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Cycle XXXI**

**Role of $\alpha V\beta 3$ integrin in cortical
synaptic transmission:
relevance for Autism Spectrum Disorder
and Epilepsy**

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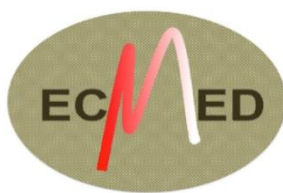


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1 Abstract

Autism Spectrum Disorder (ASD) is a group of neurodevelopmental disorders characterized by social communication impairments and restricted interests. Epilepsy is a brain disorder diagnosed after the occurrence of at least one epileptic seizure. ASD and epilepsy are often comorbid.

The integrin $\alpha V\beta 3$ is a synaptic cell adhesion molecule that binds to counter-receptors and extracellular matrix proteins. *Itgb3*, the gene encoding the $\beta 3$ subunit of integrin $\alpha V\beta 3$, has been associated to ASD in humans, and constitutive knockout (KO) mice for this gene exhibit ASD-like behaviors, while brain-specific conditional KO mice for the other subunit of integrin $\alpha V\beta 3$ (αV) have higher propensity for seizures.

Integrin $\alpha V\beta 3$ may, therefore, play important neuronal roles in brain regions relevant for ASD and epilepsy. To test this hypothesis, I expanded the behavioral characterization of *Itgb3* constitutive KO mice and characterized the behavioral phenotype of an *Itgav* conditional KO mouse specific for excitatory neurons of the forebrain. In these mouse models, I also investigated the synaptic dysfunctions of excitatory synapses onto layer V pyramidal neurons of the medial prefrontal cortex. I find that loss of integrin $\alpha V\beta 3$ leads to deficits in social novelty preference, hyper-reactivity to acoustic stimuli of high intensity, and reduces the threshold for pharmacologically induced seizures. This correlates with a reduction in the content of GluA2-lacking AMPA receptors in excitatory inputs to layer V pyramidal neurons.

2 Introduction

According to Eric Kandel, receiver of the Nobel Prize in Physiology and Medicine in 2000, the most prominent challenge current neuroscientists face is to unify the study of behavior (the science of the mind) and neural science (the science of the brain) (Kandel et al., 2013). Indeed, it is believed that all behavior, including complex cognitive processes, is the result of brain function. In this optics, all psychiatric illnesses, result from disturbances in brain function (Kandel et al., 2013).

Brain development and function depend on the correct differentiation and organization of cells within the brain structures forming functional circuits and connections (Dityatev et al., 2014). Cell adhesion, including cell-cell interaction and cellular interaction with the extracellular matrix (ECM), is an essential mechanism for correct brain development and network activity modulation. The interaction of brain cells with ECM allows for the regulation of their polarity, shape, survival, and proliferation. Moreover, ECM provides a scaffold for the highly complex brain architecture, regulating neuronal migration, neurite projection, synapse location, glial and vascular organization, among others. ECM plays, therefore, an active role regulating synaptic activity, circuit function, with implications in animal behavior and disease (Dityatev et al., 2014; Kandel et al., 2013; Scheiffele, 2003; Togashi et al., 2009).

In this thesis, I investigated the role of $\alpha V\beta 3$ integrin, a receptor for the ECM and counter-receptors that contain one or more RGD sequences, in brain function. By combining behavioral and electrophysiological experiments in mice with genetic

deletion of the genes coding αV and $\beta 3$ integrins, I provide evidence that the function of $\alpha V\beta 3$ integrin in the brain is relevant for ASD and epilepsy.

This work is part of ECMED consortium (Marie Skłodowska-Curie grant agreement No 642881) that aims at understanding the dynamics of ECM in epilepsy, finding new biomarkers for epileptogenesis, and designing new therapeutics targeting ECM molecules.

2.1 Autism Spectrum Disorders

ASD is a group of neurodevelopmental disorders characterized by two core domains: social communication impairment and restricted interests/repetitive behaviors (American Psychiatric Association, 2013; Sharma et al., 2018). More specifically, ASD individuals show deficits in social-emotional reciprocity, deficits in nonverbal communication, and deficits in developing, maintaining, and understanding relationships. Regarding restricted and repetitive patterns of behavior, children with ASD may show stereotyped or repetitive motor movements, insistence on sameness (inflexible adherence to routines or ritualized patterns of behavior), or highly restricted interests with abnormally increased focus. Additionally, ASD individuals may show hyper- or hyporeactivity to sensory input, such as indifference to pain/temperature, adverse response to specific sounds or textures, and excessive smelling or touching objects. Associated features supporting diagnosis include intellectual impairments, motor deficits (including odd gait), self-injury, increased anxiety and depression, for example (American Psychiatric Association, 2013).

As reported by the World Health Organization¹, one in 160 children worldwide has a form of ASD. In the United States, prevalence can be as high as 1 patient in 36 children (Zablotsky et al., 2017), and it is four times higher in boys than in girls (Baio et al., 2018). While most of the cases begin in childhood, they persist into adolescence and adulthood (Sharma et al., 2018).

ASDs have been typically classified into syndromic and nonsyndromic based exclusively on clinical criteria. Syndromic ASD refers to conditions in which ASD co-occurs with additional phenotypes or dysmorphic features, and account for around 10% of the cases. In most cases of syndromic conditions, a genetic etiology is already known (Sztainberg and Zoghbi, 2016). However, non-syndromic ASD has unknown etiology. For non-syndromic ASD, risk factors include genetic factors (Hallmayer et al., 2011), parental history of psychiatric disorders (Jokiranta et al., 2013), pre-term birth (Schendel and Bhasin, 2008), maternal immune activation during pregnancy (Estes and McAllister, 2016), and fetal exposure to psychotropic drugs (Gardener et al., 2009), among others (see Sharma et al., 2018). However, it is well established that there is a very strong genetic component in ASD (Folstein and Rosen-Sheidley, 2001; De Rubeis et al., 2014; Sestan and State, 2018).

¹ <http://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders>

The majority of high-confidence risk genes to ASD encode synaptic proteins, chromatin remodeling, and transcriptional regulators, as represented in **Fig. 1** (De Rubeis et al., 2014; Sestan and State, 2018). Many cell adhesion molecules (CAMs) and components of the ECM are ASD risk genes, such as $\beta 3$ integrin (O'Roak et al., 2012), reelin (Holt et al., 2010) and neuroligin 3 (Ylisaukko-oja et al., 2005).

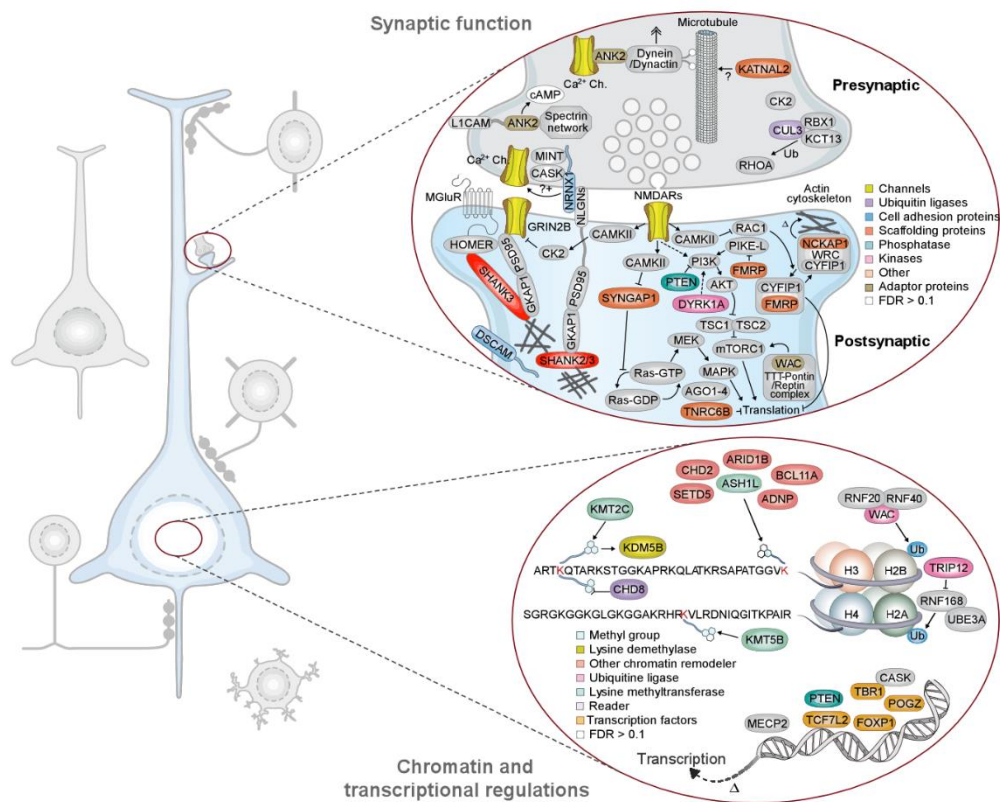


Figure 1. High-confidence nonsyndromic (nsASD) and selected syndromic risk genes for ASD. High-confidence ASD risk genes encode mainly synaptic proteins, chromatin, and transcriptional regulators. The diverse intracellular distribution and pleiotropic roles of nsASD genes and selected syndromic genes is shown inside the red outlined circles (Sestan and State, 2018).

Neurobiological studies point to altered brain connectivity in ASD individuals, namely a long distance cortical and subcortical under-connectivity with compensatory

shorter circuit over-connectivity (Kana et al., 2009; Sharma et al., 2018). Increased connectivity was also found between sensory cortices, the thalamus and basal ganglia (Cerliani et al., 2015), and between mentalizing and mirror neurons (Fishman et al., 2014). All these results indicate poor signal to noise discrimination in ASD children. The over-connectivity of certain circuits may be a compensatory mechanism for the incapacity of the brain to integrate multi-sensory stimuli (Sharma et al., 2018). Additionally, excitation and inhibition imbalances are often observed in animal models of ASD, and their correction normalizes ASD-like phenotypes in these animals (Bozdagi et al., 2013; Lee et al., 2017; Won et al., 2012).

Previously, evaluating the spatial and temporal pattern of expression of nine high-confidence ASD-risk genes, in the developing human brain, Willsey and colleagues provided robust evidence that glutamatergic projection neurons in layers V and VI of human midfetal prefrontal and primary motor-somatosensory cortex are a key point of convergence of these nine ASD-risk genes (Sestan and State, 2018; Willsey et al., 2013). This observation was corroborated by a cell type-specific expression analysis (CSEA) of ASD candidate genes performed by XU and colleagues. Output of CSEA reveals a substantial and consistent over-representation of layer V cortical neurons when considering different input data sources (cortical transcriptomic data from postmortem human tissue, and from the autism database AutDB) (Sestan and State, 2018; Xu et al., 2014).

Additionally, comorbidities occur in more than 70% of ASD cases and include epilepsy, language deficits, intellectual disability, motor abnormalities, anxiety, and gastrointestinal problems (Sharma et al., 2018; Sztainberg and Zoghbi, 2016).

2.2 Epilepsy

Epilepsy is a brain disorder diagnosed after the occurrence of at least one epileptic seizure and is characterized by a predisposition to the generation of epileptic seizures, and the neurobiological, cognitive, psychological, and social consequences from this condition (Fisher et al., 2005). According to the World Health Organization, about 50 million people worldwide suffer from this disease, making it one of the most common neurological disorders².

Not all epilepsies are the same, and their classification was revised recently considering three main levels. The starting point is the classification of the Seizure Type observed in the patient (Scheffer et al., 2017). Seizures may have focal, generalized or unknown onset (depending on the extensiveness of the brain area leading to the seizure initiation), and may have motor or non-motor features, as well as loss of consciousness (Fisher, 2017). The second level is that of Epilepsy Type, which includes focal (including unifocal and multifocal disorders, and seizures involving one brain hemisphere), generalized (generalized spike-wave activity on EEG), combined generalized and focal (patients who have both focal and generalized seizures), and unknown (when it is not possible to identify the type based on clinical data) categories. One type of epilepsy may include multiple types of seizures. The third level is the Epilepsy Syndrome diagnosis. A syndrome refers to clusters of features that incorporate seizure types, EEG and imaging data that tend to occur together (Scheffer et al., 2017).

² <http://www.who.int/news-room/fact-sheets/detail/epilepsy>

Epileptogenesis is a dynamic process of development and extension of tissue capable of generating spontaneous seizures, leading to an epileptic condition or the progression after the condition is established (Pitkänen et al., 2013). Many cellular mechanisms regarding structure organization such as neurodegeneration, neurogenesis, axonal damage or sprouting, and dendritic plasticity, are important for epileptogenesis (Dityatev et al., 2014; Pitkänen and Lukasiuk, 2009).

Epilepsies may have 6 different etiologies: genetic (result of known or presumed genetic mutations), structural, metabolic, immune, and currently unknown etiology (Myers et al., 2018; Scheffer et al., 2017). Although there are more than twenty antiepileptic drugs available in the market, still 30% of the patients remain unresponsive to current therapies. Furthermore, there are no treatments to combat epileptogenesis itself (Dityatev et al., 2014).

More than 500 loci have been associated with epilepsy, and the rapidly expanding array of genetic etiologies is helping to delineate the mechanisms and new treatments of this disease (Myers et al., 2018; Noebels, 2015; Ran et al., 2015; Staley, 2015). According to experimental work, epilepsy may arise from uncommonly high levels of network activity, or from vulnerabilities to activity-dependent shifts in the balance of inhibition and excitation (Staley, 2015). Indeed, it is possible to block seizures by increasing inhibition or decreasing excitation (Staley, 2015). However, analyses of the genetic etiology have only occasionally found direct causality between genetic mutations and imbalanced inhibition and excitation (Staley, 2015). Although none of the genes directly explains how a seizure is generated, spreads and stops, knowing the genetic identity of patients help to provide a molecular

diagnosis for the physician and offers a critical opportunity to develop new drugs (Noebels, 2015).

Regarding the molecular mechanisms of seizure generation (ictogenesis), seizures seem to be originated by impairments in synaptic transmission (Fukata and Fukata, 2017; Staley, 2015). Indeed, mutations in presynaptic proteins have been shown to be associated with epilepsy (Lazarevic et al., 2013; Rajakulendran et al., 2012), as well as in proteins important for postsynaptic mechanisms of synaptic plasticity (**Fig. 2**) (Fukata et al., 2006; Goodkin et al., 2008; Naylor et al., 2013).

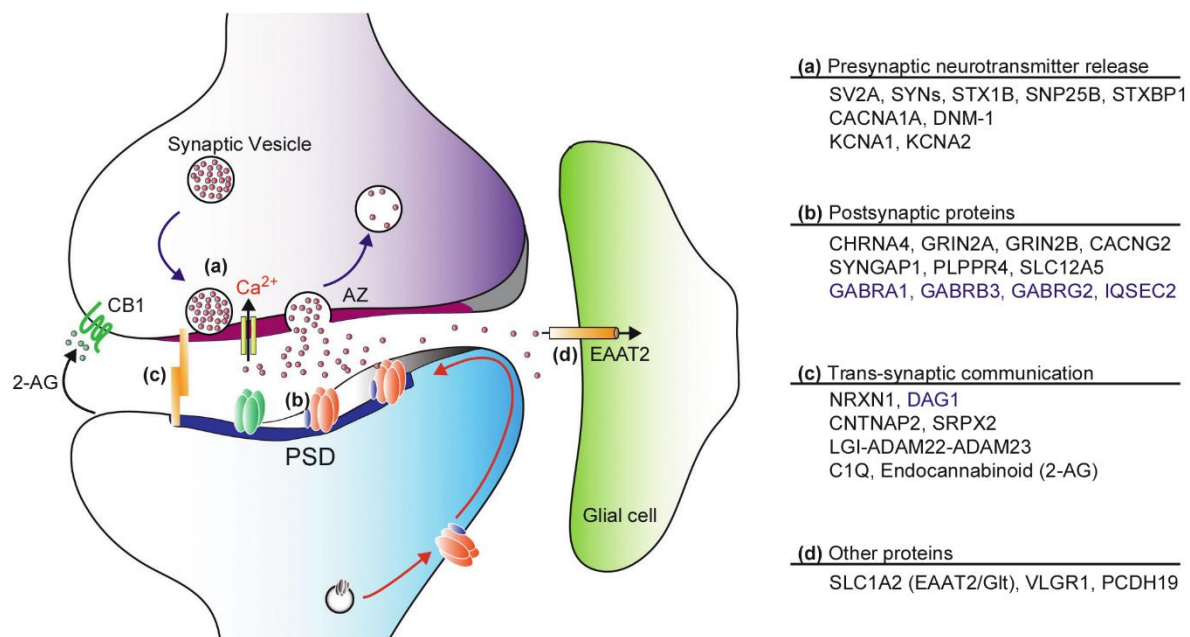


Figure 2. Selection of synaptic proteins implicated in epilepsy. Various synaptic proteins have been implicated in epilepsy etiology. These can be divided in four groups based in their synaptic localization and functions: (a) presynaptic neurotransmitter release machinery, (b) postsynaptic proteins, including ion channel subunits and scaffolding proteins, (c) *trans*-synaptic adhesion molecules and secreted proteins, (d) other epilepsy-related proteins including glial glutamate transporter (EAAT2), very large G protein-coupled receptor 1 (VLGR1), and protocadherin 19 (PCDH19). AZ, active zone; PSD, postsynaptic density (Fukata and Fukata, 2017).

2.3 Comorbidity between ASD and Epilepsy

Since ASD and epilepsy frequently coexist in the same individual, ASD and epilepsy are considered comorbid brain disorders (Bozzi et al., 2018). It is estimated that up to 40% of ASD patients also suffer from epilepsy and seizures (Canitano, 2007; Mannion and Leader, 2014). Moreover, ASD patients with no clinical epilepsy may still show epileptiform EEG activity (Chez et al., 2006; Rossi et al., 1995). Inversely, seizures halt the brain from normal activity and provoke long-lasting effects on emotional and cognitive functions. That is the case of individuals with temporal lobe epilepsy (TLE) showing hippocampal degeneration, and consequent learning and memory deficits (Ono and Galanopoulou, 2012), or patients with frontal lobe epilepsy (FLE) struggling in tasks that demand executive functions and decision making (Patrikelis et al., 2009). Notably, antiepileptic drugs such as topiramate, besides reducing seizures, also introduce side effects of cognitive impairment (Sommer et al., 2013).

Whether seizures during early development lead to later ASD phenotypes, or if seizures are just a marker of the severity of brain injury, is still an open question (Bozzi et al., 2018; Stafstrom and Benke, 2015). However, several studies reported that epilepsy can both precede or follow the manifestation of ASD, and a diagnosis of ASD-epilepsy comorbidity is usually obtained during early childhood or adolescence (Jeste and Tuchman, 2015).

The presence of comorbidities suggests a common anatomical, cellular, genetic, and molecular mechanisms underlying the pathological manifestations (Bozzi et al., 2018; Brooks-Kayal, 2011; Jeste and Tuchman, 2015). Indeed, histopathological

studies found malformations of cortical development (focal cortical dysplasia and heterotopias) in both ASD and epilepsy brain tissues (Bozzi et al., 2018). Moreover, genetic mutations discovered in ASD and epilepsy point to abnormalities in synapse function that alter the balance between neuronal excitation and inhibition (Bozzi et al., 2018; Stafstrom and Benke, 2015).

Several factors have been shown to impact on excitation and inhibition balance, such as synapse formation, development, maintenance and function (Bozzi et al., 2018; Stafstrom and Benke, 2015). In one hand, neurodevelopmental deficits related to inhibitory circuits seem to be prevalent. Impairments include defects in GABA receptors, GABAergic synaptic transmission (decreased GABA synthesis or increased GABA degradation, for example), or maturation of GABAergic interneurons and their connections. In the other hand, increased activity of glutamate receptor signaling leading to hyperexcitability has been found in cases of epilepsy and ASD (Bozzi et al., 2018; Oberman, 2012; Stafstrom and Benke, 2015). Accordingly, increased excitation is attributed to dysfunctions in depolarizing ion channels (sodium or calcium channelopathy, for example), or enhanced glutamate neurotransmission (more glutamatergic synapses, greater glutamate release, changes in glutamate receptor subunits, among others) (Stafstrom and Benke, 2015). Additionally, metabolic, immune and environmental disturbances may also compromise the balance between neuronal excitation and inhibition, as summarized in **Fig. 3** (Bozzi et al., 2018; Stafstrom and Benke, 2015).

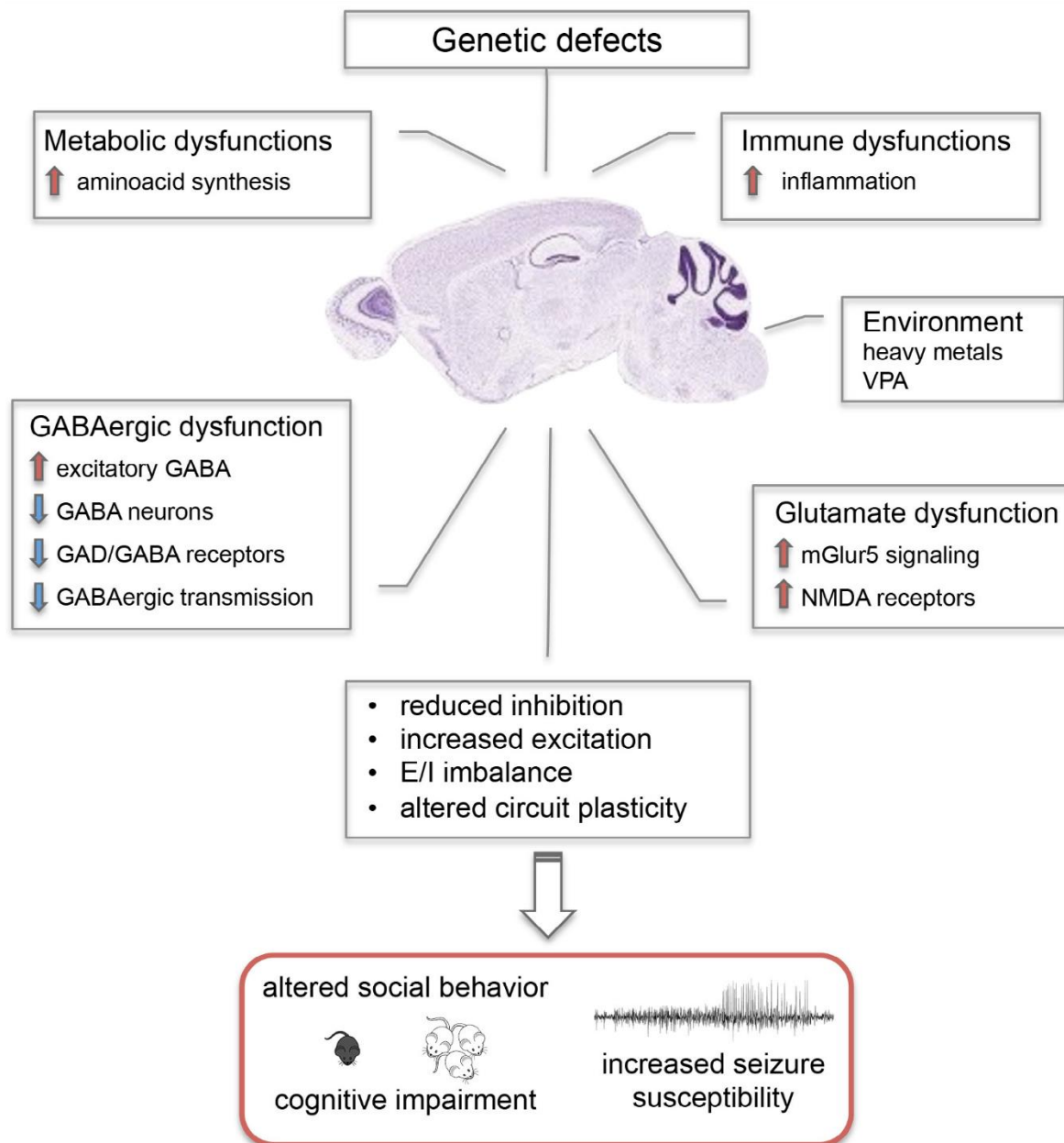


Figure 3. ASD and epilepsy share many neural substrates in rodent models. ASD-like and epileptic behaviors in rodents are a result of many factors, in which GABAergic and glutamatergic signaling deficits contribute largely to the comorbidity origin. Many ASD genes code for proteins involved in synaptic signaling, transcriptional regulation, and cell adhesion. Malfunction of these genes may result in decreased inhibition by impairing GABA signaling or increased excitation (by enhanced mGlu5/NMDAR signaling, for example). Metabolic, immune and environmental causes cannot be excluded. Experimental evidence suggests that similar mechanisms may be found in human patients (Bozzi et al., 2018).

While traditionally the GABAergic and glutamatergic systems have been the focus of research in ASD and epilepsy, genetic mutations that involve other aspects of neuronal function have been lately found and characterized (Stafstrom and Benke, 2015). It is now accepted that several structural and scaffold proteins that anchor synaptic machinery, regulate vesicle release, control subcellular signaling pathways, and govern neuronal migration and network connectivity, have important roles in neuronal excitability and are associated with ASD and epilepsy (Ebrahimi-Fakhari and Sahin, 2015; Stafstrom and Benke, 2015; Volk et al., 2015).

Accordingly, several CAMs and ECM elements have been implicated in both ASD and epilepsy. Several modifications in these molecules leads to phenotypes resembling both conditions. For example, knockout (KO) mice for *Reln* (the gene coding for reelin), display increased susceptibility to seizures and social deficits (Patrylo and Willingham, 2006; Patrylo et al., 2006; Salinger et al., 2003). This parallels observations in humans where reelin is genetically associated to both ASD (Chen et al., 2017; Persico et al., 2001) and epilepsy (Michelucci et al., 2017). Another example is the gene *PLAUR*, which codes for uPAR and has been associated with ASD (Campbell et al., 2008) and epilepsy (Royer-Zemmour et al., 2008) in humans. Mice lacking *Plaur* gene, replicate abnormal EEGs (Ndode-Ekane and Pitkänen, 2013) and impaired cognition (Bissonette et al., 2014) found in humans.

In summary, the study of the comorbidity between epilepsy and ASD with animal models allows the characterization of the genetic, molecular, physiological, and behavioral features of these disorders, which can be compared with human studies

(Stafstrom and Benke, 2015). Moreover, the study of the comorbidity between ASD and epilepsy is relevant because treating one disorder might benefit the other as well, as suggested by the improvement of ASD phenotype in patients and animal models after anticonvulsant administration (Robinson, 2012; Stafstrom and Benke, 2015).

2.4 Mouse models for ASD and epilepsy research and therapy design

The ideal animal model should have face (show the phenotypic features of the human condition), construct (recapitulate the causal mechanism underlying the disease in humans) and predictive validity (replicate the treatment responses seen clinically) (Grone and Baraban, 2015; Sztainberg and Zoghbi, 2016). Attributing ASD to a non-human animal represents a big challenge to get face validity for this disorder. Additionally, since ASD etiology is mostly unknown and no specific treatments are available for this condition, it is also complicate to have construct and predictive validity (Sztainberg and Zoghbi, 2016).

Nevertheless, the laboratory mouse, *Mus musculus*, is a social animal that displays a rich and complex social behavior repertoire that includes reciprocal interaction, social dominance, sexual behavior, parent care, among others (Silverman et al., 2010; Stafstrom and Benke, 2015; Sztainberg and Zoghbi, 2016). Therefore, many replicable behavioral tests have been already implemented to recapitulate ASD-like behavior in mice (Silverman et al., 2010). Moreover, 95 to 98% of mouse genes have an equivalent to the human genome, and current techniques allow activating and

repressing mouse genes in specific tissues or cell populations with precise time resolution. Therefore neuroscientist are able to address precise questions about the potential involvement of different genes in neural circuits in ASD and epilepsy pathology (Grone and Baraban, 2015; Noebels, 2015; Sztainberg and Zoghbi, 2016).

In the case of epilepsy, since human epilepsies comprehend a wide variety of clinical, behavioral, and electrical manifestations, scientists have brought forward an equally wide array of animal models (Grone and Baraban, 2015). Generally, ictogenesis is studied on both acutely provoked seizures (for example acute pentylenetetrazole (PTZ) injection) and on spontaneous seizures that arise in an epileptic animal during the chronic phase of the disorder (Grone and Baraban, 2015; Krook-Magnuson and Soltesz, 2015). More specifically, electrographic seizures are studied with EEG, which are in close association with observable and quantifiable convulsive behavior (Grone and Baraban, 2015).

Even if the rodents cannot recapitulate the human brain, studies with animal models largely recapitulate findings from human patients (Silverman et al., 2010; Stafstrom and Benke, 2015). Therefore, mouse models are a powerful tool to investigate the molecular, neuroanatomical and behavioral consequences of gene dysfunction in ASD and epilepsy, and are a substrate to test potential treatments (Bozzi et al., 2018; Stafstrom and Benke, 2015). Moreover, the comparative analysis between ASD mouse models with or without epilepsy might help identify the underlying mechanisms of the ASD-epilepsy comorbidity (Bozzi et al., 2018).

Since individuals with unexplained ASD and epilepsy are under increasing genomic scrutiny, powerful bioinformatics are helping identifying candidate genes of both disorders (Noebels, 2015). However, estimating the protein damage inflicted by a novel genetic variation requires supportive functional validation (Noebels, 2015). With the advent of precision medicine, the potential scientific and clinical return from each new gene identified urges the discovery and characterization of their singular and collective neurobiological impact (Myers et al., 2018; Noebels, 2015).

Therefore, the current study of ASD and epilepsy is an ensemble of many levels of basic and clinical research. It includes the genetic screening of patients, the bioinformatics treatment of that data, and validation of the genetic variations in animal models, as represented in **Fig. 4** (Myers et al., 2018; Noebels, 2015; Sztainberg and Zoghbi, 2016).

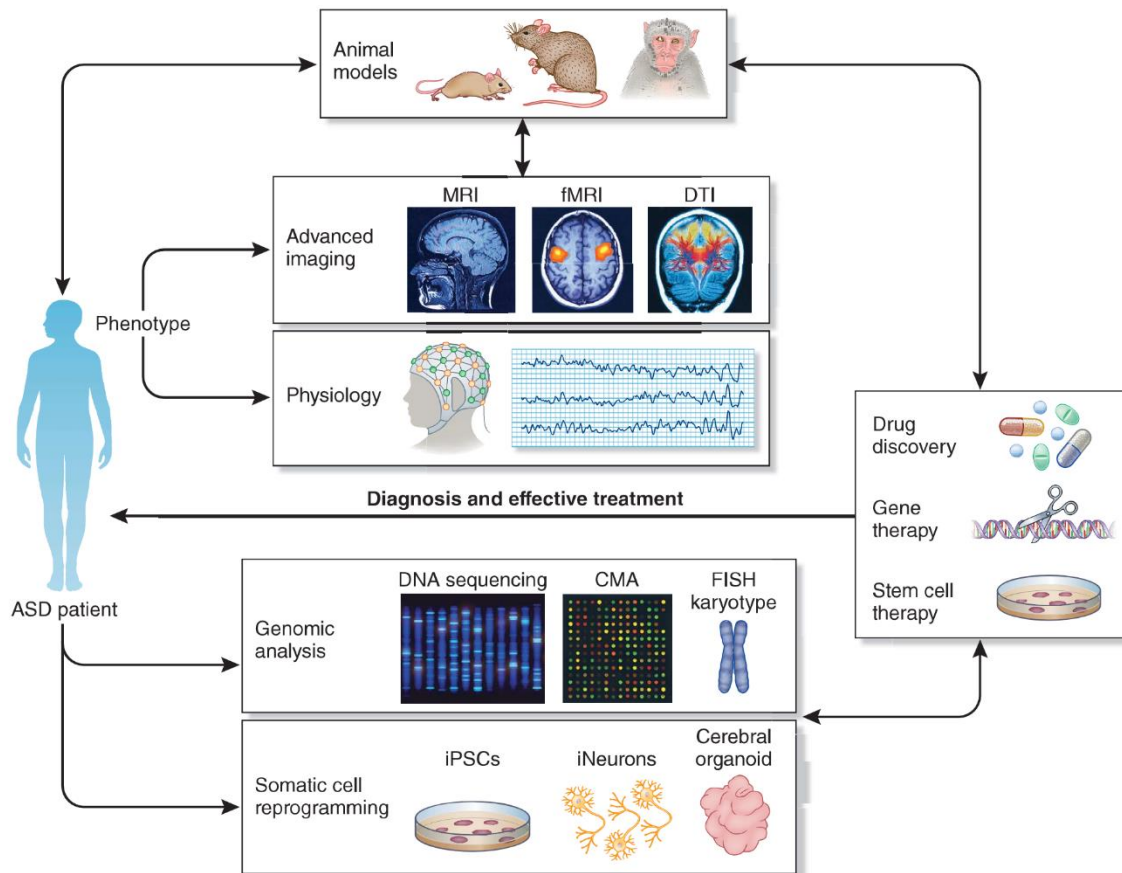


Figure 4. Animal and cellular models for ASD and epilepsy. ASD and epilepsy research today combines animal models, cells and organoids derived from patients, and advanced technology to diagnose and characterize human disease. This strategy allows scientists to see the whole picture, facilitating the development of effective diagnosis and treatment for human patients. Potential therapies are based in preclinical and clinical data, and they can be tested in animal and cellular models until approved for human use. MRI, magnetic resonance imaging; fMRI, functional MRI; DTI, diffusion tensor imaging; CMA, chromosomal microarray; FISH, fluorescence in situ hybridization; iNeurons, induced neurons derived from iPSCs. (Sztainberg and Zoghbi, 2016).

2.5 Cell adhesion and ECM in the healthy brain

Because several CAMs and proteins of the ECM have been implicated in ASD and epilepsy, I will make a brief introduction about these molecules to understand the context of my Ph.D. thesis.

The ECM functions as a structural scaffold and diffusion barrier in the brain. ECM is plastic and responsive to neuronal network activity (**Fig. 5**). By binding to cell surface receptors, or by releasing products of its activity-dependent proteolytic cleavage, ECM components activate signaling pathways in neurons. By incorporating secreted molecules and shed extracellular domains of transmembrane molecules, the ECM can change its composition, structure and functional properties (Dansie and Ethell, 2011; Dityatev et al., 2010, 2014; Frischknecht and Gundelfinger, 2012).

Elements of the ECM are secreted by both neurons and glial cells. The most conspicuous neural ECM structures are the perineuronal nets (PNNs), which have hyaluronic acid (HA) as backbone bound to chondroitin sulfate proteoglycans (CSPGs), link proteins, and tenascin-R. PNNs are found predominantly enwrapping subclasses of GABAergic inhibitory interneurons in the hippocampus and cortex (Brückner et al., 2003; Celio and Blumcke, 1994; Gundelfinger et al., 2010). Apart from PNNs, there is also a more diffuse HA-based ECM with similar composition around most of neurons in the brain, surrounding cell bodies, dendrites, the initial segment of some axons, and around synapses (Brückner et al., 2006; Dityatev et al., 2014; Gundelfinger et al., 2010).

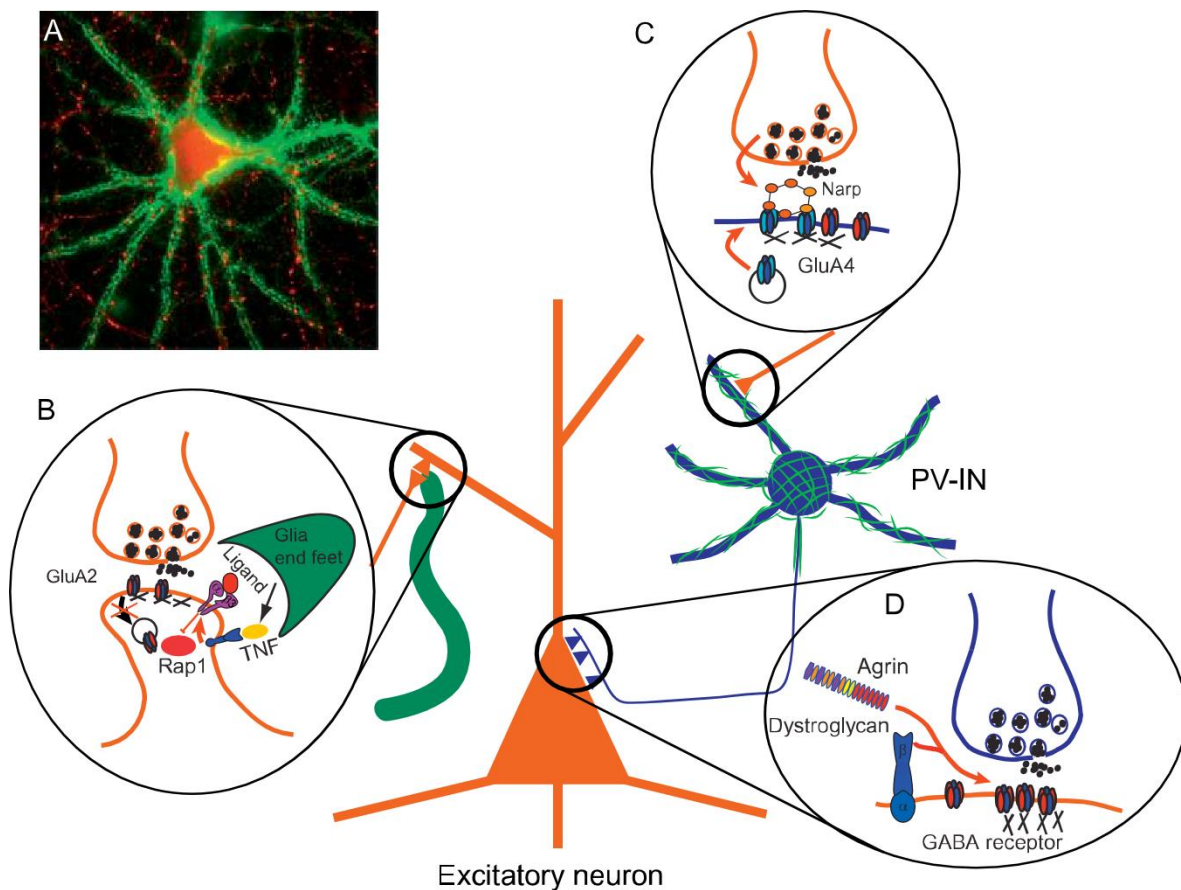


Figure 5. Perisynaptic ECM in the brain. (A) Perineuronal net ECM on an inhibitory neuron of dissociated cortical culture (green – brevican; red – GAD65) (B) TNF- α and β 3 integrin signaling in homeostatic plasticity. (C) Narp regulated excitatory synapses on parvalbumin-interneurons. (D) Dystroglycan and agrin regulate GABAergic synapse strength during network inactivation (Dityatev et al., 2014).

Based on their biochemical structure, CAMs can be grouped into families such as integrins, cadherins, immunoglobulin (Ig) superfamily, selectins, contactins, neuroligin/neurexin family, among others (Lodish et al., 2016; Sakurai, 2017; De Wit and Ghosh, 2016).

CAMs may associate laterally in homodimers or higher-order oligomers, or mediate interactions between opposing cell membranes from their extracellular domains (Lodish et al., 2016; Sakurai, 2017; Schwabe et al., 2009). The adhesion between cells can be tight and long-lasting, or relatively weak and transient (Lodish et al., 2016; Sakurai, 2017). Generally, the cytosol-facing domains of CAMs interact with sets of multifunctional adapter proteins, which directly or indirectly bind to elements of the cytoskeleton. Some CAMs also recruit intracellular molecules that belong to signaling pathways to control protein activity and gene expression (Hynes, 2002; Lodish et al., 2016). Therefore through CAMs, the surrounding environment of a cell impacts shape and functional properties of a cell in a process called “outside-in effects”, while the cellular internal signaling may also influence the cell’s surroundings in a process called “inside-out effects” (Hynes, 2002; Lodish et al., 2016).

During embryonic and post-natal development of the central nervous system (CNS), CAMs and ECM receptors are implicated in many events like axon growth and guidance, fasciculation, target recognition, synapse formation and maintenance, all together contributing for brain differentiation and wiring (Dityatev et al., 2014; Missaire and Hindges, 2015; Sakurai, 2017; Williams et al., 2010).

After synapses are established, many ECM receptors transition in function to control the stability of the connections and regulate synaptic plasticity events (Dityatev and Rusakov, 2011). ECM molecules modulate Hebbian synaptic plasticity and several forms of learning and memory. At the behavioral level, modifications of ECM

molecules facilitate reversal learning and extinction of fear memories (Dityatev et al., 2014).

In summary, interactions between CAMs and between ECM with their receptors are very important during brain development, brain wiring, and synapse physiology. Perturbations in their function impacts animal behavior, and thus they represent attractive targets for therapeutic intervention (Dityatev et al., 2014; Sakurai, 2017; Williams et al., 2010; De Wit and Ghosh, 2016).

2.6 Cell adhesion and ECM in the diseased brain

Human genetic studies using whole exome/genome sequencing have highlighted that many mutations in genes for CAMs are associated with psychiatric disorders such as ASD, epilepsy, intellectual disability (ID), Alzheimer's Disease (AD) and schizophrenia (Betancur, 2011; Redies et al., 2012; Sakurai, 2017; Zhong et al., 2015). These studies have produced extensive lists of genes (such as SFARI³ in the case of ASD, and szgene⁴ in the case of AD), including neuroligins, neurexins, contactins, and some cadherins and protocadherins, for example (Betancur, 2011; Jamain et al., 2003; Kim et al., 2008; Morrow et al., 2008).

As previously mentioned, CAMs regulate brain development including neuronal differentiation and migration, and synapse formation, maintenance, and function (Dityatev et al., 2014). Therefore, the associations of mutations in CAMs to brain

³ www.sfari.org/resources/sfari-gene

⁴ www.szgene.org

diseases do not come as a surprise. Several reasons have been given for these associations, such as changes in neurotransmission dynamics, in the formation of correct circuitries, in the excitatory/inhibitory balance, in neuromodulation, and impairments in homeostatic mechanisms (Sakurai, 2017).

Synapses allow the transmission of information across neurons. Changes in the strength and timing of synaptic transmission may impair information processing, altering neuronal network function and behavior (Titley et al., 2017). For example, neurons lacking α -neurexin show lower neurotransmitter vesicle release, possibly due to reduced $\text{Ca}_v2.1$ -mediated Ca^{2+} influx (Brockhaus et al., 2018). This shows that changes in CAMs may affect synaptic function.

As previously mentioned, the imbalance between excitation and inhibition is a putative mechanism for ASD and epilepsy (Lee et al., 2017; Staley, 2015). Several CAMs, such as neuroligins/neurexins and NCAM have been shown to regulate the formation and function of excitatory and inhibitory synapses (Brenneman et al., 2011; Tabuchi et al., 2007). Understanding in more detail how specific CAMs or ECM elements regulate this balance may allow the modulation of the stability of neuronal circuits.

Besides excitatory and inhibitory circuits, neuromodulatory transmission (including the systems of dopamine, noradrenaline, serotonin, acetylcholine, etc.) sends projections to many brain areas, thus affecting behavior (Sakurai, 2017). How adhesion regulate these systems is still relatively poorly understood. One example is cadherin 13, which has been related to addiction vulnerability and attention deficit

hyperactivity disorder (ADHD) probably due to changes in the mesocortical dopaminergic system (Drgonova and Walther, 2016).

Finally, homeostatic mechanisms are essential to keep neural activity inside physiological boundaries (Turrigiano, 2012). Brains deal constantly with multiple plastic challenges, such as developmental plasticity, learning, or lesion, and homeostatic plasticity is a mechanism to counterbalance these destabilizing stimuli. At the cellular level, homeostatic plasticity is achieved for example by altering surface receptor levels, or vesicle release rate (Sakurai, 2017). CAMs such as $\beta 3$ integrin have been implicated in these mechanisms (Cingolani et al., 2008).

Therefore, CAMs and ECM molecules are important for normal brain function and have been implicated in several brain disorders (including ASD and epilepsy) (Dityatev et al., 2014). Since ECM molecules and their receptors are easily targeted by antibodies, peptides, glycosaminoglycans, and other compounds, they are potential target of new therapies (Dityatev et al., 2014).

2.7 Cell adhesion and ECM in the brain: Integrins

Integrins are a class of adhesion receptors that connect physically and functionally the ECM and the cell cytoskeleton. Each integrin is composed of non-covalently bonded heterodimers of α and β subunits. In mammals, there are 24 different integrin heterodimers but only a subset of them is expressed in the brain. In particular, integrins $\alpha 3$, $\alpha 5$, $\alpha 8$, αV , $\beta 1$, and $\beta 3$ have been shown to play important roles in neuronal migration, synapse and dendrite development, morphogenesis and

synaptic plasticity (Gupton and Gertler, 2010; Hynes, 2002; McCarty et al., 2005; Pinkstaff et al., 1999; Wu and Reddy, 2012).

Integrin β subunits contain intracellular tails that bind to the cytoskeleton and cytoplasmic signaling proteins and activate signaling pathways, while α subunits extracellular domains bind and confer most of the ligand specificity (Dityatev et al., 2014; Hynes, 2002). Integrins containing $\alpha 5$, $\alpha 8$ or αV subunits recognize the Arg-Gly-Asp (RGD) binding motif on target proteins, such as fibronectin, vitronectin, tenascins, and thrombospondins, and are therefore called RGD integrins. Integrins with $\alpha 3$, $\alpha 6$ or $\alpha 7$ subunits bind to the laminin family of proteins (Humphries, 2006; Hynes, 2002). Additionally, other proteins may bind and cluster with integrins behaving as co-receptors, altering ligand specificity or integrin function, such as N-cadherin and tetraspanin (Bassani and Cingolani, 2012; Mortillo et al., 2012). Therefore, integrins can serve as a true hub, modulator, or amplifier of signals passing through the cell, placing them in a central position to regulate multiple cellular processes (Dityatev et al., 2014).

Integrins can assume an active and inactive conformation. When inactive, they adopt a compact conformation that hides both extracellular and intracellular binding-sites (Hynes, 2002). They can be activated bi-directionally, which causes conformational and functional changes. Generally, inside-out signaling is initiated by talin or kindling proteins. On the extracellular side, ECM proteins and soluble ligands bind to integrin extracellular domains, activating them in an outside-in fashion (Hynes, 2002).

Integrins control synapse function throughout development. At early stages of synapse formation, presynaptic $\beta 1$ integrins and post-synaptic intercellular adhesion

molecule 5 (ICAM-5) interact between filopodia tips and axonal terminals, presumably to maintain immature spines in a highly dynamic state and oppose their maturation (Ning et al., 2013). During maturation of glutamatergic synapses, there is a reduction in glutamate release probability, and a transition from GluN2B-containing to GluN2A-containing NMDA receptors (NMDAR) (Cull-Candy et al., 2001), which can be blocked by chronic inhibition of β 3-containing integrins (Chavis and Westbrook, 2001). Many studies show that integrins also control synaptic plasticity in mature synapses. Application of echistatin (a peptide that inhibits β 1 and β 3 integrins) to hippocampal slices suppresses LTP (Chun et al., 2001). Genetic ablation of β 1 integrins in mature excitatory neurons also impairs LTP (Chan et al., 2003). Notably, integrin-blocking antibodies also block LTP (LeBaron et al., 2003; Wang et al., 2008).

Complementarily, digestion of ECM by chondroitinase ABC (ChABC) in hippocampal slices, increases motility of dendritic spines and the appearance of abnormal spine protrusions, which correlates with activation of β 1 integrins and focal adhesion kinase (FAK) at synapses (Orlando et al., 2012).

Moreover, astrocytes also interact with neurons to control integrin expression by releasing secreted acidic and rich in cysteine protein (SPARC). In neurons, SPARC downregulates surface expression of β 3 integrin, and GluA1- and GluA2-containing AMPA receptors (AMPA) (Jones et al., 2011).

Together, these studies show that integrins modulate dendritic spine morphology and maturation, and synaptic plasticity and show that the surrounding environment through integrin-mediated signaling may modulate neuronal activity.

Interference with integrin signaling not only affects synaptic maturation and plasticity but also animal behavior. KO of $\beta 1$ integrin is accompanied by defects in novel object recognition tests and mitigates behavioral consequences of cocaine exposure (Warren et al., 2012). KO of $\alpha 3$ integrin yields defects in hippocampal LTP, defects in hippocampus-dependent working memory, and novel object recognition tests (Chan et al., 2003; Kerrisk et al., 2013). Triple heterozygous mice for $\alpha 3$, $\alpha 5$, and $\alpha 8$ integrin subunits exhibit impairments in hippocampal LTP and in spatial memory in the Morris Water Maze test (Chan et al., 2003).

Little is known about the roles of integrins in neuropsychiatric disorders. However indirect evidence relates integrin with pathological conditions such as memory deficits, brain tumor, Alzheimer's, stroke, ASD, and epilepsy (Wu and Reddy, 2012). In particular, $\beta 3$ integrin has been implicated in ASD (O'Roak et al., 2012). Chromosomal microdeletions, or duplications involving the genes coding for $\alpha 3$ and $\beta 1$ integrin, as well as their downstream partner Arg kinase, have been identified in cases of intellectual disability (Chaabouni et al., 2006; Preiksaitiene et al., 2012; Talkowski et al., 2012). Increased levels of $\alpha 2$ integrins in the anterior temporal neocortex were found from human tissue of intractable epilepsy (Wu et al., 2011), and $\beta 1$ integrin was shown to be increased in rat brains after seizures (Pinkstaff et al., 1998). Interestingly, RGD peptides reduced the number of bursts in rat hippocampal slices in a kindling model of epilepsy (Grooms and Jones, 1997). Together these data suggests that it is possible to modulate and attenuate epileptogenesis by targeting integrins and other ECM molecules.

2.8 α V β 3 integrin: a friend or a foe?

Integrins containing the α V subunit are RGD receptors. The α V subunit forms heterodimers with the beta subunits β 1, β 3, β 5, β 6, and β 8. On the contrary, the β 3 subunit forms heterodimers only with the alpha subunits α V and α IIb. Data from our group indicates that β 3 integrin is enriched selectively in layer V pyramidal neurons. α V integrin appears instead one of the most abundant and broadly expressed integrin subunits in the brain (Pinkstaff et al., 1999). The levels of α V are relatively high in the anterior olfactory nucleus, superficial and deep cortical layers and in Purkinje layer of the cerebellum (Pinkstaff et al., 1999).

At synapses (**Fig. 6**), β 3 integrin interacts directly with the GluA2 subunit of AMPARs, regulating AMPAR trafficking and synaptic strength in cultures of hippocampal neurons (Pozo et al., 2012), and is a central regulator of homeostatic synaptic plasticity (Thalhammer and Cingolani, 2014). Synaptic upscaling by chronic blockade of neuronal network activity is accompanied by an increase in surface levels of integrin β 3; moreover, synaptic upscaling is absent in β 3 integrin KO mice (Cingolani et al., 2008). By contrast, integrin β 3 does not interfere with Hebbian plasticity in the hippocampus (Mcgeachie et al., 2012). Additionally, surface levels of β 3 integrin are affected by astrocyte-secreted factors, such as tumor necrosis factor- α (Cingolani et al., 2008) and SPARC (Jones et al., 2011), raising the possibility that astrocytes are able to influence synaptic physiology through β 3 integrin.

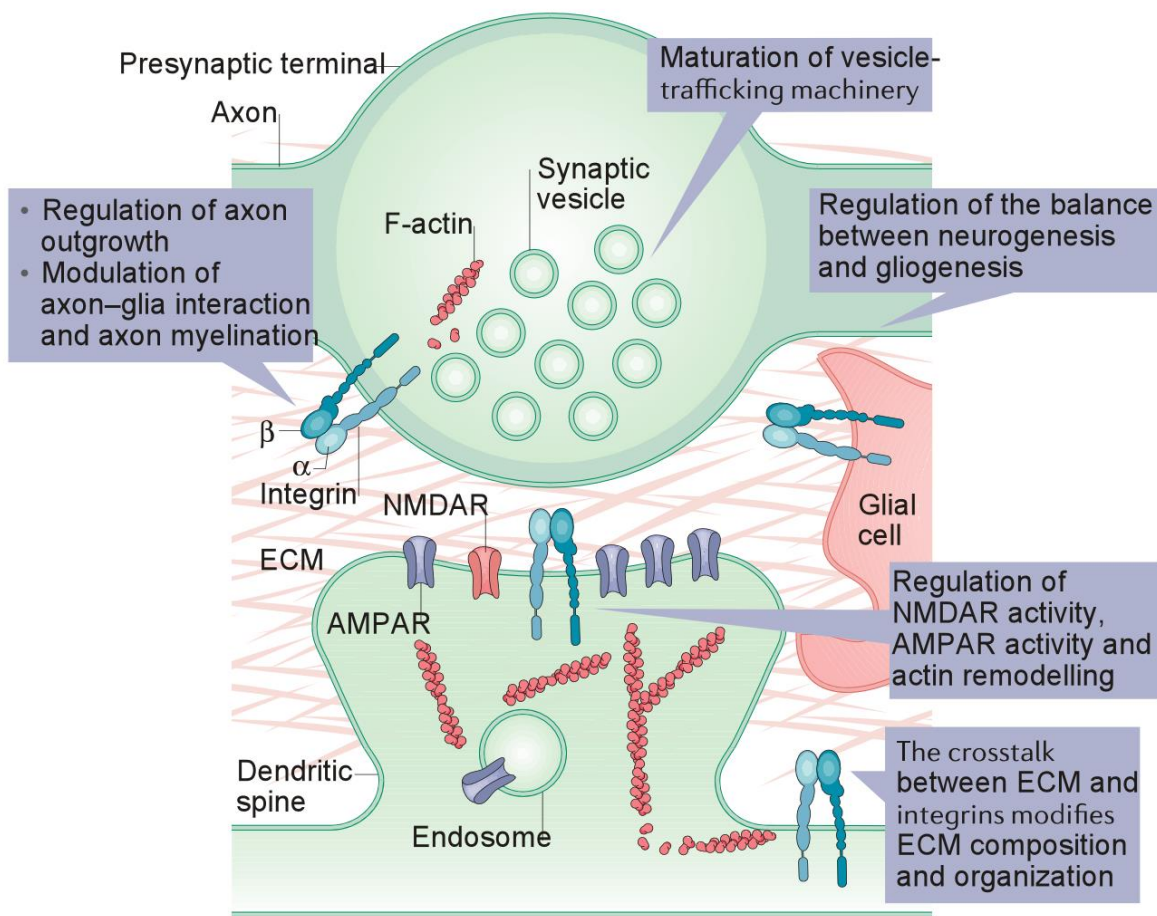


Figure 6. Integrin functions are associated with every component of the tripartite synapse. Functions of integrins in neurons include modulation of axonal outgrowth, regulate the composition of the ECM, regulation of AMPAR activity, among others (Park and Goda, 2016).

Several genetic reports have correlated ITGB3, the human gene for $\beta 3$ integrin, to ASD in a number of population cohorts (Napolioni et al., 2011; O'Roak et al., 2012; Schuch et al., 2014). In particular, one rare missense mutation in this gene was identified in an individual with ASD (O'Roak et al., 2012). Constitutive KO mice for *Itgb3* show social novelty deficits in the three-chamber test, and increased grooming in novel environments (Carter et al., 2011), mirroring the sociability defects and

presence of repetitive behaviors found in humans diagnosed with ASD (American Psychiatric Association, 2013). Additionally, ITGB3 was identified as a quantitative trait loci (QTL) for whole blood serotonin (Weiss et al., 2004, 2006). Notably, hyperserotonemia is found in about 30% of individuals with ASD (Cook and Leventhal, 1996). Recently, a knockin mouse (KI) carrying the human Pro33 variant of ITGB3 (ITGB3^{Leu33Pro}), which activates β 3 integrin, was generated. This ITGB3 polymorphism influences whole-blood serotonin levels. Mice carrying this mutation show repetitive behaviors (marble burying test) and deficits in anxiety (open-field) and social behaviors (social reciprocity- novel mouse and littermate). Additionally, the KI also has fewer serotonergic synapses in the midbrain, and reduced activity of the serotonin transporter (SERT) (Dohn et al., 2017).

Synaptic roles of α V integrin are much less known than those of β 3 integrin, but it has been implicated in some brain functions and human pathologies. In the mouse hippocampus, by electron microscopy, α V integrin was detected in dendritic spines, post-synaptic densities, and in some axon terminals and glial processes (Nishimura et al., 1998). Integrin α V is important for neuronal differentiation (Abe et al., 2018), and plays important roles in neuronal migration, particularly sustaining neuronal migration on radial glial fibers (Anton et al., 1999). Integrin α V mediates GDNF effects on dopaminergic neurons survival, outgrowth, dopamine turnover and locomotor activity in rats (Chao et al., 2003). By interacting with myelin proteolipid protein (PLP) in the cerebellum, α V integrin forms a complex with the GluA2 subunit of AMPARs (Harlow et al., 2015). In the medial prefrontal cortex (mPFC) of rats, the expression of integrin α V was changed after a protocol of post-traumatic stress

disorder (PTSD) (Li et al., 2015), and in the hippocampus, its expression changed after transient forebrain ischemia (Kang et al., 2008). Recently ITGAV, the human gene for αV integrin, was suggested as a Schizophrenia susceptible gene by bioinformatics analysis, and because this gene was found to be expressed differently in prefrontal cortex of 114 schizophrenia patients than in healthy controls (Zhang et al., 2018).

Furthermore, *Itgav* has been implicated in epilepsy in mutant mice. Full ablation of this gene leads to gestational or perinatal death, accompanied by developmental delay, intracerebral and intestinal hemorrhages (Bader et al., 1998). Restricting ablation of αV integrin to glial cells of CNS, using a hGFP-Cre conditional KO mouse line, leads to brain microhemorrhages with no additional aberrant phenotypes described. When αV integrin is knocked out in glial cells and neurons (using a Nestin-Cre conditional KO mouse line), thirty percent of the mice show behavioral defects, spontaneous convulsions and died by postnatal week four. The remaining animals survive several months, but show impaired hind limb coordination, ataxia, are physically weaker, and develop seizures by six months of age. At the cellular level, they present demyelination and axonal degeneration in the spinal cord and cerebellum (McCarty et al., 2005).

2.9 Aims of the project

Since $\beta 3$ integrin was implicated in ASD, and αV integrin was associated with epilepsy, the **overall aim** of this thesis is to identify the brain functions of $\beta 3$ and αV integrins.

The specific tasks are:

- 1) **To characterize the behavioral phenotypes** of the *Itgb3* and *Itgav* KO mice;
- 2) **To investigate the synaptic dysfunction** of the *Itgb3* and *Itgav* KO mice.

3 Methods

3.1 Mice and genotyping

All animal procedures were in accordance with the Italian Government and Italian Institute of Technology guidelines for animal welfare used in scientific research. All mice used for experimentation came from two separate colonies on C57BL/6J background. Animals were housed in a temperature- and humidity-controlled room under a 12:12 h light/dark cycle with lights on at 7am.

A constitutive knockout for the gene *Itgb3* was obtained from The Jackson Laboratory (B6.129S2-*Itgb3*^{tm1Hyn/J}) and was maintained crossing a wild type (*Itgb3*^{+/+}) or a knockout (*Itgb3*^{-/-}) male with heterozygous (*Itgb3*^{+/-}) females. In the former crossing, progeny was composed by 50% *Itgb3* WT (*Itgb3*^{+/+}, homozygous wild type) and 50% *Itgb3* Het (*Itgb3*^{+/-}, heterozygous for *Itgb3*), while in the latter crossing, progeny was composed by 50% *Itgb3* KO (*Itgb3*^{-/-}, homozygous knockout) and 50% *Itgb3* Het mice. Although we did not obtain *Itgb3* WT and KO littermates, all mothers had the same genotype (*Itgb3* Het) and were alternated between the two crossing schemes. Thus, *Itgb3* WT and KO mice shared the same mothers.

A conditional knockout for the gene *Itgav* was obtained crossing a line of *Itgav* double-floxed mice described elsewhere (McCarty et al., 2005), with *Camk2a-Cre* mice from the Jackson Laboratory (B6.Cg-Tg(CamK2a-cre)T29-1Stl/J). In this mouse line, *Itgav* is deleted from excitatory neurons of the forebrain, starting from the second-third postnatal week, following the pattern of expression of *Camk2a*. The colony was maintained crossing a control (*Itgav*^{fl/fl}) with a *Itgav* conditional knockout (*Itgav*^{fl/fl}*CamK2a-Cre*^{+/-}) mouse. The resulting progeny was composed by 50% *Itgav*

Control/Ctr (*Itgav*^{fl/fl}, the *Itgav* gene is double-floxed and expressed) and 50% *Itgav* KO (*Itgav*^{fl/fl}*CamK2a-Cre*^{+/-}, Cre is expressed in postnatal excitatory neurons of the forebrain inducing deletion of *Itgav* in these neuron types) mice, which could all be used for experimentation. For electrophysiology experiments, these mice were additionally crossed with a *tdTomato* Cre-dependent mouse line obtained from The Jackson Laboratory to express tdTomato, a red-fluorescent protein, in Cre-positive cells. To obtain mice for electrophysiology one mouse *Itgav*^{fl/fl}; *tdTomato*^{fl/fl} (the *Itgav* gene is double-floxed and expressed, the *tdTomato* transgene is double-floxed and not expressed) was crossed with a *Itgav*^{fl/fl}; *CamK2a-Cre*^{+/-} mouse (Cre is expressed in postnatal excitatory neurons of the forebrain inducing deletion of *Itgav*). The progeny was 50% *Itgav* Ctr (*Itgav*^{fl/fl}; *tdTomato*^{fl/-}, the *Itgav* gene is double-floxed and expressed, the transgene *tdTomato* is in heterozygosis and not expressed), and 50% *Itgav* KO (*Itgav*^{fl/fl}; *tdTomato*^{fl/-}; *CamK2a-Cre*^{+/-}, Cre is expressed in postnatal excitatory neurons of the forebrain inducing deletion of *Itgav* and expression of the *tdTomato* transgene in these neuron types). Since I performed whole-cell patch-clamp recordings at an age when *CamK2a-Cre* is not yet expressed in every pyramidal neuron, the TdTomato fluorescence allowed me to visualize and patch exclusively neurons that expressed *CamK2a-Cre* and that therefore had, presumably, reduced expression levels of integrin α V.

The genotype of the progeny was confirmed by standard PCR-based genotyping of DNA isolated from ear snips. All animals were re-genotyped after experimentation. The primer sequences used for PCR genotyping and the size of the PCR products can be seen in **table 1**.

Table 1. List of primers used for genotyping and their correspondent PCR products.

Gene	Primer sequence	PCR Product
<i>Itgb3</i>	5'-CACGAGACTAGTGAGACGTG-3' 5'-CTTAGACACCTGCTACGGGC-3' 5'-CCTGCCTGAGGCTGAGTG-3'	WT – 4127 bp KO – 533 bp Het – both bands
<i>Itgav floxed</i>	5'-CACAAATCAAGGATGACCAAAGTCTGAG-3' 5'-TTCAGGACGGCACAAGACCGTTG-3'	WT – 250 bp <i>Itgav</i> double floxed – 400 bp
<i>Camk2a-Cre</i>	5'-GCGGTCTGGCAGTAAAACTATC-3' 5'-GTGAAACAGCATTGCTGTCACTT-3' 5'-CTAGGCCACAGAATTGAAAGATCT-3' 5'-GTAGGTGGAAATTCTAGCATCATCC-3'	Control – 324 bp Cre – 100 bp
<i>tdTomato-floxed</i>	5'-AAGGGAGCTGCAGTGGAGTA-3' 5'-CCGAAAATCTGTGGGAAGTC-3' 5'-GGCATTAAAGCAGCGTATCC-3' 5'-CTGTTCTGTACGGCATGG-3'	WT – 297 bp <i>tdTomato^{ff}</i> - 196 bp <i>tdTomato^{f/-}</i> - both bands

3.2 Substances

The list of all substances used for experimentation and the supplier for each substance is present in **table 2**.

Table 2. List of substances used for experimentation and their supplier.

Supplier	Substance
Sigma-Aldrich	CsCl, CsMeSO ₃ , EGTA, D-(+)-Glucose, HEPES, KCl, MgATP, MgCl ₂ , MgSO ₄ , NaCl, Na ₂ CreatinineP, NaGTP, NaHCO ₃ , NaH ₂ PO ₄ , NaOH, Pentylenetetrazole (PTZ), (+)-Sodium L-Ascorbate, Sodium Pyruvate, TEACl
Tocris	D-AP5, (+)-Bicuculline, NBQX, Qx-314Cl, Ro 25-6981, Spermine tetrahydrochloride
Fluka	CaCl ₂ 1 M solution

3.3 Behavioral Tests

For behavioral experiments, only male animals aged between 2 and 5 months were used. In the case of PTZ test, the age of the mice ranged from 7 to 9 months. Mice were caged in groups of 2-4 siblings. Cages were enriched with a red plastic house and a card cylinder. Experimentation and analysis were performed under blind conditions. During three-chambered social approach task and olfactory habituation/dishabituation test, all animals from each cage were tested before testing the following cage. The order in which each cage was tested was randomly determined. In other experiments, the animal order of testing was also randomized. For each day of experiment, all genotypes were tested. All experiments were performed during light phase.

Open-field: Mice habituated for at least 30 min in an adjacent room to the testing room. After habituation, mice were placed in an open arena of 40 x 40 cm and video recorded for 15 min from above. Using Any-maze software, a central (20 x 20 cm)

and border areas of the open field were virtually defined. Total distance traveled, average speed and time in central area versus border area were automatically calculated from the videos, by tracking the center of the mouse body with Any-maze software.

Rotarod: An accelerating rotarod for mice from Jones & Roberts model 7650 was used to measure motor coordination. Mice were moved to testing area and allowed to habituate for at least 30 min. After habituation, animals were placed on the rotarod at minimum speed. When the test started, acceleration increased from 4 to 40 rpm over 5 min. The latency period to fall from the rod was measured for each mouse in 6 trials for two days (3 trials per day, 1 hour of rest between trials). Trials were ended if the mouse fell from the rod, if the mouse clung to the rod without walking for two revolutions, or if the mouse successfully walked on the rod for 6 min.

Three-chambered social approach task: Social preference over an object and preference for social novelty was tested using a three-chamber apparatus. The test consists in four phases of 10 min each. In the first phase, the tested mouse is restricted to the central chamber to habituate to the environment. In the second phase, the gates are opened and the mouse is allowed to explore and habituate to the empty lateral chambers. At the end of second phase, the mouse is temporarily restricted to the central chamber and the gates are closed. For the third phase, which tests sociability, one inverted empty pencil cup (object) is placed in one lateral chamber, and a stimulus mouse (stranger 1) is placed under a second inverted pencil cup in the second lateral chamber. Once the empty cup and stimulus mouse are positioned, the gates are opened and starts the third phase. During this phase, the time that the test mouse spends interacting with the stimulus mouse or with the

object is quantified with silent stop-watches. Interaction includes sniffing, body contact with the cup, or eye contact to the object/mouse in very proximity. At the end of third phase, the tested mouse is temporarily restricted to the central chamber once again. The fourth phase, which tests social novelty preference and social memory, consists in maintaining the first stimulus mouse (stranger 1) in the same chamber as before, so it can now be recognized as a familiar mouse, while a second stimulus mouse (stranger 2) is positioned under the previously empty pencil cup. Once the second stimulus mouse is positioned, the gates are open and starts the fourth phase. Time interacting with the now familiar mouse (stranger 1) or novel mouse (stranger 2) is measured like in the previous stage. The lateral chambers where the empty cup/stranger 2 and the stranger 1 are positioned are randomly attributed. The stimulus mice are C57BL/6J (obtained from Charles River) males, aged-matched to tested mice and have never been in contact with tested mice.

Olfactory habituation/dishabituation test: Each mouse was habituated to a closed testing cage containing fresh bedding and a plain cotton swab, for at least 30 min. The cotton swabs were placed 4-5 cm above the bedding. For olfactory testing, subjects were tested for time spent sniffing or nibbling cotton swabs saturated in different odors, which was quantified with a silent stopwatch. The test consisted in 15 trials of 2 min each and interval of about 1 min. Each odor was presented in three consecutive trials to allow habituation, and each trial corresponds to a new cotton swab. Habituation is characterized by a gradual reduction of interaction time with the cotton swab, while discrimination is identified by a reinstatement of a high level of interaction when a new odor is presented. Mice were exposed to water as a control, almond and banana scents for non-social odors, and two different social scents,

presented in this order. Almond and banana scents were obtained by diluting almond and banana extracts (TRS, UK) 1:100 in water, while social scents were obtained by sweeping the bottom of two different cages containing only stranger individuals to the testing mice. The cages used to obtain the social scents were not changed for at least 3 days before testing and contained 3-4 aged-matched males to the tested subjects.

Repetitive behaviors in a novel environment - self-grooming, vertical rearings and jumps: Self-grooming behavior was scored by placing each mouse directly from its home-cage in a new empty cage, without bedding, food or water. Mice were then transported to the experimental area and video recorded for 20 min. Mice were allowed to habituate to the new environment for the first 10 min. Then I measured the number of vertical rearings and jumps during the following 10 min. Grooming during the total duration of the test (20 min) was later measured in slots of 5 min using the video recordings.

Startle response and Paired-pulse Inhibition of Startle Response (PPI): Startle response and PPI were measured using Startle Response System from TSE Systems, consisting of an individual cage that restrain the animal, a noise stimulus generator, and a transducer platform, positioned inside a soundproof box. Mice were first habituated to the restraining cage for 5 min, one and two weeks prior to the startle protocol, and the PPI protocol, respectively. In each testing day, mice were weighed and then allowed to habituate for at least 30 min in a room adjacent to testing area. To measure the startle response, mice were exposed to noise stimuli of 70, 82, 90, 100, 110 and 120 dB, six times each stimulus, in a pseudo-randomized order and random inter-stimulus interval between 10 and 20 s. To measure the PPI,

mice were exposed to trials of a single stimulation of 120 dB, a stimulus of 120 dB preceded by a prepulse of 74, 78, 82, 86 or 90 dB, and trials with no noise stimulation. Each trial was repeated 6 times on a fully randomized order and inter-stimulus interval (between 10 and 20 s). Each stimulus and prepulse had a duration of 40 ms. The interval between each prepulse and the 120 dB stimulation was fixed at 100 ms. Startle response was measured automatically by the Startle Response System. The maximum startle amplitude recording during the 65 ms sampling window was used as testing variable.

Induced Seizures by Pentylentetrazole (PTZ): A fresh solution of PTZ was prepared each day of experimentation with a concentration of 4 mg/ml PTZ for *Itgb3* WT, Het and KO animals and 3.5 mg/ml PTZ for *Itgav* Ctr and KO animals, because the two colonies showed different sensibilities to PTZ in the pilot test. Tested mice were then moved to experimental area, weighted and transferred to a new empty cage. Each subject received consecutive intraperitoneal injections of 100 µl PTZ / 35 g with 10 min intervals for observation. During the 10 min of observation, the behavior outcome was scored according to the following scale: 0- no abnormal behavior, 1- reduced motility and prostate position, 2- partial clonus (neck and body jerk), 3- generalized clonus (including extremities) and stronger (loss of balance and wild jumping). For each injection, it was considered the highest score presented by the animal. The test proceeded until mice lost balance or showed wild jumps, after which was humanely sacrificed by cervical dislocation.

3.4 Electrophysiology

Ex-vivo brain slice preparation: Brain slices were prepared from males and females with 3 weeks of age when not mentioned otherwise. Slice preparation was done in the morning, and experiments were carried out in the afternoon (light period). Mice were anesthetized with CO₂ and sacrificed by cervical dislocation. Brains were immediately transferred in an ice-cold dissecting artificial cerebrospinal fluid (aCSF) containing (in mM): 100 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 5 NaAscorbate, 3 NaPyruvate, 25 D-Glucose, 10 MgSO₄, 6 NaOH, and 0.5 CaCl₂, aerated with 95% O₂ and 5% CO₂, and adjusted to 7.4 pH and 300 mOsm. Coronal sections (300 µm thick) of the frontal lobe were obtained using a vibratome VT1200 S (Leica). Slices were allowed to equilibrate for 30 min at 33 °C in recording aCSF and then at room temperature for more than 30 min before being transferred to the recording chamber.

Whole-cell patch clamp: During experiments, slices were continuously superfused at a rate of 2 ml / min at 30 ± 1 °C with a recording aCSF containing (in mM): 120 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 12.5 D-Glucose, 1.5 MgSO₄, 6 NaOH, and 2.5 CaCl₂, aerated with 95% O₂ and 5% CO₂, and adjusted to 7.4 pH and 300 mOsm. To block GABA_A receptors, all experiments were performed in the presence of (+)-bicuculline (10 µM) in the recording aCSF. When mentioned, NBQX (10 µM), D-AP5 (100 µM), and Ro 25-6981 (3 µM) was added to the recording aCSF as well. Whole-cell recordings were obtained from pyramidal neurons of layer V of the prelimbic and cingulate regions of the medial prefrontal cortex (mPFC). Glass micropipettes (3 - 4.5 MΩ) were filled with a solution containing (in mM): 120 CsMeSO₃, 17 CsCl, 1 TEACl, 10 Hepes, 0.5 EGTA, 1 MgCl₂, 4 MgATP, 0.5 NaGTP,

20 Na₂CreatinineP, 5 Qx-314Cl, adjusted to 7.29 pH and 290 mOsm. For AMPAR-mediated current rectification protocol, this solution was complemented with 0.5 mM Spermine.

Data were acquired using a double patch clamp EPC 10 USB amplifier (HEKA) controlled by Patchmaster (v2 x 73.1) software and sampled at 50 kHz.

For afferent electric stimulation of basal dendrites, theta-glass stimulating electrodes were placed in deep layer V (>100 μ m from the cell body of the recorded neuron, **Fig. 7**). Electric stimulation was generated by a A36 Stimulus Isolator (WPI) controlled by Patchmaster software. The intensity of the electric pulses (2 – 12 μ A) was regulated in order to evoke excitatory postsynaptic currents (EPSC) of similar amplitudes across slices and had a duration of 0.5 ms. All protocols included a depolarizing step of -5 mV during 100 ms to estimate input resistance (R_{in}). Data were excluded when R_{in} changed >20% during the course of the experiment.

Paired-pulse ratio was measured holding the patched neurons at -70 mV and evoking AMPAR EPSCs by a double electric stimulation with inter-stimulus interval of 10, 25, 50, 100 and 200 ms. Each pair of stimuli was repeated 6 times with 20 s interval.

NMDAR EPSCs were measured by holding the patched neurons at +60 mV in the presence of NBQX (10 μ M) to block AMPAR-mediated currents and evoked by a single electric pulse every 20 s. A baseline was determined for 15 sweeps / 5 min followed by application of Ro 25-6981 (3 μ M) for 20 min. The GluN2B component was estimated comparing the average of the NMDAR EPSC amplitude of the last three sweeps of the incubation period with Ro 25-6981 with that of the baseline. In subset of cells, subsequent application of D-AP5 (100 μ M) completely blocked the

remaining currents, confirming that it was NMDAR-mediated. Using the same averaged traces, it was calculated the NMDAR EPSC decay tau during baseline and after application of Ro 25-6981. The NMDAR EPSC decay tau was calculated dividing the maximum amplitude of the EPSC by the area below the curve from the moment of the maximum amplitude until it reduced to 10% of that value.

The NMDAR/AMPA ratio was estimated by comparing the average NMDAR EPSCs amplitude during baseline with the average of the first AMPA EPSCs evoked during the paired-pulse ratio protocol.

To measure the rectification of AMPA-mediated currents, patched neurons were hold sequentially at -70, -40, 0, and +40 mV in the presence of D-AP5 (100 μ M), and AMPA EPSCs were evoked by a single electric pulse at each membrane holding potential. The cycle was repeated 6 times with 20 s between stimulations. Patched neurons were hold at -50 mV between stimulations. Subsequent NBQX (10 μ M) application blocked all remaining current, confirming that it was AMPA-mediated. Data were excluded when currents at 0 mV after NBQX (10 μ M) application were measurable. The rectification index was calculated by dividing the AMPA-EPSC amplitudes measured at +40 mV and -70 mV.

For all experiments of electrophysiology, traces were analyzed with Clampfit and statistical analysis performed in GraphPad.

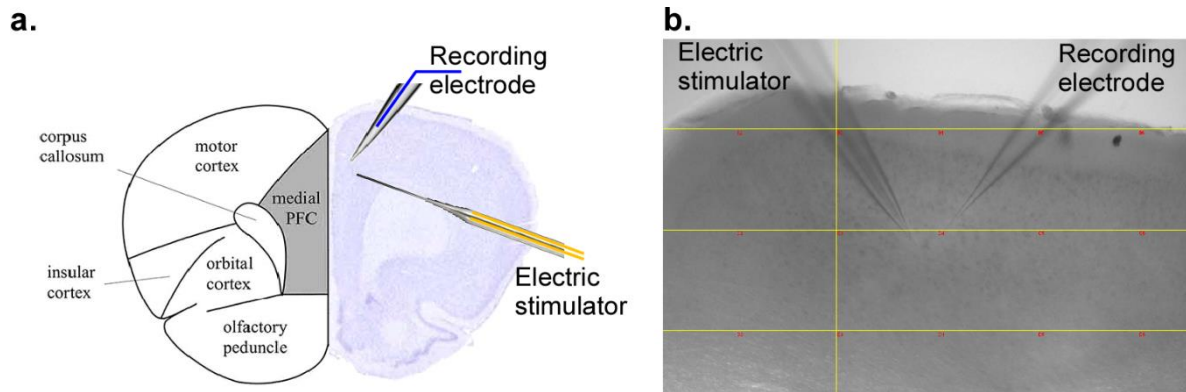


Figure 7. Schematics of the electrophysiology experiments. **a.** Schematics of the brain areas (mPFC) where the recording electrode and the electric stimulator were positioned. **b.** Image of a brain slice showing the position of the recording electrode and the electric stimulator. (scheme in **a.** adapted from (Grüter et al., 2015)).

3.5 Statistical analysis

Statistical analysis was performed with paired or unpaired Student's t-test, or with One- or Two-way analysis of variance (ANOVA) and Bonferroni post hoc test, using Prism GraphPad, as indicated. Box plots show median, 25 and 75 percentiles, with whiskers showing 10 and 90 percentiles. Dots represent individual samples. Data in bar charts and line graphs are shown as mean $\pm$ standard error of the mean (sem).

4 Results

I investigated the behavioral phenotype and excitatory synaptic transmission of the mPFC in two genetic models: a constitutive KO for $\beta 3$ integrin and a conditional KO for αV integrin obtained by crossing an *Itgav* double-floxed mouse with a *CamK2a-Cre* mouse, resulting in the ablation of *Itgav* from excitatory neurons of the forebrain starting from the second-third postnatal week.

Behavioral characterization of these mouse models focused on tasks involving sociability, repetitive behaviors, and sensorial sensibility (aspects related to ASD), and the measurement of threshold for seizures (propensity for epilepsy).

In order to understand how changes in the levels of $\beta 3$ and αV integrins lead to alterations in behavior, I investigated the roles of these integrins in excitatory synaptic transmission of the mPFC. Using acute coronal slices of this brain region, I performed recordings in whole-cell patch-clamp on pyramidal neurons of layer V of the mPFC and characterized the excitatory synaptic currents onto these neurons.

4.1 Knockout mice for $\beta 3$ or αV integrins display no defects in the Open-field test

Changed anxiety-levels is an associated symptom found in a subset of ASD patients. In mice, changed anxiety can introduce artifacts that could confound the interpretation of a mouse phenotype. For example, mice with anxiety-like traits engage less in exploratory activities, resulting in less entries in the lateral chambers of a three-chamber apparatus, which could render social approach data difficult to

interpret (Silverman et al., 2010). Locomotor impairments also introduce artifacts in tests that rely in mouse ambulatory behavior.

Since previous findings are contrasting, with data showing unaltered (Carter et al., 2011), or reduced anxiety (Mcgeachie et al., 2012), I started testing our mice in an open-field test.

Because mice tend to avoid open spaces, when they are placed in an open-field, they tend to spend more time in the peripheral area and only sporadically explore the central area. Mice with increased anxiety avoid even more the central area, while mice with risk assessment impairments tend to spend more time in the central area than normal (Seibenhener and Wooten, 2015).

Total ambulatory distance and average speed were calculated as measures of general motor activity and exploratory propensity of the mice. Here, I did not find any difference between *Itgb3* WT, Het and KO for total distance moved (*Itgb3* WT 57.3 ± 3.5 m, n = 12; Het 49.7 ± 2.6 m, n = 16; KO 50.0 ± 3.1 m, n = 19; p=0.1947 One-way ANOVA, **Fig. 8 a.**) and for average speed (*Itgb3* WT 9.2 ± 1.5 cm/s, n = 12, Het 8.2 ± 1.3 cm/s, n = 16; KO 9.5 ± 1.3 cm/s, n = 19; p=0.2728 One-way ANOVA, **Fig. 8 b.**). Similarly, *Itgav* KO mice also showed no abnormal total distance moved (*Itgav* Ctr 45.3 ± 1.4 m, n = 23; KO 48.9 ± 2.1 m, n = 20; p=0.1593 unpaired t-test, **Fig.9 a.**) and average speed (*Itgav* Ctr 5.0 ± 0.2 cm/s, n = 23; KO 5.4 ± 0.2 cm/s, n = 20; p=0.1424 unpaired t-test, **Fig.9 a.**).

Finally, the time spent in the center of the arena (20 x 20 cm) was calculated as a report of anxiety levels. Once again, I did not find differences in time spent in the

center between *Itgb3* WT, Het and KO (*Itgb3* WT 100.8 ± 15.3 s, n = 12; Het 76.6 ± 7.4 s, n = 16; KO 79.5 ± 9.7 s, n = 19; p=0.2728 One-way ANOVA, **Fig. 8 c.**), and between *Itgav* Ctr and KO mice (*Itgav* Ctr 96.9 ± 5.7 s, n = 23; KO 92.9 ± 10.6 s, n = 20; p=0.7357 unpaired t-test, **Fig 9 c.**)

In conclusion, KO mice for $\beta 3$ and αV integrins did not show abnormal general motor activity and anxiety levels.

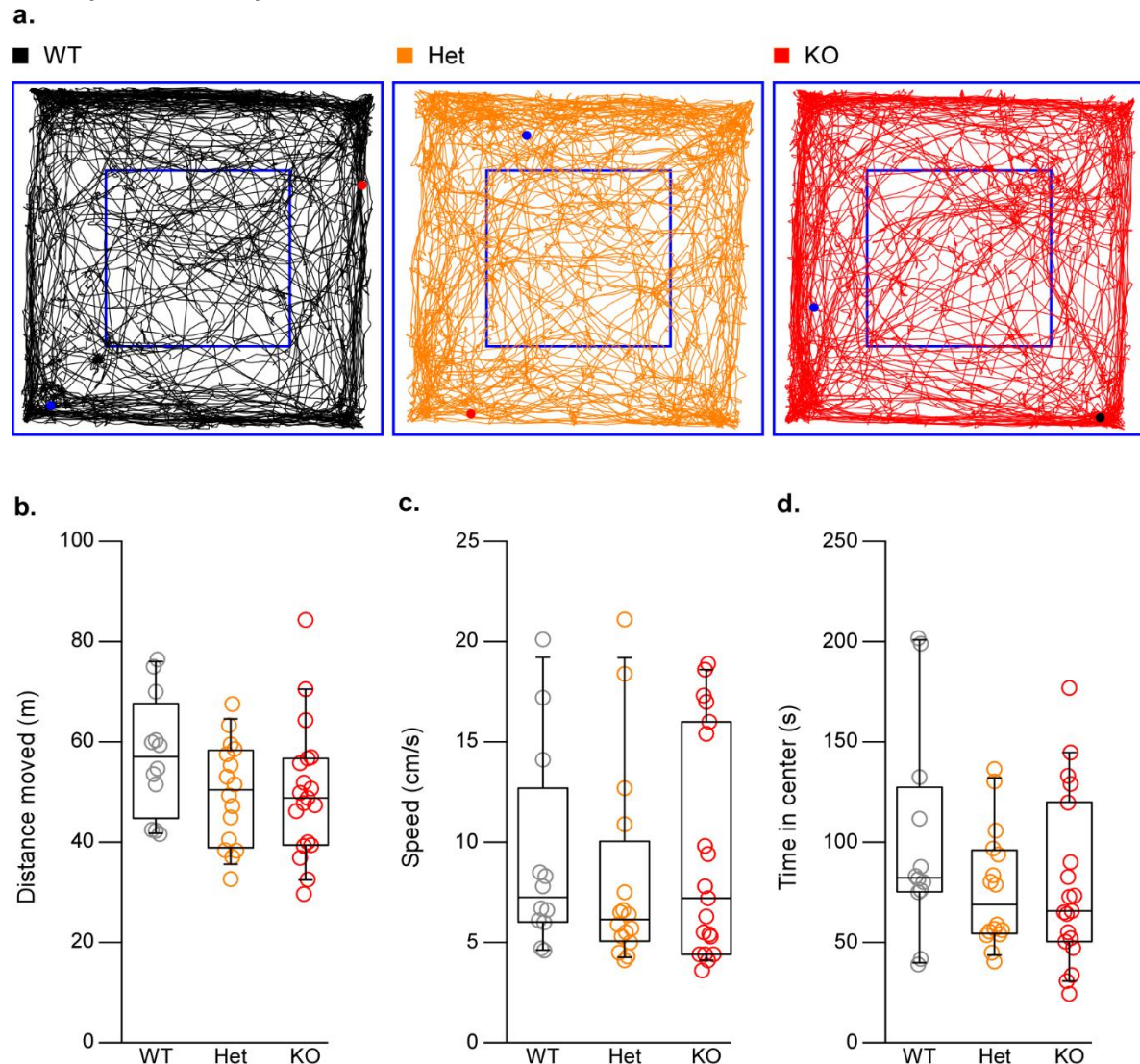


Figure 8. KO and Het mice for *Itgb3* exhibit no abnormal behaviors in the open-field test. **a.** Tracking plots of the center of the body of a WT mouse in black, an Het mouse in orange and a KO mouse in red. The external blue square shows the limits of the arena (40

x 40 cm), the smaller blue square represents the central area (20 x 20 cm) that was defined with ANY-maze. Dots represent the initial and final standing points of each animal. **b.** Total distance moved during the 15 min of observation ($p=0.1947$ One-way ANOVA). **c.** Average speed ($p=0.2728$ One-way ANOVA). **d.** Time in the center ($p=0.2728$ One-way ANOVA). Box-plots show the median, 25 and 75 percentiles, whiskers show 10 and 90 percentiles (*Itgb3* WT n = 12; Het n = 16; KO n = 19).

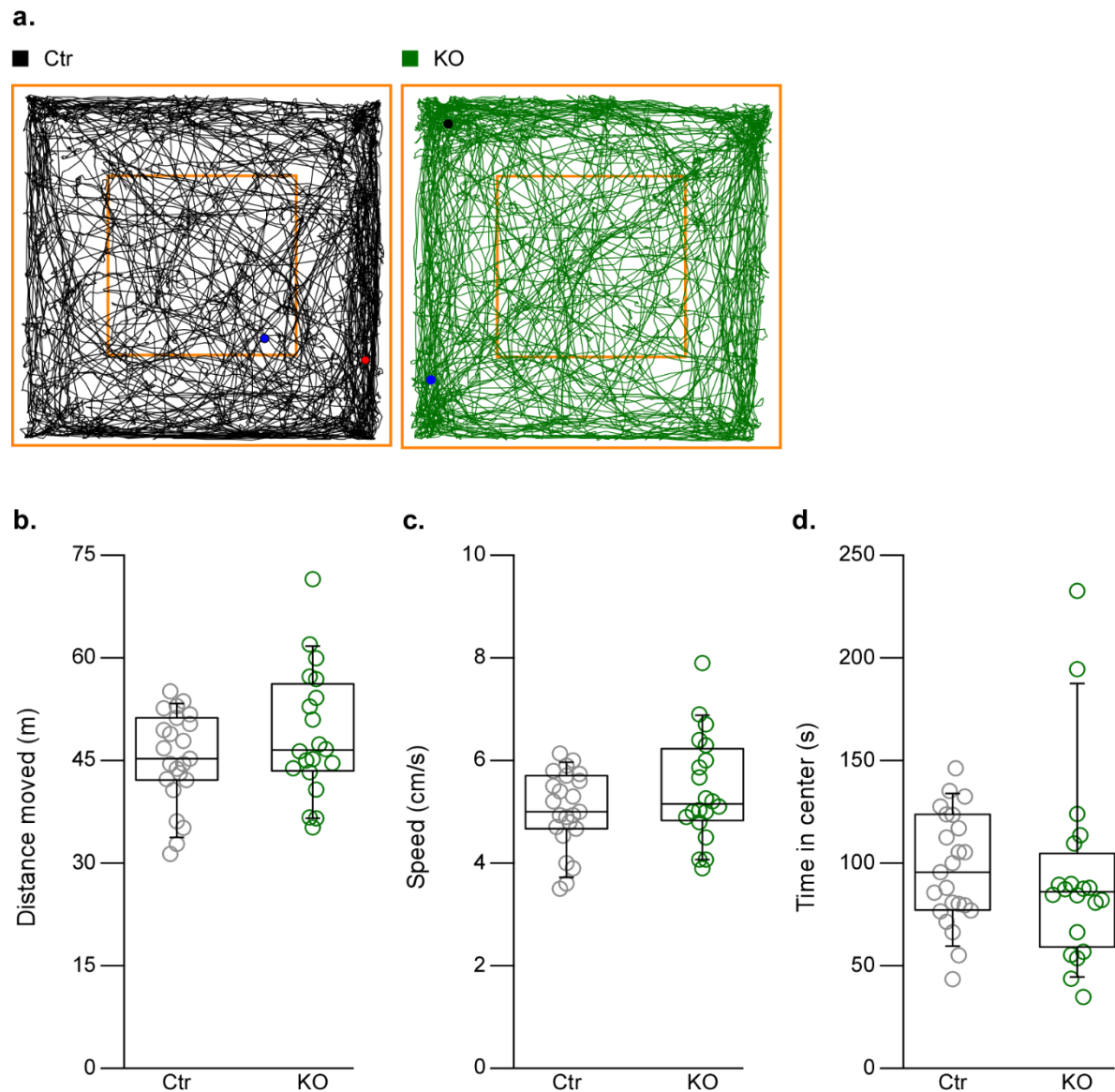


Figure 9. Conditional KO mice for *Itgav* exhibit no abnormal behavior in the open-field test. **a.** Tracking plots of the center of the body of a Ctr mouse in black, and a KO mouse in green. The external orange square shows the limits of the arena (40 x 40 cm), the smaller orange square represents the central area (20 x 20 cm) that was defined with ANY-maze.

Dots represent the initial and final standing points of each animal. **b.** Total distance moved during the 15 min of observation ($p=0.1593$ unpaired t-test). **c.** Average speed ($p=0.1424$ unpaired t-test). **d.** Time in center ($p=0.7357$ unpaired t-test). Box-plots show the median, 25 and 75 percentiles, whiskers show 10 and 90 percentiles (*Itgav* Ctr $n = 23$; KO $n = 20$).

4.2 Knockout of $\beta 3$ or αV integrins has no effects in motor balance and coordination

In a previous study, Nestin-Cre conditional KO mice for *Itgav* showed motor deficits, most probably due to demyelination of caudal cerebellum white matter, of the spinal cord white matter, and axonal dystrophy at the level of the fasciculus gracilis (McCarty et al., 2005). Although we did not expect to observe the same phenotype of Nestin-Cre *Itgav* KO mice in our *Camk2a Itgav* KO, because of the selective expression of Camk2a-Cre in postnatal pyramidal neurons of the forebrain, I tested our colonies for motor coordination and balance using an accelerating rotarod.

An accelerating rotarod tests the ability of mice to walk and to adapt to faster speeds. Mice with motor impairments tend to fall precociously from the rotating rod. Therefore, the latency to fall is a measure used to assess mouse motor function.

As seen in **Fig. 10**, mice were able to learn the task over the trials, but no differences between genotypes were found (*Itgb3*: WT $n = 11$, Het $n = 14$, KO $n = 11$, trial $p<0.0001$, genotypes $p=0.6717$, interaction $p=0.9309$, Two-way ANOVA, **Fig 10 a.** *Itgav*: Ctr $n = 12$, KO $n = 10$, trial $p=0.0002$, genotype $p=0.1297$, interaction $p=0.6685$, Two-way ANOVA, **Fig 10 b.**).

Thus, there was an initial phase of learning in which the latency to fall increases over time, but in all trials, there was no difference between genotypes of the two colonies.

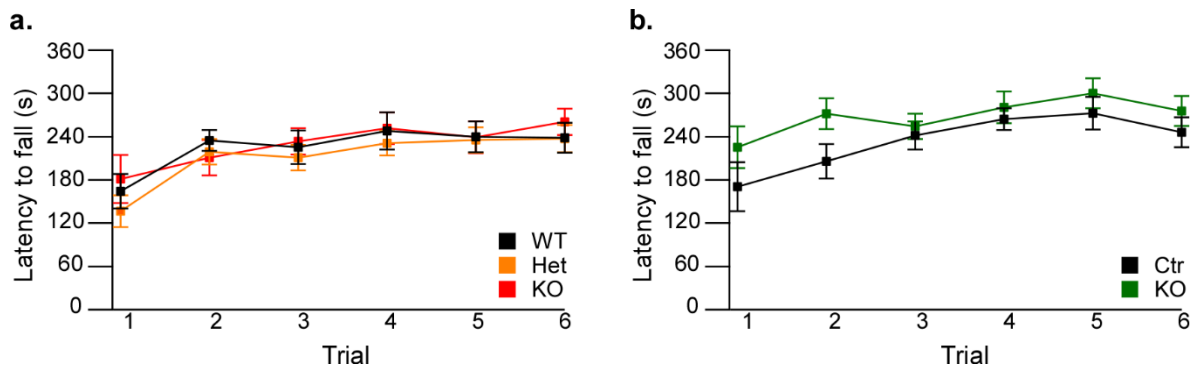


Figure 10. Ablation of *Itgb3* and *Itgav* does not affect the latency to fall from an accelerating rotarod. **a.** Average time standing on the rotarod for *Itgb3* KO colony (*Itgb3* WT n = 11, Het n = 14, KO n = 11, trial p<0.0001, genotypes p=0.6717, interaction p=0.9309, Two-way ANOVA). **b.** As in **a.** but for *Itgav* KO colony (*Itgav* Ctr in n = 12, KO n = 10 trial p=0.0002, genotype p=0.1297, interaction p=0.6685, Two-way ANOVA). Data are shown as mean $\pm$ sem.

4.3 Knockout mice for $\beta 3$ or αV integrins exhibit impairments in social novelty preference

Sociability impairment is a hallmark of ASD prognostics, and impairments in social preference and social recognition are commonly found in animal models of ASD (Silverman et al., 2010). Moreover, it was previously shown with a three-chamber test that *Itgb3* constitutive KO mice have unchanged sociability but they fail to prefer a novel mouse versus a familiar mouse (Carter et al., 2011). For that reason, I tested if our *Itgb3* KO mice would reproduce that phenotype and if *Itgav* KO mice also show aberrant social behavior.

In order to test if *Itgb3* and *Itgav* KO show alterations in sociability and social novelty preference, I used a three-chamber test following the same protocol used by Carter and colleagues (Carter et al., 2011).

In **Fig. 11 b.**, it is possible to observe how much time each animal spent on the left and on the right chamber (*Itgb3* WT n = 14, p=0.8594; Het n = 15, p=0.5777; KO n = 13, p=0.2945; paired t-test left versus right). Since the habituation index was very close to 0, all genotypes showed no preference for any of the lateral chambers (habituation index: *Itgb3* WT 0.009 ± 0.045 , n = 14; Het -0.016 ± 0.036 , n = 15; KO 0.063 ± 0.054 , n = 13; p=0.4680 One-way ANOVA, **Fig. 11 c.**). During the following phase (testing social preference), *Itgb3* WT, Het and KO mice highly preferred to interact with a stranger mouse instead of an object (*Itgb3* WT n = 14, p<0.0001; Het n = 15, p<0.0001; KO n = 12, p<0.0001; paired t-test mouse versus object, **Fig 11 e.**). A positive value of sociability index further confirmed a strong preference for a mouse versus an object, while no differences were found between genotypes (sociability index: WT 0.531 ± 0.040 , n = 14; Het 0.479 ± 0.056 , n = 15; KO 0.527 ± 0.041 , n = 13; p=0.6774 One-way ANOVA, **Fig. 11 f.**). Finally, in the last phase of the test (testing social novelty preference) mice from all genotypes preferred to interact with a novel mouse than with a familiar mouse (*Itgb3* WT n = 14, p<0.0001; Het n = 15, p=0.0011; KO n = 13, p=0.0273; paired t-test familiar mouse vs novel mouse, **Fig. 11 h.**). However, as expected, *Itgb3* KO mice showed a significantly lower value of novelty index in comparison with WT (novelty index: WT 0.409 ± 0.032 , n = 14; Het 0.284 ± 0.054 , n = 15; KO 0.138 ± 0.059 , n = 13; p=0.0022 One-way ANOVA; p<0.001 Tukey WT versus KO, **Fig 11 i.**).

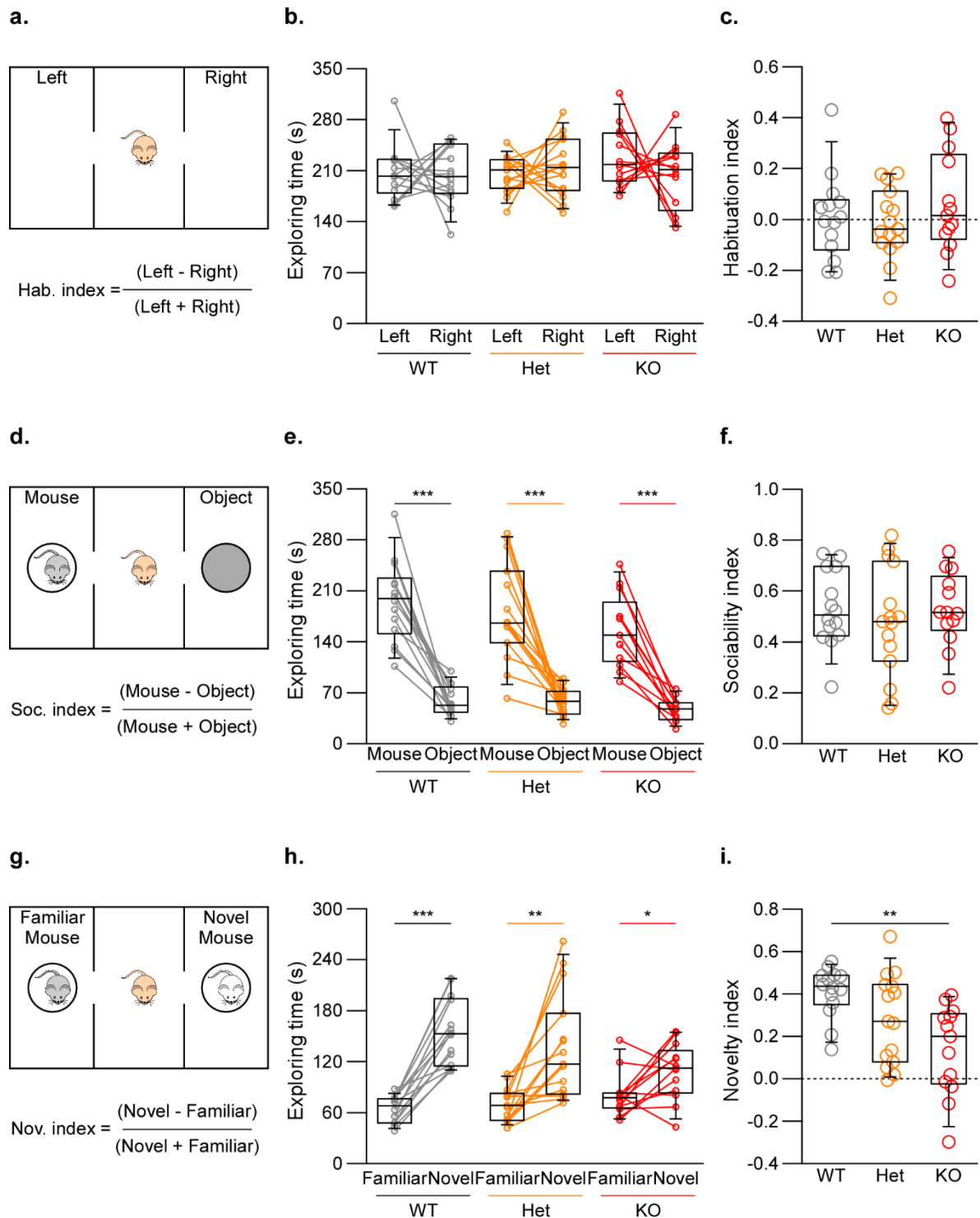


Figure 11. Knockout mice for *Itgb3* show social recognition deficits, as assessed by the three-chamber test. a., b., c. schematics of the behavioral task and the mathematical formula used to calculate habituation, sociability, and novelty indexes; **b.** time spent exploring the left and right chambers during habituation; **c.** habituation index; **e.** time spent exploring a stranger mouse and an object; **f.** sociability index; **h.** time exploring the same

mouse from previous stage (familiar mouse) and a second stranger mouse (novel); **i.** novelty index. Box plots show median, 25 and 75 percentiles, whiskers show 10 and 90 percentiles. (*Itgb3* WT n = 14, Het n = 15, KO n = 13). *p<0.05, **p<0.001, ***p<0.0001, **b.**, **e.**, **h.** paired t-test; **c.**, **f.**, **i.** One-way ANOVA.

Therefore, since we could validate the test and reproduce the observations previously made by Carter and colleagues (Carter et al., 2011), I also tested *Itgav* KO mice in the three-chamber test.

Itgav Ctr and KO mice showed no preference for any of the lateral chambers (*Itgav* Ctr n = 13, p=0.9532; KO n = 13, p=0.4103; paired t-test left versus right, **Fig. 12 b.**), as confirmed by a habituation index close to 0 (habituation index: *Itgav* Ctr 0.002 ± 0.030 , n = 13; KO 0.023 ± 0.031 , n = 13; p=0.6343 unpaired t-test Ctr versus KO, **Fig. 12 c.**). In the following phase (testing sociability), both *Itgav* Ctr and KO showed a strong preference for the mouse versus the object (*Itgav* Ctr n = 13, p<0.0001; KO n = 13, p=0.0037; paired t-test mouse versus object, **Fig. 12 e.**). However, *Itgav* KO showed reduced sociability index in comparison to Ctr (sociability index: *Itgav* Ctr 0.586 ± 0.035 , n = 13; KO 0.300 ± 0.082 , n = 13; p=0.0037 unpaired t-test Ctr versus KO, **Fig. 12 f.**). Lastly, both *Itgav* Ctr and KO mice showed preference for the novel mouse versus the familiar mouse (*Itgav* Ctr n = 13, p<0.0001, KO n = 13, p=0.0288; paired t-test familiar versus novel, **Fig. 12 h.**). However, a substantial difference of novelty index was found between Ctr and KO mice (novelty index: Ctr 0.464 ± 0.038 , n = 13, KO 0.130 ± 0.052 , n = 13; p<0.0001 unpaired t-test Ctr versus KO, **Fig. 12 i.**).

In summary, both *Itgb3* and *Itgav* KO mice showed strong impairments in social novelty preference, as evidenced by the novelty index. Moreover, *Itgav* KO also show reduced preference for a mouse vs an object, as evidenced by the difference of sociability index between *Itgav* Ctr and KO.

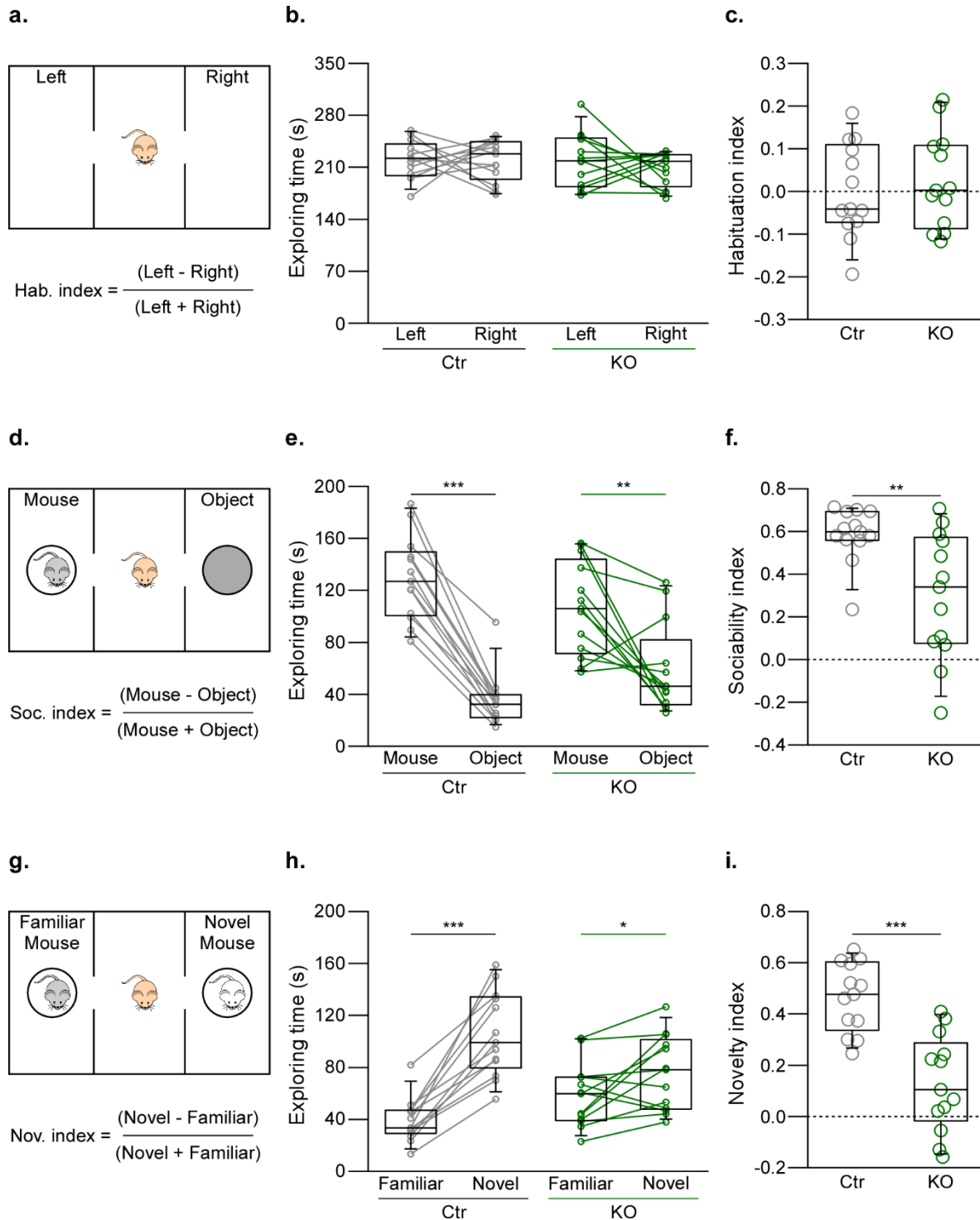


Figure 12. Knockout mice for *Itgav* show social preference and social recognition deficits, as assessed by the three-chamber test. a., b., c. schematics of the behavioral task and the mathematical formula used for calculate habituation, sociability, and novelty indexes; **b.** time spent exploring the left and right chambers during habituation; **c.** habituation index; **e.** time spent exploring a stranger mouse and an object; **f.** sociability index; **h.** time spent exploring the same mouse from previous stage (familiar mouse) and a second stranger mouse (novel mouse); **i.** novelty index. Box plots show median, 25 and 75 percentiles, whiskers show 10 and 90 percentiles. (*Itgav* Ctr n = 13, KO n = 13). *p<0.05, **p<0.001, ***p<0.0001, **b., e., h.** paired t-test; **c., f., i.** unpaired t-test,

4.4 Knockout mice for $\beta 3$ or αV integrins have no defects in odor discrimination and habituation

Olfactory cues are very important for communication in mice. Therefore abnormalities in odor discrimination can underlie impairments in social behavior (Silverman et al., 2010). Since the defects in social novelty preference found with the three-chamber test could be due to olfactory impairments, I controlled if our animals are able to discriminate and habituate to different odors. Previously, it was shown that *Itgb3* KO exhibit normal olfactory response (Carter et al., 2011).

Here I exposed the tested mice to two non-social odors (almond and banana) and to two different social odors. Each odor was presented three times in order to allow habituation. After habituation, the increment of interaction is indicative of odor discrimination (Yang and Crawley, 2009).

As expected, *Itgb3* WT, Het and KO mice were able to discriminate the different odors and to habituate to each odor when it was presented multiple times, with no significant differences between genotypes (*Itgb3* WT n = 17, Het n = 18, KO n = 15, odor p<0.0001, genotype p=0.9486, interaction p=0.8148, Two-way ANOVA, **Fig.**

13 a.). Similarly, *Itgav* Ctr and KO were also able to discriminate different odors and habituate to each odor when presented multiple times, with no differences between the two genotypes (*Itgav* Ctr n = 14, KO n = 14, odor p<0.0001, genotype p=0.2573, interaction p=0.2673, Two-way ANOVA, **Fig. 13 b.).**

Therefore, KO mice for $\beta 3$ and αV integrin were able to identify different non-social and social odors, and to habituate to them.

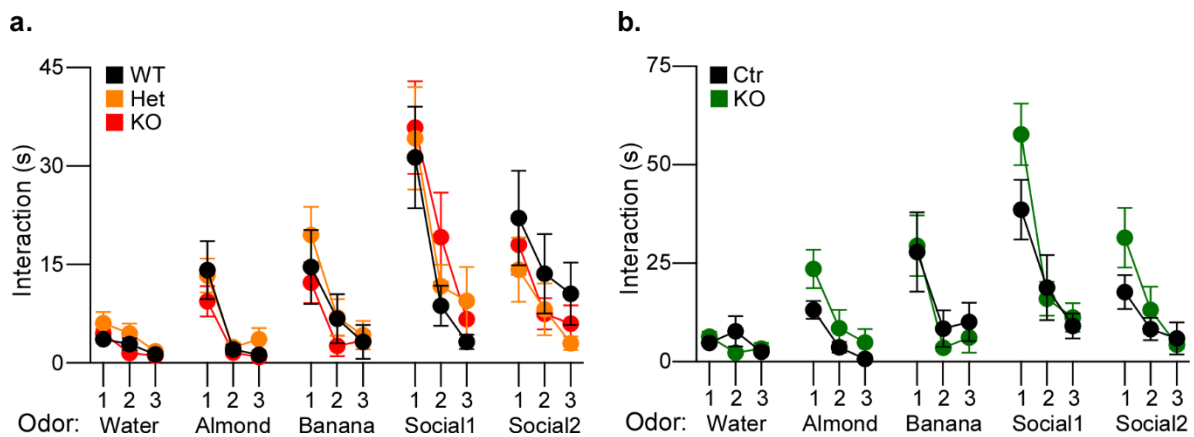


Figure 13. Knockout and heterozygous mice for *Itgb3* and KO mice for *Itgav* exhibit normal olfactory responses, as assessed by olfactory habituation/dishabituation task. a. time *Itgb3* WT, Het and KO mice interact with cotton swabs embedded in water, or containing non-social (almond and banana) or social scents (social 1, social 2) odors (*Itgb3* WT n = 17, Het n = 18, KO n = 15; odor p<0.0001, genotype p=0.9486, interaction p=0.8148, Two-way ANOVA). **b.** as in **a.** but for *Itgav* Ctr and KO (*Itgav* Ctr n = 14, KO n = 14, odor p<0.0001, genotype p=0.2573, interaction p=0.2673, Two-way ANOVA). Data are shown as mean $\pm$ sem.

4.5 Knockout mice for $\beta 3$ or αV integrins do not exhibit abnormal repetitive behaviors

Mice exhibit spontaneous motor stereotypies that include grooming, standing in rear legs and jumping. In models of ASD, even though repetitive motor behaviors are generally normal in their microstructure, they usually persist for unusually long periods of time (Silverman et al., 2010).

Since it was previously shown that *Itgb3* KO mice exhibit increased duration of grooming when placed in novel environments (Carter et al., 2011), I tested if this phenotype is maintained in our *Itgb3* KO colony.

Surprisingly, I did not find any difference in grooming duration between *Itgb3* WT and KO in the 10 min period after habituation (WT 81.9 ± 14.3 s, $n = 10$; Het 69.5 ± 9.7 s, $n = 13$; KO 71.9 ± 16.1 s, $n = 10$; $p=0.7814$ One-way ANOVA, **Fig. 14 a.**), following the protocol from Carter et al., 2011. For that reason, I then analyzed grooming duration in 5 min slots including the habituation period. Once again, although grooming duration increased over time, all genotypes showed similar levels of grooming at all time-points (*Itgb3* WT $n = 10$, Het $n = 13$, KO $n = 10$, time-point $p=0.0002$, genotype $p=0.8625$, interaction $p=0.8472$, Two-way ANOVA, **Fig. 14 a.**).

Afterward, since *Itgav* KO mice were never tested for repetitive behaviors, I did the same analysis with this colony. In line with our observations from *Itgb3* constitutive KO mice, *Itgav* KO mice also exhibited similar grooming levels as Ctr animals at all time-points (*Itgav* Ctr $n = 12$, KO $n = 12$, time-point $p=0.0033$, genotype $p=0.6343$, interaction $p=0.5401$, Two-way ANOVA, **Fig. 14 a.**).

Since other stereotypic behaviors were observed during this test, I also quantified the number of times the animals stood on their rear legs and jumped during the last 10 min of observation. For both parameters, no differences were found between *Itgb3* WT, Het and KO mice (number of vertical rearings: WT 77.7 ± 5.0 , $n = 10$; Het 71.4 ± 6.0 , $n = 13$; KO 79.6 ± 5.9 , $n = 10$; $p=0.5564$ One-way ANOVA; number of jumps: WT 2.8 ± 1.1 , $n = 10$; Het 3.5 ± 1.5 , $n = 13$; KO 2.0 ± 1.3 , $n = 10$; $p=0.7272$ One-way ANOVA, **Fig. 14 b., c.**). Once more, *Itgav* KO mice showed similar number of vertical rearings and jumps as control animals (number of vertical rearings: Ctr 68.2 ± 4.3 , $n = 12$; KO 73.3 ± 3.8 , $n = 12$; $p=0.3853$ unpaired t-test; number of jumps: Ctr 11.1 ± 3.5 , $n = 12$; KO 19.1 ± 4.4 , $n = 12$, $p=0.1705$ unpaired t-test Ctr versus KO; **Fig 14 e., f.**).

In conclusion, KO mice for $\beta 3$ or αV integrins did not exhibit abnormal repetitive behaviors (grooming, vertical rearings, and jumps) when placed in a novel environment.

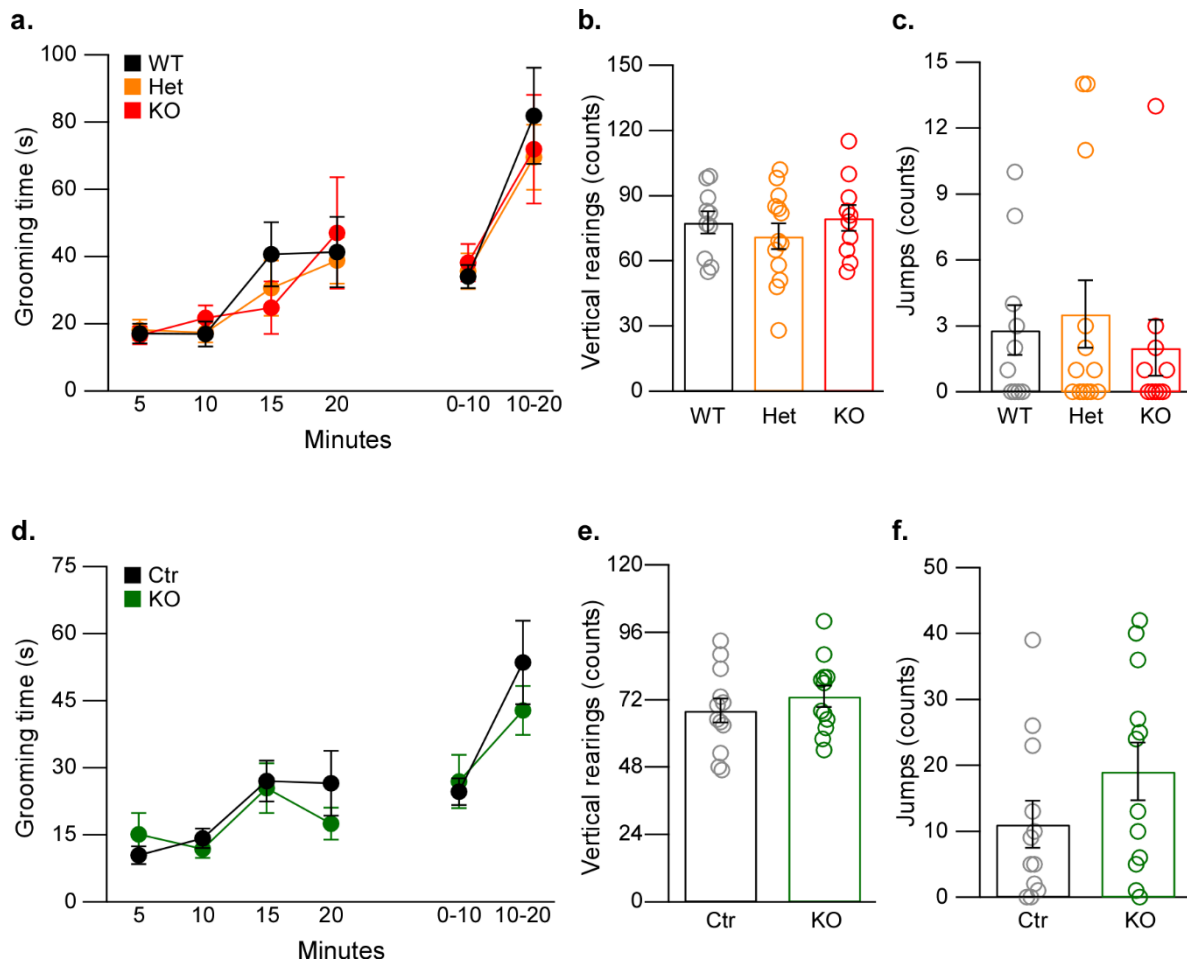


Figure 14. Knockout and heterozygous mice for *Itgb3* and KO mice for *Itgav* do not present altered stereotypic behaviors. **a.** total grooming time over slots of 5 and 10 min for a total time of observation of 20 min for *Itgb3* KO colony (time-point $p=0.0002$, genotype $p=0.8625$, interaction $p=0.8472$, Two-way ANOVA). **b.** number of vertical rearings ($p=0.5564$ One-way ANOVA) and **c.** jumps ($p=0.7272$ One-way ANOVA) shown by *Itgb3* WT, Het, and KO mice; **d.** as in **a.** but for *Itgav* KO colony (time-point $p=0.0033$, genotype $p=0.6343$, interaction $p=0.5401$, Two-way ANOVA). **b.** number of vertical rearings ($p=0.3853$ unpaired t-test) and **c.** jumps ($p=0.1705$ unpaired t-test) shown by *Itgav* Ctr, and KO mice. (*Itgb3* WT $n = 10$, Het $n = 13$, KO $n = 10$; *Itgav* Ctr $n = 12$, KO $n = 12$). Data are shown as mean $\pm$ sem.

4.6 Knockout for $\beta 3$ or αV integrins leads to hyper-reactivity to acoustic stimuli

In addition to sociability defects and repetitive behaviors, ASD is characterized by a series of associated symptoms including changed reactivity to sensory stimuli (Silverman et al., 2010). For that reason, I tested our animals in a startle test to measure reactivity to acoustic stimulus.

Additionally, the ability to gate the sensorimotor reflex to a strong acoustic stimulus by a preceding stimulus of lower intensity is a robust and highly conserved behavior across species. This inhibition of startle response by a prepulse (PPI) relies on brain areas relevant for ASD (such as the limbic cortex and the striatum) and is commonly found in patients and animal models of schizophrenia (Swerdlow et al., 2001, 2008). Accordingly, I also subjected our animals to a prepulse inhibition (PPI) test.

In order to assess the reactivity of *Itgb3* and *Itgav* KO mice to acoustic stimuli, I measured the startle response to acoustic stimuli using a Startle Response System from TSE. Using this system, I identified a hyper-reactivity to strong acoustic stimulus of 120 dB in *Itgb3* Het and KO mice (*Itgb3* WT 61.9 ± 5.7 g., $n = 27$; Het 76.1 ± 5.6 g, $n = 30$; KO 74.9 ± 6.8 g, $n = 30$; stimulus $p < 0.0001$, genotype $p = 0.2217$, interaction $p = 0.2395$, Two-way ANOVA, Bonferroni posttest WT_{120dB} vs Het_{120dB} $p < 0.01$, WT_{120dB} vs KO_{120dB} $p < 0.05$, **Fig. 15 a.**). Moreover, I also found a hyper-reactivity to strong acoustic stimulus of 120 dB in *Itgav* KO mice (*Itgav* Ctr 88.5 ± 3.3 g, $n = 30$; KO 104.3 ± 4.6 g, $n = 30$; stimulus $p < 0.0001$, genotype $p = 0.0584$, interaction $p = 0.0006$, Two-way ANOVA, Bonferroni posttests Ctr_{120dB} vs KO_{120dB} $p < 0.001$, **Fig. 15 b.**).

Although *Itgb3* Het, KO, and *Itgav* KO exhibit increased startle response to strong acoustic stimuli, they have normal PPI to an acoustic stimulus of 120 dB (*Itgb3* WT n = 27, Het n = 30, KO n = 30, stimulus p<0.0001, genotype p=0.9289, interaction p=0.9077, Two-way ANOVA, **Fig. 15 c.**; *Itgav* Ctr n = 30, KO n = 30, stimulus p<0.0001, genotype p=0.5284, interaction p=0.7756, Two-way ANOVA, **Fig. 15 d.**).

Thus, all mutant genotypes tested showed increased reactivity to strong acoustic stimuli (120 dB), but they were still able to gate their motor reflex in accordance to the intensity of a prepulse.

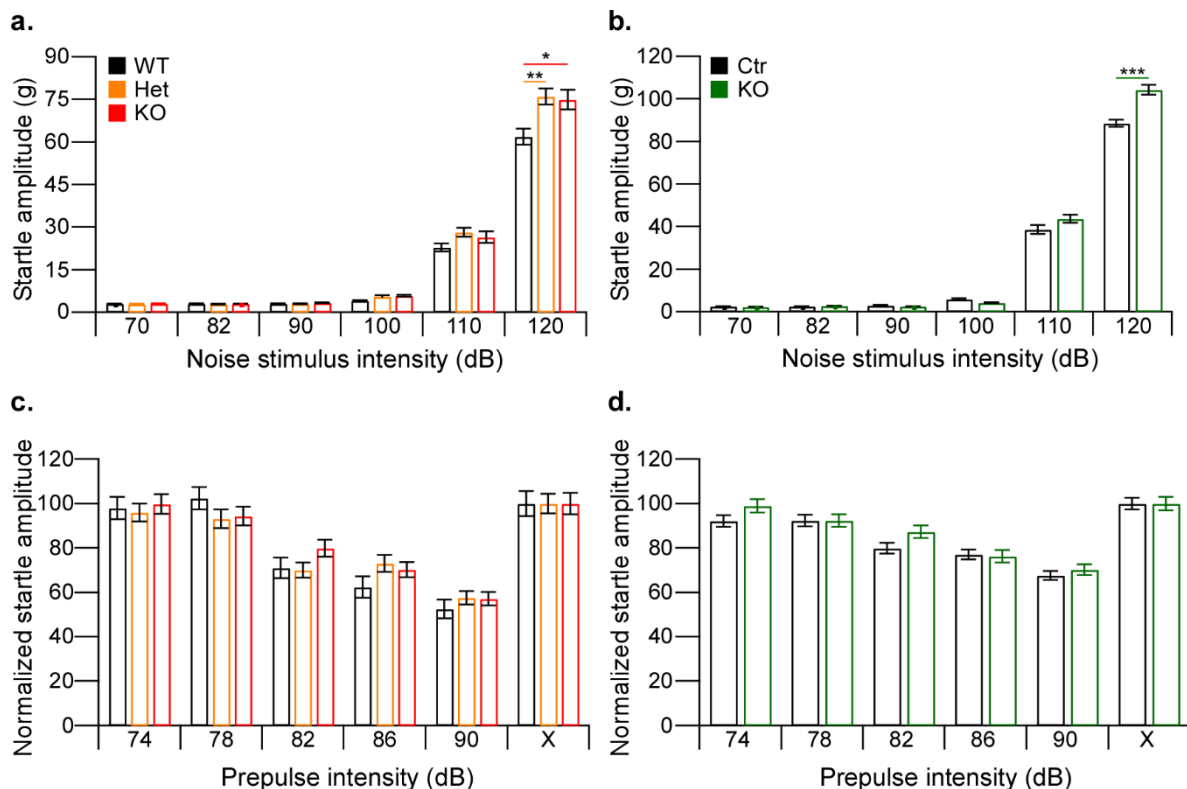


Figure 15. Knockout for $\beta 3$ or αV integrins leads to hyperreactivity to acoustic stimuli, but unchanged prepulse inhibition. Startle response of *Itgb3* WT, Het, and KO mice (stimulus p<0.0001, genotype p=0.2217, interaction p=0.2395, Two-way ANOVA) (**a.**), and *Itgav* Ctr and KO mice (stimulus p<0.0001, genotype p=0.0584, interaction p=0.0006, Two-way ANOVA) (**b.**) to acoustic stimulus of diverse intensities. Prepulse inhibition for *Itgb3* WT,

Het, and KO mice (stimulus $p < 0.0001$, genotype $p = 0.9289$, interaction $p = 0.9077$, Two-way ANOVA) (**c.**), and *Itgav* Ctr and KO mice (stimulus $p < 0.0001$, genotype $p = 0.5284$, interaction $p = 0.7756$, Two-way ANOVA) (**d.**), when a stimulus of 120 dB is preceded by a prepulse of different intensities as indicated, or no prepulse (X). (*Itgb3* WT $n = 27$, Het $n = 30$, KO $n = 30$, *Itgav* Ctr $n = 30$, KO $n = 30$). Data are shown as mean $\pm$ sem, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Bonferroni posttest.

4.7 Knockout mice for $\beta 3$ or αV integrins decreases the threshold for seizures

There is a high degree of overlap of genes and brain areas implicated in ASD and epilepsy, and a high number of patients with manifestations of both disorders (Bozzi et al., 2018). Moreover, a previous study suggested that the ablation of *Itgav* from neurons and glial cells from the CNS (Nestin-Cre *Itgav* KO) leads to increased seizure propensity in mice (McCarty et al., 2005).

Therefore, I tested the threshold for seizures induced by PTZ in our animal models. Each injection was followed by a period of 10 min of observation, in which the strongest behavioral outcome was ranked according to **table 3**. Successive injections were given until mice lost their balance or started jumping.

Table 3. Scale of intensity of the epileptic phenotype

Outcome:	No effect	Reduced motility	Partial clonus	Generalized clonus (including tail curving) and stronger
Rank:	0	1	2	3

In **Fig. 16**, we can see that the number of injections needed for *Itgb3* KO and *Itgav* KO to reach rank 2 is significantly lower than their respective controls (*Itgb3*: WT n = 11, Het n = 10, KO n = 12, rank $p < 0.0001$, genotype $p = 0.0142$, interaction $p = 0.2346$, Two-way ANOVA, Bonferroni posttest WT_{rank2} vs KO_{rank2} $p < 0.01$, **Fig. 16 a.**, *Itgav*: Ctr n = 15, KO n = 15, rank $p < 0.0001$, genotype $p = 0.0762$, interaction $p = 0.0065$, Two-way ANOVA, Bonferroni posttest Ctr_{rank2} vs KO_{rank2} $p < 0.01$, **Fig. 16 b.**).

Moreover, in plots showing the severity of phenotype per injection (**Fig. 16 c. – h.**), there is a significant difference between genotypes at fourth injection (*Itgb3*: WT n = 11, Het n = 10, KO n = 12, $p = 0.0498$ Fisher exact probability test; *Itgav*: Ctr n = 15, KO n = 15, $p = 0.0077$ Fisher exact probability test). For that reason, other members of the laboratory have given a single injection of 45 mg / kg (correspondent to the concentration of PTZ given until injection 4) and measured the outcome for 10 min. In that experiment, *Itgb3* Het and KO mice showed a significantly higher severity score than WT animals. Accordingly, *Itgav* KO mice also showed significantly higher severity score than Ctr animals.

Hence, constitutive KO for *Itgb3* and a postnatal conditional KO for *Itgav* restricted to excitatory neurons of forebrain, displayed increased susceptibility to PTZ-induced seizures.

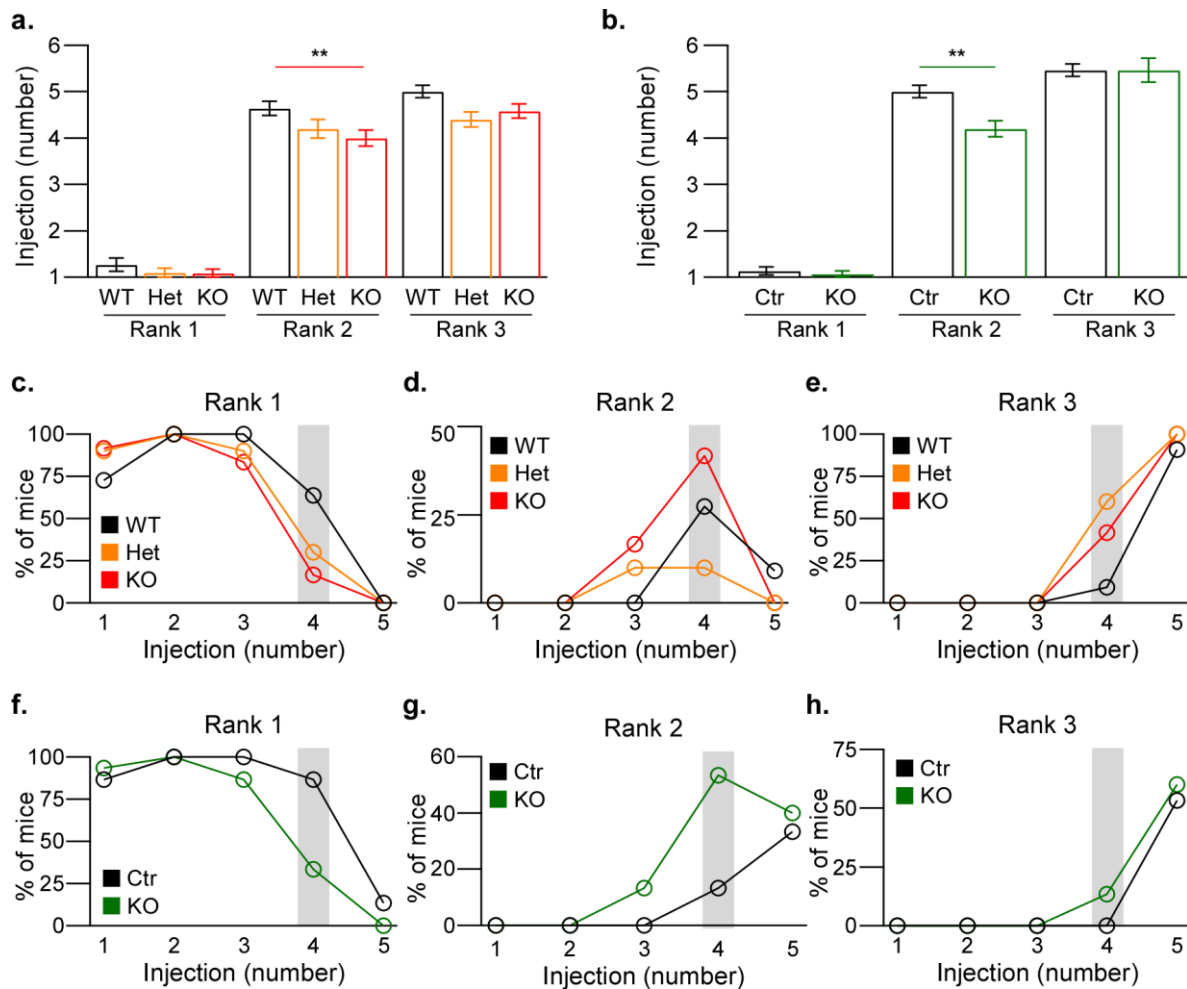


Figure 16. KO mice for *Itgb3* and KO mice for *Itgav* show lower threshold for seizures induced by PTZ. Number of injections needed to reach each rank of intensity for (a.) *Itgb3* WT, Het and KO (WT n = 11, Het n = 10, KO n = 12, rank $p < 0.0001$, genotype $p = 0.0142$, interaction $p = 0.2346$, Two-way ANOVA), and for (b.) *Itgav* Ctr, and KO (Ctr n = 15, KO n = 15, rank $p < 0.0001$, genotype $p = 0.0762$, interaction $p = 0.0065$, Two-way ANOVA) mice. c. Percentage (%) of *Itgb3* strain mice per injection showing a rank 1 phenotype. d. Percentage (%) of *Itgb3* strain mice per injection showing a rank 2 phenotype. e. Percentage (%) of *Itgb3* strain mice per injection showing a rank 3 phenotype. f. Percentage (%) of *Itgav* strain mice per injection showing a rank 1 phenotype. g. Percentage (%) of *Itgav* strain mice per injection showing a rank 2 phenotype. h. Percentage (%) of *Itgav* strain mice per injection showing a rank 3 phenotype. a., b. bars show mean $\pm$ sem. a., b. $**p < 0.01$ Bonferroni posttest. c.-h. data tested with Fisher exact probability test, a difference was found at injection 4 in both strains (*Itgb3* $p = 0.0498$; *Itgav* $p = 0.0077$).

4.8 Knockout of $\beta 3$ integrins does not affect presynaptic efficacy

Since KO mice for $\beta 3$ and αV integrins exhibit alterations in behaviors that depend on the mPFC, and because $\beta 3$ integrin is specifically enriched in layer V pyramidal neurons, I next characterized excitatory synaptic transmission onto layer V pyramidal neurons of the mPFC.

Paired-pulse ratio is often used to detect dysfunctions of the presynaptic side of synapses (Zucker and Regehr, 2002). Presynaptic fibers are stimulated with two electric pulses of the same intensity, and the ratio between the second and first peak of the evoked synaptic currents is taken as an indirect measure of neurotransmitter release probability. Synapses with high release probability generally exhibit paired-pulse depression (PPD; PPR <1), mainly because most synaptic vesicles are released in response to the first stimulation. By contrast, synapses with low release probability tend to facilitate in response to repetitive stimulation (paired-pulse facilitation, PPF; PPR >1). This latter process is mainly due to a build-up of Ca^{2+} in the presynaptic boutons with repetitive stimulation, which facilitates the release of neurotransmitter vesicles (Zucker and Regehr, 2002).

Here, I patched layer V pyramidal neurons of the mPFC in acute brain slices of three week old *Itgb3* WT and KO mice and stimulated them using a PPR protocol.

As shown in **Fig 17**, shorter intervals between the two electric pulses yielded a strong potentiation of the second pulse up to approximately 37% in both *Itgb3* WT and KO mice (for a paired-pulse interval of 10 ms). However, there were no statistical differences between neurons from *Itgb3* WT and KO mice (*Itgb3* WT n = 25 cells /

13 mice, KO n = 22 cells / 10 mice; interpulse interval $p < 0.0001$, genotype $p = 0.7970$, interaction $p = 0.6681$, Two-way ANOVA).

These results suggests that loss of $\beta 3$ integrin had no major effects on release probability at excitatory synapses onto layer V pyramidal neurons of the mPFC.

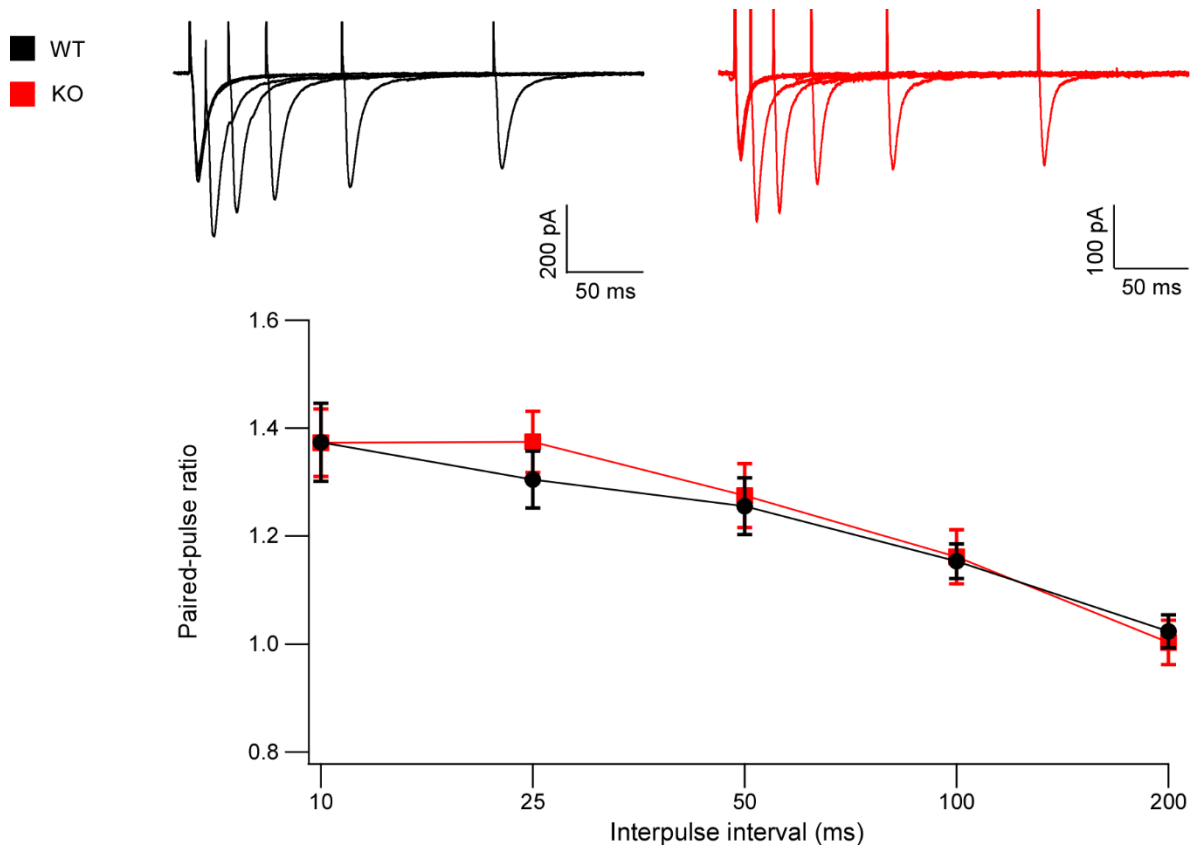


Figure 17. Knockout of $\beta 3$ integrin does not affect presynaptic efficacy. Layer V pyramidal neurons of the mPFC were patched and presynaptic fibers to their basal dendrites stimulated with a paired-pulse ratio protocol. Top, average of 5 traces of a representative cell are shown, in black *Itgb3* WT and in red *Itgb3* KO. Bottom, pooled data showing the ratios between the second and first pulse. Data are shown as means $\pm$ sem. (*Itgb3* WT n = 25 cells / 13 mice; KO n = 22 cells / 10 mice; interpulse interval $p < 0.0001$, genotype $p = 0.7970$, interaction $p = 0.6681$, Two-way ANOVA).

4.9 Knockout of $\beta 3$ integrins does not affect the ratio between NMDAR- and AMPAR-mediated currents

Since $\beta 3$ integrin has been shown to regulate AMPAR subunit composition at hippocampal excitatory synapses in culture (Cingolani et al., 2008; Pozo et al., 2012), I next investigated whether $\beta 3$ integrin affect the composition of glutamate receptors in the mPFC. First, I measured the ratio between NMDAR- and AMPAR-mediated synaptic currents. Both glutamate receptors are of primary importance for basal synaptic transmission and for synaptic plasticity, and changes in their relative abundance could implicate dysfunctions in synaptic computation.

Using the recording configuration explained for the previous experiment, I isolated AMPAR- and NMDAR-mediated synaptic currents. The AMPAR- and NMDAR-mediated currents were elicited by stimulating presynaptic fibers to the basal dendrites of patched neurons AMPAR-mediated currents were isolated by holding the patched neurons at -70 mV, while NMDAR-mediated currents were measured by holding the patched neurons at +60 mV in the presence of NBQX (10 μ M) to block AMPAR currents. The NMDA/AMPA receptor ratio was estimated by dividing the averaged amplitudes of NMDAR EPSCs by the averaged amplitudes of AMPAR EPSCs.

As shown **Fig. 18**, NMDAR-mediated currents are much larger than AMPAR-mediated currents at 3 postnatal weeks in both *Itgb3* WT and KO mice. Moreover, the ratio between the two current types revealed no major differences in terms of relative abundance of AMPAR- and NMDAR-mediated synaptic currents (*Itgb3* WT

1.44 ± 0.15 , $n = 20$ cells / 11 mice ; KO 1.41 ± 0.14 , $n = 18$ cells / 11 mice; $p=0.8734$ unpaired t-test).

Therefore, the loss of $\beta 3$ integrin had no major effects on the AMPAR- and NMDAR-mediated synaptic current ratio.

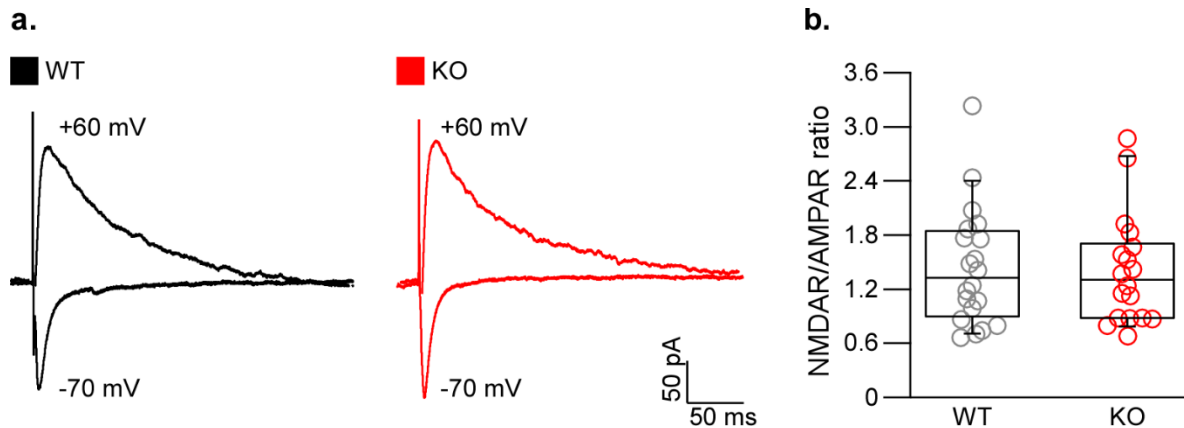


Figure 18. Knockout of $\beta 3$ integrin does not affect NMDAR/AMPA ratio. **a.** Averages of 5 AMPAR- and NMDAR-mediated currents from representative cells. **b.** Box-plot showing median, 25 and 75 percentiles, and whiskers showing 10 and 90 percentiles of data distribution. (*Itgb3* WT $n = 20$ cells / 11 mice, KO $n = 18$ cells / 11 mice, $p=0.8734$ unpaired t-test).

4.10 Knockout of $\beta 3$ integrins does not affect the subunit composition of NMDARs

The GluN2B subunit of NMDARs has been implicated in ASD and epilepsy (Pan et al., 2015; Szczurowska and Mareš, 2015). Moreover, RGD peptides, which target integrins, were shown to induce NMDAR-dependent increases in intracellular Ca^{2+} levels in cortical neurons (Watson et al., 2007; but see Cingolani et al., 2008). Thus, changes in expression or activity of GluN2B-containing NMDARs could contribute to the behavioral phenotypes here observed.

To assess the contribution of the GluN2B-containing NMDARs to the overall NMDAR-mediated current, I elicited NMDAR-mediated currents before and after application of Ro 25-6981 (3 μ M), which is a highly specific antagonist of the subunit GluN2B (Szczurowska and Mareš, 2015). As shown in **Fig. 19 c.**, 15 min incubation with this drug leads to a reduction of $57.0 \pm 7.8\%$ and $58.4 \pm 5.3\%$ in *Itgb3* WT and KO neurons, respectively. Statistical analysis showed a strong effect of the drug (time $p < 0.0001$) but no effect of genotype (genotype $p = 0.358$, interaction $p = 0.9991$, Two-way ANOVA; *Itgb3* WT $n = 6$ cells / 5 mice, KO $n = 8$ cells / 5 mice, **Fig. 19 c.**). In subset of cells (3 in WT and 2 in KO) subsequent application of D-AP5 (100 μ M) completely blocked the remaining current, confirming that it was NMDAR-mediated. Additionally, I calculated the decay time constant of the NMDAR-mediated currents, which depends in part on the subunit composition of the NMDARs. NMDARs composed of GluN1/GluN2A subunits have faster activation and deactivation rates than those composed of GluN1/GluN2B subunits (Shipton and Paulsen, 2014). Application of Ro 25-6981 accelerated the decay time constant of synaptic NMDAR currents, confirming the specificity of the pharmacological treatment. In *Itgb3* WT, the decay time constant was reduced from a mean value of 65.96 ± 8.21 ms during baseline to 37.52 ± 6.83 ms in the presence of Ro 25-6981. Similarly, in *Itgb3* KO, the decay time constant was reduced from a mean value of 73.60 ± 7.30 ms during baseline to 45.64 ± 4.36 ms in the presence of Ro 25-6981 (*Itgb3* WT $n = 6$ cells / 5 mice; KO $n = 8$ cells / 5 animals; paired t-test WT $p = 0.0007$, KO $p = 0.0048$, **Fig. 19 b.**).

In summary, the subunit composition of synaptic NMDARs in layer V pyramidal neurons of the mPFC was not affected by loss of $\beta 3$ integrin.

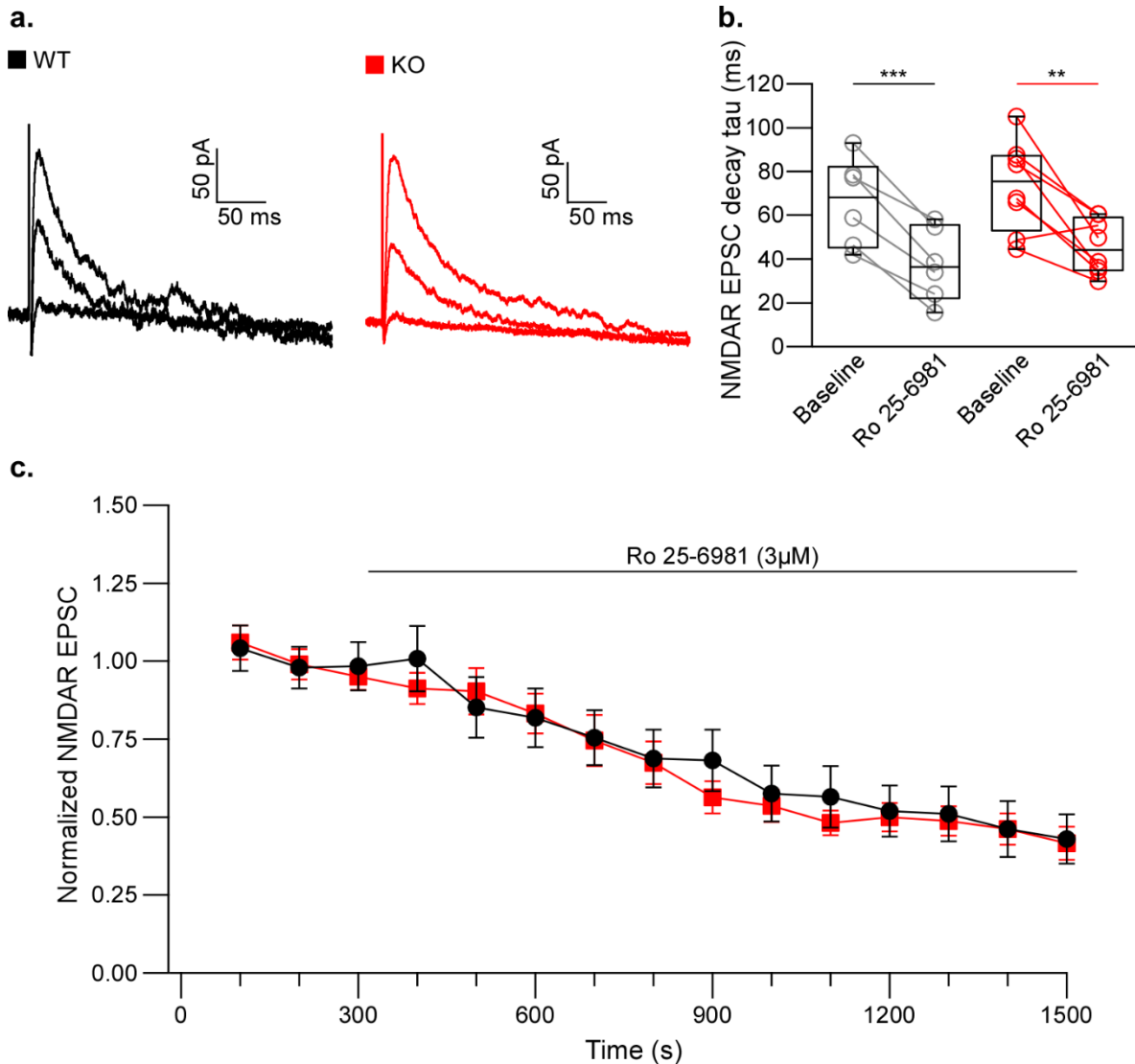


Figure 19. GluN2B-containing NMDARs are present in the same proportion in *Itgb3* WT and KO neurons. **a.** representative NMDAR EPSCs (each trace is an average of 5 trials) during baseline, after application of Ro 25-6981 (3 μ M), and after subsequent application of D-AP5 (100 μ M); **b.** NMDAR EPSC decay time constant (tau in ms) measured during baseline and after 15 min of incubation with Ro25-5981; **c.** time course of NMDAR EPSC normalized to baseline, each point corresponds to the average of 5 traces. Data are shown as mean $\pm$ sem. (*Itgb3* WT n = 6 cells / 5 mice, KO n = 8 cells / 5 mice). **b.** paired t-test **p<0.01, ***p<0.001, **c.** (time p<0.0001, genotype p=0.3578, interaction p=0.9991, Two-way ANOVA).

4.11 Knockout of $\beta 3$ integrin increases synaptic GluA2-containing AMPARs

Since the GluA2 subunit of AMPARs interacts with $\beta 3$ integrin (Pozo et al., 2012), I next investigated the subunit composition of AMPARs.

AMPARs play a crucial role in excitatory synaptic transmission, and like NMDARs, their biophysical properties depend on their subunit composition. Namely, GluA2A confers impermeability to Ca^{2+} while AMPARs lacking this subunit are permeable to Ca^{2+} (Kumar et al., 2002). In fact, more than 90% of the GluA2 mRNA is edited in such a way that a glutamine is replaced by an arginine, a positively-charged amino acid, which blocks the passage of highly charged molecules, such as Ca^{2+} and spermine. Spermine can be used to distinguish GluA2-containing from GluA2-lacking AMPARs because, if included in the intracellular pipette solution, it blocks specifically GluA2-lacking AMPARs at positive holding potentials, thus causing an inward rectification (Pellegrini-Giampietro, 2003). Rectification of AMPAR-mediated currents is, therefore, a method used to estimate the subunit composition of synaptic AMPARs.

Here, I calculated the rectification index as the ratio of the amplitudes of the AMPAR-mediated currents recorded at holding potentials of +40mV and -70mV.

As shown in **Fig. 20**, pyramidal neurons of layer V of mPFC from P9-P11 mice, exhibit a stronger rectification in WT than in *Itgb3* KO mice. Specifically, the rectification index was 0.33 ± 0.02 and 0.44 ± 0.03 , in *Itgb3* WT and KO mice, respectively (*Itgb3* WT n = 10 cells / 8 mice, KO n = 10 cells / 6 mice; $p=0.0054$

unpaired t-test, **Fig 20 b.**), which corresponds to an increase of approximately 33% in the KO relative to WT.

In pyramidal neurons of the rat frontal cortex, the rectification index increases during development from approximately 0.7 at P13-P15 animals to 1.2 at P16-P21 (Kumar et al., 2002). This indicates that the content of the GluA2 subunit increases in the rat frontal cortex during development, as confirmed also by immunohistochemistry (Kumar et al., 2002).

Next, I asked whether the differences in the degree of rectification of the synaptic AMPAR currents between WT and KO mice were limited to the early developmental stages I had investigated (P9-P11) or whether they were maintained at later developmental stages. In P17-P21 *Itgb3* WT mice, the rectification of synaptic AMPAR current tends to be higher than at P9-P11 but it is still pronounced (0.39 ± 0.03 , WT n = 9 cells / 8 mice,). Notably, *Itgb3* KO mice exhibit a significantly higher rectification index than WT mice also at this developmental stage (0.48 ± 0.02 , n = 9 cells / 8 mice; unpaired t-test $p=0.0301$, **Fig. 20 d.**). At this age, the rectification index is therefore 24% higher in KO than in WT.

Altogether, these data suggest that *Itgb3* KO pyramidal neurons have increased levels of GluA2-containing AMPARs than WT, at both developmental stages.

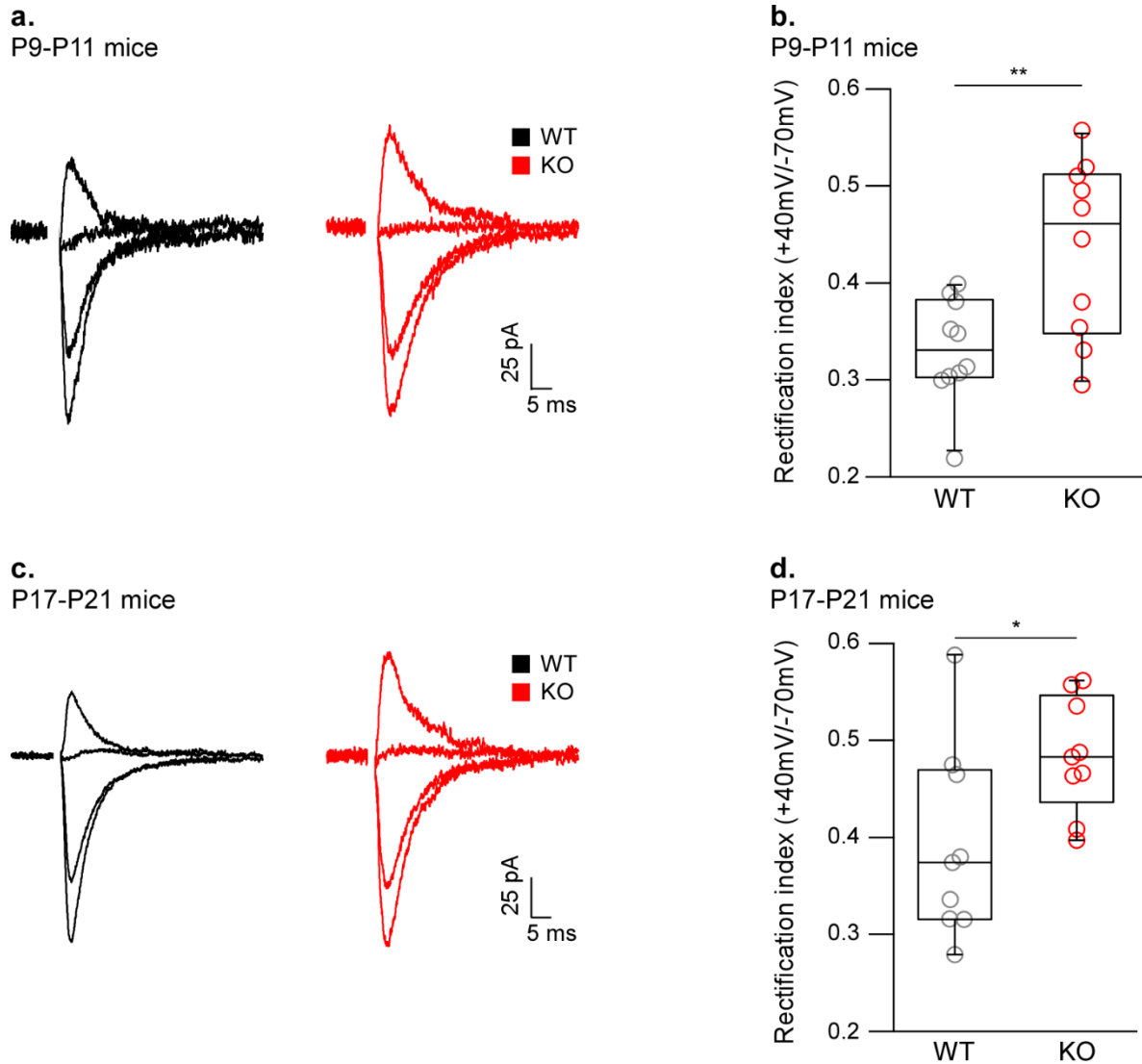


Figure 20. Knockout of $\beta 3$ integrin leads to an increase of synaptic GluA2-containing AMPARs. **a.** representative traces from P9-P11 mice recorded at different holding membrane potentials (-70, -40, 0 and +40mV); **b.** rectification index of P9-P11 mice (*Itgb3* WT n = 10 cells / 8 mice, KO n = 10 cells / 6 mice); **c.** representative traces from P17-P21 mice recorded at different holding membrane potentials (-70, -40, 0 and +40mV); **d.** rectification index of P17-P21 mice (*Itgb3* WT n = 9 cells / 8 mice, KO n = 9 cells / 8 mice); box-plot shows median, 25 and 75 percentiles, whiskers showing 10 and 90 percentiles. * $p < 0.05$, ** $p < 0.01$ unpaired t-test. The stimulation artifact is blanked.

Since the full KO for *Itgb3* leads to a change in AMPAR subunit composition, I next investigated whether the *Itgav* conditional KO (*Itgav^{fl/fl}*; *tdTomato^{fl/fl}*; *CamK2a-Cre^{+/-}*) exhibited a similar phenotype. Because the *CamK2a* promotor of the conditional KO begins to be active during the second-third postnatal week in a mosaic fashion, I used the tdTomato fluorescence of this mouse line to select the neurons for patching. This experimental design allowed me to patch exclusively neurons that expressed Cre and that, therefore, had presumably reduced levels of the integrin α V (see methods for more details).

In three weeks old mice, I did not detect any difference in rectification between *Itgav* Ctr and KO mice (*Itgav* Ctr 0.40 ± 0.02 , $n = 10$ cells / 5 mice, KO 0.40 ± 0.04 ; $n = 10$ cells / 6 mice; $p=0.9630$ unpaired t-test, **Fig. 21 b.**).

Since expression of *CamK2a* starts postnatally, it is possible that there are no differences between the two genotypes at three weeks because not enough time has elapsed to allow a significant reduction of α V integrins. Indeed, the reduction of the mRNA for integrin α V is still very modest at this developmental stage, as assessed by quantitative real-time PCR, but more pronounced at one month of age (Cingolani laboratory, unpublished data).

I, therefore, recorded *Itgav* KO mice at one month of age and quantified the level of rectification. As reported in **Fig. 21 d.**, I detected no significant differences between *Itgav* Ctr and KO (*Itgav* Ctr 0.37 ± 0.02 , $n = 9$ cells / 6 mice, KO 0.43 ± 0.03 , $n = 9$ cells / 6 mice, $p=0.0899$ unpaired t-test, **Fig 21 d.**). Because the individual rectification indexes in *Itgav* KO were very variable and because a trend towards higher average rectification levels for *Itgav* KO seemed to be present, other

members of the laboratory have repeated these experiments in adult mice (2 months). In the adult, *Itgav* KO mice had a significant higher level of rectification than *Itgav* Ctr. Moreover, adult *Itgb3* KO mice showed as well a significant higher level of rectification than WT adult mice (Cingolani laboratory, unpublished data).

My data, together with the findings of other members of the laboratory, suggest therefore that loss of integrin $\alpha V\beta 3$ increases the synaptic content of GluA2-containing AMPARs at excitatory synapses on layer V pyramidal neurons of the mPFC in a development-independent manner.

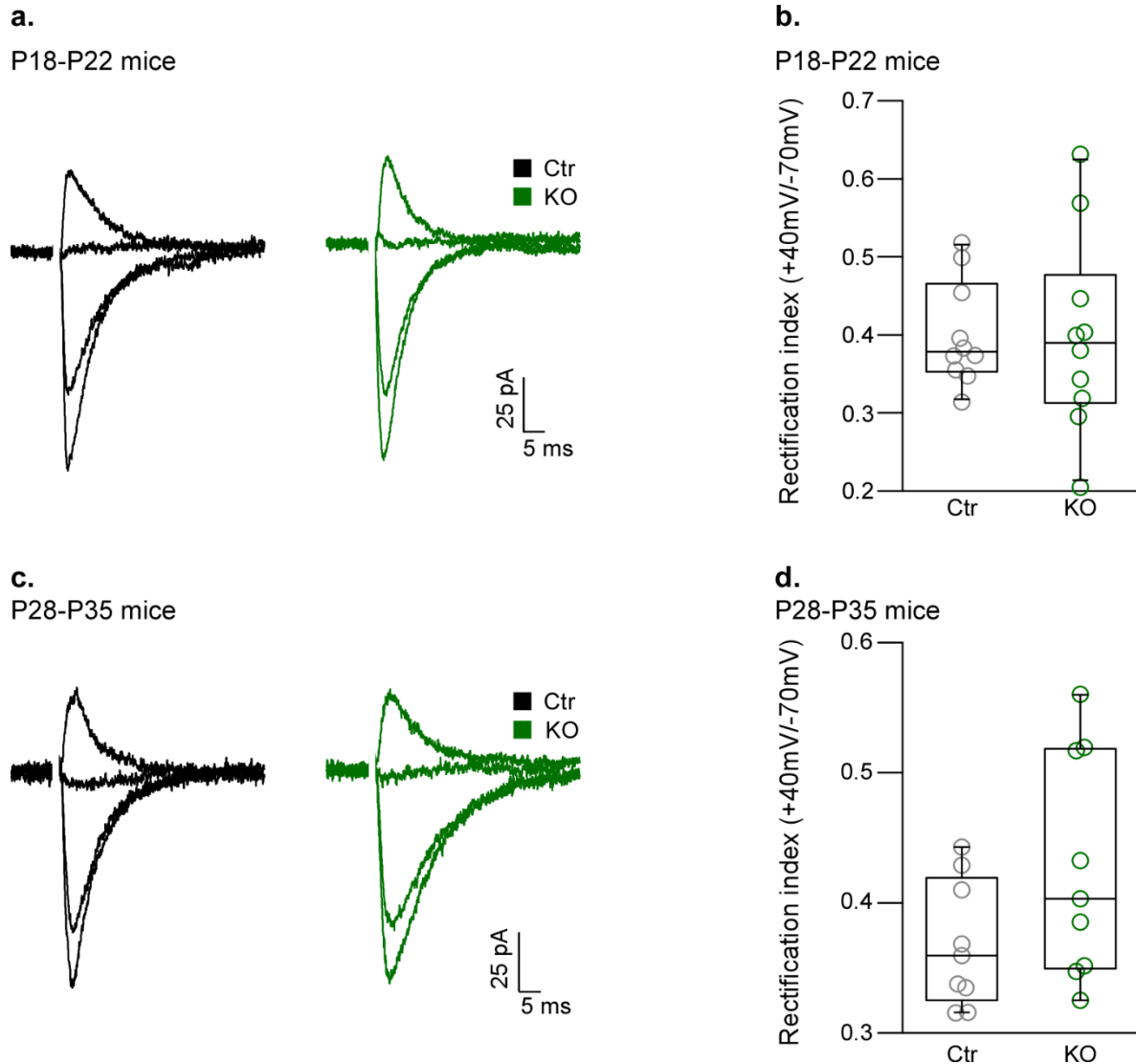


Figure 21. Postnatal knockout of αV integrin does not affect AMPAR subunit composition in developing mice. **a.** representative traces from P18-P22 mice recorded at different holding membrane potentials (-70, -40, 0 and +40mV); **b.** rectification index of P18-22 mice (*Itgav* Ctr n = 10 cells / 5 mice, KO n = 10 cells / 6 mice); **c.** representative traces from P28-P35 mice recorded at different holding membrane potentials (-70, -40, 0 and +40mV); **d.** rectification index of P28-P35 mice (*Itgav* Ctr n = 9 cells / 6 mice, KO n = 9 cells / 6 mice); box-plot shows median, 25 and 75 percentiles, whiskers showing 10 and 90 percentiles. The stimulation artifact is blanked.

5 Discussion

The integrin $\alpha V\beta 3$ is a cell adhesion molecule that binds to counter-receptors and extracellular matrix proteins that contain one or more RGD sequences. Using RGD peptides and KO models, previous studies have shown that the integrin $\alpha V\beta 3$ is important for synaptic transmission and plasticity *in vitro*, in particular for AMPAR trafficking and homeostatic synaptic scaling (Cingolani et al., 2008; Pozo et al., 2012). Knockout of $\beta 3$ integrin leads to abnormalities in social novelty preference, increased grooming and, possibly, reduced anxiety (Carter et al., 2011; Mcgeachie et al., 2012). Moreover, one point mutation in ITGB3, the gene coding $\beta 3$ integrin in humans, has been identified in one ASD male patient (O'Roak et al., 2012). Concerning integrin αV , several brain-specific conditional knockout models of *Itgav* have been generated but only one has been characterized at the behavioral level. More specifically, the Nestin-Cre *Itgav* KO mice (ablation from CNS precursor cells that give rise to both neurons and glia) develop seizures, motor dysfunction, and die prematurely (McCarty et al., 2005).

During my Ph.D., I have extended the behavioral characterization of the constitutive KO for $\beta 3$ integrin, I have carried out a behavioral characterization of a new conditional KO for αV integrin specific for excitatory neurons of the forebrain, and characterized excitatory synaptic transmission of mPFC in the two mouse models. Common phenotypes between the two mouse models are likely due to the loss of the dimer $\alpha V\beta 3$ integrin, while different phenotypes might result because of spatiotemporal differences in the impairment of $\alpha V\beta 3$ integrin, and because, in the *Itgav* KO mice, other integrin heterodimers, besides $\alpha V\beta 3$, are likely to be affected.

Constitutive *Itgb3* KO deletes $\beta 3$ integrin throughout development and in all tissues. However, in the brain, $\beta 3$ integrin dimers only with αV integrin, so other integrins are not directly affected. While, in the conditional KO for *Itgav* under the *CamK2a* promotor, αV integrin is removed only from excitatory neurons of the forebrain starting from second-third postnatal week, thus not interfering with early developmental stages, but other dimers containing αV integrin (such as $\alpha V\beta 5$ and $\alpha V\beta 8$) expressed in these neurons are likely to be affected as well.

Using a comprehensive set of behavioral assays for social interaction, repetitive behaviors, and other associated symptoms to ASD, I confirmed that global KO for *Itgb3*, and showed that our conditional KO for *Itgav*, present behavioral phenotypes relevant for the symptoms of ASD.

Using a three-chamber test, I show that *Itgav* KO mice have reduced preference for mouse versus object than control mice, indicating reduced sociability. While both *Itgb3* KO and *Itgav* KO mice showed reduced preference for a novel mouse versus a familiar mouse, indicating impairments in social novelty preference or social memory. Therefore, I replicated previous observations that global KO *Itgb3* induce deficits in social novelty preference (Carter et al., 2011), and showed for the first time that αV integrin is also necessary for normal social novelty behavior. Therefore, even an exclusively postnatal ablation of αV integrin is able to mimic symptoms of ASD, a developmental disorder.

Importantly, this phenotype is not due to olfactory impairments, since both *Itgb3* KO and *Itgav* KO were able to successfully distinguish and habituate to different non-social and social odors.

Surprisingly, I found no differences in repetitive behaviors (grooming, vertical rearings, and jumps) between *Itgb3* and *Itgav* KO mice and their specific controls. This result is in apparent contrast with previous observations of increased grooming in *Itgb3* KO mice (Carter et al., 2011). Divergent results are not uncommon for genetic models of ASD when the same or similar lines of mice are tested in different laboratories. For example, adult mice lacking neuroligin 4 were shown at first to present social approach deficits and reduced ultrasonic vocalizations (Jamain et al., 2008). However, in later characterization of the same animal line, such phenotypes were lost (EY et al., 2012). Such divergences were attributed to the contribution of environmental, generational and/or procedural factors on behavioral phenotypes (EY et al., 2012). Since I used the same lineage of mice than Carter and colleagues, the divergent findings are most probably due to environmental or generational differences. Procedural factors are probably not the cause of the discrepancies, since I used the same methodology as described in Carter and colleagues' work, and ran several pilot trials testing for several experimental conditions such as cage-type and illumination.

In addition to test for social and repetitive behaviors, I studied associated symptoms which are present in subsets of patients with ASD, including abnormal reactivity to sensory stimulation and anxiety (Silverman et al., 2010). Regarding sensory reactivity, I found a hyper-reaction to acoustic stimuli of high intensity in *Itgb3* Het, KO and *Itgav* KO mice comparing to their controls. Nevertheless, the ability to gate the motor response of a strong acoustic stimulus by a weaker acoustic prepulse is not altered by loss of either $\beta 3$ or αV integrin.

In the open field test, I did not find any sign of altered exploratory behavior pointing towards normal anxiety levels for both *Itgb3* and *Itgav* KO mice. Previous studies presented opposing results regarding anxiety levels in *Itgb3* KO. Carter and colleagues failed to find differences in open-field and elevated plus-maze (Carter et al., 2011), in line with my observations. While McGeachi and colleagues found decreased anxiety levels using the elevated plus-maze (Mcgeachie et al., 2012). As for the repetitive behaviors, we may assume these are not the strongest phenotypes induced by impairment of $\alpha V\beta 3$ integrin, and that these abnormalities may be more sensitive to external factors.

Remarkably, a protocol of post-traumatic stress in rats induces a biphasic regulation of the levels of αV mRNA and protein levels in the mPFC (Li et al., 2015). While *Itgb3* KO mice have exaggerated vulnerability to unpredictable chronic mild stress, since stereotypic behaviors (including grooming) and entries in center area in the open-field were significantly reduced specifically in *Itgb3* KO mice (Varney et al., 2015). In this view, a reduction of stress in animals tested by Carter and colleagues, or a very slight increase in stress in our animals (because I find no changes in the open-field) could underlie the differences observed in these two studies.

Regardless, $\alpha V\beta 3$ integrin function appears to be essential for normal social behavior because in both models of αV and $\beta 3$ integrin KO, even with all the differences between them already discussed, it is possible to replicate the impairments in social novelty preference.

Motor coordination and balance was further shown to be unaffected by loss of either $\beta 3$ or αV integrin using the accelerating rotarod. Additionally, the lack of differences in average speed and total distance measured in the open-field points towards

normal ambulatory capacities in both mouse lines. In previous works, the full KO for *Itgb3* presented similar values of distance traveled and ambulatory time as WT in the open-field (Carter et al., 2011), but a conditional KO for *Itgav* (under the Nestin-Cre system) from CNS precursor cells that give rise both to neurons and glia, showed signs of ataxia (McCarty et al., 2005). These deficits of locomotion in Nestin-Cre *Itgav* KO were attributed to demyelination and axonal degeneration in the spinal cord and cerebellum (McCarty et al., 2005). Since $\beta 3$ integrin is not expressed in the cerebellum and the conditional KO for *Itgav* I used (under *CamK2a* promotor) spare these brain regions, normal ambulatory capacities, as I observed, were expected. Altogether, the behavioral data clearly indicate that both *Itgb3* and *Itgav* KO mice present autistic-like impairments in social novelty and in associated symptoms such as increased hyperreactivity to sensory stimuli. This is the first time integrin αV was implicated in the neurogenetics of ASD. Since the same impairments are found in both models, it is reasonable to attribute these phenotypes to a specific and developmental-independent lack of $\alpha V\beta 3$ integrin. Indeed, even if $\alpha V\beta 3$ integrin is present during early development (like in *Itgav* KO under *CamK2a* promotor), normal social behavior still requires $\alpha V\beta 3$ integrin expression in excitatory neurons later in development.

Epilepsy is often comorbid with ASD and integrin αV was previously suggested to be implicated with higher propensity for seizures (McCarty et al., 2005; Silverman et al., 2010). My data show that both *Itgb3* KO and *Itgav* KO have reduced threshold for seizures induced by the convulsive compound PTZ. Whereas, with Nestin-Cre KO for *Itgav*, is not possible to attribute the higher propensity for seizures to a lack of αV integrin in a specific cell type (because Nestin promotor is expressed in

progenitor cells of both neurons and glial cells), here I show that ablating αV integrin exclusively from the excitatory neurons of the forebrain is enough to reduce the threshold for seizures. Suggesting that lack of $\alpha V\beta 3$ integrin in excitatory neurons of the forebrain, even later in development, confers higher susceptibility for seizures. Overall, my results show that both models present autistic-like behaviors and a reduced threshold for seizures. The reciprocity of the behavioral impairments suggests that these deficits are due specifically to an impairment in $\alpha V\beta 3$ integrin. Moreover, considering that $\beta 3$ integrin, in the brain, is enriched in layer V pyramidal neurons and that, in the conditional *Itgav* KO, αV integrin is ablated selectively from postnatal excitatory neurons of the forebrain, mature excitatory circuits of the cortex are likely to underlie such impairments.

To understand the synaptic changes defects that could account for the deficits found in behavior, I studied excitatory synaptic transmission onto layer V pyramidal neurons of the mPFC in *ex vivo* cortical slices. I focused on the mPFC because deep-layer projecting neurons from prefrontal and primary motor-somatosensory cortex are consistently involved in these pathologies (Sestan and State, 2018; Willsey et al., 2013), and $\beta 3$ is specifically enriched in this region.

I found that GluA2-containing AMPARs are more abundant in *Itgb3* KO than in WT mice at all developmental stages I considered (P9-P11 and P17-P21), which was maintained in adulthood. These effects are specific because other aspects of synaptic transmission, short-term synaptic plasticity, NMDAR/AMPA ratio, and NMDAR subunit composition were unchanged in the *Itgb3* KO mice.

To note that AMPAR subunit composition undergo a developmental switch in layer V pyramidal neurons of the frontal cortex. GluA2-lacking AMPARs are broadly

detected in synapses in early development, but after P14 they are rapidly lost from synapses and replaced by GluA2-containing AMPARs (Diering and Huganir, 2018; Kumar et al., 2002). Later in development, GluA2-lacking AMPARs are expressed at synapses mainly to sustain certain forms of synaptic plasticity. But it was reported in mice, at excitatory synapses on cortical layer V neurons, a circadian insertion and removal of GluA2-lacking AMPARs during their active phase (at night) and during the sleep phase (during the day), respectively (Diering and Huganir, 2018; Lante et al., 2011). Since I found increased GluA2-containing AMPARs content at synapses of layer V pyramidal neurons of the mPFC of *Itgb3* KO mice at early development (during the day), it is possible that lack of $\beta 3$ integrin is shortening the developmental switch period of AMPAR subunit composition or is impairing the daily cycle of incorporation of GluA2-lacking AMPARs.

Interestingly, the behavioral phenotypes of constitutive *Itgb3* and conditional *Itgav* (under *CamK2a* promotor) KO mice are similar to that of the *Nlgn3* KO mice. *Nlgn3* KO mice exhibit normal sociability but impaired social novelty preference (Bariselli et al., 2018; Radyushkin et al., 2009). Additionally, since epilepsy is often comorbid with ASD, Hill-Yardin and colleagues tested if a point mutation in neuroligin 3 gene (*NLGN3*^{R451C}) found in patients with ASD would change PTZ-induced seizure susceptibility. Mice carrying this mutation (*Nlgn3*^{R451C} mice) are more resistant to generalized convulsive seizures (Hill-Yardin et al., 2015), which is the opposite effect seen in our conditional KO for *Itgav* and constitutive KO for *Itgb3*.

KO mice for other cell adhesion molecules show aberrant behaviors, which are only partially overlapping with the phenotype exhibited by mice with impaired $\alpha V\beta 3$ integrin. For example, neuroligin 4 KO mice show impaired sociability and social

novelty preference (Jamain et al., 2008). The ablation of neuroligin 1 or neurexin 1 α leaves sociability and social novelty preference intact but leads to increased repetitive behaviors (Blundell et al., 2010; Etherton et al., 2009). Therefore, cell adhesion molecules seem to play important roles in the neural circuits underlying ASD, in a non-redundant way.

Remarkably, the correct function of dopaminergic neurons of the ventral tegmental area (VTA) and the insertion of GluA2-lacking AMPARs in these neurons, are essential for normal social novelty preference. Social interaction with an unfamiliar congenital mouse leads to an enrichment in GluA2-lacking AMPARs, which is maintained after repeated and sustained conspecific interaction. Chemogenetic blockage of the activity of dopaminergic neurons of the VTA, or NASPM (a GluA2-lacking AMPAR blocker) infusion in the VTA occludes the preference for social novelty. Intriguingly, constitutive KO or postnatal knockdown of *Nlgn3* induces an aberrant increase of GluA2-lacking AMPARs (Bariselli et al., 2018). The authors suggest this baseline (i.e. before social interaction with unknown conspecifics) aberrant increase in GluA2-lacking AMPARs may occlude synaptic plasticity during social interaction, and account for the social novelty deficits characteristic of *Nlgn3* KO mice.

In *Itgb3* and *Itgav* KO mice, I found reduced levels of GluA2-lacking AMPARs in layer V pyramidal neurons of the mPFC. Although I did not measure the rectification of AMPAR currents before and after social interaction with conspecifics, it is tempting to speculate that, if the same synaptic signature is present in the mPFC as in the VTA, we are in a situation opposed to that observed in the *Nlgn3* KO mice. Without integrin α V β 3, layer V pyramidal neurons of the mPFC may be incapable to

upregulate GluA2-lacking AMPARs during social interaction with unknown conspecifics, thus causing the observed behavior deficits. Therefore, it would be interesting to evaluate, whether the rectification of AMPAR currents changes after social interaction in the mPFC, and, if this is the case, whether those changes are absent in *Itgb3* and *Itgav* KO mice.

It is worth remembering that the VTA sends projections mainly to the mPFC and the nucleus accumbens (NAc). It was shown by optogenetics that the circuit VTA-to-mPFC accounts for anxiety-like effects but not sociability. By contrast, stimulation of the circuit VTA-to-NAc affected sociability (Gunaydin et al., 2014). Nevertheless, this study did not address possible roles of the VTA-to-mPFC circuit in social novelty.

I investigated only basal excitatory synaptic transmission onto the mPFC but differences in the content of the GluA2 subunit at the synapse is involved in many synaptic plasticity mechanisms such as LTP, LTD and homeostatic plasticity (Diering and Huganir, 2018). Moreover, $\beta 3$ integrin has been previously implicated in homeostatic synaptic scaling (Cingolani et al., 2008; Thalhammer and Cingolani, 2014). It would, therefore, be important to study these types of synaptic plasticity in the mPFC of $\alpha V\beta 3$ integrin-deficient mice.

6 Conclusions and future perspectives

In this work, I provide evidence that $\alpha\text{V}\beta 3$ integrin plays important roles in synapses of layer V pyramidal neurons of the mPFC and that ablation of this integrin leads to ASD-like behavior and lower threshold for seizures.

Impairment in $\alpha\text{V}\beta 3$ integrin leads to aberrant social novelty preference and hyper-reactivity to acoustic stimuli of high intensity. However, I did not find increased grooming when *Itgb3* KO mice are placed in a novel environment, unlike a previous report (Carter et al., 2011). Since *Itgb3* KO mice are particularly sensitive to stress (Varney et al., 2015), it would be interesting to repeat the analysis of grooming in novel environments after a protocol of chronic stress, and after administration of anxiolytic drugs.

I also showed that impairment in $\alpha\text{V}\beta 3$ integrin leads to reduced threshold for PTZ-induced seizures. It would be very interesting to perform electroencephalogram (EEG) recordings in our models to test for spontaneous epileptiform activity.

Lastly, I found an increased expression of synaptic GluA2-containing AMPARs content in *Itgb3* KO mice. The implications of this observation in synaptic plasticity is still to be elucidated. Particularly, it would be important to test LTP and homeostatic synaptic plasticity in the mPFC of *Itgb3* KO mice. Considering that changes in the rectification of AMPAR currents occur in the VTA after social interaction (Bariselli et al., 2018), and that the VTA connects to the mPFC (Gunaydin et al., 2014), it would be interesting to study AMPAR subunit composition in the mPFC before and after social interaction with a stranger mouse.

7 Bibliography

Abe, A., Hashimoto, K., Akiyama, A., Iida, M., Ikeda, N., Hamano, A., Watanabe, R., Hayashi, Y., and Miyamoto, Y. (2018). $\alpha v\beta 5$ integrin mediates the effect of vitronectin on the initial stage of differentiation in mouse cerebellar granule cell precursors. *Brain Res.* 1691, 94–104.

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders : DSM-5*. American Psychiatric Association (Arlington, VA).

Anton, E., Kreidberg, J.A., and Rakic, P. (1999). Distinct Functions of $\alpha 3$ and αV Integrin Receptors in Neuronal Migration and Laminar Organization of the Cerebral Cortex. *Neuron* 22, 277–289.

Bader, B.L., Rayburn, H., Crowley, D., and Hynes, R.O. (1998). Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell* 95, 507–519.

Baio, J., Wiggins, L., Christensen, D.L., Maenner, M.J., Daniels, J., Warren, Z., Kurzius-Spencer, M., Zahorodny, W., Robinson, C., Rosenberg, et al. (2018). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR. Surveill. Summ.* 67, 1–23.

Bariselli, S., Hörnberg, H., Prévost-Solié, C., Musardo, S., Hatstatt-Burklé, L., Scheiffele, P., and Bellone, C. (2018). Role of VTA dopamine neurons and neuroligin 3 in sociability traits related to nonfamiliar conspecific interaction. *Nat. Commun.* 9, 1–15.

Bassani, S., and Cingolani, L.A. (2012). Tetraspanins: Interactions and interplay with

integrins. *Int. J. Biochem. Cell Biol.* 44, 703–708.

Betancur, C. (2011). Etiological heterogeneity in autism spectrum disorders: More than 100 genetic and genomic disorders and still counting. *Brain Res.* 1380, 42–77.

Bissonette, G.B., Bae, M.H., Suresh, T., Jaffe, D.E., and Powell, E.M. (2014). Prefrontal cognitive deficits in mice with altered cerebral cortical GABAergic interneurons. *Behav. Brain Res.* 259, 143–151.

Blundell, J., Blaiss, C.A., Etherton, M.R., Espinosa, F., Tabuchi, K., Walz, C., Bolliger, M.F., Sudhof, T.C., and Powell, C.M. (2010). Neuroligin-1 Deletion Results in Impaired Spatial Memory and Increased Repetitive Behavior. *J. Neurosci.* 30, 2115–2129.

Bozdagi, O., Tavassoli, T., and Buxbaum, J.D. (2013). Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay. *Mol. Autism* 4, 1–4.

Bozzi, Y., Provenzano, G., and Casarosa, S. (2018). Neurobiological bases of autism–epilepsy comorbidity: a focus on excitation/inhibition imbalance. *Eur. J. Neurosci.* 47, 534–548.

Brenneman, L.H., Kochlamazashvili, G., Stoenica, L., Nonneman, R.J., Moy, S.S., Schachner, M., Dityatev, A., and Maness, P.F. (2011). Transgenic mice overexpressing the extracellular domain of NCAM are impaired in working memory and cortical plasticity. *Neurobiol. Dis.* 43, 372–378.

Brockhaus, J., Schreitmüller, M., Repetto, D., Klatt, O., Reissner, C., Elmslie, K., Heine, M., and Missler, M. (2018). α -Neurexins together with $\alpha 2\delta$ -1 auxiliary subunits regulate Ca^{2+} influx through $\text{CaV}2.1$ channels. *J. Neurosci.* 38, 8277–8294.

Brooks-Kayal, A. (2011). Molecular mechanisms of cognitive and behavioral

comorbidities of epilepsy in children. *Epilepsia* 52, 13–20.

Brückner, G., Grosche, J., Hartlage-Rübsamen, M., Schmidt, S., and Schachner, M. (2003). Region and lamina-specific distribution of extracellular matrix proteoglycans, hyaluronan and tenascin-R in the mouse hippocampal formation. *J. Chem. Neuroanat.* 26, 37–50.

Brückner, G., Szeöke, S., Pavlica, S., Grosche, J., and Kacza, J. (2006). Axon initial segment ensheathed by extracellular matrix in perineuronal nets. *Neuroscience* 138, 365–375.

Campbell, D.B., Li, C., Sutcliffe, J.S., Persico, A.M., and Levitt, P. (2008). Genetic evidence implicating multiple genes in the met receptor tyrosine kinase pathway in autism spectrum disorder. *Autism Res.* 1, 159–168.

Canitano, R. (2007). Epilepsy in autism spectrum disorders. *Eur. Child Adolesc. Psychiatry* 16, 61–66.

Carter, M.D., Shah, C.R., Muller, C.L., Crawley, J.N., Carneiro, A.M.D., and Veenstra-VanderWeele, J. (2011). Absence of preference for social novelty and increased grooming in integrin $\beta 3$ knockout mice: Initial studies and future directions. *Autism Res.* 4, 57–67.

Celio, M.R., and Blumcke, I. (1994). Perineuronal nets - a specialized form of extracellular matrix in the adult nervous system. *Brain Res. Rev.* 19, 128–145.

Cerliani, L., Mennes, M., Thomas, R.M., Di Martino, A., Thioux, M., and Keyzers, C. (2015). Increased functional connectivity between subcortical and cortical resting-state networks in Autism spectrum disorder. *JAMA Psychiatry* 72, 767–777.

Chaabouni, M., Martinovic, J., Sanlaville, D., Attié-Bittach, T., Caillat, S., Turleau, C., Vekemans, M., and Morichon, N. (2006). Prenatal diagnosis and molecular

characterization of an interstitial 1q24.2q25.2 deletion. *Eur. J. Med. Genet.* 49, 487–493.

Chan, C.-S., Weeber, E.J., Kurup, S., Sweatt, J.D., and Davis, R.L. (2003). Integrin requirement for hippocampal synaptic plasticity and spatial memory. *J. Neurosci.* 23, 7107–7116.

Chao, C.C., Ma, Y.L., Chu, K.Y., and Lee, E.H.Y. (2003). Integrin α_v and NCAM mediate the effects of GDNF on DA neuron survival, outgrowth, DA turnover and motor activity in rats. *Neurobiol. Aging* 24, 105–116.

Chavis, P., and Westbrook, G. (2001). Integrins mediate functional pre- and postsynaptic maturation at a hippocampal synapse. *Nature* 411, 317–321.

Chen, N., Bao, Y., Xue, Y., Sun, Y., Hu, D., Meng, S., Lu, L., and Shi, J. (2017). Meta-analyses of RELN variants in neuropsychiatric disorders. *Behav. Brain Res.* 332, 110–119.

Chez, M.G., Chang, M., Krasne, V., Coughlan, C., Kominsky, M., and Schwartz, A. (2006). Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. *Epilepsy Behav.* 8, 267–271.

Chun, D., Gall, C.M., Bi, X., and Lynch, G. (2001). Evidence that integrins contribute to multiple stages in the consolidation of long term potentiation in rat hippocampus. *Neuroscience* 105, 815–829.

Cingolani, L.A., Thalhammer, A., Yu, L.M.Y., Catalano, M., Ramos, T., Colicos, M.A., and Goda, Y. (2008). Activity-Dependent Regulation of Synaptic AMPA Receptor Composition and Abundance by β_3 Integrins. *Neuron* 58, 749–762.

Cook, E.H., and Leventhal, B.L. (1996). The serotonin system in autism. *Curr. Opin.*

Pediatr. 8, 348–354.

Cull-Candy, S., Brickley, S., and Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11, 327–335.

Dansie, L.E., and Ethell, I.M. (2011). Casting a net on dendritic spines: The extracellular matrix and its receptors. *Dev. Neurobiol.* 71, 956–981.

Diering, G.H., and Huganir, R.L. (2018). The AMPA Receptor Code of Synaptic Plasticity. *Neuron* 100, 314–329.

Dityatev, A., and Rusakov, D.A. (2011). Molecular signals of plasticity at the tetrapartite synapse. *Curr. Opin. Neurobiol.* 21, 353–359.

Dityatev, A., Schachner, M., and Sonderegger, P. (2010). The dual role of the extracellular matrix in synaptic plasticity and homeostasis. *Nat. Rev. Neurosci.* 11, 735–746.

Dityatev, A., Wehrle-Haller, B., and Pitkänen, A. (2014). *Brain Extracellular Matrix in Health and Disease*, First edition (Amsterdam: Elsevier B.V.).

Dohn, M.R., Kooker, C.G., Bastarache, L., Jessen, T., Rinaldi, C., Varney, S., Mazaloukas, M.D., Pan, H., Oliver, K.H., Velez Edwards, D.R., et al. (2017). The Gain-of-Function Integrin $\beta 3$ Pro33 Variant Alters the Serotonin System in the Mouse Brain. *J. Neurosci.* 37, 11271–11284.

Drgonova, J., and Walther, D. (2016). Cadherin 13: Human cis-Regulation and Selectively Altered Addiction Phenotypes and Cerebral Cortical Dopamine in Knockout Mice. *Mol. Med.* 22, 537–547.

Ebrahimi-Fakhari, D., and Sahin, M. (2015). Autism and the synapse: Emerging mechanisms and mechanism-based therapies. *Curr. Opin. Neurol.* 28, 91–102.

Estes, M.L., and McAllister, A.K. (2016). Maternal immune activation: Implications

for neuropsychiatric disorders. *Science* (80-.). 353, 772–777.

Etherton, M.R., Blaiss, C.A., Powell, C.M., and Sudhof, T.C. (2009). Mouse neurexin-1 deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. *Proc. Natl. Acad. Sci.* 106, 17998–18003.

EY, E., M.Yang, Katz, A.M., Wolderyohannes, L., Silverman, J.L., Leblond, C.S., Faure, P., Torquet, N., Sourd, A.M. Le, Bourgeron, T., et al. (2012). Absence of deficits in social behaviors and ultrasonic vocalizations in later generations of mice lacking neuroligin4. *Genes Brain Behav.* 11, 928–941.

Fisher, R.S. (2017). The New Classification of Seizures by the International League Against Epilepsy 2017. *Curr. Neurol. Neurosci. Rep.* 17, 1–6.

Fisher, R.S., Van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P., and Engel, J. (2005). Epileptic seizures and epilepsy: Definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 46, 470–472.

Fishman, I., Keown, C.L., Lincoln, A.J., Pineda, J.A., and Müller, R.A. (2014). Atypical cross talk between mentalizing and mirror neuron networks in autism spectrum disorder. *JAMA Psychiatry* 71, 751–760.

Folstein, S.E., and Rosen-Sheidley, B. (2001). Genetics of autism: Complex aetiology for a heterogeneous disorder. *Nat. Rev. Genet.* 2, 943–955.

Frischknecht, R., and Gundelfinger, E.D. (2012). The Brain's Extracellular Matrix and Its Role in Synaptic Plasticity. In *Synaptic Plasticity*, (Springer US), pp. 153–171.

Fukata, Y., and Fukata, M. (2017). Epilepsy and synaptic proteins. *Curr. Opin. Neurobiol.* 45, 1–8.

Fukata, Y., Adesnik, H., Iwanaga, T., Bredt, D.S., Nicoll, R.A., and Fukata, M. (2006).

Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. *Science* (80-.). *313*, 1792–1795.

Gardener, H., Spiegelman, D., and Buka, S.L. (2009). Prenatal risk factors for autism: Comprehensive meta-analysis. *Br. J. Psychiatry* *195*, 7–14.

Goodkin, H.P., Joshi, S., Mtchedlishvili, Z., Brar, J., and Kapur, J. (2008). Subunit-Specific Trafficking of GABAA Receptors during Status Epilepticus. *J. Neurosci.* *50*, 2011–2018.

Grone, B.P., and Baraban, S.C. (2015). Animal models in epilepsy research: legacies and new directions. *Nat. Neurosci.* *18*, 339–343.

Grooms, S.Y., and Jones, L.S. (1997). RGDS tetrapeptide and hippocampal in vitro kindling in rats: Evidence for integrin-mediated physiological stability. *Neurosci. Lett.* *231*, 139–142.

Grüter, T., Wiescholleck, V., Dubovyk, V., Aliane, V., and Manahan-Vaughan, D. (2015). Altered neuronal excitability underlies impaired hippocampal function in an animal model of psychosis. *Front. Behav. Neurosci.* *9*, 1–15.

Gunaydin, L.A., Grosenick, L., Finkelstein, J.C., Kauvar, I. V., Fenno, L.E., Adhikari, A., Lammel, S., Mirzabekov, J.J., Airan, R.D., Zalocusky, K.A., et al. (2014). Natural neural projection dynamics underlying social behavior. *Cell* *157*, 1535–1551.

Gundelfinger, E.D., Frischknecht, R., Choquet, D., and Heine, M. (2010). Converting juvenile into adult plasticity: A role for the brain's extracellular matrix. *Eur. J. Neurosci.* *31*, 2156–2165.

Gupton, S.L., and Gertler, F.B. (2010). Integrin signaling switches the cytoskeletal and exocytic machinery that drives neuritogenesis. *Dev. Cell* *18*, 725–736.

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe, T., Miller, J.,

Fedele, A., Collins, J., Smith, K., et al. (2011). Genetic heritability and shared environmental factors among twin pairs with autism. *Arch. Gen. Psychiatry* 68, 1095–1102.

Harlow, D.E., Saul, K.E., Komuro, H., and Macklin, W.B. (2015). Myelin Proteolipid Protein Complexes with α 5 Integrin and AMPA Receptors In Vivo and Regulates AMPA-Dependent Oligodendrocyte Progenitor Cell Migration through the Modulation of Cell-Surface GluR2 Expression. *J. Neurosci.* 35, 12018–12032.

Hill-Yardin, E.L., Argyropoulos, A., Hosie, S., Rind, G., Anderson, P., Hannan, A.J., and O'Brien, T.J. (2015). Reduced susceptibility to induced seizures in the Neuroligin-3R451C mouse model of autism. *Neurosci. Lett.* 589, 57–61.

Holt, R., Barnby, G., Maestrini, E., Bacchelli, E., Brocklebank, D., Sousa, I., Mulder, E.J., Kantojärvi, K., Järvelä, I., Klauck, S.M., et al. (2010). Linkage and candidate gene studies of autism spectrum disorders in European populations. *Eur. J. Hum. Genet.* 18, 1013–1020.

Humphries, J.D. (2006). Integrin ligands at a glance. *J. Cell Sci.* 119, 3901–3903.

Hynes, R.O. (2002). Integrins: Bidirectional, allosteric signaling machines. *Cell* 110, 673–687.

Jamain, S., Quach, H., Betancur, C., Råstam, M., Colineaux, C., Gillberg, C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C., et al. (2003). Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat. Genet.* 34, 27–29.

Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., Varoqueaux, F., Ramanantsoa, N., Gallego, J., Ronnenberg, A., Winter, D., et al. (2008). Reduced social interaction and ultrasonic communication in a mouse model

of monogenic heritable autism. *Proc. Natl. Acad. Sci. U. S. A.* 105, 1710–1715.

Jeste, S.S., and Tuchman, R. (2015). Autism Spectrum Disorder and Epilepsy: Two Sides of the Same Coin? *J. Child Neurol.* 30, 1963–1971.

Jokiranta, E., Brown, A.S., Heinimaa, M., Cheslack-Postava, K., Suominen, A., and Sourander, A. (2013). Parental psychiatric disorders and autism spectrum disorders. *Psychiatry Res.* 207, 203–211.

Jones, E. V., Bernardinelli, Y., Tse, Y.C., Chierzi, S., Wong, T.P., and Murai, K.K. (2011). Astrocytes Control Glutamate Receptor Levels at Developing Synapses through SPARC- Integrin Interactions. *J. Neurosci.* 31, 4154–4165.

Kana, R.K., Keller, T., Cherkassky, V.L., Minshew, N.J., and Just, M.A. (2009). Atypical frontal-posterior synchronization of Theory of Mind regions in autism during mental state attribution. *Soc. Neurosci.* 4, 135–152.

Kandel, E.R., Schwartz, J.H., and Jessell, T.M. (2013). *Principles of Neural Science*, Fifth Edition (McGraw-Hill Medical).

Kang, W., Choi, J., Shin, Y., Kim, H., Cha, J., Lee, J., Chun, M., and Lee, M. (2008). Differential regulation of osteopontin receptors , CD44 and the αv and $\beta 3$ integrin subunits , in the rat hippocampus following transient forebrain ischemia. *Brain Res.* 8, 0–8.

Kerrisk, M.E., Greer, C.A., and Koleske, A.J. (2013). Integrin $\beta 3$ Is Required for Late Postnatal Stability of Dendrite Arbors, Dendritic Spines and Synapses, and Mouse Behavior. *J. Neurosci.* 33, 6742–6752.

Kim, H.-G., Kishikawa, S., Higgins, A.W., Seong, I.-S., Donovan, D.J., Shen, Y., Lally, E., Weiss, L.A., Najm, J., Kutsche, K., et al. (2008). Disruption of Neurexin 1 Associated with Autism Spectrum Disorder. *Am. J. Hum. Genet.* 82, 199–207.

Krook-Magnuson, E., and Soltesz, I. (2015). Beyond the hammer and the scalpel: selective circuit control for the epilepsies. *Nat. Neurosci.* *18*, 331–338.

Kumar, S.S., Bacci, A., Kharazia, V., and Huguenard, J.R. (2002). A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. *J Neurosci* *22*, 3005–3015.

Lante, F., Toledo-Salas, J.-C., Ondrejcek, T., Rowan, M.J., and Ulrich, D. (2011). Removal of Synaptic Ca²⁺-Permeable AMPA Receptors during Sleep. *J. Neurosci.* *31*, 3953–3961.

Lazarevic, V., Pothula, S., Andres-Alonso, M., and Fejtova, A. (2013). Molecular mechanisms driving homeostatic plasticity of neurotransmitter release. *Front. Cell. Neurosci.* *7*, 244.

LeBaron, R.G., Hernandez, R. V., Orfila, J.E., and Martinez, J.L. (2003). An integrin binding peptide reduces rat CA1 hippocampal long-term potentiation during the first few minutes following theta burst stimulation. *Neurosci. Lett.* *339*, 199–202.

Lee, E., Lee, J., and Kim, E. (2017). Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders. *Biol. Psychiatry* *81*, 838–847.

Li, Y., Han, F., and Shi, Y. (2015). Changes in integrin α_v , vinculin and connexin43 in the medial prefrontal cortex in rats under single-prolonged stress. *Mol. Med. Rep.* *11*, 2520–2526.

Lodish, H.F., Berk, A., Kaiser, C.A., Kreiger, M., Bretscher, A., Ploegh, H., Angelika, A., and Kesley C., M. (2016). *Molecular Cell Biology*, 8th edition (New York: W.H. Freeman and Company).

Mannion, A., and Leader, G. (2014). Epilepsy in autism spectrum disorder. *Res. Autism Spectr. Disord.* *8*, 354–361.

McCarty, J.H., Lacy-Hulbert, A., Charest, A., Bronson, R.T., Crowley, D., Housman, D., Savill, J., Roes, J., and Hynes, R.O. (2005). Selective ablation of α 5 integrins in the central nervous system leads to cerebral hemorrhage, seizures, axonal degeneration and premature death. *Development* 132, 165–176.

Mcgeachie, A.B., Skrzypiec, A.E., Cingolani, L.A., Letellier, M., Pawlak, R., and Goda, Y. (2012). β 3 Integrin Is Dispensable for Conditioned Fear and Hebbian Forms of Plasticity in the Hippocampus. *Eur. J. Neurosci.* 36, 2461–2469.

Michelucci, R., Pulitano, P., Di Bonaventura, C., Binelli, S., Luisi, C., Pasini, E., Striano, S., Striano, P., Coppola, G., La Neve, A., et al. (2017). The clinical phenotype of autosomal dominant lateral temporal lobe epilepsy related to reelin mutations. *Epilepsy Behav.* 68, 103–107.

Missaire, M., and Hindges, R. (2015). The role of cell adhesion molecules in visual circuit formation: From neurite outgrowth to maps and synaptic specificity. *Dev. Neurobiol.* 75, 569–583.

Morrow, E.M., Yoo, S.Y., Flavell, S.W., Kim, T.K., Lin, Y., Hill, R.S., Mukaddes, N.M., Balkhy, S., Gascon, G., Hashmi, A., et al. (2008). Identifying autism loci and genes by tracing recent shared ancestry. *Science* (80-.). 321, 218–223.

Mortillo, S., Elste, A., Ge, Y., Patil, S.B., Hsiao, K., Huntley, G.W., Davis, R.L., and Benson, D.L. (2012). Compensatory redistribution of neuroligins and N-cadherin following deletion of synaptic β 1-integrin. *J. Comp. Neurol.* 520, 2041–2052.

Myers, K.A., Johnstone, D.L., and Dymment, D.A. (2018). Epilepsy genetics: Current knowledge, applications, and future directions. *Clin. Genet.* 95, 95–111.

Napolioni, V., Lombardi, F., Sacco, R., Curatolo, P., Manzi, B., Alessandrelli, R., Militerni, R., Bravaccio, C., Lenti, C., Sacconi, M., et al. (2011). Family-based

association study of ITGB3 in autism spectrum disorder and its endophenotypes. *Eur. J. Hum. Genet.* *19*, 353–359.

Naylor, D.E., Liu, H., Niquet, J., and Wasterlain, C.G. (2013). Rapid surface accumulation of NMDA receptors increases glutamatergic excitation during status epilepticus. *Neurobiol. Dis.* *54*, 225–238.

Ndode-Ekane, X.E., and Pitkänen, A. (2013). Urokinase-type plasminogen activator receptor modulates epileptogenesis in mouse model of temporal lobe epilepsy. *Mol. Neurobiol.* *47*, 914–937.

Ning, L., Tian, L., Smirnov, S., Vihinen, H., Llano, O., Vick, K., Davis, R.L., Rivera, C., and Gahmberg, C.G. (2013). Interactions between ICAM-5 and $\beta 1$ integrins regulate neuronal synapse formation. *J. Cell Sci.* *126*, 77–89.

Nishimura, S.L., Boylen, K.P., Einheber, S., Milner, T.A., Ramos, D.M., and Pytela, R. (1998). Synaptic and glial localization of the integrin $\alpha v \beta 8$ in mouse and rat brain. *Brain Res.* *791*, 271–282.

Noebels, J. (2015). Pathway-driven discovery of epilepsy genes. *Nat. Neurosci.* *18*, 344–350.

O'Roak, B.J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B.P., Levy, R., Ko, A., Lee, C., Smith, J.D., et al. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* *485*, 246–250.

Oberman, L.M. (2012). mGluR antagonists and GABA agonists as novel pharmacological agents for the treatment of autism spectrum disorders. *Expert Opin. Investig. Drugs* *21*, 1819–1825.

Ono, T., and Galanopoulou, A.S. (2012). Epilepsy and epileptic syndrome. *Adv. Exp. Med. Biol.* *724*, 99–113.

- Orlando, C., Ster, J., Gerber, U., Fawcett, J.W., and Raineteau, O. (2012). Perisynaptic Chondroitin Sulfate Proteoglycans Restrict Structural Plasticity in an Integrin-Dependent Manner. *J. Neurosci.* 32, 18009–18017.
- Pan, Y., Chen, J., Guo, H., Ou, J., Peng, Y., Liu, Q., Shen, Y., Shi, L., Liu, Y., Xiong, Z., et al. (2015). Association of genetic variants of GRIN2B with autism. *Sci. Rep.* 5, 8296.
- Park, Y.K., and Goda, Y. (2016). Integrins in synapse regulation. *Nat. Rev. Neurosci.* 17, 745–756.
- Patrikelis, P., Angelakis, E., and Gatzonis, S. (2009). Neurocognitive and behavioral functioning in frontal lobe epilepsy: A review. *Epilepsy Behav.* 14, 19–26.
- Patrylo, P.R., and Willingham, A. (2006). Anatomic and electrophysiologic evidence for a proconvulsive circuit in the dentate gyrus of reeler mutant mice, an animal model of diffuse cortical malformation. *Dev. Neurosci.* 29, 73–83.
- Patrylo, P.R., Browning, R.A., and Cranick, S. (2006). Reeler homozygous mice exhibit enhanced susceptibility to epileptiform activity. *Epilepsia* 47, 257–266.
- Pellegrini-Giampietro, D.E. (2003). An activity-dependent spermine-mediated mechanism that modulates glutamate transmission. *Trends Neurosci.* 26, 9–11.
- Persico, A.M., D'agruma, L., Maiorano, N., Totaro, A., Militeri, R., Bravaccio, C., Wassink, T.H., Schneider, C., Melmed, R., Trillo, S., et al. (2001). Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol. Psychiatry* 6, 150–159.
- Pinkstaff, J.K., Lynch, G., and Gall, C.M. (1998). Localization and seizure-regulation of integrin $\beta 1$ mRNA in adult rat brain. *Mol. Brain Res.* 55, 265–276.
- Pinkstaff, J.K., Detterich, J., Lynch, G., and Gall, C. (1999). Integrin subunit gene

expression is regionally differentiated in adult brain. *J Neurosci* 19, 1541–1556.

Pitkänen, A., and Lukasiuk, K. (2009). Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav.* 14, 16–25.

Pitkänen, A., Nehlig, A., Brooks-Kayal, A.R., Dudek, F.E., Friedman, D., Galanopoulou, A.S., Jensen, F.E., Kaminski, R.M., Kapur, J., Klitgaard, H., et al. (2013). Issues related to development of antiepileptogenic therapies. *Epilepsia* 54, 35–43.

Pozo, K., Cingolani, L.A., Bassani, S., Laurent, F., Passafaro, M., and Goda, Y. (2012). $\beta 3$ integrin interacts directly with GluA2 AMPA receptor subunit and regulates AMPA receptor expression in hippocampal neurons. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1323–1328.

Preiksaitiene, E., Männik, K., Dirse, V., Utkus, A., Ciuladaite, Z., Kasnauskiene, J., Kurg, A., and Kučinskas, V. (2012). A novel de novo 1.8 Mb microdeletion of 17q21.33 associated with intellectual disability and dysmorphic features. *Eur. J. Med. Genet.* 55, 656–659.

Radyushkin, K., Hammerschmidt, K., Boretius, S., Varoqueaux, F., El-Kordi, A., Ronnenberg, A., Winter, D., Frahm, J., Fischer, J., Brose, N., et al. (2009). Neuroligin-3-deficient mice: Model of a monogenic heritable form of autism with an olfactory deficit. *Genes, Brain Behav.* 8, 416–425.

Rajakulendran, S., Kaski, D., and Hanna, M.G. (2012). Neuronal P/Q-type calcium channel dysfunction in inherited disorders of the CNS. *Nat. Rev. Neurol.* 17, 86–96.

Ran, X., Li, J., Shao, Q., Chen, H., Lin, Z., Sun, Z.S., and Wu, J. (2015). EpilepsyGene: A genetic resource for genes and mutations related to epilepsy. *Nucleic Acids Res.* 43, 893–899.

Redies, C., Hertel, N., and Hübner, C.A. (2012). Cadherins and neuropsychiatric disorders. *Brain Res.* 1470, 130–144.

Robinson, S.J. (2012). Childhood epilepsy and autism spectrum disorders: Psychiatric problems, phenotypic expression, and anticonvulsants. *Neuropsychol. Rev.* 22, 271–279.

Rossi, P.G., Parmeggiani, A., Bach, V., Santucci, M., and Visconti, P. (1995). EEG features and epilepsy in patients with autism. *Brain Dev.* 17, 169–174.

Royer-Zemmour, B., Ponsole-Lenfant, M., Gara, H., Roll, P., Lévêque, C., Massacrier, A., Ferracci, G., Cillario, J., Robaglia-Schlupp, A., Vincentelli, R., et al. (2008). Epileptic and developmental disorders of the speech cortex: Ligand/ receptor interaction of wild-type and mutant SRPX2 with the plasminogen activator receptor uPAR. *Hum. Mol. Genet.* 17, 3617–3630.

De Rubeis, S., He, X., Goldberg, A.P., Poultney, C.S., Samocha, K., Cicek, A.E., Kou, Y., Liu, L., Fromer, M., Walker, S., et al. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature* 515, 209–215.

Sakurai, T. (2017). The role of cell adhesion molecules in brain wiring and neuropsychiatric disorders. *Mol. Cell. Neurosci.* 81, 4–11.

Salinger, W.L., Ladrow, P., and Wheeler, C. (2003). Behavioral Phenotype of the Reeler Mutant Mouse: Effects of Reln Gene Dosage and Social Isolation. *Behav. Neurosci.* 117, 1257–1275.

Scheffer, I.E., Berkovic, S., Capovilla, G., Connolly, M.B., French, J., Guilhoto, L., Hirsch, E., Jain, S., Mathern, G.W., Moshé, S.L., et al. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 58, 512–521.

Scheiffele, P. (2003). Cell-cell signaling during synapse formation in the CNS. *Annu Rev Neurosci* 26, 485–508.

Schendel, D., and Bhasin, T.K. (2008). Birth Weight and Gestational Age Characteristics of Children With Autism, Including a Comparison With Other Developmental Disabilities. *Pediatrics* 121, 1155–1164.

Schuch, J.B., Muller, D., Endres, R.G., Bosa, C.A., Longo, D., Schuler-Faccini, L., Ranzan, J., Becker, M.M., dos Santos Riesgo, R., and Roman, T. (2014). The role of $\beta 3$ integrin gene variants in autism spectrum disorders - diagnosis and symptomatology. *Gene* 553, 24–30.

Schwabe, T., Gontang, A.C., and Clandinin, T.R. (2009). More than just glue: The diverse roles of cell adhesion molecules in the *Drosophila* nervous system. *Cell Adhes. Migr.* 3, 36–42.

Seibenhener, M.L., and Wooten, M.C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *J. Vis. Exp.* 6, 1–6.

Sestan, N., and State, M.W. (2018). Lost in Translation: Traversing the Complex Path from Genomics to Therapeutics in Autism Spectrum Disorder. *Neuron* 100, 406–423.

Sharma, S.R., Gonda, X., and Tarazi, F.I. (2018). Autism Spectrum Disorder: Classification, diagnosis and therapy. *Pharmacol. Ther.* 190, 91–104.

Shipton, O.A., and Paulsen, O. (2014). GluN2A and GluN2B subunit-containing NMDA receptors in hippocampal plasticity. *Philos. Trans. R. Soc. B Biol. Sci.* 369, 1–17.

Silverman, J.L., Yang, M., Lord, C., and Crawley, J.N. (2010). Behavioural phenotyping assays for mouse models of autism. *Nat. Rev. Neurosci.* 11, 490–502.

Sommer, B.R., Mitchell, E.L., and Wroolie, T.E. (2013). Topiramate: Effects on cognition in patients with epilepsy, migraine headache and obesity. *Ther. Adv. Neurol. Disord.* 6, 211–227.

Stafstrom, C.E., and Benke, T.A. (2015). Autism and epilepsy: Exploring the relationship using experimental models. *Epilepsy Curr.* 15, 206–210.

Staley, K. (2015). Molecular mechanisms of epilepsy. *Nat. Neurosci.* 18, 367–372.

Swerdlow, N.R., Geyer, M.A., and Braff, D.L. (2001). Neural circuit regulation of prepulse inhibition of startle in the rat: Current knowledge and future challenges. *Psychopharmacology (Berl)*. 156, 194–215.

Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., and Braff, D.L. (2008). Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology (Berl)*. 199, 331–388.

Szczurowska, E., and Mareš, P. (2015). Different action of a specific NR2B/NMDA antagonist Ro 25-6981 on cortical evoked potentials and epileptic afterdischarges in immature rats. *Brain Res. Bull.* 111, 1–8.

Sztainberg, Y., and Zoghbi, H.Y. (2016). Lessons learned from studying syndromic autism spectrum disorders. *Nat. Neurosci.* 19, 1408–1417.

Tabuchi, K., Blundell, J., Etherton, M.R., Hammer, R.E., Liu, X., Powell, C.M., and Südhof, T.C. (2007). A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science (80-.)*. 318, 71–76.

Talkowski, M.E., Rosenfeld, J.A., Blumenthal, I., Pillalamarri, V., Chiang, C., Heilbut, A., Ernst, C., Hanscom, C., Rossin, E., Lindgren, A.M., et al. (2012). Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell* 149, 525–537.

Thalhammer, A., and Cingolani, L.A. (2014). Cell adhesion and homeostatic synaptic plasticity. *Neuropharmacology* 78, 23–30.

Titley, H.K., Brunel, N., and Hansel, C. (2017). Toward a Neurocentric View of Learning. *Neuron* 95, 19–32.

Togashi, H., Sakisaka, T., and Takai, Y. (2009). Cell adhesion molecules in the central nervous system. *Cell Adh.Migr.* 3, 29–35.

Turrigiano, G. (2012). Homeostatic synaptic plasticity: Local and global mechanisms for stabilizing neuronal function. *Cold Spring Harb. Perspect. Biol.* 4, 1–17.

Varney, S., Polston, K.F., Jessen, T., and Carneiro, A.M.D. (2015). Mice lacking integrin $\beta 3$ expression exhibit altered response to chronic stress. *Neurobiol. Stress* 2, 51–58.

Volk, L., Chiu, S.-L., Sharma, K., and Huganir, R.L. (2015). Glutamate Synapses in Human Cognitive Disorders. *Annu. Rev. Neurosci.* 38, 127–149.

Wang, X., Bozdagi, O., Nikitczuk, J.S., Zhai, Z.W., Zhou, Q., and Huntley, G.W. (2008). Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. *Proc. Natl. Acad. Sci. U. S. A.* 105, 19520–19525.

Warren, M.S., Bradley, W.D., Gourley, S.L., Lin, Y.-C., Simpson, M.A., Reichardt, L.F., Greer, C.A., Taylor, J.R., and Koleske, A.J. (2012). Integrin $\alpha 1$ Signals through Arg to Regulate Postnatal Dendritic Arborization, Synapse Density, and Behavior. *J. Neurosci.* 32, 2824–2834.

Watson, P.M.D., Humphries, M.J., Relton, J., Rothwell, N.J., Verkhatsky, A., and Gibson, R.M. (2007). Integrin-binding RGD peptides induce rapid intracellular calcium increases and MAPK signaling in cortical neurons. *Mol. Cell. Neurosci.* 34,

147–154.

Weiss, L.A., Veenstra-VanderWeele, J., Newman, D.L., Kim, S.J., Dytch, H., McPeck, M.S., Cheng, S., Ober, C., Cook, E.H., and Abney, M. (2004). Genome-wide association study identifies ITGB3 as a QTL for whole blood serotonin. *Eur. J. Hum. Genet.* *12*, 949–954.

Weiss, L.A., Kosova, G., Delahanty, R.J., Jiang, L., Cook, E.H., Ober, C., and Sutcliffe, J.S. (2006). Variation in ITGB3 is associated with whole-blood serotonin level and autism susceptibility. *Eur. J. Hum. Genet.* *14*, 923–931.

Williams, M.E., de Wit, J., and Ghosh, A. (2010). Molecular mechanisms of synaptic specificity in developing neural circuits. *Neuron* *68*, 9–18.

Willsey, A.J., Sanders, S.J., Li, M., Dong, S., Tebbenkamp, A.T., Muhle, R.A., Reilly, S.K., Lin, L., Fertuzinhos, S., Miller, J.A., et al. (2013). Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. *Cell* *155*, 997–1007.

De Wit, J., and Ghosh, A. (2016). Specification of synaptic connectivity by cell surface interactions. *Nat. Rev. Neurosci.* *17*, 22–35.

Won, H., Lee, H.R., Gee, H.Y., Mah, W., Kim, J.I., Lee, J., Ha, S., Chung, C., Jung, E.S., Cho, Y.S., et al. (2012). Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature* *486*, 261–265.

Wu, X., and Reddy, D.S. (2012). Integrins as receptor targets for neurological disorders. *Pharmacol. Ther.* *134*, 68–81.

Wu, Y., Wang, X.F., Mo, X.A., Li, J.M., Yuan, J., Zheng, J.O., Feng, Y., and Tang, M. (2011). Expression of laminin $\beta 1$ and integrin $\alpha 2$ in the anterior temporal neocortex tissue of patients with intractable epilepsy. *Int. J. Neurosci.* *121*, 323–328.

- Xu, X., Wells, A.B., O'Brien, D.R., Nehorai, A., and Dougherty, J.D. (2014). Cell Type-Specific Expression Analysis to Identify Putative Cellular Mechanisms for Neurogenetic Disorders. *J. Neurosci.* *34*, 1420–1431.
- Yang, M., and Crawley, J.N. (2009). Simple behavioral assessment of mouse olfaction. *Curr. Protoc. Neurosci.* *8*, 1–14.
- Ylisaukko-oja, T., Rehnström, K., Auranen, M., Vanhala, R., Alen, R., Kempas, E., Ellonen, P., Turunen, J.A., Makkonen, I., Riikonen, R., et al. (2005). Analysis of four neuroligin genes as candidates for autism. *Eur. J. Hum. Genet.* *13*, 1285–1292.
- Zablotsky, B., Black, L.I., and Blumberg, S.J. (2017). Estimated Prevalence of Children With Diagnosed Developmental Disabilities in the United States, 2014-2016. *NCHS Data Brief* 1–8.
- Zhang, T., Zhu, L., Ni, T., Liu, D., Chen, G., Yan, Z., Lin, H., Guan, F., and Rice, J.P. (2018). Voltage-gated calcium channel activity and complex related genes and schizophrenia: A systematic investigation based on Han Chinese population. *J. Psychiatr. Res.* *106*, 99–105.
- Zhong, X., Drgonova, J., Li, C.Y., and Uhl, G.R. (2015). Human cell adhesion molecules: Annotated functional subtypes and overrepresentation of addiction-associated genes. *Ann. N. Y. Acad. Sci.* *1349*, 83–95.
- Zucker, R.S., and Regehr, W.G. (2002). Short-Term Synaptic Plasticity. *Annu. Rev. Physiol.* *64*, 355–405.