatal exposure to VPA reduces social discrimination ability

VPA-exposed animals showed impaired social discrimination ability as they had worse performance in the social discrimination test compared with SAL-exposed animals ($t = 2.27$, $p = 0.044$, $df = 14$; Fig. 12D).



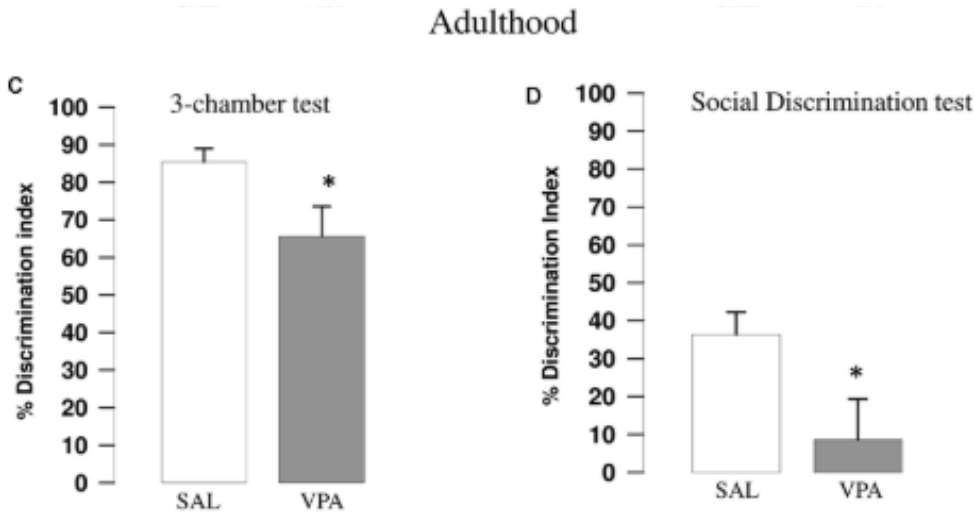


Figure 12: VPA prenatal exposure induced impairment in social domain. VPA-exposed rats showed reduced response to play solicitation (A) and increased frequency of partial rotations (B). VPA-exposed rats also showed reduced sociability in the three-chamber test (C), since they showed lower discrimination index indicating (i.e., they spent less time sniffing the stimulus animal compared to SAL-exposed rats). VPA-exposed rats also showed impaired social discrimination as they displayed a lower discrimination index compared to SAL-exposed animals in the social discrimination task (D). Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, vs. SAL group (Student's t-test).

12.4. Dopamine receptors analysis in VPA exposed rats

Biochemical analyses were performed to evaluate the expression of D1 and D2 dopamine receptors in the PFC, DS, NAc, and HIPP since these brain areas play an important role in the modulation of the rewarding properties of social interactions. During adolescence, VPA-exposed rats show a significative increase in D2 dopamine receptor expression in the NAc (SAL-exposed animals: MFI = 2,891; VPA-exposed animals: MFI = 5,272, $p < 0.05$; Fig. 13A, B). At adulthood, VPA-exposed rats showed a significative increase also in D1 dopamine receptor expression in the NAc (SAL-exposed animals: MFI = 4,789; VPA-exposed animals: MFI = 6,690, $p = 0.025$; Fig. 13C) and HIPP (SAL-exposed animals: MFI = 3,987; VPA group: MFI = 5,299, $p = 0.008$; Fig. 13C), whereas D2 dopamine receptors were significative increased only in the NAc (SAL-exposed animals: MFI = 1,697; VPA-exposed animals: MFI = 2,152, $p = 0.022$; Fig. 13D).

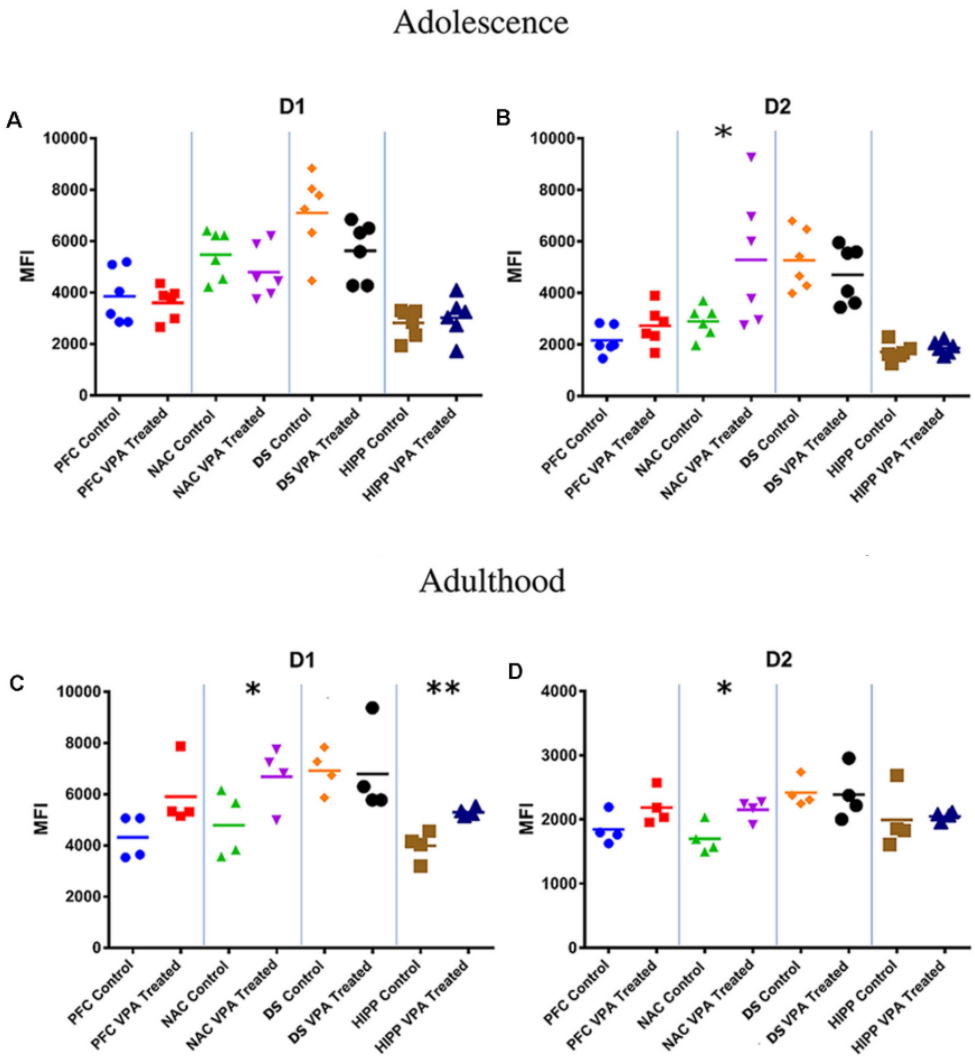


Figure 13: Flow cytometric analysis of dopamine receptors. No differences between VPA-exposed and SAL-exposed rats were found in D1 dopamine receptor expression in PFC, NAc, DS, and HIPP (A). At PNDs 30–35, VPA-exposed rats showed a significant increase in D2 dopamine receptor expression in the NAc (B) compared to SAL-exposed rats. At PNDs 90–95, VPA-exposed rats showed increased expression of D1 dopamine receptor expression in NAc and HIPP (C), while D2 receptor expression was increased only in the NAc (D). Data represent mean values ± SEM; $*p < 0.05$, $**p < 0.01$ vs. SAL group (Student's t-test).

12.5. Electrophysiological alterations of accumbal MSNs in VPA exposed rats

Whole-cell patch-clamp recordings were obtained from NAc MSNs in acute coronal slices prepared from SAL- and VPA-exposed rats at PNDs 30–45 (N = 25 and 15, respectively, Fig. 14A, B). We found that VPE significantly changes the passive membrane properties of MSNs in VPA-exposed rats (VPA MSNs) compared to SAL-exposed rats (SAL MSNs) (input resistance: SAL MSNs = 157.3 ± 12.89 MΩ, n = 39; VPA MSNs = 109.1 ± 7.23 MΩ, n = 49 ; p = 0.0028; membrane capacitance: SAL MSNs = 68.27 ± 4.84 pF, n = 39; VPA MSNs = 47.49 ± 3.37 pF n = 49; p = 0.0017; threshold: SAL MSNs = −38.96 ± 0.88 mV, n = 58; VPA MSNs = −38.62 ± 0.81 mV, n = 54; p = n.s.; all; Fig. 14C). Futhermore, VPA MSNs showed significant depolarization of

the resting membrane potential as compared to SAL MSNs (SAL MSNs = −76.71 ± 0.60 mV, n = 60; VPA MSNs = −72.21 ± 0.89 mV, n = 53; p < 0.0001; Fig. 14C).

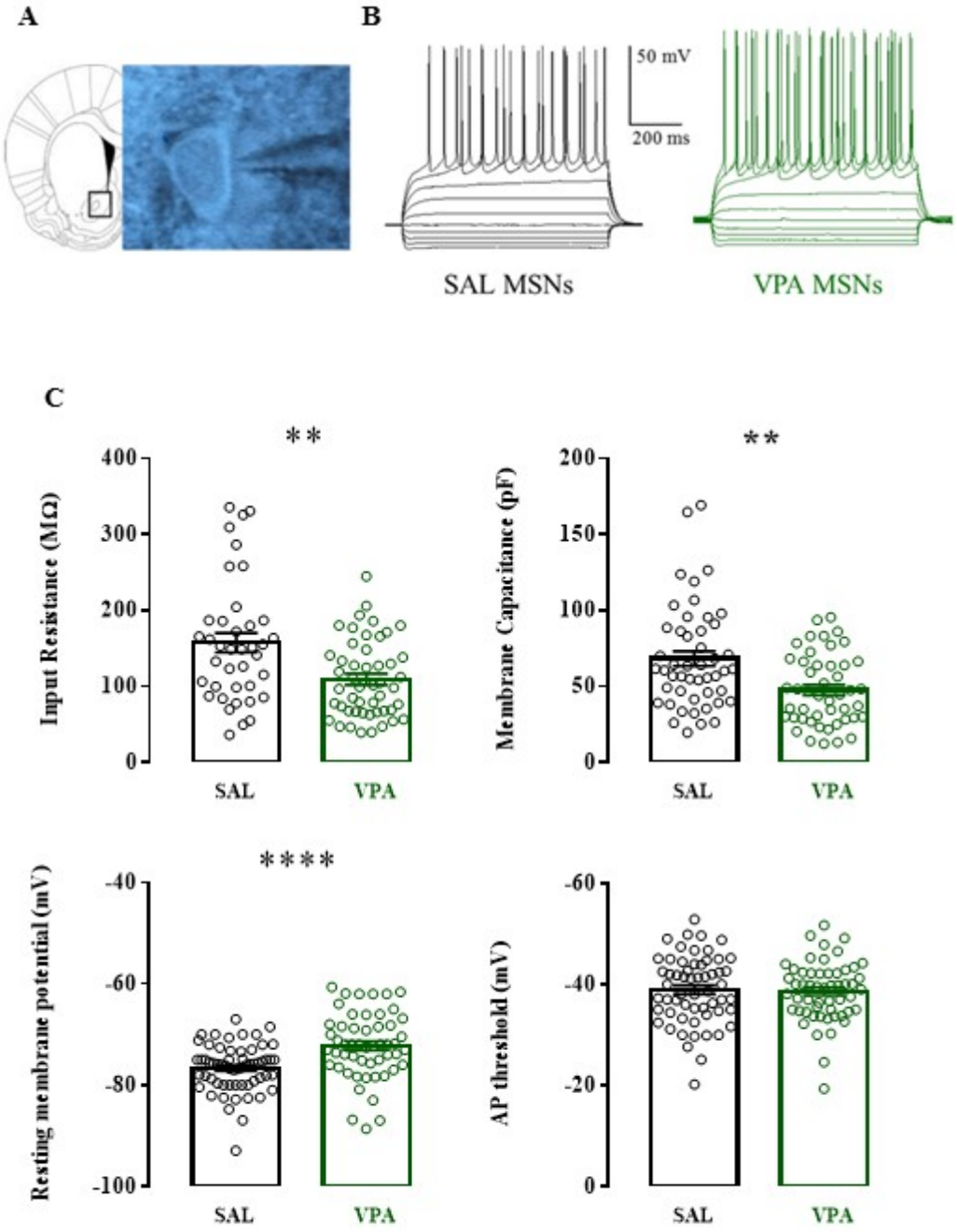


Figure 14: Electrophysiological properties of SAL and VPA medium spiny neurons (MSNs). (A) NAc-MSN visualized with infrared video microscopy (left) and schematic representation of a coronal brain slice at the level of the NAc (right). (B) Representative voltage traces of SAL MSNs and VPA MSNs evoked by injection of hyperpolarizing and depolarizing current pulses (800 ms from −200 pA to 250 pA in 50 pA steps). (C) Passive membrane properties of SAL MSNs and VPA MSNs: input resistance, membrane capacitance, resting membrane potential, and AP threshold. Data represent mean values ± SEM; ∗∗∗p < 0.0001 vs. SAL group (Mann-Whitney U test).

12.6. VPE increases the intrinsic excitability of NAc-MSNs

To evaluate the possible consequences of the depolarized resting membrane potential on the electrical activity of MSNs, we determined the intrinsic excitability of these neurons in acute slices containing the NAc by measuring the number of action potentials elicited by depolarizing current steps of increasing amplitude. Figure 15 shows a direct comparison of representative voltage responses obtained for the corresponding value of imposed current from SAL and VPA MSNs. The minimal value of current amplitude required to elicit an action potential in VPA MSNs was significantly lower compared to SAL MSNs (100 pA vs. 200 pA). However, as the amplitude of the depolarizing current increased (≥250 pA), while the number of APs fired by SAL MSNs increased linearly, VPA MSNs progressively lost the ability to fire action potentials. The plot in Figure 15 reports the mean number of APs ± SEM fired by SAL (black) and VPA (green) MSNs vs. imposed current (SAL MSNs, n = 55, vs. VPA MSNs n = 59; 50 pA p = 0.001; 100 pA p = 0.001; 150 pA; p = 0.008 ; 200 pA; p = 0.01; 350 pA; p = 0.002; ≥ 400 pA; p < 0. 0001).

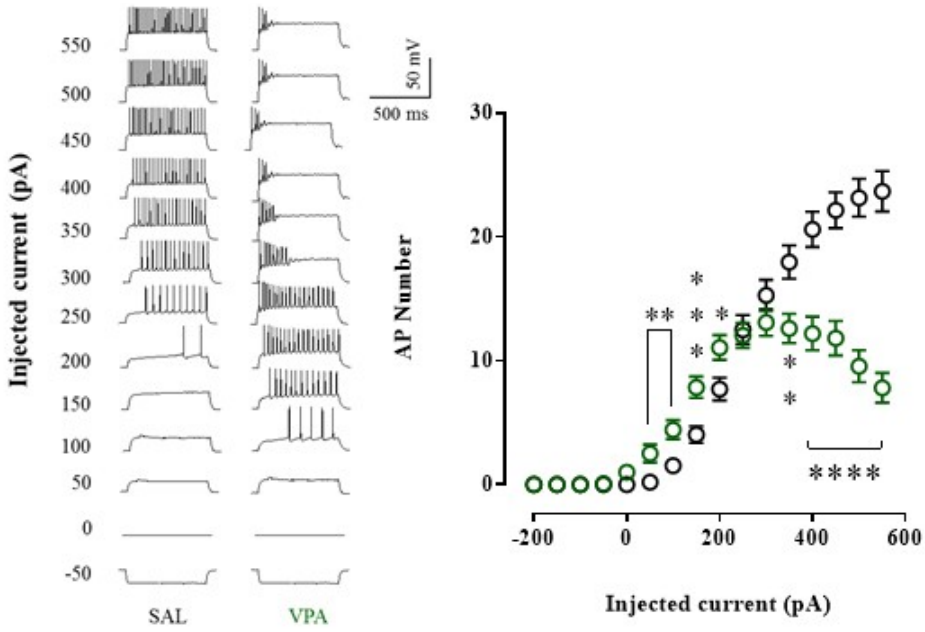


Figure 15: Intrinsic excitability of SAL MSNs and VPA MSNs. Representative voltage traces obtained in response to–50 to 550 pA current steps (800 ms duration, 50 pA amplitude) from resting potential, recorded from SAL and VPA MSNs (left). Number of APs fired plotted against current steps of increasing value (right). Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001 vs. SAL group (Student's t-test).

12.7. VPE induces a loss-of-function of Kir channels

The resting membrane potential of MSNs is strongly influenced by the activity of Kir channels. Indeed, at rest, Kir channels are responsible for their characteristic negative resting potential. Moreover, at the rest, Kir channels limit membrane depolarization in response to excitatory synaptic inputs (Kreitzer 2009). Based on this evidence a reduction in available Kirs in MSNs could be responsible of the significant increase in excitability. To investigate this possibility in our

experimental model, we measured whole-cell currents elicited at hyperpolarizing potentials in control aCSF and in presence of 1 mM Cs+, a IKir blocker (Cazorla et al., 2012). Figure 16 shows representative inward currents obtained in control solution (I_{aCSF}) and in presence of 1 mM Cs+. IKir is obtained as the Cs-sensitive component in IaCSF. Current-voltage relationship reveals a significant reduction of IKir current density in VPA MSNs (SAL MSNs, n = 7, VPA MSNs, n = 15; $p < 0.05$ where indicated).

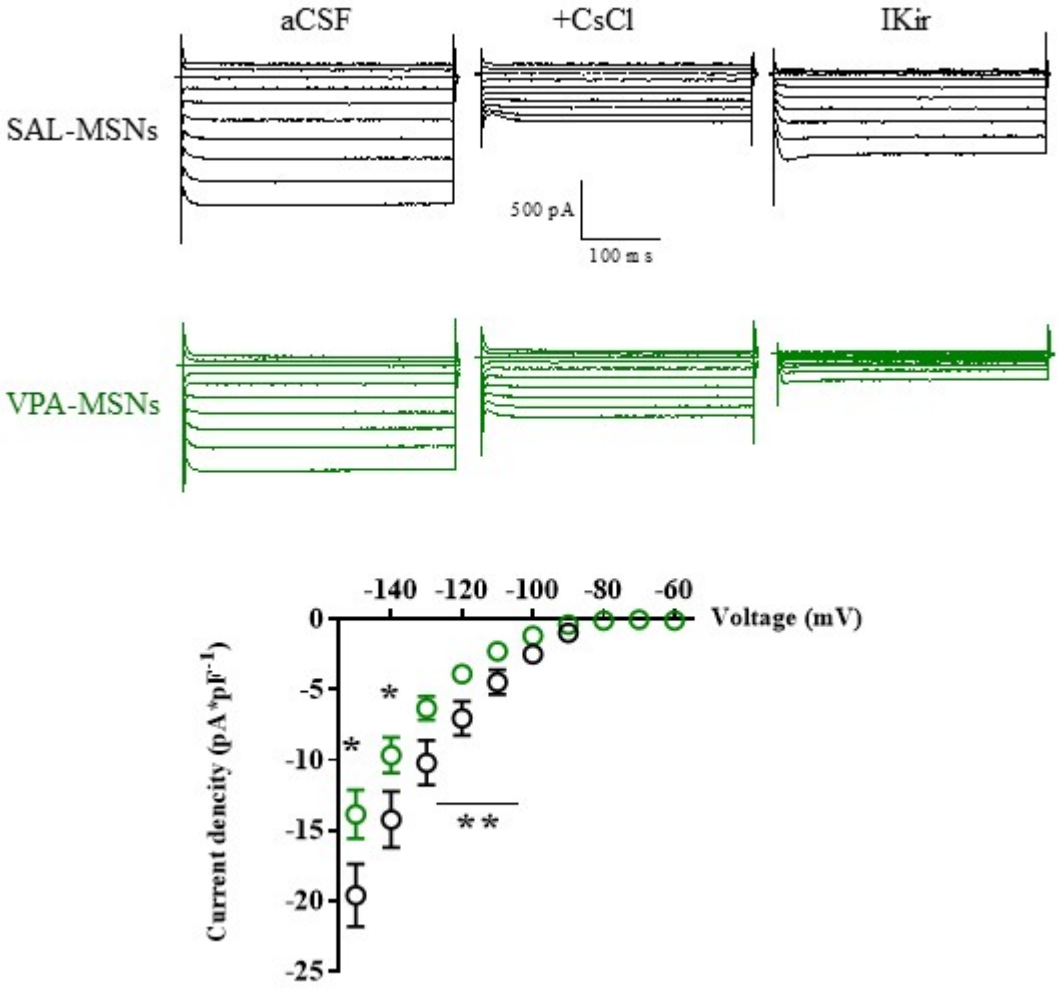


Figure 16: Inwardly rectifying potassium current (IKir) density in SAL MSNs and VPA MSNs. (A) Representative IKir traces obtained at hyperpolarized membrane potentials (−60 to −150 mV in 10 mV steps) obtained from SAL and VPA MSNs. IKir is obtained by subtracting ICs from IaCSF. Current-voltage relationship of IKir current density in SAL and VPA MSNs. (B) Current-voltage relationship of IKir current density in SAL and VPA MSNs. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001 vs. SAL group (Student's t-test).

The disruption in the excitatory and inhibitory (E/I) balance has been proposed among one of the possible mechanisms involved in ASD etiology and symptoms. Indeed, VPA has been found to lead to modulation of the synaptic neurotransmission in different brain areas (Banerjee et al. 2013; Martin and Manzoni 2014; Wang et al. 2012). As this phenomenon has not yet been explored in the

NAc, we decided to investigate the excitatory synaptic transmission in VPA- and SAL-exposed rats (Fig. 17A). Spontaneous excitatory postsynaptic currents were recorded during whole-cell voltage-clamp recordings with a holding potential of -70 mV, in the presence of gabazine10 μM to block GABAergic synaptic inputs. We found that VPE significantly decreases the amplitude of sEPSCs of VPA MSNs compared to SAL MSNs in adolescent rats (SAL MSNs = -15.87 ± 0.79 pA n = 26; VPA MSNs = -12.24 ± 0.40 pA n = 25; p = <0.0001 *Mann-Whitney U test*; Fig. 17B) and the cumulative distribution of sEPSC amplitudes was shifted toward higher values in SAL MSNs (Fig. 17B). In contrast, the frequency of spontaneous events among VPA MSNs and SAL MSNs (SAL MSNs = 2.32 ± 0.29 Hz n = 26; VPA MSNs = 1.73 ± 0.28 Hz n = 25; p = n.s. *Mann-Whitney U test*; Fig. 17C). Interestingly, we also found that VPA changes the kinetics of AMPA-mediated events. Indeed, as shown in figure 17B., sEPSCs in VPA MSNs are characterized by slower rise time compared to SAL MSNs (SAL MSNs = 1.07 ± 0.03 ms n= 26; VPA MSNs = 1.23 ± 0.04 ms n = 25; p = 0.004 *Mann-Whitney U test*).

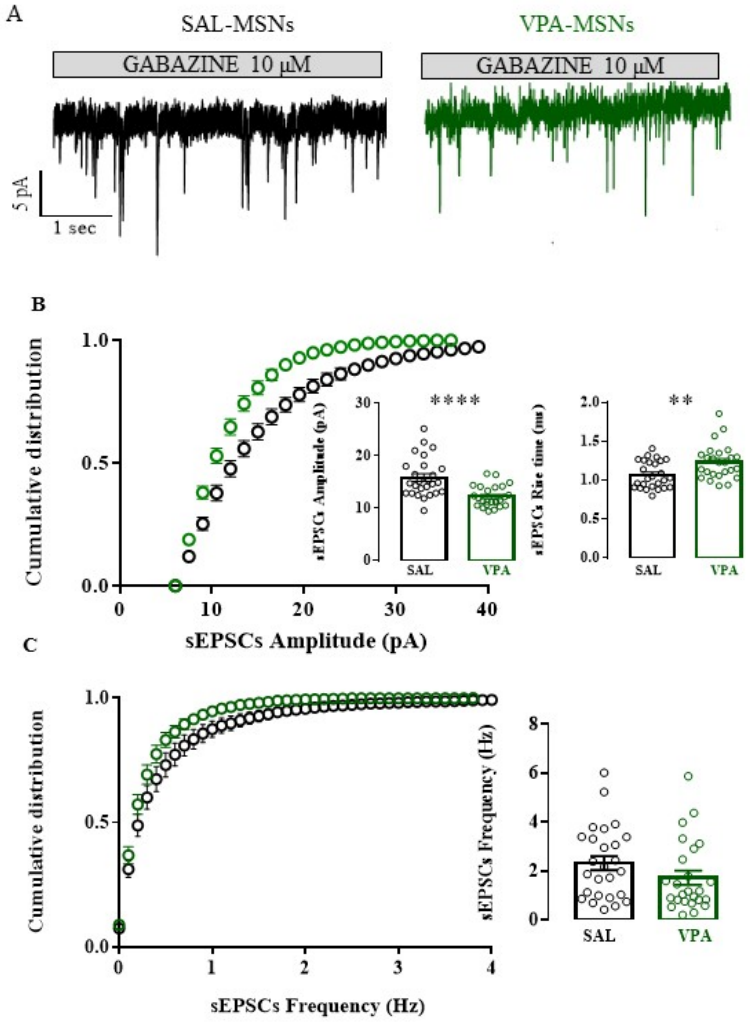


Figure 17: Altered Synaptic Transmission in NAc-MSNs of VPA-exposed rats (A) Representative traces from the whole-cell voltage-clamp recording of sEPSCs in the NAc-MSNs from SAL MSNs (left) and VPA MSNs (right). (B) Cumulative distribution of sEPSCs amplitude, plotted averaged amplitudes, and rise time

from each recorded neuron (insert figure). (C) Same as (B) but for the instantaneous frequency and plotted averaged frequency of sEPSCs. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001 vs. SAL group (Mann-Whitney U test; Kolmogorov-Smirnov test).

Moreover, we investigated whether the effect of VPA prenatal exposure affected the release probability of synaptic vesicles in an action potential-independent way. To clarify this point, we performed whole-cell voltage-clamp recordings in presence of TTX 1µM, a voltage-gated Na+ channels blocker (Fig. 18A). As shown in figure 18B, VPE does not cause any significant modification of either mEPSCs amplitude (SAL MSNs = -14.16 ± 0.67 n = 20; VPA MSNs = -13.06 ± 0.49 n= 22; p = n.s. *Mann-Whitney U test*) or rise time (SAL MSNs = 1.17 ± 0.04 n = 20; VPA MSNs = 1.24 ± 0.06 n= 22; p = n.s. *Mann-Whitney U test*) in VPA MSNs compared to SAL MSNs in adolescent rats. Interestingly, we found that VPE increases the frequency of mEPSCs in VPA MSNs compared to SAL MSNs (SAL MSNs = 2.05 ± 0.23 Hz n = 20; VPA MSNs = 2.91 ± 0.31 Hz n= 22; p = 0.03. *Mann-Whitney U test*, Fig. 18C), suggesting an increase in release probability or in the number of functional synaptic sites.

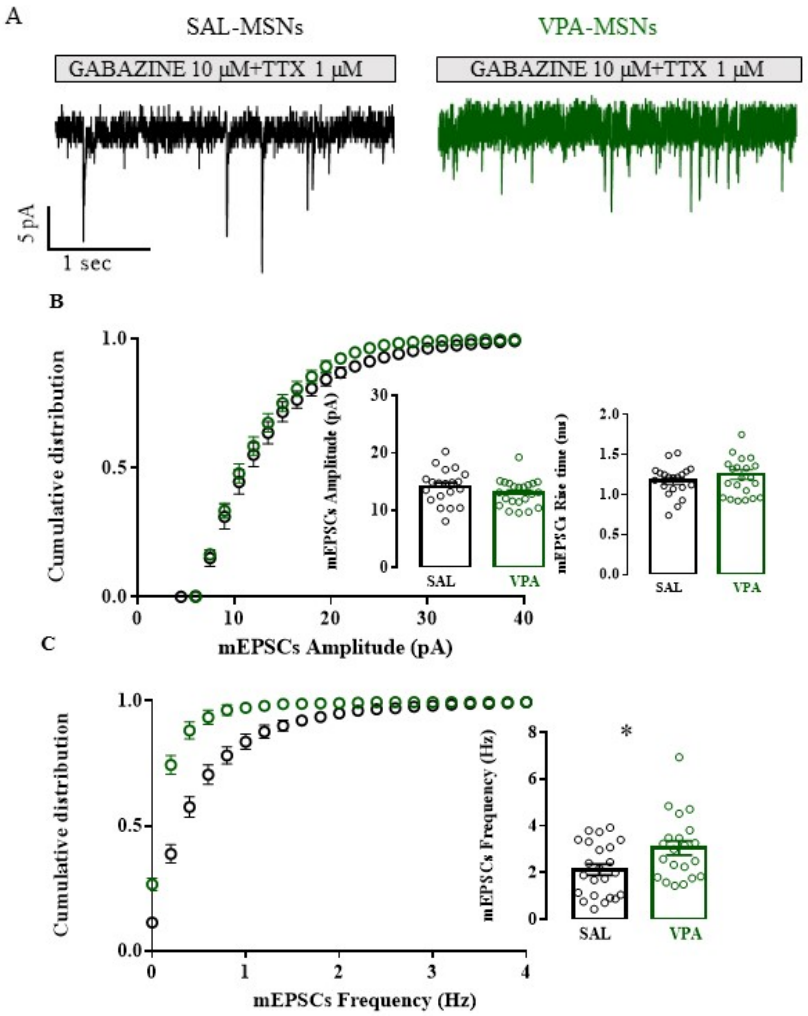


Figure 18: Altered Synaptic Transmission in NAc-MSNs of VPA-exposed rats (A) Representative traces from the whole-cell voltage-clamp recording of mEPSCs in the NAc-MSNs from SAL MSNs (left) and VPA MSNs (right). (B) Cumulative distribution of mEPSCs amplitude, plotted averaged amplitudes and rise time

from each recorded neuron (insert figure). (C) Same as (B) but for the instantaneous frequency and plotted averaged frequency of mEPSCs. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001 vs. SAL group (Mann-Whitney U test; Kolmogorov-Smirnov test).

12.9. VPA could alter inhibitory neurotransmission in NAc-MSNs

Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded during whole-cell voltage-clamp recordings using a high KCl intracellular solution. In these experimental conditions, sIPSCs appear as inward currents (Fig. 19A). Holding potential was held at -70 mV in the presence of NBQX 10 μM and DL-APV 50 μM to block glutamatergic synaptic inputs. Preliminary data shown no significant differences in sIPSCs amplitude between MSNs from SAL- and VPA-exposed rats (SAL MSNs = -36.63 ± 4.6 n = 12; VPA MSNs = -34.04 ± 9.87 n= 6; p = n.s. *Mann-Whitney U test* Fig. 19B). Instead, VPE increases the rise time of GABA-mediated events (SAL MSNs = 0.60 ± 0.05 ms n = 12; VPA MSNs = 1.007 ± 0.07 ms n= 6; p = 0.001. *Mann-Whitney U test* Fig. 19B). Finally, as shown in figure 19C prenatally exposed to VPA significantly decreases the frequency of sIPSCs events (SAL MSNs = 1.73 ± 0.18 Hz n = 12; VPA MSNs = 0.70 ± 0.16 Hz n= 6; p = 0.003. *Mann-Whitney U test*). Of note, these are preliminary data, requiring additional recordings to confirm the effect of VPE on the inhibitory neurotransmission.

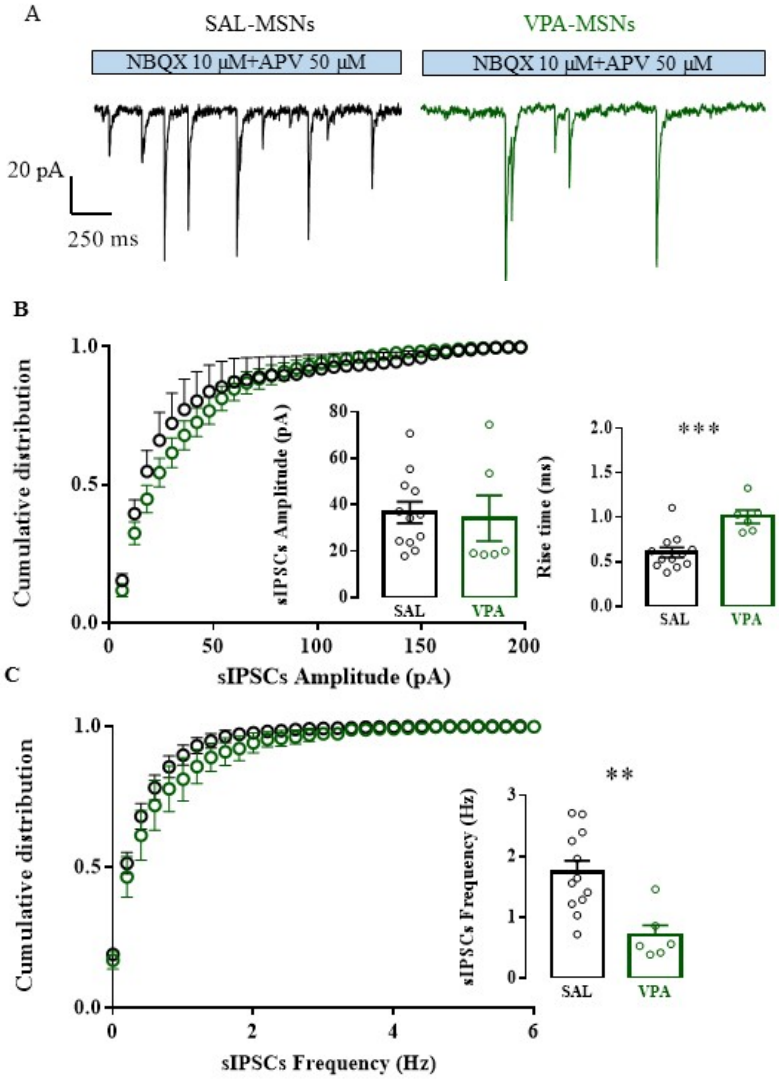


Figure 19: VPA could alter inhibitory neurotransmission in NAc-MSNs. (A) Representative traces from whole-cell voltage-clamp recordings of sIPSCs in the NAc-MSNs from SAL MSNs (left) and VPA MSNs (right). (B) Cumulative distribution of sIPSCs amplitude, plotted averaged amplitudes, and rise time from each recorded neuron (insert figure). (C) Same as (B) but for the instantaneous frequency and plotted averaged frequency of sIPSCs. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001 vs. SAL group (Mann-Whitney U test; Kolmogorov-Smirnov test).

12.10. Acute rapamycin application normalizes the resting membrane potential of VPA-MSNs

As shown by recent evidence, the overactivation of mTOR pathway causes diseases that are syndromic with ASD (Costa-Mattioli and Monteggia 2013). The hyperactivation of this pathway has been demonstrated in VPA-exposed mice. Moreover, rapamycin treatment improves the social deficit induced by VPE (Kotajima-Murakami et al. 2019) suggesting a causative role of this pathway in the behavioral alteration. Based on these arguments, we decided to investigate if the cellular alterations observed in VPA-exposed rats could be caused by mTOR dysregulation, by testing the electrophysiological effects caused by acute bath application of rapamycin. Recordings were obtained from NAc MSNs in acute coronal slices prepared from SAL- and VPA-exposed rats at PNDs 30–45 (N = 6 and 6). Preliminary data shows that acute administration of rapamycin (200 nM)

does not change membrane capacitance of NAc-MSNs (membrane capacitance: SAL-aCSF = 104.6 ± 6.35 pF n = 14; SAL-RAPA = 83.98 ± 5.95 pF n = 8; VPA-aCSF = 85.53 ± 7.75 pF n = 16; VPA-RAPA = 75.78 ± 8.95 pF n = 12; n.s. *Two-way Anova*; Fig. 20A). On the contrary, rapamycin affects the input resistance of NAc-MSNs. Indeed, *Post-hoc* analysis shows that rapamycin increases the IR of SAL-RAPA compared to SAL-aCSF (input resistance: SAL-aCSF = 109.6 ± 17.86 MΩ, n = 14; SAL-RAPA = 291.4 ± 24.01 MΩ, n = 7; p = 0.001 *Two-way Anova*; Fig. 20B). The same effect is observed between VPA-RAPA and VPA-aCSF neurons (input resistance: VPA-aCSF = 136.6 ± 15.22 MΩ, n = 16; VPA-RAPA = 267.5 ± 30.5 MΩ, n = 12; p = 0.001 *Two-way Anova*; Fig. 20B). Interestingly, rapamycin reverts the effet of VPE on the resting membrane potential of VPA-MSNs. As shown in figure 20C, VPA-RAPA neurons are characterized by a more hyperpolarized resting membrane potential compared to VPA-aCSF neurons (resting membrane potential: VPA-aCSF = -75.74 ± 0.89 mV, n = 14; VPA-RAPA = -84.31 ± 2.19 mV, n = 13; p = 0.001 *Two-way Anova*). Moreover, no difference was found between SAL-aCSF and SAL-RAPA neurons (resting membrane potential: SAL-aCSF = -80.71 ± 0.77 mV, n = 14; SAL-RAPA = -81.43 ± 0.99 mV, n = 10; n.s. *Two-way Anova*; Fig. 20C).

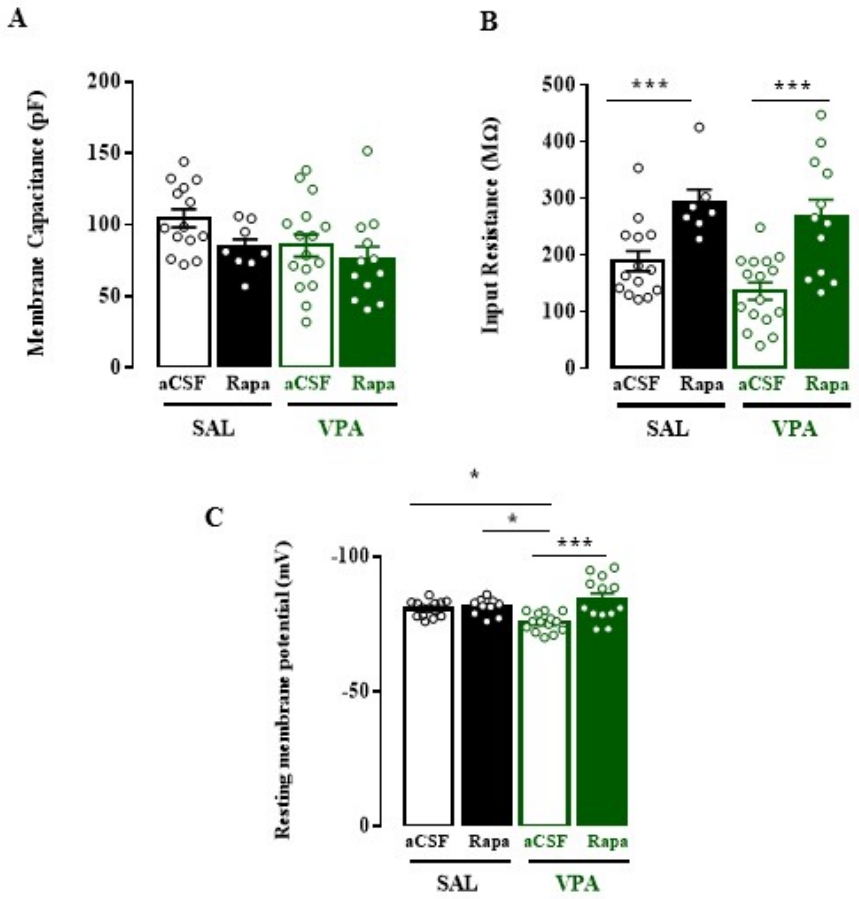


Figure 20: Effect of acute rapamycin on the passive membrane properties of SAL and VPA NAc-MSNs. (A) membrane capacitance; (B) input resistance and (C) resting membrane potential. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001, *Two-way Anova*, Student-Newman-Keuls *post-hoc* test.

12.11. Effect of rapamycin on NAc-MSNs excitability

Given the effect of rapamycin on resting membrane potential, we investigated if the repolarization observed in VPA-RAPA neurons could normalize the enhanced intrinsic excitability of VPA-MSNs induced by VPE. To address this hypothesis, we measured the number of action potentials elicited by depolarizing current steps of increasing amplitude before and after acute application of rapamycin. Preliminary data shows that rapamycin does not induce a significant difference in the firing properties of NAc-MSN (SAL-aCSF n = 6; SAL-RAPA n = 7; VPA-aCSF n = 13; VPA-aCSF n = 13; *Two-way Anova*, Tukey *post-hoc* test; Fig. 21C). Interestingly, if we consider the effect of rapamycin on SAL-exposed rats only, we can observe an increase in MSNs excitability similar to the one caused by VPE. Anyway, other recordings are required to confirm the effect of rapamycin on MSNs excitability.

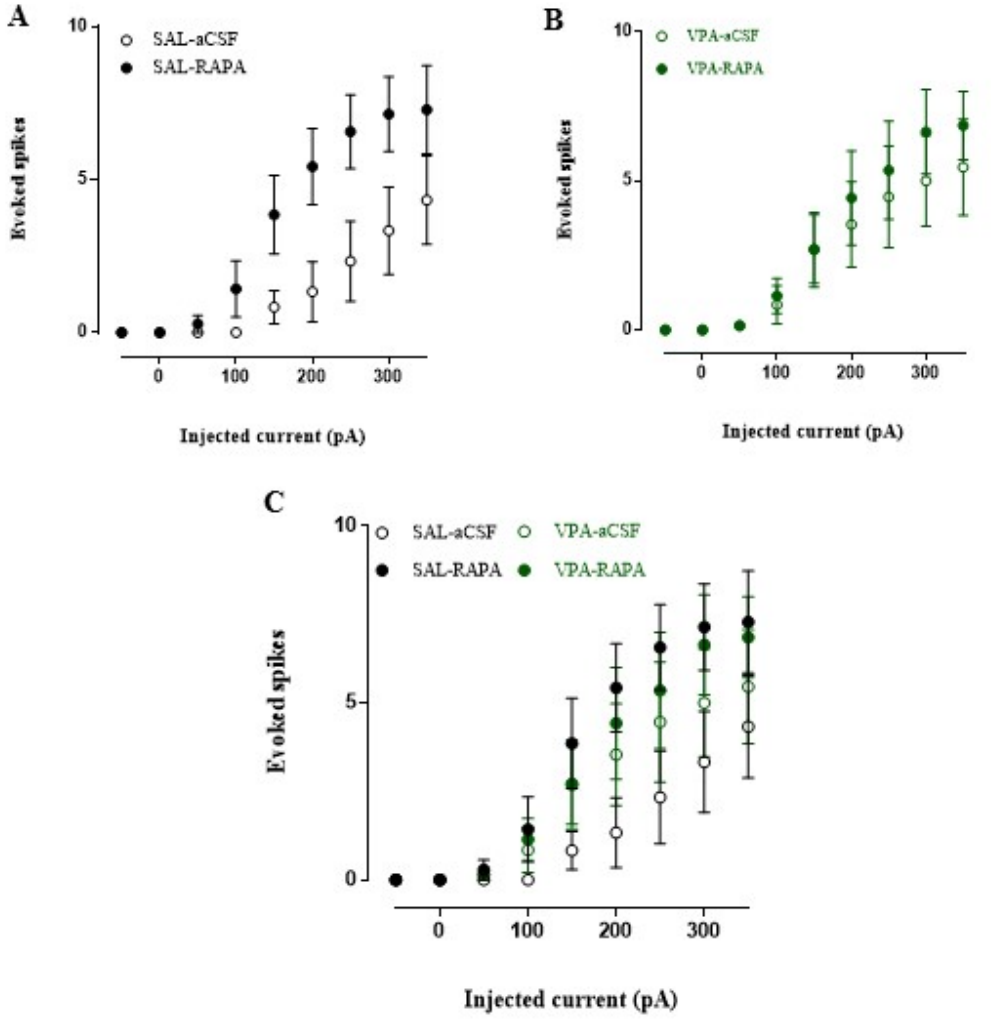


Figure 21: Effect of rapamycin on NAc-MSNs excitability. Number of APs fired plotted against current steps of increasing value. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001, *Two-way Anova*, Tukey *post-hoc* test.

12.12. The effect of rapamycin on excitatory synaptic neurotransmission

Finally, we investigated the effect of rapamycin on synaptic excitability and network activity by performing sEPSCs and mEPSCs recordings as previously described in paragraph 12.8. The effect on post-synaptic currents was analyzed after 30-minute bath application and during excitatory synapct activity isolation. As shown in figure 22 rapamycin does not influence the amplitude (SAL-aCSF = 11.8 ± 0.46 pA n = 16; SAL-RAPA = 10.93 ± 0.48 pA n = 14; VPA-aCSF = 11.78 ± 0.40 pA n = 16; VPA-aCSF = 7.40 ± 2.5 pA n = 15; n.s. *Two-way Anova*, Fig. 22B) or the frequency (SAL-aCSF = 2.32 ± 0.24 Hz n = 16; SAL-RAPA = 1.96 ± 2.29 Hz n = 14; VPA-aCSF = 2.20 ± 0.27 Hz n = 16; VPA-aCSF = 2.04 ± 0.26 Hz n = 15; n.s. *Two-way Anova,* Fig. 22C) of sEPSCs recorded from NAc-MSNs. Moreover, rapamycin does not change the kinetict of AMPA mediated current (SAL-aCSF = 1.48 ± 0.09 ms n = 16; SAL-RAPA = 1.51 ± 0.09 ms n = 14; VPA-aCSF = 1.23 ± 0.08 ms n = 16; VPA-aCSF = 1.38 ± 0.08 ms n = 15; n.s. *Two-way Anova*; Fig. 22D).

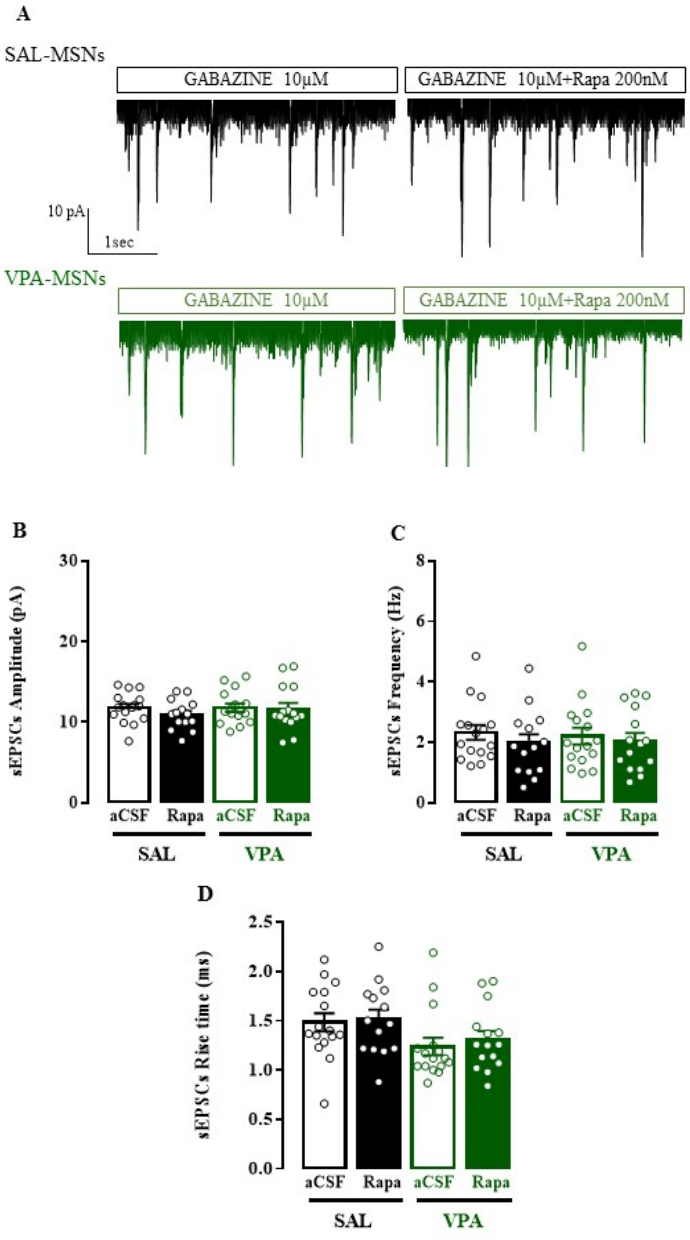


Figure 22: Effect of rapamycin on synaptic neurotransmission (A) Representative traces from whole-cell voltage-clamp recording of sEPSCs in the NAc-MSNs before (left) and afrer (right) acute rapamycin application. (B) Plotted averaged amplitudes, (C) frequency, and (D) rise time of sEPSCs. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001, *Two-way Anova*, Student-Newman-Keuls *post-hoc* test.

Based on our previously results we added TTX 1μM to record miniature events in order to investigate the effect of rapamycin on neurotransmitter release (Fig. 23A). As shown in figure 23 we found that the amplitude of these events is lower in SAL-RAPA than in SAL-aCSF neurons (SAL-aCSF = 14.16 ±0.67 pA n = 20; SAL-RAPA = 10.52 ± 0.82 pA n = 10; p = 0.005 *Two-way Anova*, Fig. 23B). Moreover, rapamycin reduce the amplitude of mEPSCs also between SAL-aCSF and VPA-RAPA neurons (SAL-aCSF = 14.16 ±0.67 pA n = 20; VPA-RAPA = 10.91 ± 0.52 pA n = 13; p = 0.003 *Two-way Anova*, Fig. 23B). Furthermore, we observed the same results when comparing SAL-RAPA with VPA-aCSF neurons (SAL-RAPA = 10.52 ± 0.82 pA n = 10; VPA-aCSF = 13.06 ± 0.49 pA n = 22; p = 0.01 *Two-way Anova*, Fig. 23B). Likewise, in VPA-exposed rats rapamycin application reduced the amplitude of miniature excitatory events (VPA-aCSF = 13.06 ± 0.49 pA n = 22; VPA-RAPA = 10.91 ± 0.52 pA n = 13; p = 0.01 *Two-way Anova*, Fig. 23B), while no difference were found in frequency (SAL-aCSF = 2.37 ± 0.29 Hz n = 20; SAL-RAPA = 2.75 ± 0.47 Hz n = 10; VPA-aCSF = 2.91 ± 0.31 Hz n = 16; VPA-aCSF = 2.08 ± 0.31 Hz n = 13; n.s. *Two-way Anova,* Fig. 23C) or rise time of mEPSCs (SAL-aCSF = 1.17 ± 0.04 ms n = 20; SAL-RAPA = 1.43 ± 0.09 ms n = 14; VPA-aCSF = 1.23 ± 0.06 ms n = 16; VPA-aCSF = 1.23 ± 0.08 ms n = 13; n.s. *Two-way Anova*; Fig. 23D).

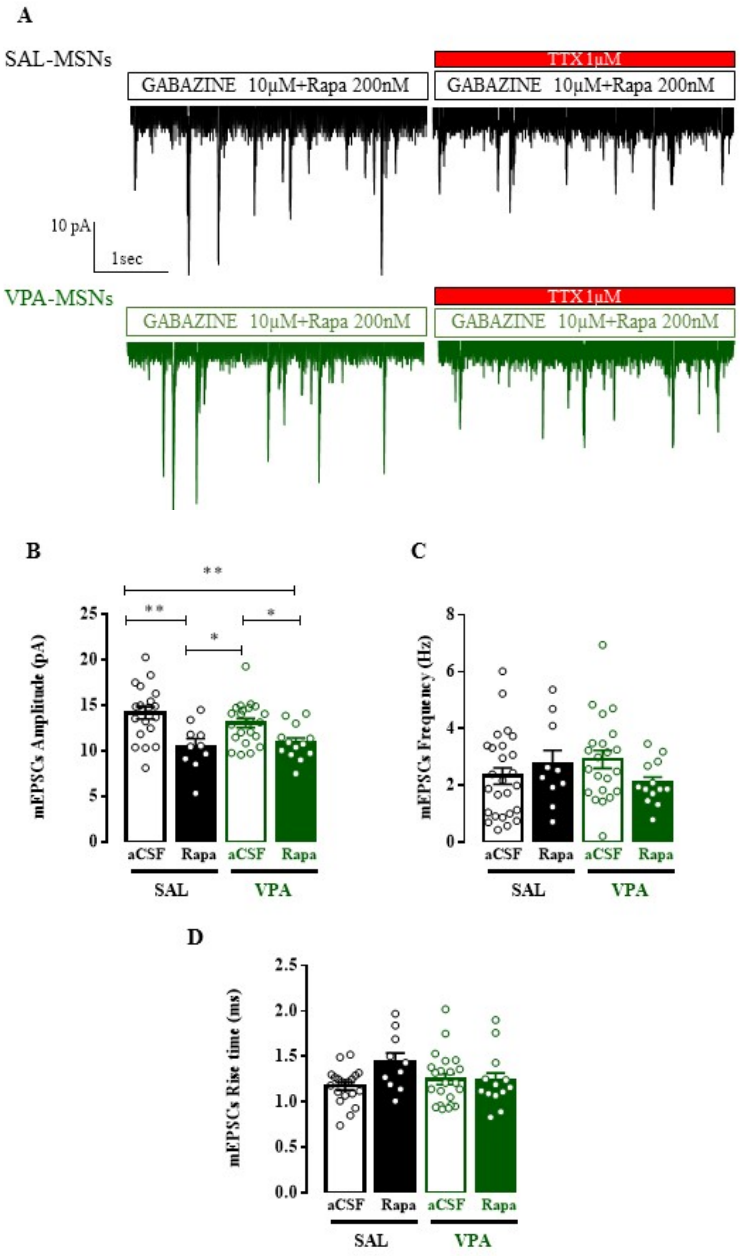


Figure 23: effect of rapamycin on synaptic neurotransmission (A) Representative traces from the whole-cell voltage-clamp recording of mEPSCs in the NAc-MSNs before (left) and afrer (right) TTX application and during rapamycin perfusion. (B) Plotted averaged amplitudes, (C) frequency, and (D) rise time of mEPSCs. Data represent mean values ± SEM; ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, <0.0001, *Two-way Anova*, Student-Newman-Keuls *post-hoc* test.

13. DISCUSSION AND FUTURE PROSPECTIVE

13.1. Identification of a novel electrophysiological endophenotype in the VPA rat model of autism

Prenatal exposure to the antiepileptic drug valproic acid (VPA) induces autism spectrum disorder (ASD) in humans and autistic-like behaviors in rodents, which makes it a good model to study the neural underpinnings of ASD (Bromley et al. 2008; Gail Williams and Hersh 1997; Roullet et al. 2013). According to the DSM-5 diagnostic criteria, persistent deficits in social communication and social interaction are key features of ASD. The pervasive social deficits found in autistic patients have been initially explained in terms of cognitive impairments and inability to infer others' mental states. More recently, they have been related to blunted social reward processing, i.e., inability to enjoy and prolong reciprocal social interactions, which has been hypothesized to be the consequence of the abnormal activity of the brain reward circuit in social contexts (C. Chevallier et al. 2012; Pellissier et al. 2018). In agreement, functional MRI studies in both children and adults with ASD, showing significant impairment in social skills measured by the ADI-R subscale, demonstrate the reduction in mesolimbic (ML) reward pathway functional connectivity (Supekar et al. 2018). Along this line, the social dysfunctions displayed by VPA-exposed rats may be caused by either their inability to properly understand and respond to social signals by the social partner or by a failure of their reward system to assign a positive value to the social experience. Thus, we hypothesize that the social impairment displayed by VPA-exposed rats could be caused by a dysfunction in the mesolimbic reward pathway.

Therefore, to test this hypothesis, in the first part of this thesis, we performed behavioral, neurochemical, and electrophysiological experiments in the VPA rat model. To confirm that the offspring of VPA-treated rat dams show ASD-like behavioral phenotype, we performed validated behavioral tests to evaluate social skills. In line with the results reported in many studies (Schneider and Przewłocki 2005; Schneider, Turczak, and Przewłocki 2006; Felix-Ortiz and Febo 2012; Servadio et al. 2016 and Melancia et al. 2018), we confirmed that adolescent rats prenatally exposed to VPA show decreased responsiveness to play solicitation since they respond to play solicitation mainly by performing partial rotations rather than reciprocating the playful interaction. Notably, these social deficits were long-lasting, since VPA exposed rats showed social deficits also when tested at adulthood in the three-chamber and social discrimination tests. Therefore, behavioral characterization confirms that our VPA exposed rats have impaired social skills. This is of fundamental importance for the subsequent electrophysiological interrogation of relevant brain areas.

Brain regions involved in the control of social behavior include corticolimbic structures, such as the prefrontal cortex (PFC) and the NAc. Therefore, their altered functionality may represent one neural substrate contributing to the social impairments characteristic of ASD (Scott-Van Zeeland et al., 2010; Chevallier et al., 2012; Ameis and Catani, 2015; Supekar et al., 2018). As these regions are strongly modulated by dopaminergic transmission, it has been suggested that a dysfunction of the latter leads to the social deficits observed in ASD (Paval et al., 2017). In autistic subjects,

dopamine imbalances manifested as either hyperactivity or hypoactivity of midbrain dopaminergic pathways have been detected, highlighting the heterogeneity of the disease (Paval et al., 2017). Recently, it has also been demonstrated that altered VTA dopamine neuron function represents a key mechanism by which insufficiency of SHANK3, encoding the synapse scaffolding protein SHANK3, generates impaired social preference in mice (Bariselli et al., 2016). Furthermore, mice knockout for genes strongly associated with ASD, such as Cntnap4 (Karayannis et al., 2014) and neuroligin-3 (Rothwell et al., 2014) mutant mice, show changes in NAc dopaminergic neurotransmission together with deficits in the core ASD behavioral domains. Modulation by dopamine in reward-related areas is mainly mediated by activation of D1 and D2 receptors in corticolimbic regions (Gunaydin et al. 2014; Kopec et al. 2018; Robinson, Heien, and Wightman 2002). Therefore, we studied the expression of D1 and D2 receptors in the NAc of VPA exposed versus SAL rats, focusing in the PFC, DS, NAc and HIPP since these brain areas play not only an important role in the modulation of the rewarding properties of social interactions but have also a key role in cognitive aspects of the social repertoire (e.g., social cognition). Indeed, the PFC and HIPP have also been found to be deeply involved in cognitive aspects of social behavior (Montagrin, Saiote, and Schiller 2018; Vanderschuren et al. 2016). We found that adolescent VPA exposed rats show increased expression of D2 dopamine receptors in the NAc. Augmented D2 expression levels in the NAc are persistent in adulthood and, in addition, we found increased expression also of D1 both in the NAc and HIPP. These results may imply that prenatal VPA exposure may disrupt dopaminergic transmission in the corticolimbic pathway, thus explaining the deficit in social reward, but also in the established cognitive disabilities in the model and in other ASD models as well as in human subjects (Gao, Wang, et al. 2016; Gao, Wu, et al. 2016; Hara et al. 2016). In line with our results, changes in hippocampal dopamine and D1 receptor levels associated with social deficits have been demonstrated in a genetic mouse model exhibiting autism-relevant behavioral abnormalities (Liu et al. 2017).

Building on the evidence of altered D1/D2 expression profile, we studied the basic electrophysiological properties of NAc MSNs in acute striatal brain slices of VPA- and SAL-exposed offspring. We found that MSNs of VPA-exposed rats have significantly reduced membrane capacitance. Membrane capacitance is classically considered a measure of soma size and morphological complexity. This change is in agreement with recent evidence associating reduced morphological complexity, including dendritic arborization, in reprogrammed cortical excitatory neurons exposed to VPA in vitro (Soham Chanda et al. 2019). Chanda et al., demonstrated the involvement of MARCKSL1, a cytoskeletal protein playing a major role in dendrite and synapse formation through the regulation of F-actin bundling (Soham Chanda et al. 2019).

Morphological properties of NAc-MSNs are known to change during physiological maturation. In particular, membrane capacitance during the juvenile to adulthood transition, as a result of enlargement of the dendritic compartment, which accompanies increased density of dendritic spines, and the elaboration of synaptic inputs (Kasanetz and Manzoni 2009). Thus, reduced

membrane capacitance observed in VPA-NAc-MSNs is consistent with an immature neuronal profile.

We further found that NAc MSNs of VPA-exposed animals show a significant depolarization of the resting membrane potential and increased excitability in the lower part of the excitability curve, indicating higher firing probability in conditions of normal synaptic excitation. As previously described for membrane capacitance, resting membrane potential and neuronal excitability also decrease during the neuronal maturation as a consequence of developmentally-regulated changes in ion channels expression in the NAc and HIPP (Kasanetz and Manzoni 2009; Mongiat et al. 2009). Alterations in the intrinsic excitability were reported in other neuronal types in the VPA model with ex vivo recordings. Brumback et al. described defects in intrinsic excitability which was selective for L5 pyramidal neurons. Importantly, the electrical alteration was conserved across different animal models including CNTNAP2 KO and FMR1 KO mice (Brumback et al. 2017), suggesting that changes in neuronal excitability could underlie the shared ASD-like components characterizing distinct syndromes.

Current recordings suggest that alterations of intrinsic excitability in NAc-MSNs may be caused by reduced Kir current density. Kir channels are critical to determine the hyperpolarized value of membrane potential (∼−85 mV) in normal MSNs and to affect their firing discharge pattern (Cazorla et al. 2012; Kreitzer 2009). Kir current is mainly mediated by Kir 2.1 channels in NAc-MSNs, highly expressed in the somatodendritic compartment (Prüss et al. 2003). In this regard, a genetic analysis of ASD children led to the identification of twelve tag single nucleotide polymorphisms (SNPs) in the genes encoding Kir 2.1 and Kir 4.1 channels, strengthening the notion that their intrinsic dysfunction may play a central role in ASD pathogenesis (Sun et al. 2018). Interestingly, Sun et al. further reported lower Kir2.1 and Kir4.1 expression in VPA-exposed rats (Sun et al. 2018). In line with our results, the loss of function of Kir channels could be indicating that these channels play an important role in ASD susceptibility. Thus, changes in passive membrane properties, together with the reduced Kir currents we observed, likely drive increased excitability in VPA exposed rats.

Overall, intrinsic electrophysiological alterations described in this thesis are in perfect agreement with similar approaches in the VPA and other animal models and point to the persistence of an immature morphological and electrophysiological phenotype of NAc MSNs. Besides representing additional evidence in support of a critical role played by the mesolimbic pathway in ASD pathophysiology, these data propose additional cellular and/or molecular targets for the design of ASD-specific medications, which are currently lacking.

Disturbances of excitatory/inhibitory synaptic balance are thought to be involved in the ASD etiology and symptoms. E/I imbalance is associated with normal development and functioning of the brain. It represents a key process for optimal tuning of the circuits to respond to salient inputs (Canitano and Pallagrosi 2017; Shew et al. 2011). In addition, the E/I balance undergoes profound changes during neuronal development, therefore alterations in the proper acquisition of E/I balance

was suggested as an underlying cause of ASD traits (Banerjee et al. 2013; Gao and Penzes 2015; Lee, Lee, and Kim 2017; Rubenstein and Merzenich 2003; Walcott, Higgins, and Desai 2011). We here demonstrate that VPA causes changes in both excitatory and inhibitory synaptic neurotransmission also in the NAc. Recordings of synaptic activity onto MSNs point to an overall decrease of synaptic connectivity in both excitatory and inhibitory components. In light of the increased intrinsic firing capabilities of MSNs, the reduction in functional synaptic connectivity may be regarded as a homeostatic mechanism. However, it is not possible with slice recordings, to estimate the consequences on the resulting NAc output towards downstream neural areas.

In the context of the extensive literature describing local and distal hyperconnectivity with increased E/I ratio in multiple areas, the NAc seems to make an exception. This finding is particularly relevant as it points to heterogeneous, are-specific alterations in functional connectivity, rather than a generalized increase of synaptic excitation. In contrast, the reduction in the strength of inhibitory inputs observed in the NAc in this work largely converges with reports of reduced density in parvalbumin-, calbindin- and calretinin-positive GABAergic interneurons in multiple areas in VPA and other animal models of ASD (Dendrinos, Hemelt, and Keller 2011; Gogolla et al. 2009; Lawrence et al. 2010). Further support to the hypothesis of an impaired inhibitory system comes from changes in the distribution of GABAergic neurons in autoptic tissue from idiopathic ASD patients (Lawrence et al. 2010) and fragile X syndrome patients (Selby, Zhang, and Sun 2007). Abnormalities in the operation of GABAergic interneurons might be caused by a failure in proper differentiation, migration, and/or proliferation (Van Kooten et al. 2005). To summarize, alterations in synaptic connectivity in the VPA model is consistent with an immature phenotype of neural areas implied in the genesis of ASD symptoms.

13.2. Modulation of the mTOR pathway as a strategy to restore the electrophysiological phenotype of MSNs

The mTOR pathway is disrupted in multiple types of syndromic ASD, such as Tuberous sclerosis, Phelan McDermid, Hamartoma, Engelmann and Rett syndrome, and in related animal models (Crino 2016; Nicolini et al. 2015; Takei and Nawa 2014; Winden et al. 2018). Overactivation of the mTOR pathway was demonstrated also in the VPA rat model, and administration of the selective mTOR inhibitor rapamycin rescues social impairment (Kotajima-Murakami et al. 2019; Qin et al. 2016; Sato 2016). Interestingly, aberrant activation of mTOR pathway was associated with increased excitability of dorsal MSNs (Benthall, Ong, and Bateup 2018), suggesting that the modulation of the mTOR pathway could represent a useful pharmacological intervention. However, the action of rapamycin was only tested at behavioural level (Kotajima-Murakami et al. 2019; Sato et al. 2012). This work reports, for the first time, the effects of acute application of rapamycin during patch-clamp recordings in NAc MSNs from VAP-exposed rats. Acute application of selective rapamycin concentrations during patch-clamp recordings changed electrophysiological parameters

in a complex manner. No change was observed in membrane capacitance. This is not surprising considering that the timescale of an acute challenge is likely not consistent with changes in morphology. In contrast, membrane potential, which is relatively depolarized in MSNs from VPA-exposed rats, was normalized by acute rapamycin. As for intrinsic excitability, preliminary data, not achieving statistical significance, point to an increase in excitability in both VPA and SAL animals following bath application of rapamycin. Finally, rapamycin caused a small but significant reduction in the amplitude, but not in the frequency, of excitatory inputs. Further investigation is required before establishing a causal link between mTOR pathway and the abnormal electrical phenotype of NAc MSNs and validation of this signaling pathway as a useful target for the treatment of ASD behavioral features.

14. CONCLUSION

Although more levels of analysis are needed to shed light on the mechanisms underlying the aberrant behavior found in VPA-exposed rats, based on biochemical, behavioral, and electrophysiological results, we can hypnotize the effective involvement of the mesolimbic reward system in ASD-like phenotype displayed in our animal model.

15. LIST OF ABBREVIATIONS

AC	Anterior Commissure
aCSF	artificial cerebrospinal fluid
AED	Anti-Epileptic Drug
AHP	afterhyperpolarization
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	action potential
ASD	autistic spectrum disorder
BDNF	Brain-Derived Neurotrophic Factor
Cm	membrane capacitance
CNS	Central Nervous System
DA	dopamine
DR	dopamine receptor
DS	Dorsal Striatum
DSB	Double Strand Break
DSM	Diagnostic and Statistical Manual of Mental Disorders
E/I	Excitatory/Inhibitory balance
E12	Embryonic Day 12
EPSC	Excitatory postsynaptic current
FMR1	Fragile X Mental Retardation 1 gene
FXS	Fragile X Syndrome
GABA	γ-aminobutyric acid
HDAC	Histone Deacetilase
HIPP	Hippocampus
ID	Intellectual Disability
IKir	Inwardly rectifying potassium currents
IPSCs	Inhibitory postsynaptic currents
ISI	interspike interval
K^+	Potassium ione
Kir	Inward-Rectifier Potassium Channel

KO	Knock-Out
LTD	Long-Term Depression
LTP	Long-Term Potentiation
MeCP2	X-linked gene Methyl CpG binding protein 2
mEPSC	miniature Excitatory Post-Synaptic Current
ML	Mesolimbic
mTOR	mammalian Target of Rapamycin
mTORC1	mammalian Target of Rapamycin Complex 1
mTORC2	mammalian Target of Rapamycin Complex 2
MRI	magnetic resonance imaging
MSN	medium spiny neuron
NAc	nucleus accumbens
NLGN	neuroligin
NMDA	N-methyl-D-aspartate
NRXN	Neuroxin
NSC	Neural Stem Cell
PFC	Prefrontal Cortex
PMS	Phelan McDermid Syndrome
PND	post-natal day
PSD	Post-Synaptic Density
PTEN	Phosphatase and Tensin homolog gene
RELN	Reelin
RMP	resting membrane potential
Ri	input resistance
SAL	saline
SHANK	multiple ankyrin repeat domain
SNP	Single Nucleotide Polymorphism
SYN	Synuclein
ToM	theory of mind

Tsc1	tuberous sclerosis 1
Tsc2	tuberous sclerosis 2
TTX	tetodrotoxin
USV	Ultrasonic vocalization
VPA	valproic acid
VS	Ventral Striaum
VTA	Ventral Tegmental Area
WNT2	Wingless-type member 2

16. Bibliography

Albani, F., R. Riva, M. Contin, A. Baruzzi, M. Altomare, GP Merlini, and E. Perucca. 1984. "Differential Transplacental Binding of Valproic Acid: Influence of Free Fatty Acids." *British Journal of Clinical Pharmacology* 17(6):759–62.

Amaral, David G., Cynthia Mills Schumann, and Christine Wu Nordahl. 2008. "Neuroanatomy of Autism." *Trends in Neurosciences* 31(3):137–45.

Amir, Ruthie E., Ignatia B. Van Den Veyver, Mimi Wan, Charles Q. Tran, Uta Francke, and Huda Y. Zoghbi. 1999. "Rett Syndrome Is Caused by Mutations in X-Linked MECP2, Encoding Methyl- CpG-Binding Protein 2." *Nature Genetics* 23(2):185–88.

Antion, Marcia D., Maayan Merhav, Charles A. Hoeffer, Gerald Reis, Sara C. Kozma, George Thomas, Erin M. Schuman, Kobi Rosenblum, and Eric Klann. 2008. "Removal of S6K1 and S6K2 Leads to Divergent Alterations in Learning, Memory, and Synaptic Plasticity." *Learning and Memory* 15(1):29–38.

Auerbach, Benjamin D., Emily K. Osterweil, and Mark F. Bear. 2011. "Mutations Causing Syndromic Autism Define an Axis of Synaptic Pathophysiology." *Nature* 480(7375):63–68.

Baltz, Thomas, Ana D. de Lima, and Thomas Voigt. 2010. "Contribution of GABAergic Interneurons to the Development of Spontaneous Activity Patterns in Cultured Neocortical Networks." *Frontiers in Cellular Neuroscience* 4(JUN):15.

Banerjee, Anwesha, Francisco García-Oscos, Swagata Roychowdhury, Luis C. Galindo, Shawn Hall, Michael P. Kilgard, and Marco Atzori. 2013. "Impairment of Cortical GABAergic Synaptic Transmission in an Environmental Rat Model of Autism." *International Journal of Neuropsychopharmacology* 16(6):1309–18.

Banko, Jessica L., Lingfei Hou, Francis Poulin, Nahum Sonenberg, and Eric Klann. 2006. "Regulation of Eukaryotic Initiation Factor 4E by Converging Signaling Pathways during Metabotropic Glutamate Receptor-Dependent Long-Term Depression." *Journal of Neuroscience* 26(8):2167–73.

Banko, Jessica L., Maayan Merhav, Elad Stern, Nahum Sonenberg, Kobi Rosenblum, and Eric Klann. 2007. "Behavioral Alterations in Mice Lacking the Translation Repressor 4E-BP2." *Neurobiology of Learning and Memory* 87(2):248–56.

Baretić, Domagoj, and Roger L. Williams. 2014. "The Structural Basis for MTOR Function." *Seminars in Cell and Developmental Biology* 36:91–101.

Bargas, J., G. X. Ayala, C. Vilchis, J. C. Pineda, and E. Galarraga. 1999. "Ca2+-Activated Outward Currents in Neostriatal Neurons." *Neuroscience* 88(2):479–88.

Baron-Cohen, Simon, Alan M. Leslie, and Uta Frith. 1985. “Does the Autistic Child Have a ‘Theory of Mind’ ?” *Cognition* 21(1):37–46.

Barrett, Nicholas. 2017. “The Role of ECMO in Acute Interstitial Lung Disease.” *Qatar Medical Journal* 2017(1):42.

Baudouin, Stéphane J., Julien Gaudias, Stefan Gerharz, Laetitia Hatstatt, Kuikui Zhou, Pradeep Punnakkal, Kenji F. Tanaka, Will Spooren, Rene Hen, Chris I. De Zeeuw, Kaspar Vogt, and Peter Scheiffele. 2012. “Shared Synaptic Pathophysiology in Syndromic and Nonsyndromic Rodent Models of Autism.” *Science* 338(6103):128–32.

Bean, Bruce P. 2007. “The Action Potential in Mammalian Central Neurons.” *Nature Reviews Neuroscience* 8(6):451–65.

Ben-Ari, Yehezkel, Jean Luc Gaiarsa, Roman Tyzio, and Rustem Khazipov. 2007. “GABA: A Pioneer Transmitter That Excites Immature Neurons and Generates Primitive Oscillations.” *Physiological Reviews* 87(4):1215–84.

Ben-Sahra, Issam, Jessica J. Howell, John M. Asara, and Brendan D. Manning. 2013. “Stimulation of de Novo Pyrimidine Synthesis by Growth Signaling through MTOR and S6K1.” *Science* 339(6125):1323–28.

Benarroch, Eduardo E. 2009. “Potassium Channels: Brief Overview and Implications in Epilepsy.” *Neurology* 72(7):664–69.

Benayed, Rym, Neda Gharani, Ian Rossman, Vincent Mancuso, Gloria Lazar, Silky Kamdar, Shannon E. Bruse, Samuel Tischfield, Brett J. Smith, Raymond A. Zimmerman, Emanuel DiCicco-Bloom, Linda M. Brzustowicz, and James H. Millonig. 2005. “Support for the Homeobox Transcription Factor Gene ENGRAILED 2 as an Autism Spectrum Disorder Susceptibility Locus.” *American Journal of Human Genetics* 77(5):851–68.

Benthall, Katelyn N., Stacie L. Ong, and Helen S. Bateup. 2018. “Corticostriatal Transmission Is Selectively Enhanced in Striatonigral Neurons with Postnatal Loss of Tsc1.” *Cell Reports* 23(11):3197–3208.

Bernardet, Maude, and Wim E. Crusio. 2006. “Fmr1 KO Mice as a Possible Model of Autistic Features.” *TheScientificWorldJournal* 6:1164–76.

Berridge, Kent C., and Morten L. Kringelbach. 2015. “Pleasure Systems in the Brain.” *Neuron* 86(3):646–64.

Berridge, Kent C., Terry E. Robinson, and J. Wayne Aldridge. 2009. “Dissecting Components of Reward: ‘Liking’, ‘Wanting’, and Learning.” *Current Opinion in Pharmacology* 9(1):65–73.

Bescoby-Chambers, Nicholas, Pauline Forster, and Gordon Bates. 2001. “Foetal Valproate Syndrome and Autism: Additional Evidence of an Association.” *Developmental Medicine and Child Neurology* 43(12):847.

Betancur, Catalina, Takeshi Sakurai, and Joseph D. Buxbaum. 2009. “The Emerging Role of Synaptic Cell-Adhesion Pathways in the Pathogenesis of Autism Spectrum Disorders.” *Trends in Neurosciences* 32(7):402–12.

Bichet, Delphine, Friederike A. Haass, and Lily Yeh Jan. 2003. “Merging Functional Studies with Structures of Inward-Rectifier K + Channels.” *Nature Reviews Neuroscience* 4(12):957–67.

Bidinosti, Michael, Paolo Botta, Sebastian Kröttner, Catia C. Proenca, Natacha Stoehr, Mario Bernhard, Isabelle Fruh, Matthias Mueller, Debora Bonenfant, Hans Voshol, Walter Carbone, Sarah J. Neal, Stephanie M. McTighe, Guglielmo Roma, Ricardo E. Dolmetsch, Jeffrey A. Porter, Pico Caroni, Tewis Bouwmeester, Andreas Löthi, and Ivan Galimberti. 2016. “CLK2 Inhibition Ameliorates Autistic Features Associated with SHANK3 Deficiency.” *Science* 351(6278):1199–1203.

Bienvenu, Thierry, and Jamel Chelly. 2006. “Molecular Genetics of Rett Syndrome: When DNA Methylation Goes Unrecognized.” *Nature Reviews Genetics* 7(6):415–26.

Bozdagi, Ozlem, Takeshi Sakurai, Danae Papapetrou, Xiaobin Wang, Dara L. Dickstein, Nagahide Takahashi, Yuji Kajiwara, Mu Yang, Adam M. Katz, Maria Scattoni, Mark J. Harris, Roheeni Saxena, Jill L. Silverman, Jacqueline N. Crawley, Qiang Zhou, Patrick R. Hof, and Joseph D. Buxbaum. 2010. “Haploinsufficiency of the Autism-Associated Shank3 Gene Leads to Deficits in Synaptic Function, Social Interaction, and Social Communication.” *Molecular Autism* 1(1):15.

Bradbury, C. A., F. L. Khanim, R. Hayden, C. M. Bunce, D. A. White, M. T. Drayson, C. Craddock, and B. M. Turner. 2005. “Histone Deacetylases in Acute Myeloid Leukaemia Show a Distinctive Pattern of Expression That Changes Selectively in Response to Deacetylase Inhibitors.” *Leukemia* 19(10):1751–59.

Bremer, Anna, MaiBritt Giacobini, Mats Eriksson, Peter Gustavsson, Viviann Nordin, Elisabeth Fernell, Christopher Gillberg, Ann Nordgren, Åsa Uppströmer, Britt-Marie Anderlid, Magnus Nordenskjöld, and Jacqueline Schoumans. 2011. “Copy Number Variation Characteristics in Subpopulations of Patients with Autism Spectrum Disorders.” *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 156(2):115–24.

Bromberg-Martin, Ethan S., Masayuki Matsumoto, and Okihide Hikosaka. 2010. “Dopamine in Motivational Control: Rewarding, Aversive, and Alerting.” *Neuron* 68(5):815–34.

Bromley, R. L., G. Mawer, J. Clayton-Smith, and G. A. Baker. 2008. “Autism Spectrum Disorders Following in Utero Exposure to Antiepileptic Drugs.” *Neurology* 71(23):1923–24.

Browne, David L., Stephen T. Gancher, John G. Nutt, Ewout R. P. Brunt, Eric A. Smith, Patricia Kramer, and Michael Litt. 1994. “Episodic Ataxia/Myokymia Syndrome Is Associated with Point Mutations in the Human Potassium Channel Gene, KCNA1.” *Nature Genetics* 8(2):136–40.

Bruining, Hilgo, Marinus Jc Eijkemans, Martien Jh Kas, Sarah R. Curran, Jacob As Vorstman, and Patrick F. Bolton. 2014. “Behavioral Signatures Related to Genetic Disorders in Autism.” *Molecular Autism* 5(1).

Brumback, A. C., I. T. Ellwood, C. Kjaerby, J. Iafrati, S. Robinson, A. T. Lee, T. Patel, S. Nagaraj, F. Davatolhagh, and V. S. Sohal. 2017. “Identifying Speci Fi c Prefrontal Neurons That Contribute to Autism-Associated Abnormalities in Physiology and Social Behavior.” (August):1–12.

Brunet, Isabelle, Christine Weinl, Michael Piper, Alain Trembleau, Michel Volovitch, William Harris, Alain Prochiantz, and Christine Holt. 2005. “The Transcription Factor Engrailed-2 Guides Retinal Axons.” *Nature* 438(7064):94–98.

C. Chevallier, G. Kohls, V. Troiani, E. S. Brodkin, and R. T. Schultz. 2012. “The Social Motivation Theory.” *Trends in Cognitive Sciences* 16(4):231–39.

Campbell, Daniel B., Rosanna D’Oronzio, Krassi Garbett, Philip J. Ebert, Karoly Mirnics, Pat Levitt, and Antonio M. Persico. 2007. “Disruption of Cerebral Cortex MET Signaling in Autism Spectrum Disorder.” *Annals of Neurology* 62(3):243–50.

Canitano, Roberto, and Mauro Pallagrosi. 2017. “Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories.” *Frontiers in Psychiatry* 8(MAY).

Cartocci, Veronica, Martina Catallo, Massimo Tempestilli, Marco Segatto, Frank W. Pfrieger, Maria Rosanna Bronzuoli, Caterina Scuderi, Michela Servadio, Viviana Trezza, and Valentina Pallottini. 2018. “Altered Brain Cholesterol/Isoprenoid Metabolism in a Rat Model of Autism Spectrum Disorders.” *Neuroscience* 372:27–37.

Cazorla, Maxime, Mariya Shegda, Bhavani Ramesh, Neil L. Harrison, and Christoph Kellendonk. 2012. “Striatal D2 Receptors Regulate Dendritic Morphology of Medium Spiny Neurons via Kir2 Channels.” *Journal of Neuroscience* 32(7):2398–2409.

Cepeda, Carlos, Véronique M. André, Irene Yamazaki, Nanping Wu, Max Kleiman-Weiner, and Michael S. Levine. 2008. “Differential Electrophysiological Properties of Dopamine D1 and

D2 Receptor-Containing Striatal Medium-Sized Spiny Neurons." *European Journal of Neuroscience* 27(3):671–82.

Chang, Jonathan, Sarah R. Gilman, Andrew H. Chiang, Stephan J. Sanders, and Dennis Vitkup. 2015. "Genotype to Phenotype Relationships in Autism Spectrum Disorders." *Nature Neuroscience* 18(2):191–98.

Chastel, Xavier; Axel Esside. n.d. *Enquête Relative Aux Spécialités Pharmaceutiques Contenant Du Valproate de Sodium*.

CHEN, Cui Ping, Takako YOKOZAWA, and Kenichi KITANI. 1999. "Beneficial Effects of Sanguisorbae Radix in Renal Dysfunction Caused by Endotoxin in Vivo." *Biological & Pharmaceutical Bulletin* 22(12):1327–30.

Chenn, Anjen. 2008. "Wnt/β-Catenin Signaling in Cerebral Cortical Development." *Organogenesis* 4(2):76–80.

Choi, Yong Jin, Alessia Di Nardo, Ioannis Kramvis, Lynsey Meikle, David J. Kwiatkowski, Mustafa Sahin, and Xi He. 2008. "Tuberous Sclerosis Complex Proteins Control Axon Formation." *Genes and Development* 22(18):2485–95.

Christianson, Arnold L., Nicole Chester, and Jennifer G. R. Kromberg. 1994. "Fetal Valproate Syndrome: Clinical and Neuro-developmental Features in Two Sibling Pairs." *Developmental Medicine & Child Neurology* 36(4):361–69.

Cipriani, Chiara, Laura Ricceri, Claudia Matteucci, Alessia De Felice, Anna Maria Tartaglione, Ayele Argaw-Denboba, Francesca Pica, Sandro Grelli, Gemma Calamandrei, Paola Sinibaldi Vallebona, and Emanuela Balestrieri. 2018. "High Expression of Endogenous Retroviruses from Intrauterine Life to Adulthood in Two Mouse Models of Autism Spectrum Disorders." *Scientific Reports* 8(1):629.

Clayton-Smith, J., and D. Donnai. 1995. "Fetal Valproate Syndrome." *Journal of Medical Genetics* 32(9):724–27.

Costa-Mattioli, Mauro, and Lisa M. Monteggia. 2013. "MTOR Complexes in Neurodevelopmental and Neuropsychiatric Disorders." *Nature Neuroscience* 16(11):1537–43.

Courchesne, Eric, Tiziano Pramparo, Vahid H. Gazestani, Michael V. Lombardo, Karen Pierce, and Nathan E. Lewis. 2019. "The ASD Living Biology: From Cell Proliferation to Clinical Phenotype." *Molecular Psychiatry* 24(1):88–107.

Crino, Peter B. 2016. "The MTOR Signalling Cascade: Paving New Roads to Cure Neurological Disease." *Nature Reviews Neurology* 12(7):379–92.

Cristina D'adamo, Maria, Francesca Moro, Paola Imbrici, Dianda Martino, Mauro Roscini, Maria Santorelli, Federico Sicca, and Mauro Pessia. 2011. *The Emerging Role of the Inwardly Rectifying K + Channels in Autism Spectrum Disorders and Epilepsy*. Vol. 23.

Cruz, Fabio C., Eisuke Koya, Danielle H. Guez-Barber, Jennifer M. Bossert, Carl R. Lupica, Yavin Shaham, and Bruce T. Hope. 2013. "New Technologies for Examining the Role of Neuronal Ensembles in Drug Addiction and Fear." *Nature Reviews Neuroscience* 14(11):743–54.

Dafny, N., and G. C. Rosenfeld. 2017. "Neurobiology of Drugs of Abuse." *Conn's Translational Neuroscience* 715–22.

Dalens, Bernard, Elie Jean Raynaud, and Jacqueline Gaulme. 1980. "Teratogenicity of Valproic Acid." *The Journal of Pediatrics* 97(2):332–33.

Darnell, Jennifer C., and Eric Klann. 2013. "The Translation of Translational Control by FMRP: Therapeutic Targets for FXS." *Nature Neuroscience* 16(11):1530–36.

Dendrinos, Georgia, Marie Hemelt, and Asaf Keller. 2011. "Prenatal VPA Exposure and Changes in Sensory Processing by the Superior Colliculus." *Frontiers in Integrative Neuroscience* 5.

Diederich, Marc, Sébastien Chateauvieux, Franck Morceau, and Mario Dicato. 2010. "Molecular and Therapeutic Potential and Toxicity of Valproic Acid." *Journal of Biomedicine and Biotechnology* 2010.

Dietert, Rodney R., Janice M. Dietert, and Jamie C. DeWitt. 2011. "Environmental Risk Factors for Autism." *Emerging Health Threats Journal* 4(1):0–11.

Doupnik, Craig A. 2017. "Venom-Derived Peptides Inhibiting Kir Channels: Past, Present, and Future." *Neuropharmacology* 127:161–72.

Dufour-Rainfray, Diane, Patrick Vourc'h, Anne Marie Le Guisquet, Lucette Garreau, David Ternant, Sylvie Bodard, Emilie Jaumain, Zuhal Gulhan, Catherine Belzung, Christian R. Andres, Sylvie Chalon, and Denis Guilloteau. 2010. "Behavior and Serotonergic Disorders in Rats Exposed Prenatally to Valproate: A Model for Autism." *Neuroscience Letters* 470(1):55–59.

Ebert, Daniel H., and Michael E. Greenberg. 2013. "Activity-Dependent Neuronal Signalling and Autism Spectrum Disorder." *Nature* 493(7432):327–37.

Ehninger, D., Y. Sano, P. J. De Vries, K. Dies, D. Franz, D. H. Geschwind, M. Kaur, Y. S. Lee, W. Li, J. K. Lowe, J. A. Nakagawa, M. Sahin, K. Smith, V. Whittemore, and A. J. Silva. 2012. "Gestational Immune Activation and Tsc2 Haploinsufficiency Cooperate to Disrupt Fetal Survival and May Perturb Social Behavior in Adult Mice." *Molecular Psychiatry* 17(1):62–70.

Ehninger, Dan, Sangyeul Han, Carrie Shilyansky, Yu Zhou, Weidong Li, David J. Kwiatkowski,

Vijaya Ramesh, and Alcino J. Silva. 2008. “Reversal of Learning Deficits in a Tsc2+/- Mouse Model of Tuberous Sclerosis.” *Nature Medicine* 14(8):843–48.

Etherton, Mark R., Cory A. Blaiss, Craig M. Powell, and Thomas C. Südhof. 2009. “Mouse Neurexin-1α Deletion Causes Correlated Electrophysiological and Behavioral Changes Consistent with Cognitive Impairments.” *Proceedings of the National Academy of Sciences of the United States of America* 106(42):17998–3.

Evergren, Emma, F. Benfenati, and O. Shupliakov. 2007. “The Synapsin Cycle: A View from the Synaptic Endocytic Zone.” Pp. 2648–56 in *Journal of Neuroscience Research*. Vol. 85. J Neurosci Res.

Fassio, Anna, Lysanne Patry, Sonia Congia, Franco Onofri, Amelie Piton, Julie Gauthier, Davide Pozzi, Mirko Messa, Enrico Defranchi, Manuela Fadda, Anna Corradi, Pietro Baldelli, Line Lapointe, Judith St-Onge, Caroline Meloche, Laurent Mottron, Flavia Valtorta, Dang Khoa Nguyen, Guy A. Rouleau, Fabio Benfenati, and Patrick Cossette. 2011. “SYN1 Loss-of-Function Mutations in Autism and Partial Epilepsy Cause Impaired Synaptic Function.” *Human Molecular Genetics* 20(12):2297–2307.

Felix-Ortiz, Ada C., and Marcelo Febo. 2012. “Gestational Valproate Alters BOLD Activation in Response to Complex Social and Primary Sensory Stimuli” edited by M. Sakakibara. *PLoS ONE* 7(5):e37313.

Felix, John P., Jessica Liu, William A. Schmalhofer, Timothy Bailey, Maria A. Bednarek, Stephanie Kinkel, Adam B. Weinglass, Martin Kohler, Gregory J. Kaczorowski, Birgit T. Priest, and Maria L. Garcia. 2006. “Characterization of Kir1.1 Channels with the Use of a Radiolabeled Derivative of Tertiapin.” *Biochemistry* 45(33):10129–39.

Feyder, Michael, Rose Marie Karlsson, Poonam Mathur, Matthew Lyman, Roland Bock, Reza Momenan, Jeeva Munasinghe, Maria Luisa Scattoni, Jessica Ihne, Marguerite Camp, Carolyn Graybeal, Douglas Strathdee, Alison Begg, Veronica A. Alvarez, Peter Kirsch, Marcella Rietschel, Sven Cichon, Henrik Walter, Andreas Meyer-Lindenberg, Seth G. N. Grant, and Andrew Holmes. 2010. “Association of Mouse Dlg4 (PSD-95) Gene Deletion and Human DLG4 Gene Variation with Phenotypes Relevant to Autism Spectrum Disorders and Williams’ Syndrome.” *American Journal of Psychiatry* 167(12):1508–17.

Floresco, Stan B. 2015. “The Nucleus Accumbens: An Interface Between Cognition, Emotion, and Action.” *Annual Review of Psychology* 66(1):25–52.

Fonseca, Bruno D., and Christopher G. Proud. 2009. “Downstream Targets of MTORC1.” 179–200.

Frank, Michael J., Lauren C. Seeberger, and Randall C. O’Reilly. 2004. “By Carrot or by Stick:

Cognitive Reinforcement Learning in Parkinsonism." *Science* 306(5703):1940–43.

Frith, U. 1996. "Cognitive Explanations of Autism." Pp. 63–68 in *Acta Paediatrica, International Journal of Paediatrics, Supplement*. Vol. 85. Acta Paediatr Suppl.

Frith, U., and F. Happé. 1994. "Language and Communication in Autistic Disorders." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 346(1315):97–104.

Fuccillo, Marc V. 2016. "Striatal Circuits as a Common Node for Autism Pathophysiology." *Frontiers in Neuroscience* 10(FEB).

Fuller, Leah C., Shannon K. Cornelius, Charles W. Murphy, and Darrell J. Wiens. 2002. "Neural Crest Cell Motility in Valproic Acid." *Reproductive Toxicology* 16(6):825–39.

G.E., Von Unruh, Froescher W., Hoffmann F., and Niesen M. 1984. "Valproic Acid in Breast Milk: How Much Is Really There?" *Therapeutic Drug Monitoring*.

Gail Williams, P., and Joseph H. Hersh. 1997. "A Male with Fetal Valproate Syndrome and Autism." *Developmental Medicine and Child Neurology* 39(9):632–34.

Gandal, Michael J., J. Christopher Edgar, Richard S. Ehrlichman, Mili Mehta, Timothy P. L. Roberts, and Steven J. Siegel. 2010. "Validating γ Oscillations and Delayed Auditory Responses as Translational Biomarkers of Autism." *Biological Psychiatry* 68(12):1100–1106.

Gao, Jingquan, Xuelai Wang, Hongli Sun, Yonggang Cao, Shuang Liang, Han Wang, Yanming Wang, Feng Yang, Fengyu Zhang, and Lijie Wu. 2016. "Neuroprotective Effects of Docosahexaenoic Acid on Hippocampal Cell Death and Learning and Memory Impairments in a Valproic Acid-Induced Rat Autism Model." *International Journal of Developmental Neuroscience* 49:67–78.

Gao, Jingquan, Hongmei Wu, Yonggang Cao, Shuang Liang, Caihong Sun, Peng Wang, Ji Wang, Hongli Sun, and Lijie Wu. 2016. "Maternal DHA Supplementation Protects Rat Offspring against Impairment of Learning and Memory Following Prenatal Exposure to Valproic Acid." *Journal of Nutritional Biochemistry* 35:87–95.

Gao, R., and P. Penzes. 2015. "Common Mechanisms of Excitatory and Inhibitory Imbalance in Schizophrenia and Autism Spectrum Disorders." *Current Molecular Medicine* 15(2):146–67.

Garcia-Junco-Clemente, Pablo, and Peyman Golshani. 2014. "PTEN." *Communicative & Integrative Biology* 7(2):e28358.

Gargus, J. Jay. 2009. "Genetic Calcium Signaling Abnormalities in the Central Nervous System: Seizures, Migraine, and Autism." *Annals of the New York Academy of Sciences* 1151(1):133–56.

Gharani, N., R. Benayed, V. Mancuso, L. M. Brzustowicz, and J. H. Millonig. 2004. “Association of the Homeobox Transcription Factor, ENGRAILED 2, 3, with Autism Spectrum Disorder.” *Molecular Psychiatry* 9(5):474–84.

Ghodke-Puranik, Yogita, Caroline F. Thorn, Jatinder K. Lamba, J. Steven Leeder, Wen Song, Angela K. Birnbaum, Russ B. Altman, and Teri E. Klein. 2013. “Valproic Acid Pathway: Pharmacokinetics and Pharmacodynamics.” *Pharmacogenetics and Genomics* 23(4):236–41.

Gilbert, James, and Heng Ye Man. 2017. “Fundamental Elements in Autism: From Neurogenesis and Neurite Growth to Synaptic Plasticity.” *Frontiers in Cellular Neuroscience* 11(November):1–25.

Gingras, Anne Claude, Steven P. Gygi, Brian Raught, Roberto D. Polakiewicz, Robert T. Abraham, Merl F. Hoekstra, Ruedi Aebersold, and Nahum Sonenberg. 1999. “Regulation of 4E-BP1 Phosphorylation: A Novel Two Step Mechanism.” *Genes and Development* 13(11):1422–37.

Go, Hyo Sang, Ki Chan Kim, Chang Soon Choi, Se Jin Jeon, Kyung Ja Kwon, Seol Heui Han, Jongmin Lee, Jae Hoon Cheong, Jong Hoon Ryu, Chong Hyun Kim, Kwang Ho Ko, and Chan Young Shin. 2012. “Prenatal Exposure to Valproic Acid Increases the Neural Progenitor Cell Pool and Induces Macrocephaly in Rat Brain via a Mechanism Involving the GSK-3β/β-Catenin Pathway.” *Neuropharmacology* 63(6):1028–41.

Goffin, Aleide, Lies H. Hoefsloot, Ermanno Bosgoed, Ann Swillen, and Jean-Pierre Fryns. 2001. “PTEN Mutation in a Family with Cowden Syndrome and Autism.” *American Journal of Medical Genetics* 105(6):521–24.

Gogolla, Nadine, Jocelyn J. LeBlanc, Kathleen B. Quast, Thomas C. Südhof, Michela Fagiolini, and Takao K. Hensch. 2009. “Common Circuit Defect of Excitatory-Inhibitory Balance in Mouse Models of Autism.” *Journal of Neurodevelopmental Disorders* 1(2):172–81.

Goorden, Susanna M. I., Geeske M. Van Woerden, Louise Van Der Weerd, Jeremy P. Cheadle, and Ype Elgersma. 2007. “Cognitive Deficits in Tsc1+/- Mice in the Absence of Cerebral Lesions and Seizures.” *Annals of Neurology* 62(6):648–55.

Göttlicher, Martin, Saverio Minucci, Ping Zhu, Oliver H. Krämer, Annemarie Schimpf, Sabrina Giavara, Jonathan P. Sleeman, Francesco Lo Coco, Clara Nervi, Pier Giuseppe Pelicci, and Thorsten Heinzel. 2001. “Valproic Acid Defines a Novel Class of HDAC Inhibitors Inducing Differentiation of Transformed Cells.” *EMBO Journal* 20(24):6969–78.

Götze, Silke, Monique Coersmeyer, Oliver Müller, and Sonja Sievers. 2014. “Histone Deacetylase Inhibitors Induce Attenuation of Wnt Signaling and TCF7L2 Depletion in Colorectal Carcinoma Cells.” *International Journal of Oncology* 45(4):1715–23.

Greco, Barbara, Francesca Managò, Valter Tucci, Hung Teh Kao, Flavia Valtorta, and Fabio Benfenati. 2013. “Autism-Related Behavioral Abnormalities in Synapsin Knockout Mice.” *Behavioural Brain Research* 251:65–74.

Gunaydin, Lisa A., Logan Grosenick, Joel C. Finkelstein, Isaac V. Kauvar, Lief E. Fenno, Avishek Adhikari, Stephan Lammel, Julie J. Mirzabekov, Raag D. Airan, Kelly A. Zalocusky, Kay M. Tye, Polina Anikeeva, Robert C. Malenka, and Karl Deisseroth. 2014. “Natural Neural Projection Dynamics Underlying Social Behavior.” *Cell* 157(7):1535–51.

Gutierrez, H. 2004. “HGF Regulates the Development of Cortical Pyramidal Dendrites.” *Development* 131(15):3717–26.

Hagerman, Randi, Gry Hoem, and Paul Hagerman. 2010. “Fragile X and Autism: Intertwined at the Molecular Level Leading to Targeted Treatments.” *Molecular Autism* 1(1):1–14.

Haghparast, Abbas, Zahra Fatahi, Reza Arezoomandan, Sara Karimi, Zahra Taslimi, and Shahram Zarrabian. 2017. *Functional Roles of Orexin/Hypocretin Receptors in Reward Circuit*. Vol. 235. 1st ed. Elsevier B.V.

Hall, Anita C., Angela Brennan, Robert G. Goold, Karen Cleverley, Fiona R. Lucas, Phillip R. Gordon-Weeks, and Patricia C. Salinas. 2002. “Valproate Regulates GSK-3-Mediated Axonal Remodeling and Synapsin I Clustering in Developing Neurons.” *Molecular and Cellular Neuroscience* 20(2):257–70.

Hara, Yuta, Yukio Ago, Atsuki Taruta, Keisuke Katashiba, Shigeru Hasebe, Erika Takano, Yusuke Onaka, Hitoshi Hashimoto, Toshio Matsuda, and Kazuhiro Takuma. 2016. “Improvement by Methylphenidate and Atomoxetine of Social Interaction Deficits and Recognition Memory Impairment in a Mouse Model of Valproic Acid-Induced Autism.” *Autism Research* 9(9):926–39.

Harris, Thurl E., and John C. Lawrence. 2003. “TOR Signaling.” *Science’s STKE : Signal Transduction Knowledge Environment* 2003(212):re15–re15.

Hay, Nissim, and Nahum Sonenberg. 2004. “Upstream and Downstream of MTOR.” *Genes and Development* 18(16):1926–45.

Heimer, L., and G. F. Alheid. 1991. “Piecing Together the Puzzle of Basal Forebrain Anatomy.” *Advances in Experimental Medicine and Biology* 295:1–42.

Hentges, Kathryn E., Baheya Sirry, Anne Claude Gingeras, Dos Sarbassov, Nahum Sonenberg, David Sabatini, and Andrew S. Peterson. 2001. “FRAP/MTOR Is Required for Proliferation and Patterning during Embryonic Development in the Mouse.” *Proceedings of the National Academy of Sciences of the United States of America* 98(24):13796–801.

Hibino, Hiroshi, Atsushi Inanobe, Kazuharu Furutani, Shingo Murakami, Ian Findlay, and Yoshihisa Kurachi. 2010. "Inwardly Rectifying Potassium Channels: Their Structure, Function, and Physiological Roles." *Physiological Reviews* 90(1):291–366.

Hill, Denise S., Bogdan J. Wlodarczyk, Ana M. Palacios, and Richard H. Finnell. 2010. "Teratogenic Effects of Antiepileptic Drugs." *Expert Review of Neurotherapeutics* 10(6):943–59.

Hille, B. 1978. "Ionic Channels in Excitable Membranes. Current Problems and Biophysical Approaches." *Biophysical Journal* 22(2):283–94.

Hou, Lingfei, and Eric Klann. 2004. "Activation of the Phosphoinositide 3-Kinase-Akt-Mammalian Target of Rapamycin Signaling Pathway Is Required for Metabotropic Glutamate Receptor-Dependent Long-Term Depression." *Journal of Neuroscience* 24(28):6352–61.

Huber, Kimberly M., Sean M. Gallagher, Stephen T. Warren, and Mark F. Bear. 2002. "Altered Synaptic Plasticity in a Mouse Model of Fragile X Mental Retardation." *Proceedings of the National Academy of Sciences of the United States of America* 99(11):7746–50.

Huber, Kimberly M., John C. Roder, and Mark F. Bear. 2001. "Chemical Induction of MGluR5- and Protein Synthesis-Dependent Long-Term Depression in Hippocampal Area CA1." *Journal of Neurophysiology* 86(1):321–25.

Hunt, Carol A., Leslie J. Schenker, and Mary B. Kennedy. 1996. "PSD-95 Is Associated with the Postsynaptic Density and Not with the Presynaptic Membrane at Forebrain Synapses." *Journal of Neuroscience* 16(4):1380–88.

Imbrici, Paola, Diana Conte Camerino, and Domenico Tricarico. 2013. "Major Channels Involved in Neuropsychiatric Disorders and Therapeutic Perspectives." *Frontiers in Genetics* 4(MAY):76.

Imredy, John P., Chinfei Chen, and Roderick MacKinnon. 1998. "A Snake Toxin Inhibitor of Inward Rectifier Potassium Channel ROMK1." *Biochemistry* 37(42):14867–74.

James Surmeier, D., J. Bargas, and S. T. Kitai. 1989. "Two Types of A-Current Differing in Voltage-Dependence Are Expressed by Neurons of the Rat Neostriatum." *Neuroscience Letters* 103(3):331–37.

Jaworski, Jacek, and Morgan Sheng. 2006. "The Growing Role of MTOR in Neuronal Development and Plasticity." 34(3):205–19.

Jeste, Shafali S., Kandice J. Varcin, Gerhard S. Hellemann, Amanda C. Gulsrud, Rujuta Bhatt, Connie Kasari, Joyce Y. Wu, Mustafa Sahin, and Charles A. Nelson. 2016. "Symptom Profiles of Autism Spectrum Disorder in Tuberous Sclerosis Complex." *Neurology* 87(8):766–72.

Jeste, Shafali, Mustafa Sahin, Patrick Bolton, George Ploubidis, and Ayla Humphrey. 2008. "Characterization of Autism in Young Children with Tuberous Sclerosis Complex." *Journal of Child Neurology* 23(5):520–25.

Jin, Weili, Angela M. Klem, John H. Lewis, and Zhe Lu. 1999. "Mechanisms of Inward-Rectifier K+ Channel Inhibition by Tertiapin-Q." *Biochemistry* 38(43):14294–301.

Jin, Weili, and Zhe Lu. 1998. "A Novel High-Affinity Inhibitor for Inward-Rectifier K+ Channels." *Biochemistry* 37(38):13291–99.

Johannessen, Cecilie U., and Svein I. Johannessen. 2003. *Valproate: Past, Present, and Future*.

Johannessen, Svein I. 1992. "Pharmacokinetics of Valproate in Pregnancy: Mother-Foetus-Newborn." *Pharmaceutisch Weekblad Scientific Edition* 14(3):114–17.

Joyner, Alexandra L. 1996. "Engrailed, Wnt and Pax Genes Regulate Midbrain-Hindbrain Development." *Trends in Genetics* 12(1):15–20.

Kandel, Eric R. 2001. "The Molecular Biology of Memory Storage: A Dialog between Genes and Synapses." Pp. 565–611 in *Bioscience Reports*. Vol. 21. Biosci Rep.

Kaneko, S., D. Battino, E. Andermann, K. Wada, R. Kan, A. Takeda, Y. Nakane, Y. Ogawa, G. Avanzini, C. Fumarola, T. Granata, F. Molteni, G. Pardi, L. Minotti, R. Canger, L. Dansky, M. Oguni, I. Lopes-Cendas, A. Sherwin, F. Andermann, M. H. Seni, M. Okada, and T. Teranishi. 1999. "Congenital Malformations Due to Antiepileptic Drugs." *Epilepsy Research* 33(2–3):145–58.

Kanjhan, Refik, Elizabeth J. Coulson, David J. Adams, and Mark C. Bellingham. 2005. "Tertiapin-Q Blocks Recombinant and Native Large Conductance K^+ Channels in a Use-Dependent Manner." *Journal of Pharmacology and Experimental Therapeutics* 314(3):1353–61.

Kasanetz, Fernando, and Olivier J. Manzoni. 2009. "Maturation of Excitatory Synaptic Transmission of the Rat Nucleus Accumbens From Juvenile to Adult." *Journal of Neurophysiology* 101(5):2516–27.

Kataoka, Shunsuke, Kazuhiro Takuma, Yuta Hara, Yuko Maeda, Yukio Ago, and Toshio Matsuda. 2013. "Autism-like Behaviours with Transient Histone Hyperacetylation in Mice Treated Prenatally with Valproic Acid." *International Journal of Neuropsychopharmacology* 16(1):91–103.

Kawaguchi, Yasuo, Charles J. Wilson, Sarah J. Augood, and Piers C. Emson. 1995. "Striatal Interneurones: Chemical, Physiological and Morphological Characterization." *Trends in Neurosciences* 18(12):527–35.

Ketzef, Maya, and Daniel Gitler. 2014. “Epileptic Synapsin Triple Knockout Mice Exhibit Progressive Long-Term Aberrant Plasticity in the Entorhinal Cortex.” *Cerebral Cortex* 24(4):996–1008.

Kim, Ji-Woon, Hana Seung, Kyung Ja Kwon, Mee Jung Ko, Eun Joo Lee, Hyun Ah Oh, Chang Soon Choi, Ki Chan Kim, Edson Luck Gonzales, Jueng Soo You, Dong-Hee Choi, Jongmin Lee, Seol-Heui Han, Sung Min Yang, Jae Hoon Cheong, Chan Young Shin, and Geon Ho Bahn. 2014. “Subchronic Treatment of Donepezil Rescues Impaired Social, Hyperactive, and Stereotypic Behavior in Valproic Acid-Induced Animal Model of Autism” edited by I. Silman. *PLoS ONE* 9(8):e104927.

Kim, Ki Chan, Chang Soon Choi, Ji-Woon Kim, Seol-Heui Han, Jae Hoon Cheong, Jong Hoon Ryu, and Chan Young Shin. 2014. “MeCP2 Modulates Sex Differences in the Postsynaptic Development of the Valproate Animal Model of Autism.” *Molecular Neurobiology 2014 53:1* 53(1):40–56.

Kim, Ki Chan, Pitna Kim, Hyo Sang Go, Chang Soon Choi, Jin Hee Park, Hee Jin Kim, Se Jin Jeon, Ike Campomayor Dela Pena, Seol Heui Han, Jae Hoon Cheong, Jong Hoon Ryu, and Chan Young Shin. 2013. “Male-Specific Alteration in Excitatory Post-Synaptic Development and Social Interaction in Pre-Natal Valproic Acid Exposure Model of Autism Spectrum Disorder.” *Journal of Neurochemistry* 124(6):832–43.

Kim, Ki Chan, Pitna Kim, Hyo Sang Go, Chang Soon Choi, Sung Il Yang, Jae Hoon Cheong, Chan Young Shin, and Kwang Ho Ko. 2011. “The Critical Period of Valproate Exposure to Induce Autistic Symptoms in Sprague-Dawley Rats.” *Toxicology Letters* 201(2):137–42.

Kim, Ki Chan, Dong Keun Lee, Hyo Sang Go, Pitna Kim, Chang Soon Choi, Ji Woon Kim, Se Jin Jeon, Mi Ryoung Song, and Chan Young Shin. 2014a. “Pax6-Dependent Cortical Glutamatergic Neuronal Differentiation Regulates Autism-like Behavior in Prenatally Valproic Acid-Exposed Rat Offspring.” *Molecular Neurobiology* 49(1):512–28.

Kim, Ki Chan, Dong Keun Lee, Hyo Sang Go, Pitna Kim, Chang Soon Choi, Ji Woon Kim, Se Jin Jeon, Mi Ryoung Song, and Chan Young Shin. 2014b. “Pax6-Dependent Cortical Glutamatergic Neuronal Differentiation Regulates Autism-like Behavior in Prenatally Valproic Acid-Exposed Rat Offspring.” *Molecular Neurobiology* 49(1):512–28.

Kini, U., N. Adab, J. Vinten, A. Fryer, and J. Clayton-Smith. 2006. “Dysmorphic Features: An Important Clue to the Diagnosis and Severity of Fetal Anticonvulsant Syndromes.” *Archives of Disease in Childhood: Fetal and Neonatal Edition* 91(2):F90–95.

Kneussel, Matthias, Johann Helmut Brandstätter, Bodo Laube, Sabine Stahl, Ulrike Müller, and Heinrich Betz. 1999. “Loss of Postsynaptic GABA(A) Receptor Clustering in Gephyrin-

Deficient Mice." *Journal of Neuroscience* 19(21):9289–97.

Kolevzon, Alexander, Raz Gross, and Abraham Reichenberg. 2007. "Prenatal and Perinatal Risk Factors for Autism: A Review and Integration of Findings." *Archives of Pediatrics and Adolescent Medicine* 161(4):326–33.

Kondo, T., K. Otani, T. Hirano, and S. Kaneko. 1987. "Placental Transfer and Neonatal Elimination of Mono-unsaturated Metabolites of Valproic Acid [Letter]." *British Journal of Clinical Pharmacology* 24(3):401–3.

Konopko, Melissa A., Allison L. Densmore, and Bruce K. Krueger. 2017. "Sexually Dimorphic Epigenetic Regulation of Brain-Derived Neurotrophic Factor in Fetal Brain in the Valproic Acid Model of Autism Spectrum Disorder." *Developmental Neuroscience* 39(6):507–18.

van Kooten, Imke A. J., Patrick R. Hof, Herman van Engeland, Harry W. M. Steinbusch, Paul H. Patterson, and Christoph Schmitz. 2005. "Autism: Neuropathology, Alterations of the GABAergic System, and Animal Models." *International Review of Neurobiology* 71:1–26.

Kopec, Ashley M., Caroline J. Smith, Nathan R. Ayre, Sean C. Sweat, and Staci D. Bilbo. 2018. "Microglial Dopamine Receptor Elimination Defines Sex-Specific Nucleus Accumbens Development and Social Behavior in Adolescent Rats." *Nature Communications* 9(1):1–16.

Kotajima-Murakami, Hiroko, Toshiyuki Kobayashi, Hirofumi Kashii, Atsushi Sato, Yoko Hagino, Miho Tanaka, Yasumasa Nishito, Yukio Takamatsu, Shigeo Uchino, and Kazutaka Ikeda. 2019. "Effects of Rapamycin on Social Interaction Deficits and Gene Expression in Mice Exposed to Valproic Acid in Utero." *Molecular Brain* 12(1):1–14.

Kozma, Chahira. 2001. "Valproic Acid Embryopathy: Report of Two Siblings with Further Expansion of the Phenotypic Abnormalities and a Review of the Literature." *American Journal of Medical Genetics* 98(2):168–75.

Kravitz, Alexxai V., and Anatol C. Kreitzer. 2012. "Striatal Mechanisms Underlying Movement, Reinforcement, and Punishment." *Physiology* 27(3):167–77.

Kreitzer, Anatol C. 2009. "Physiology and Pharmacology of Striatal Neurons." *Annual Review of Neuroscience* 32(1):127–47.

Kullmann, Dimitri M. 2010. "Neurological Channelopathies." *Annual Review of Neuroscience* 33:151–72.

Kultima, Kim, Anna-Maja Nyström, Birger Scholz, Anne-Lee Gustafson, Lennart Dencker, and Michael Stigson. 2004. "Valproic Acid Teratogenicity: A Toxicogenomics Approach." *Environmental Health Perspectives* 112(12):1225–35.

Kumar, Baldeep, Ajay Prakash, Rakesh K. Sewal, Bikash Medhi, and Manish Modi. 2012. “Drug Therapy in Autism : A Present and Future Perspective.” 1291–1304.

Kumari, Daman, Aditi Bhattacharya, Jeffrey Nadel, Kristen Moulton, Nicole M. Zeak, Anne Glicksman, Carl Dobkin, David J. Brick, Philip H. Schwartz, Carolyn B. Smith, Eric Klann, and Karen Usdin. 2014. “Identification of Fragile X Syndrome Specific Molecular Markers in Human Fibroblasts: A Useful Model to Test the Efficacy of Therapeutic Drugs.” *Human Mutation* 35(12):1485–94.

Kuo, Hsiao Ying, and Fu Chin Liu. 2018. “Molecular Pathology and Pharmacological Treatment of Autism Spectrum Disorder-like Phenotypes Using Rodent Models.” *Frontiers in Cellular Neuroscience* 12(November).

Kwon, Chang Hyuk, Bryan W. Luikart, Craig M. Powell, Jing Zhou, Sharon A. Matheny, Wei Zhang, Yanjiao Li, Suzanne J. Baker, and Luis F. Parada. 2006a. “Pten Regulates Neuronal Arborization and Social Interaction in Mice.” *Neuron* 50(3):377–88.

Kwon, Chang Hyuk, Bryan W. Luikart, Craig M. Powell, Jing Zhou, Sharon A. Matheny, Wei Zhang, Yanjiao Li, Suzanne J. Baker, and Luis F. Parada. 2006b. “Pten Regulates Neuronal Arborization and Social Interaction in Mice.” *Neuron* 50(3):377–88.

Lawrence, Y. A., T. L. Kemper, M. L. Bauman, and G. J. Blatt. 2010. “Parvalbumin-, Calbindin-, and Calretinin-Immunoreactive Hippocampal Interneuron Density in Autism.” *Acta Neurologica Scandinavica* 121(2):99–108.

Lee, Eunee, Jiseok Lee, and Eunjoon Kim. 2017. “Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders.” *Biological Psychiatry* 81(10):838–47.

Leppik, Ilo E., and Angela K. Birnbaum. 2010. “Epilepsy in the Elderly.” *Annals of the New York Academy of Sciences* 1184(1):208–24.

Lim, Carmay, and Todor Dudev. 2016. “Potassium Versus Sodium Selectivity in Monovalent Ion Channel Selectivity Filters.” Pp. 325–47 in *Metal ions in life sciences*. Vol. 16. Met Ions Life Sci.

Lionel, Anath C., Andrea K. Vaags, Daisuke Sato, Matthew J. Gazzellone, Elyse B. Mitchell, Hong Yang Chen, Gregory Costain, Susan Walker, Gerald Egger, Bhooma Thiruvahindrapuram, Daniele Merico, Aparna Prasad, Evdokia Anagnostou, Eric Fombonne, Lonnie Zwaigenbaum, Wendy Roberts, Peter Szatmari, Bridget A. Fernandez, Lyudmila Georgieva, Linda M. Brzustowicz, Katharina Roetzer, Wolfgang Kaschnitz, John B. Vincent, Christian Windpassinger, Christian R. Marshall, Rosario R. Trifiletti, Salman Kirmani, George Kirov, Erwin Petek, Jennelle C. Hodge, Anne S. Bassett, and Stephen W. Scherer. 2013. “Rare Exonic

Deletions Implicate the Synaptic Organizer Gephyrin (GPHN) in Risk for Autism, Schizophrenia and Seizures." *Human Molecular Genetics* 22(10):2055–66.

Lipton, Jonathan O., and Mustafa Sahin. 2014. "The Neurology of MTOR." *Neuron* 84(2):275–91.

Liu, Qiang, Jieyun Shi, Rongfei Lin, and Tieqiao Wen. 2017. "Dopamine and Dopamine Receptor D1 Associated with Decreased Social Interaction." *Behavioural Brain Research* 324:51–57.

Lord, Catherine, Traolach S. Brugha, Tony Charman, James Cusack, Guillaume Dumas, Thomas Frazier, Emily J. H. Jones, Rebecca M. Jones, Andrew Pickles, Matthew W. State, Julie Lounds Taylor, and Jeremy Veenstra-VanderWeele. 2020. "Autism Spectrum Disorder." *Nature Reviews Disease Primers* 6(1).

Lotter, Victor. 1966. "Epidemiology of Autistic Conditions in Young Children - 1. Prevalence." *Social Psychiatry* 1(3):124–35.

Lu, Ping, Xiaolong Chen, Yun Feng, Qiao Zeng, Cizhong Jiang, Xianmin Zhu, Guoping Fan, and Zhigang Xue. 2016. "Integrated Transcriptome Analysis of Human IPS Cells Derived from a Fragile X Syndrome Patient during Neuronal Differentiation." *Science China Life Sciences* 59(11):1093–1105.

Luca, Guglielmi, Servettini Ilenio, Caramia Martino, Catacuzzeno Luigi, Franciolini Fabio, Cristina D'Adamo1 Maria, and Pessia Mauro. 2015. "Update on the Implication of Potassium Channels in Autism: K+ Channelautism Spectrum Disorder." *Frontiers in Cellular Neuroscience* 9(MAR).

MacDonald, Jessica L., and A. Jane Roskams. 2008. "Histone Deacetylases 1 and 2 Are Expressed at Distinct Stages of Neuro-Glial Development." *Developmental Dynamics* 237(8):2256–67.

Maenner, Matthew J., Kelly A. Shaw, Jon Baio, Anita Washington, Mary Patrick, Monica DiRienzo, Deborah L. Christensen, Lisa D. Wiggins, Sydney Pettygrove, Jennifer G. Andrews, Maya Lopez, Allison Hudson, Thaer Baroud, Yvette Schwenk, Tiffany White, Cordelia Robinson Rosenberg, Li Ching Lee, Rebecca A. Harrington, Margaret Huston, Amy Hewitt, Amy Esler, Jennifer Hall-Lande, Jenny N. Poynter, Libby Hallas-Muchow, John N. Constantino, Robert T. Fitzgerald, Walter Zahorodny, Josephine Shenouda, Julie L. Daniels, Zachary Warren, Alison Vehorn, Angelica Salinas, Maureen S. Durkin, and Patricia M. Dietz. 2020. "Prevalence of Autism Spectrum Disorder among Children Aged 8 Years-Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016." *MMWR Surveillance Summaries* 69(4):1–12.

Magnuson, Brian, Bilgen Ekim, and Diane C. Fingar. 2012. "Regulation and Function of Ribosomal Protein S6 Kinase (S6K) within MTOR Signalling Networks." *Biochemical Journal* 441(1):1–

21.

Markram, Kamila, Tania Rinaldi, Deborah La Mendola, Carmen Sandi, and Henry Markram. 2008. "Abnormal Fear Conditioning and Amygdala Processing in an Animal Model of Autism." *Neuropsychopharmacology* 33(4):901–12.

Marson, Anthony G., Asya M. Al-Kharusi, Muna Alwaidh, Richard Appleton, Gus A. Baker, David W. Chadwick, Celia Cramp, Oliver C. Cockerell, Paul N. Cooper, Julie Doughty, Barbara Eaton, Carrol Gamble, Peter J. Goulding, Stephen JL Howell, Adrian Hughes, Margaret Jackson, Ann Jacoby, Mark Kellett, Geoffrey R. Lawson, John Paul Leach, Paola Nicolaides, Richard Roberts, Phil Shackley, Jing Shen, David F. Smith, Philip EM Smith, Catrin Tudur Smith, Alessandra Vanoli, and Paula R. Williamson. 2007. "The SANAD Study of Effectiveness of Valproate, Lamotrigine, or Topiramate for Generalised and Unclassifiable Epilepsy: An Unblinded Randomised Controlled Trial." *Lancet* 369(9566):1016–26.

Martin, Henry G. S., and Olivier J. Manzoni. 2014. "Late Onset Deficits in Synaptic Plasticity in the Valproic Acid Rat Model of Autism." *Frontiers in Cellular Neuroscience* 8(JAN).

Meador, Kimford J., Gus A. Baker, Nancy Browning, Morris J. Cohen, Jill Clayton-Smith, Laura A. Kalayjian, Andres Kanner, Joyce D. Liporace, Page B. Pennell, Michael Privitera, and David W. Loring. 2011. "Foetal Antiepileptic Drug Exposure and Verbal versus Non-Verbal Abilities at Three Years of Age." *Brain* 134(2):396–404.

Meador, Kimford, Matthew W. Reynolds, Sheila Crean, Kyle Fahrbach, and Corey Probst. 2008. "Pregnancy Outcomes in Women with Epilepsy: A Systematic Review and Meta-Analysis of Published Pregnancy Registries and Cohorts." *Epilepsy Research* 81(1):1–13.

Mehta, Mili V., Michael J. Gandal, and Steven J. Siegel. 2011. "MGluR5-Antagonist Mediated Reversal of Elevated Stereotyped, Repetitive Behaviors in the VPA Model of Autism" edited by M. Coleman. *PLoS ONE* 6(10):e26077.

Melancia, Francesca, Sara Schiavi, Michela Servadio, Veronica Cartocci, Patrizia Campolongo, Maura Palmery, Valentina Pallottini, and Viviana Trezza. 2018. "Sex-Specific Autistic Endophenotypes Induced by Prenatal Exposure to Valproic Acid Involve Anandamide Signalling." *British Journal of Pharmacology* 175(18):3699–3712.

MEUNIER, H., G. CARRAZ, Y. NEUNIER, P. EYMARD, and M. AIMARD. 1963. "Pharmacodynamic Properties of N-Dipropylacetic Acid." *Thérapie* 18:435–38.

Meyer, Daniel, Tobias Bonhoeffer, and Volker Scheuss. 2014. "Balance and Stability of Synaptic Structures during Synaptic Plasticity." *Neuron* 82(2):430–43.

Missler, Markus, Weiqi Zhang, Astrid Rohlmann, Gunnar Kattenstroth, Robert E. Hammer, Kurt

Gottmann, and Thomas C. Südhof. 2003. “α-Neurexins Couple Ca 2+ Channels to Synaptic Vesicle Exocytosis.” *Nature* 423(6943):939–48.

Miyazaki, Kaoru, Naoko Narita, and Masaaki Narita. 2005a. “Maternal Administration of Thalidomide or Valproic Acid Causes Abnormal Serotonergic Neurons in the Offspring: Implication for Pathogenesis of Autism.” *International Journal of Developmental Neuroscience* 23(2–3):287–97.

Miyazaki, Kaoru, Naoko Narita, and Masaaki Narita. 2005b. “Maternal Administration of Thalidomide or Valproic Acid Causes Abnormal Serotonergic Neurons in the Offspring: Implication for Pathogenesis of Autism.” *International Journal of Developmental Neuroscience* 23(2–3):287–97.

Moldrich, Randal X., Gayeshika Leanage, David She, Elliot Dolan-Evans, Michael Nelson, Nargis Reza, and David C. Reutens. 2013. “Inhibition of Histone Deacetylase in Utero Causes Sociability Deficits in Postnatal Mice.” *Behavioural Brain Research* 257:253–64.

Mongiat, Lucas A., M. Soledad Espósito, Gabriela Lombardi, and Alejandro F. Schinder. 2009. “Reliable Activation of Immature Neurons in the Adult Hippocampus.” *PLoS ONE* 4(4).

Montagrin, Alison, Catarina Saiote, and Daniela Schiller. 2018. “The Social Hippocampus.” *Hippocampus* 28(9):672–79.

Munji, Roeben N., Youngshik Choe, Guangnan Li, Julie A. Siegenthaler, and Samuel J. Pleasure. 2011. “Wnt Signaling Regulates Neuronal Differentiation of Cortical Intermediate Progenitors.” *Journal of Neuroscience* 31(5):1676–87.

Murakami, Mirei, Tomoko Ichisaka, Mitsuyo Maeda, Noriko Oshiro, Kenta Hara, Frank Edenhofer, Hiroshi Kiyama, Kazuyoshi Yonezawa, and Shinya Yamanaka. 2004. “MTOR Is Essential for Growth and Proliferation in Early Mouse Embryos and Embryonic Stem Cells.” *Molecular and Cellular Biology* 24(15):6710–18.

Muscarella, Lucia Anna, Vito Guarnieri, Roberto Sacco, Roberto Militerni, Carmela Bravaccio, Simona Trillo, Cindy Schneider, Raun Melmed, Maurizio Elia, Maria Lucia Mascia, Emanuela Rucci, Maria Rosaria Piemontese, Leonardo D’Agruma, and Antonio M. Persico. 2007. “HOXA1 Gene Variants Influence Head Growth Rates in Humans.” *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics* 144(3):388–90.

Nadebaum, C., V. A. Anderson, F. Vajda, D. C. Reutens, S. Barton, and A. G. Wood. 2011. “Language Skills of School-Aged Children Prenatally Exposed to Antiepileptic Drugs.” *Neurology* 76(8):719–26.

Nadebaum, Caroline, Vicki Anderson, Frank Vajda, David Reutens, Sarah Barton, and Amanda

Wood. 2011. “The Australian Brain and Cognition and Antiepileptic Drugs Study: Iq in School-Aged Children Exposed to Sodium Valproate and Polytherapy.” *Journal of the International Neuropsychological Society* 17(1):133–42.

Nagode, Daniel A., Xiangying Meng, Daniel E. Winkowski, Ed Smith, Hamza Khan-Tareen, Vishnupriya Kareddy, Joseph P. Y. Kao, and Patrick O. Kanold. 2017. “Abnormal Development of the Earliest Cortical Circuits in a Mouse Model of Autism Spectrum Disorder.” *Cell Reports* 18(5):1100–1108.

Narita, Naoko, Michiko Kato, Mami Tazoe, Kaoru Miyazaki, Masaaki Narita, and Nobuo Okado. 2002a. “Increased Monoamine Concentration in the Brain and Blood of Fetal Thalidomide- and Valproic Acid–Exposed Rat: Putative Animal Models for Autism.” *Pediatric Research* 52(4):576–79.

Narita, Naoko, Michiko Kato, Mami Tazoe, Kaoru Miyazaki, Masaaki Narita, and Nobuo Okado. 2002b. “Increased Monoamine Concentration in the Brain and Blood of Fetal Thalidomide- and Valproic Acid–Exposed Rat: Putative Animal Models for Autism.” *Pediatric Research* 52(4):576–79.

Nau, H. 1994. “Valproic Acid-Induced Neural Tube Defects.” *Ciba Foundation Symposium* 181.

Newschaffer, Craig J., Lisa A. Croen, Julie Daniels, Ellen Giarelli, Judith K. Grether, Susan E. Levy, David S. Mandell, Lisa A. Miller, Jennifer Pinto-Martin, Judy Reaven, Ann M. Reynolds, Catherine E. Rice, Diana Schendel, and Gayle C. Windham. 2007. “The Epidemiology of Autism Spectrum Disorders.” Pp. 235–58 in *Annual Review of Public Health*. Vol. 28. Annu Rev Public Health.

Nicolini, Chiara, Younghee Ahn, Bernadeta Michalski, Jong M. Rho, and Margaret Fahnestock. 2015. “Decreased MTOR Signaling Pathway in Human Idiopathic Autism and in Rats Exposed to Valproic Acid.” *Acta Neuropathologica Communications* 3:3.

Nicolini, Chiara, and Margaret Fahnestock. 2018. “The Valproic Acid-Induced Rodent Model of Autism.” *Experimental Neurology* 299:217–27.

Nisenbaum, E. S., and C. J. Wilson. 1995. “Potassium Currents Responsible for Inward and Outward Rectification in Rat Neostriatal Spiny Projection-Neurons.” *Journal of Neuroscience* 15(6):4449–63.

Nisenbaum, Eric S., Charles J. Wilson, Robert C. Foehring, and D. James Surmeier. 1996. “Isolation and Characterization of a Persistent Potassium Current in Neostriatal Neurons.” *Journal of Neurophysiology* 76(2):1180–94.

Omtzigt, Juliette G. C., Frans J. Los, Diederick E. Grobbee, Leen Pijpers, Milena G. J. Jahoda, Helen

Brandenburg, Patricia A. Stewart, Hans L. J. Gaillard, Eva S. Sachs, Juri W. Wladimiroff, and Dick Lindhout. 1992. “The Risk of Spina Bifida Aperta after First-Trimester Exposure to Valproate in a Prenatal Cohort.” *Neurology* 42(4):119–25.

Ornoy, A. 2003. “The Impact of Intrauterine Exposure versus Postnatal Environment in Neurodevelopmental Toxicity: Long-Term Neurobehavioral Studies in Children at Risk for Developmental Disorders.” Pp. 171–81 in *Toxicology Letters*. Vols. 140–141. Elsevier Ireland Ltd.

Ornoy, Asher. 2009a. “Valproic Acid in Pregnancy: How Much Are We Endangering the Embryo and Fetus?” *Reproductive Toxicology* 28(1):1–10.

Ornoy, Asher. 2009b. “Valproic Acid in Pregnancy: How Much Are We Endangering the Embryo and Fetus?” *Reproductive Toxicology* 28(1):1–10.

Park, Hye Ran, Jae Meen Lee, Hyo Eun Moon, Dong Soo Lee, Bung Nyun Kim, Jinhyun Kim, Dong Gyu Kim, and Sun Ha Paek. 2016. “A Short Review on the Current Understanding of Autism Spectrum Disorders.” *Experimental Neurobiology* 25(1):1–13.

Pavăl, Denis. 2017. “A Dopamine Hypothesis of Autism Spectrum Disorder.” *Developmental Neuroscience* 39(5):355–60.

Peça, João, Cátia Feliciano, Jonathan T. Ting, Wenting Wang, Michael F. Wells, Talaignair N. Venkatraman, Christopher D. Lascola, Zhanyan Fu, and Guoping Feng. 2011. “Shank3 Mutant Mice Display Autistic-like Behaviours and Striatal Dysfunction.” *Nature* 472(7344):437–42.

Peckman, Howard. 2010. “The U.S. Food and Drug Adminstration (FDA) Has Assigned Pregnancy Category D to Valproate.” *North Carolina Medical Journal* 71(4):396–98.

Pellissier, Lucie P., Jorge Gandía, Thibaut Laboute, Jérôme A. J. Becker, and Julie Le Merrer. 2018. “M Opioid Receptor, Social Behaviour and Autism Spectrum Disorder: Reward Matters.” *British Journal of Pharmacology* 175(14):2750–69.

Phelan, Mary C. 2008a. “Deletion 22q13.3 Syndrome.” *Orphanet Journal of Rare Diseases* 3(1):14.

Phelan, Mary C. 2008b. “Deletion 22q13.3 Syndrome.” *Orphanet Journal of Rare Diseases* 3(1).

Phiel, Christopher J., Fang Zhang, Eric Y. Huang, Matthew G. Guenther, Mitchell A. Lazar, and Peter S. Klein. 2001. “Histone Deacetylase Is a Direct Target of Valproic Acid, a Potent Anticonvulsant, Mood Stabilizer, and Teratogen.” *Journal of Biological Chemistry* 276(39):36734–41.

Ploeger, Annemie, Maartje E. J. Raijmakers, Han L. J. van der Maas, and Frietson Galis. 2010. “The Association Between Autism and Errors in Early Embryogenesis: What Is the Causal

Mechanism?" *Biological Psychiatry* 67(7):602–7.

Porstmann, Thomas, Claudio R. Santos, Beatrice Griffiths, Megan Cully, Mary Wu, Sally Leevers, John R. Griffiths, Yuen Li Chung, and Almut Schulze. 2008. "SREBP Activity Is Regulated by MTORC1 and Contributes to Akt-Dependent Cell Growth." *Cell Metabolism* 8(3):224–36.

Portmann, Thomas, Mu Yang, Rong Mao, Georgia Panagiotakos, Jacob Ellegood, Gul Dolen, Patrick L. Bader, Brad A. Grueter, Carleton Goold, Elaine Fisher, Katherine Clifford, Pavitra Rengarajan, David Kalikhman, Darren Loureiro, Nay L. Saw, Zhou Zhengqui, Michael A. Miller, Jason P. Lerch, R. Mark Henkelman, Mehrdad Shamloo, Robert C. Malenka, Jacqueline N. Crawley, and Ricardo E. Dolmetsch. 2014. "Behavioral Abnormalities and Circuit Defects in the Basal Ganglia of a Mouse Model of 16p11.2 Deletion Syndrome." *Cell Reports* 7(4):1077–92.

Prüss, Harald, Mareike Wenzel, Dirk Eulitz, Achim Thomzig, Andreas Karschin, and Rüdiger W. Veh. 2003. "Kir2 Potassium Channels in Rat Striatum Are Strategically Localized to Control Basal Ganglia Function." *Molecular Brain Research* 110(2):203–19.

Qin, Liyan, Xufang Dai, and Yunhong Yin. 2016. "Valproic Acid Exposure Sequentially Activates Wnt and MTOR Pathways in Rats." *Molecular and Cellular Neuroscience* 75:27–35.

Rasalam, A. D., H. Hailey, J. H. G. Williams, S. J. Moore, P. D. Turnpenny, D. J. Lloyd, and J. C. S. Dean. 2005. "Characteristics of Fetal Anticonvulsant Syndrome Associated Autistic Disorder." *Developmental Medicine and Child Neurology* 47(8):551–55.

Reimann, F., P. Proks, and F. M. Ashcroft. 2001. "Effects of Mitiglinide (S 21403) on Kir6.2/SUR1, Kir6.2/SUR2A and Kir6.2/SUR2B Types of ATP-Sensitive Potassium Channel." *British Journal of Pharmacology* 132(7):1542–48.

Richards, Caroline, Christopher Jones, Laura Groves, Jo Moss, and Chris Oliver. 2015. "Prevalence of Autism Spectrum Disorder Phenomenology in Genetic Disorders: A Systematic Review and Meta-Analysis." *The Lancet Psychiatry* 2(10):909–16.

Rinaldi, Tania. 2008. "Hyper-Connectivity and Hyper-Plasticity in the Medial Prefrontal Cortex in the Valproic Acid Animal Model of Autism." *Frontiers in Neural Circuits* 2(OCT):4.

Rinaldi, Tania, Karina Kulangara, Katia Antoniello, and Henry Markram. 2007. "Elevated NMDA Receptor Levels and Enhanced Postsynaptic Long-Term Potentiation Induced by Prenatal Exposure to Valproic Acid." *Proceedings of the National Academy of Sciences of the United States of America* 104(33):13501–6.

Rinaldi, Tania, Gilad Silberberg, and Henry Markram. 2008. "Hyperconnectivity of Local Neocortical Microcircuitry Induced by Prenatal Exposure to Valproic Acid." *Cerebral Cortex*

18(4):763–70.

Robert, Elisabeth, and Pierre Guibaud. 1982. “MATERNAL VALPROIC ACID AND CONGENITAL NEURAL TUBE DEFECTS.” *The Lancet* 320(8304):937.

Robinson, Donita L., Michael L. A. V. Heien, and R. Mark Wightman. 2002. “Frequency of Dopamine Concentration Transients Increases in Dorsal and Ventral Striatum of Male Rats during Introduction of Conspecifics.” *Journal of Neuroscience* 22(23):10477–86.

Rodier, Patricia M., Jennifer L. Ingram, Barbara Tisdale, Sarah Nelson, and John Romano. 1996a. “Embryological Origin for Autism: Developmental Anomalies of the Cranial Nerve Motor Nuclei.” *Journal of Comparative Neurology* 370(2):247–61.

Rodier, Patricia M., Jennifer L. Ingram, Barbara Tisdale, Sarah Nelson, and John Romano. 1996b. “Embryological Origin for Autism: Developmental Anomalies of the Cranial Nerve Motor Nuclei.” *Journal of Comparative Neurology* 370(2):247–61.

Rothwell, Patrick E. 2016. “Autism Spectrum Disorders and Drug Addiction: Common Pathways, Common Molecules, Distinct Disorders?” *Frontiers in Neuroscience* 10(FEB):20.

Rothwell, Patrick E., Marc V. Fuccillo, Stephan Maxeiner, Scott J. Hayton, Ozgun Gokce, Byung Kook Lim, Stephen C. Fowler, Robert C. Malenka, and Thomas C. Südhof. 2014. “Autism-Associated Neuroligin-3 Mutations Commonly Impair Striatal Circuits to Boost Repetitive Behaviors.” *Cell* 158(1):198–212.

Roullet, Florence I., Jonathan K. Y. Lai, and Jane A. Foster. 2013. “In Utero Exposure to Valproic Acid and Autism - A Current Review of Clinical and Animal Studies.” *Neurotoxicology and Teratology* 36:47–56.

Rubenstein, J. L. R., and M. M. Merzenich. 2003. “Model of Autism: Increased Ratio of Excitation/Inhibition in Key Neural Systems.” *Genes, Brain and Behavior* 2(5):255–67.

Sahin, Mustafa, and Mriganka Sur. 2015. “Genes, Circuits, and Precision Therapies for Autism and Related Neurodevelopmental Disorders.” *Science* 350(6263).

Sala, Carlo, Cinzia Vicidomini, Ilaria Bigi, Adele Mossa, and Chiara Verpelli. 2015. “Shank Synaptic Scaffold Proteins: Keys to Understanding the Pathogenesis of Autism and Other Synaptic Disorders.” *Journal of Neurochemistry* 135(5):849–58.

Salgado, Sanjay, and Michael G. Kaplitt. 2015. “E-Mail The Nucleus Accumbens: A Comprehensive Review.” *Stereotact Funct Neurosurg* 93:75–93.

Sato, Atsushi. 2016. “MTOR, a Potential Target to Treat Autism Spectrum Disorder.” *CNS & Neurological Disorders - Drug Targets* 15(5):533–43.

Sato, Atsushi, Shinya Kasai, Toshiyuki Kobayashi, Yukio Takamatsu, Okio Hino, Kazutaka Ikeda, and Masashi Mizuguchi. 2012. "Rapamycin Reverses Impaired Social Interaction in Mouse Models of Tuberous Sclerosis Complex." *Nature Communications* 3.

Saxton, Robert A., and David M. Sabatini. 2017. "MTOR Signaling in Growth, Metabolism, and Disease." *Cell* 168(6):960–76.

Schmahmann, Jeremy D., and David Caplan. 2006. "Cognition, Emotion and the Cerebellum." *Brain : A Journal of Neurology* 129(Pt 2):290–92.

Schneider, Tomasz, and Ryszard Przewłocki. 2005. "Behavioral Alterations in Rats Prenatally to Valproic Acid: Animal Model of Autism." *Neuropsychopharmacology* 30(1):80–89.

Schneider, Tomasz, Adam Roman, Agnieszka Basta-Kaim, Marta Kubera, Bogusława Budziszewska, Karolina Schneider, and Ryszard Przewłocki. 2008. "Gender-Specific Behavioral and Immunological Alterations in an Animal Model of Autism Induced by Prenatal Exposure to Valproic Acid." *Psychoneuroendocrinology* 33(6):728–40.

Schneider, Tomasz, Joanna Turczak, and Ryszard Przewłocki. 2006. "Environmental Enrichment Reverses Behavioral Alterations in Rats Prenatally Exposed to Valproic Acid: Issues for a Therapeutic Approach in Autism." *Neuropsychopharmacology* 31(1):36–46.

Selby, Leah, Chunzhao Zhang, and Qian Quan Sun. 2007. "Major Defects in Neocortical GABAergic Inhibitory Circuits in Mice Lacking the Fragile X Mental Retardation Protein." *Neuroscience Letters* 412(3):227–32.

Sernagor, Evelyne, François Chabrol, Guillaume Bony, and Laura Cancedda. 2010. "Gabaergic Control of Neurite Outgrowth and Remodeling during Development and Adult Neurogenesis: General Rules and Differences in Diverse Systems." *Frontiers in Cellular Neuroscience* 4(APR):11.

Servadio, M., F. Melancia, A. Manduca, A. Di Masi, S. Schiavi, V. Cartocci, V. Pallottini, P. Campolongo, P. Ascenzi, and V. Trezza. 2016a. "Targeting Anandamide Metabolism Rescues Core and Associated Autistic-like Symptoms in Rats Prenatally Exposed to Valproic Acid." *Translational Psychiatry* 6(9):1–11.

Servadio, M., F. Melancia, A. Manduca, A. Di Masi, S. Schiavi, V. Cartocci, V. Pallottini, P. Campolongo, P. Ascenzi, and V. Trezza. 2016b. "Targeting Anandamide Metabolism Rescues Core and Associated Autistic-like Symptoms in Rats Prenatally Exposed to Valproic Acid." *Translational Psychiatry* 6(9):902.

Servadio, Michela, Antonia Manduca, Francesca Melancia, Loris Leboffe, Sara Schiavi, Patrizia Campolongo, Maura Palmery, Paolo Ascenzi, Alessandra di Masi, and Viviana Trezza. 2018.

"Impaired Repair of DNA Damage Is Associated with Autistic-like Traits in Rats Prenatally Exposed to Valproic Acid." *European Neuropsychopharmacology* 28(1):85–96.

Shao, Jun Tang, and Erin M. Schuman. 2002. "Protein Synthesis in the Dendrite." *Philosophical Transactions of the Royal Society B: Biological Sciences* 357(1420):521–29.

Sharma, Ali, Charles A. Hoeffer, Yukihiro Takayasu, Takahiro Miyawaki, Sean M. McBride, Eric Klann, and R. Suzanne Zukin. 2010. "Dysregulation of MTOR Signaling in Fragile X Syndrome." *Journal of Neuroscience* 30(2):694–702.

Shen, Weixing, Salvador Hernandez-Lopez, Tatiana Tkatch, Joshua E. Held, and D. James Surmeier. 2004. "Kv1.2-Containing K+ Channels Regulate Subthreshold Excitability of Striatal Medium Spiny Neurons." *Journal of Neurophysiology* 91(3):1337–49.

Sheng, Morgan, and Carlo Sala. 2001. "PDZ Domains and the Organization of Supramolecular Complexes." *Annual Review of Neuroscience* 24(1):1–29.

Shew, Woodrow L., Hongdian Yang, Shan Yu, Rajarshi Roy, and Dietmar Plenz. 2011. "Information Capacity and Transmission Are Maximized in Balanced Cortical Networks with Neuronal Avalanches." *Journal of Neuroscience* 31(1):55–63.

Shieh, Char-Chang, Michael Coghlan, James P. Sullivan, and Murali Gopalakrishnan. 2000. *Potassium Channels: Molecular Defects, Diseases, and Therapeutic Opportunities*.

Shin, Jung Hwan, Dohoung Kim, and Min Whan Jung. 2018. "Differential Coding of Reward and Movement Information in the Dorsomedial Striatal Direct and Indirect Pathways." *Nature Communications* 9(1):1–14.

Shubassi, Ghadeer, Thomas Robert, Fabio Vanoli, Saverio Minucci, and Marco Foiani. 2012. "Acetylation: A Novel Link between Double-Strand Break Repair and Autophagy." *Cancer Research* 72(6):1332–35.

Silva, Guilherme Testa, Jean Vincent Le Bé, Imad Riachi, Tania Rinaldi, Kamila Markram, and Henry Markram. 2009. "Enhanced Long-Term Microcircuit Plasticity in the Valproic Acid Animal Model of Autism." *Frontiers in Synaptic Neuroscience* 1(1):1–9.

Soham Chanda, Authors, Cheen Euong Ang, Qian Yi Lee, Yohei Shibuya, Marius Wernig, and Thomas C. S. 2019. "Direct Reprogramming of Human Neurons Identifies MARCKSL1 as a Pathogenic Mediator of Valproic Acid-Induced Teratogenicity."

Soorya, Latha, Alexander Kolevzon, Jessica Zweifach, Teresa Lim, Yuriy Dobry, Lily Schwartz, Yitzchak Frank, A. Ting Wang, Guiqing Cai, Elena Parkhomenko, Danielle Halpern, David Grodberg, Benjamin Angarita, Judith P. Willner, Amy Yang, Roberto Canitano, William

Chaplin, Catalina Betancur, and Joseph D. Buxbaum. 2013. “Prospective Investigation of Autism and Genotype-Phenotype Correlations in 22q13 Deletion Syndrome and SHANK3 Deficiency.” *Molecular Autism* 4(1).

Stodgell, Christopher J., Jennifer L. Ingram, Melanie O’Bara, Barbara K. Tisdale, Heinz Nau, and Patricia M. Rodier. 2006. “Induction of the Homeotic Gene Hoxa1 through Valproic Acid’s Teratogenic Mechanism of Action.” *Neurotoxicology and Teratology* 28(5):617–24.

Strömland, K., V. Nordin, M. Miller, B. Akerström, and C. Gillberg. 1994. “AUTISM IN THALIDOMIDE EMBRYOPATHY: A POPULATION STUDY.” *Developmental Medicine & Child Neurology* 36(4):351–56.

Südhof, Thomas C. 2008. “Neuroligins and Neurexins Link Synaptic Function to Cognitive Disease.” *Nature* 455(7215):903–11.

Sun, Caihong, Mingyang Zou, Ling Li, Dexin Li, Yongjuan Ma, Wei Xia, Lijie Wu, and Huan Ren. 2018. “Association Study between Inwardly Rectifying Potassium Channels 2.1 and 4.1 and Autism Spectrum Disorders.” *Life Sciences* 213(July):183–89.

Sun, Jiandong, Yan Liu, Stephanie Moreno, Michel Baudry, and Xiaoning Bi. 2015. “Imbalanced Mechanistic Target of Rapamycin C1 and C2 Activity in the Cerebellum of Angelman Syndrome Mice Impairs Motor Function.” *Journal of Neuroscience* 35(11):4706–18.

Sun, Jiandong, Yan Liu, Jennifer Tran, Patrick O’Neal, Michel Baudry, and Xiaoning Bi. 2016. “MTORC1–S6K1 Inhibition or MTORC2 Activation Improves Hippocampal Synaptic Plasticity and Learning in Angelman Syndrome Mice.” *Cellular and Molecular Life Sciences* 73(22):4303–14.

Sun, Woong, Hiroshi Funakoshi, and Toshikazu Nakamura. 2002. “Localization and Functional Role of Hepatocyte Growth Factor (HGF) and Its Receptor c-Met in the Rat Developing Cerebral Cortex.” *Molecular Brain Research* 103(1–2):36–48.

Supekar, Kaustubh, John Kochalka, Marie Schaer, Holly Wakeman, Shaozheng Qin, Aarthi Padmanabhan, and Vinod Menon. 2018. “Deficits in Mesolimbic Reward Pathway Underlie Social Interaction Impairments in Children with Autism.” *Brain* 141(9):2795–2805.

Surmeier, D. James, Alessandro Stefani, Robert C. Foehring, and S. T. Kitai. 1991. “Developmental Regulation of a Slowly-Inactivating Potassium Conductance in Rat Neostriatal Neurons.” *Neuroscience Letters* 122(1):41–46.

Szatmari, P., M. B. Jones, L. Zwaigenbaum, and J. E. MacLean. 1998. “Genetics of Autism: Overview and New Directions.” *Journal of Autism and Developmental Disorders* 28(5):351–68.

Takei, Nobuyuki, and Hiroyuki Nawa. 2014. “MTOR Signaling and Its Roles in Normal and Abnormal Brain Development.” *Frontiers in Molecular Neuroscience* 7(1 APR).

Tartaglione, Anna Maria, Sara Schiavi, Gemma Calamandrei, and Viviana Trezza. 2019. “Prenatal Valproate in Rodents as a Tool to Understand the Neural Underpinnings of Social Dysfunctions in Autism Spectrum Disorder.” *Neuropharmacology* 159:107477.

Tavazoie, Sohail F., Veronica A. Alvarez, Dennis A. Ridenour, David J. Kwiatkowski, and Bernardo L. Sabatini. 2005. “Regulation of Neuronal Morphology and Function by the Tumor Suppressors Tsc1 and Tsc2.” *Nature Neuroscience* 8(12):1727–34.

Terbach, Nicole, and Robin S. B. Williams. 2009. “Structure-Function Studies for the Panacea, Valproic Acid.” Pp. 1126–32 in *Biochemical Society Transactions*. Vol. 37. Biochem Soc Trans.

Tkatch, Tatiana, Gytis Baranauskas, and D. James Surmeier. 2000. “Kv4.2 MRNA Abundance and A-Type K+ Current Amplitude Are Linearly Related in Basal Ganglia and Basal Forebrain Neurons.” *Journal of Neuroscience* 20(2):579–88.

Tremblay, Léon, Yulia Worbe, and Jeffrey R. Hollerman. 2009. *The Ventral Striatum. A Heterogeneous Structure Involved in Reward Processing, Motivation, and Decision-Making*. Elsevier Inc.

Tsai, Peter T., Court Hull, Yunxiang Chu, Emily Greene-Colozzi, Abbey R. Sadowski, Jarrett M. Leech, Jason Steinberg, Jacqueline N. Crawley, Wade G. Regehr, and Mustafa Sahin. 2012. “Autistic-like Behaviour and Cerebellar Dysfunction in Purkinje Cell Tsc1 Mutant Mice.” *Nature* 488(7413):647–51.

Tung, Emily W. Y., and Louise M. Winn. 2011. “Valproic Acid-Induced DNA Damage Increases Embryonic P27 KIP1 and Caspase-3 Expression: A Mechanism for Valproic-Acid Induced Neural Tube Defects.” *Reproductive Toxicology* 32(3):255–60.

Upton, Dominic, and Rhiannon Corcoran. 1995. “The Role of the Right Temporal Lobe in Card Sorting: A Case Study.” *Cortex* 31(2):405–9.

Vanderschuren, Louk J. M. J., E. J. Marijk. Achterberg, and Viviana Trezza. 2016. “The Neurobiology of Social Play and Its Rewarding Value in Rats.” *Neuroscience and Biobehavioral Reviews* 70:86–105.

Vanderschuren, Louk J. M. J., and Viviana Trezza. 2014. “What the Laboratory Rat Has Taught Us about Social Play Behavior: Role in Behavioral Development and Neural Mechanisms.” *Current Topics in Behavioral Neurosciences* 16:189–212.

Varga, Elizabeth A., Matthew Pastore, Thomas Prior, Gail E. Herman, and Kim L. McBride. 2009. "The Prevalence of PTEN Mutations in a Clinical Pediatric Cohort with Autism Spectrum Disorders, Developmental Delay, and Macrocephaly." *Genetics in Medicine* 11(2):111–17.

Vincen, John B., Jo Anne Herbrick, Hugh M. D. Gurling, Patrick F. Bolton, Wendy Roberts, and Stephen W. Scherer. 2000. "Identification of a Novel Gene on Chromosome 7q31 That Is Interrupted by a Translocation Breakpoint in an Autistic Individual." *American Journal of Human Genetics* 67(2):510–14.

Wager, Tor D., Matthew L. Davidson, Brent L. Hughes, Martin A. Lindquist, and Kevin N. Ochsner. 2008. "Prefrontal-Subcortical Pathways Mediating Successful Emotion Regulation." *Neuron* 59(6):1037–50.

Walcott, Elisabeth C., Emily A. Higgins, and Niraj S. Desai. 2011. "Development/Plasticity/Repair Synaptic and Intrinsic Balancing during Postnatal Development in Rat Pups Exposed to Valproic Acid in Utero."

Waltereit, Robert, Birte Japs, Miriam Schneider, Petrus J. De Vries, and Dusan Bartsch. 2011. "Epilepsy and Tsc2 Haploinsufficiency Lead to Autistic-like Social Deficit Behaviors in Rats." *Behavior Genetics* 41(3):364–72.

Wang, Chao Chuan, Po See Chen, Chien Wen Hsu, Shou Jung Wu, Chieh Ting Lin, and Po Wu Gean. 2012. "Valproic Acid Mediates the Synaptic Excitatory/Inhibitory Balance through Astrocytes - A Preliminary Study." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 37(1):111–20.

Wang, Xiaoming, Portia A. McCoy, Ramona M. Rodriguiz, Yanzhen Pan, H. Shawn Je, Adam C. Roberts, Caroline J. Kim, Janet Berrios, Jennifer S. Colvin, Danielle Bousquet-Moore, Isabel Lorenzo, Gangyi Wu, Richard J. Weinberg, Michael D. Ehlers, Benjamin D. Philpot, Arthur L. Beaudet, William C. Wetsel, and Yong Hui Jiang. 2011. "Synaptic Dysfunction and Abnormal Behaviors in Mice Lacking Major Isoforms of Shank3." *Human Molecular Genetics* 20(15):3093–3108.

Warburton, Pamela, Gillian Baird, Wai Chen, Kathleen Morris, Brian W. Jacobs, Shirley Hodgson, and Zoe Docherty. 2000. "Support for Linkage of Autism and Specific Language Impairment to 7q3 from Two Chromosome Rearrangements Involving Band 7q31." *American Journal of Medical Genetics - Neuropsychiatric Genetics* 96(2):228–34.

Wassink, Thomas H., Linda M. Brzustowicz, Christopher W. Bartlett, and Peter Szatmari. 2004. "The Search for Autism Disease Genes." *Mental Retardation and Developmental Disabilities Research Reviews* 10(4):272–83.

Wendy S. Biggs, MD. 2009. “Managing Pregnancy-Related Issues in Women with Epilepsy.” *NEJM Journal Watch* 2009.

Weston, Jennifer, Rebecca Bromley, Cerian F. Jackson, Naghme Adab, Jill Clayton-Smith, Janette Greenhalgh, Juliet Hounsome, Andrew J. Mckay, Catrin Tudur Smith, and Anthony G. Marson. 2016. “Monotherapy Treatment of Epilepsy in Pregnancy: Congenital Malformation Outcomes in the Child.” *Cochrane Database of Systematic Reviews* 2016(11).

Wiltse, Jeanette. 2005. “Mode of Action: Inhibition of Histone Deacetylase, Altering WNT-Dependent Gene Expression, and Regulation of Beta-Catenin - Developmental Effects of Valproic Acid.” *Critical Reviews in Toxicology* 35(8–9):727–38.

Winden, Kellen D., Darius Ebrahimi-Fakhari, and Mustafa Sahin. 2018. “Abnormal MTOR Activation in Autism.” *Annual Review of Neuroscience* 41(1):1–23.

Wink, Logan K., Sarah Fitzpatrick, Rebecca Shaffer, Sophia Melnyk, Amber H. Begtrup, Emma Fox, Tori L. Schaefer, Lauren Mathieu-Frasier, Balmiki Ray, Debomoy Lahiri, Paul A. Horn, and Craig A. Erickson. 2015. “The Neurobehavioral and Molecular Phenotype of Angelman Syndrome.” *American Journal of Medical Genetics, Part A* 167(11):2623–28.

Wolf, Uri, Mark J. Rapoport, and Tom A. Schweizer. 2009. “Evaluating the Affective Component of the Cerebellar Cognitive Affective Syndrome.” *Journal of Neuropsychiatry and Clinical Neurosciences* 21(3):245–53.

Won, Hyejung, Won Mah, and Eunjoon Kim. 2013. “Autism Spectrum Disorder Causes, Mechanisms, and Treatments: Focus on Neuronal Synapses.” *Frontiers in Molecular Neuroscience* 6(JULY):1–26.

Wood, Jacqueline N., and Jordan Grafman. 2003. “Human Prefrontal Cortex: Processing and Representational Perspectives.” *Nature Reviews Neuroscience* 4(2):139–47.

Wyszynski, D. F., M. Nambisan, T. Surve, R. M. Alsdorf, C. R. Smith, and L. B. Holmes. 2005. “Increased Rate of Major Malformations in Offspring Exposed to Valproate during Pregnancy.” *Neurology* 64(6):961–65.

Xing, Xiaoliang, Jing Zhang, Kunyang Wu, Beibei Cao, Xianfeng Li, Fang Jiang, Zhengmao Hu, Kun Xia, and Jia Da Li. 2019. “Suppression of Akt-MTOR Pathway Rescued the Social Behavior in Cntnap2-Deficient Mice.” *Scientific Reports* 9(1):1–9.

Xiong, Qiaojie, Hysell V. Oviedo, Lloyd C. Trotman, and Anthony M. Zador. 2012. “PTEN Regulation of Local and Long-Range Connections in Mouse Auditory Cortex.” *Journal of Neuroscience* 32(5):1643–52.

Zhang, Xiaoli, Ningren Cui, Zhongying Wu, Junda Su, Jyothirmayee S. Tadepalli, Sowmya Sekizar, and Chun Jiang. 2010. “Intrinsic Membrane Properties of Locus Coeruleus Neurons in *Mecp2* -Null Mice.” *American Journal of Physiology-Cell Physiology* 298(3):C635–46.

Zhou, Jing, Jacqueline Blundell, Shiori Ogawa, Chang Hyuk Kwon, Wei Zhang, Christopher Sinton, Craig M. Powell, and Luis F. Parada. 2009. “Pharmacological Inhibition of MTORCl Suppresses Anatomical, Cellular, and Behavioral Abnormalities in Neural-Specific PTEN Knock-out Mice.” *Journal of Neuroscience* 29(6):1773–83.