Title

Autistic traits in epilepsy models: Why, when and how?

Authors

Velíšková, Jana
Silverman, Jill L
Benson, Melissa
et al.

Publication Date

2018-08-01

DOI

10.1016/j.eplepsyres.2018.05.009

Peer reviewed

Published in final edited form as:

Epilepsy Res. 2018 August ; 144: 62–70. doi:10.1016/j.eplepsyres.2018.05.009.

Autistic Traits in Epilepsy Models: Why, When and How?

Jana Velíšková[1,2,3,*], **Jill L. Silverman**[4,*], **Melissa Benson**[1,*], and **Pierre-Pascal Lenck-Santini**[5,*]

[1]Department of Cell Biology & Anatomy, New York Medical College, Valhalla, NY, USA

[2]Department of Obstetrics & Gynecology, New York Medical College, Valhalla, NY, USA

[3]Department of Neurology, New York Medical College, Valhalla, NY, USA

[4]University of California Davis School of Medicine, Department of Psychiatry and Behavioral Sciences and MIND Institute, Sacramento, CA, USA, 95817

[5]INMED, Aix-Marseille University, INSERM, Marseille, France

Abstract

Autism spectrum disorder (ASD) is a common comorbidity of epilepsy and seizures and/or epileptiform activity are observed in a significant proportion of ASD patients. Current research also implies that autistic traits can be observed to a various degree in mice and rats with seizures. This suggests that there are shared mechanisms in both ASD and epilepsy syndromes. Here, we first review the standard, validated methods used to assess autistic traits in animal models as well as their limitations with regards to epilepsy models. We then discuss two of the potential pathological processes that could be shared between ASD and epilepsy. We first focus on functional implications of neuroinflammation including changes to excitable networks mediated by inflammatory regulators. Finally we examine mechanisms at the cellular and network level involved in neuronal excitability, timing and network coordination that may directly lead to behavioral disturbances present in both epilepsy and ASD. This mini-review summarizes the work first presented at an Investigators Workshop at the 2016 American Epilepsy Society meeting.

Keywords

Autism spectrum disorder; epilepsy comorbidity; seizures; translation; novel mechanisms; behavioral assays; neuroinflammation; network coordination; excitation-inhibition balance

*Corresponding Author: Jana Velíšková, MD, PhD, Department of Cell Biology & Anatomy, BSB-A21, New York Medical College, 15 Dana Rd., Valhalla, NY 10595, Phone: +1(914) 594-4840, Fax: +1(914) 594-4653, Jana_Veliskova@nymc.edu.
*All authors contributed equally to this review.

Introduction

Autism spectrum disorder (ASD) and epilepsy are prevalent and pervasive lifelong disorders for which medicinal interventions are either not readily available or need drastic improvements. ASD represents a common co-morbidity in patients with epilepsy and *vice versa,* evidenced by the prevalence statistics that show that epileptic disorders are 10 times more likely to be diagnosed with ASD (Sundelin et al., 2016). According to prevailing estimates, ~30% of patients with ASD develop epilepsy at some point of their lives, and at the same time ~30% of patients with epilepsy as a primary diagnosis fit the criteria of being diagnosed with ASD (Clarke et al., 2005; Seidenberg et al., 2009; Tuchman & Rapin, 2002).

ASD prevalence is higher in boys compared to girls (Baron-Cohen et al., 2011). However in the past two years, research suggests that the ASD sex-bias toward boys may be the result of cases of under- or misdiagnosis in girls, who present with distinct, subtler behavioral profiles than boys due to a variety of compensatory behaviors and camouflaging the symptoms (Lai et al., 2015; Park et al., 2016; Rynkiewicz et al., 2016). Interestingly, association of epilepsy and ASD has been diagnosed more often in girls than in boys (for review see (Tuchman et al., 2010b)).

Most significant indicators that predict whether patients with epilepsy will also have ASD are an early onset of epilepsy together with low cognitive functioning and intellectual disability. Thus, the highest incidence of ASD is in those patients with epilepsy suffering from severe epileptic encephalopathies of infancy and childhood, i.e., Ohtahara, West (infantile spasms) and Lennox-Gastaut syndromes, and in those children with identifiable severe structural damage (symptomatic epilepsies). In these patients, a large number of uncontrolled seizures together with the structural abnormalities also likely contribute to ASD.

Stratifying patient subgroups and focusing on genetically identifiable ASD populations with co-morbid epilepsy is a main focus in autism research (i.e. genetic disorders); therefore, use of similar tools would be advantageous to both research focus groups. In fact, several neurodevelopmental syndromes with causal genetic etiology show co-existence of epilepsy and ASD as parallel syndromes, such as Rett, Dravet, Angelman, Dup15q and/or Landau Kleffner syndromes. In these syndromes, seizures and epilepsy often occur as a sign of general hyperexcitability with EEG epileptiform abnormalities, which is also common in some children with ASD (Tuchman et al., 2010b). Besides seizures, associated symptoms often found in ASD patients can include lack of achievement of neurodevelopmental milestones, learning and memory deficits, poor motor skills, hyperactivity, hyperexcitability, changes in responsiveness, aggression, anxiety, fear, sensory processing, altered sleep patterns and gastrointestinal distress.

Assessing social abilities in children is based on standardized interviews and observations using Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview (ADI). The latest Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) recognizes two main behavioral domains for diagnostic criteria of ASD in humans: 1) impairments in reciprocal social communication (verbal and non-verbal) and 2) repetitive

behaviors, with restricted interests and behavioral inflexibility. New diagnostic criteria include a broader definition of the ASD phenotype and reflect the current consensus that the causes and clinical presentations of ASD are highly heterogeneous (Association, 2013; Lai et al., 2014). To diagnose ASD clinically, at least six symptoms have to be present with a minimum two abnormal measures in social interaction, and at least one measure of disturbed social communication with some type of repetitive behavior. The new criteria in the DSM-5 manual help more specifically diagnose the patients with ASD (Kulage et al., 2014) and assure better tailored treatments for distinct social behavior disorders (Halls et al., 2015). The new clinical benchmarks for ASD also lead to re-evaluation of the criteria for animal models of ASD and pose more strict rules for the ASD relevant behavioral phenotypes, especially to identify and study ASD as co-morbidity in animal models of epilepsy.

ASD as well as epilepsy have multifactorial etiology and the high prevalence of co-existence of ASD and epilepsy suggests some shared underlying neurological abnormalities. New research suggests that a combination of heritable and environmental factors have a strong influence on both ASD and epilepsy (Meltzer & Van de Water, 2017; Ornoy et al., 2016; Sandin et al., 2017; Shorvon, 2014; Wipfler et al., 2018). Yet etiology in the overwhelming majority of cases is still unknown. Animal models have become important tools for studying the roles of genetic and environmental factors, and their reciprocal influence on the onset and severity of disorders, including ASD and epilepsies. Here, we would like to first review standard, validated methods used to assess autistic traits in animal models consistent with the human DSM-5 criteria recommendations as well as review their limitations with regards to epilepsy models. Then we want to focus on two newly implicated mechanisms contributing to neurodevelopmental dysfunction and etiology in both ASD and epilepsy: the immune dysfunction associated with chronic neuroinflammation and the changes in network synchrony. While other mechanisms have been proposed and discussed in recent excellent reviews and meta-analysis studies (Besag, 2015; Jeste & Tuchman, 2015; Lee et al., 2015; Spence & Schneider, 2009; Strasser et al., 2018), neuroinflammation and network coordination hypothesis recently gained attraction of the research community. We believe they may also form common substrates for epilepsy and ASD and contribute to the disturbances in the excitatory-inhibitory (E-I) balance characteristic for both diseases. This mini-review summarizes the work presented at an Investigators Workshop at the 2016 American Epilepsy Society Annual Meeting held in Houston, Texas. Both clinicians and basic scientists have participated to discuss the new directions in epilepsy and ASD research.

Modeling the Social Brain: Preclinical assays for behavioral outcomes relevant to ASD and epilepsy. A cautionary tale for epilepsy models

The criteria for ASD diagnosis are purely behavioral and *in vivo* assessments in preclinical rodent models are important tools to determine the presence of autism-relevant phenotypes (Crawley, 2012; Silverman et al., 2010). Whether or not ASD-relevant behavioral phenotypes are present in an animal model of epilepsy, it is imperative that the criteria established for the endophenotypes of the human syndrome correspond to the behavioral phenotypes of the animals without confounding effects. Such strategies will ensure not collecting false positive or false negative data (Sukoff Rizzo & Silverman, 2016).

Standard, validated methods for quantifying behavior relevant to social communication and repetitive behaviors with restricted interests are available in the rodent model behavioral neuroscience literature (Kazdoba et al., 2016; Silverman et al., 2010; Sukoff Rizzo & Silverman, 2016) and summarized in Table 1.

The methods employed for behavioral phenotyping for ASD-relevant traits are complex and warrant caution with regards to confounding factors. Some behaviors seen in animal models of ASD can overlap with seizure behavior. Therefore, cautious interpretations are necessary to determine whether the behavior of the animal is the result of ASD traits or seizures (see Table 1). The ultimate goal is to identify disease-relevant and translational behavioral endpoints that are robust, reliable, and reproducible, and that can be employed to evaluate potential of novel therapeutic agents to treat disease. The impact of a competing or confounding behavior on the behavioral endpoints should not be underestimated. For example, previous experience of seizures or some mutations can cause physical impairments that limit a subject's ability to perform a task. Genetic mutations relevant to ASD that caused physical defects, including smaller body weights include the most common copy number variant in ASD, 16p11.2 deletions (Portmann et al., 2014). Motor defects in ASD models including hypo- (Copping et al., 2017; Dhamne et al., 2017) and hyper- locomotion (Kazdoba et al., 2014; Penagarikano et al., 2011; Pietropaolo et al., 2011; Spencer et al., 2011; Spencer et al., 2006; Uutela et al., 2012) can also have consequences on the behavioral outcome of interest by competing or preventing the subject from engaging in the tasks of core symptomology testing. Another example is that strains of mice with visual impairments (FVB or CE3H) may not be useful for cognitive tests that employ visual cues as reference stimuli, and, further, it is well documented that blind mice tend to be hyperactive (Dyer & Weldon, 1975). Olfactory impairments that can occur following seizures (Tiedeken et al., 2013) may abolish sociability in mice and rats. Animals with history of seizures may exhibit long-term changes in locomotor activity (Samotaeva et al., 2012). Most importantly, seizures may lead to other co-morbidities, i.e., depression-like behavior, changes in anxiety or developing aggressive behavior (Castelhano et al., 2015; Kalynchuk, 2000; Medel-Matus et al., 2017; Szyndler et al., 2002; Tiedeken & Ramsdell, 2013) and all of those may interfere with proper evaluation in complex social communication behavior. Repetitive behavior and automatisms in rodents such as wet dog shakes, excessive grooming, scratching, jumping or circling could be part of seizure behavior (Velíšková & Velíšek, 2017) and not necessarily a sign of autistic-like behavior. EEG recordings can provide the information regarding ongoing ictal activity to distinguish ASD and ongoing seizure activity.

Just as it is important to understand the limitations of a behavioral task itself, it is important to investigate, acknowledge, and report the limitations of the rodent model being tested so as not to be shortsighted in the interpretations of the data.

A Common Thread: Neuroinflammation in Epilepsy and ASD

Neuroinflammation is a pathological process actively present in both epilepsy and ASD, further linking the two diseases. The causes of neuroinflammation in both cases are poorly understood, however recent studies have begun to shine a light on how inflammation in the brain is contributing to these diseases' pathologies. In this mini review, we will focus mainly

on commonalities between epilepsy and ASD in terms of neuroinflammation and functional implications of neuroinflammation, including changes to excitable networks mediated by inflammatory regulators.

I) Inflammation in ASD

In ASD, the presence of neuroinflammation has been reported in both clinical and preclinical studies. Post-mortem brain tissue from ASD patients, ranging in age from 3 years to adult, was shown to have Allograft Inflammatory Factor 1 (Iba1) -positive microglia with an activated morphology, defined by truncated processes and an enlarged cell soma volume, compared to age matched controls (Morgan et al., 2010). However in this study, microglial activation was not specific to ASD patients with seizure presence, as data from these patients showed “normal” ramified microglial morphology. This highlights that microglial activation is not always dependent on seizure presence, nor is it a consistent marker of seizures in ASD patients.

Astrocytes, another inflammation-associated cell type in the brain, are also activated in ASD patients (Vargas et al., 2005). Increased Glial fibrillary acidic protein (GFAP; astrocytic marker) expression is observed in cerebellum and cortex and accompanied by localized expression of pro-inflammatory cytokine interleukin-6 (IL-6), and chemokine MCP-1. IL-6 drives microglial activation and promotes astrogliosis, whilst MCP-1 is a chemoattractant driving macrophage migration, both contributing to the neuro-inflammatory cycle.

A recent study found that increased serum levels of high mobility group box protein 1 (HMGB1) significantly correlated to impaired social interaction in ASD patients (Emanuele et al., 2010). This protein is a key inflammatory molecule, which can activate interleukin-1 (IL-1) mediated signaling (including IL-1β) leading to activation of toll-like receptor 4 (TLR4) through NFκB-mediated processes.

Besides the changes in neuroinflammatory markers, the brain’s immune response, in general, is compromised in ASD patients, namely the imbalance in pro-inflammatory/anti-inflammatory signaling (Ashwood et al., 2011). Adaptive immune response, primarily coordinated by T helper cells, differs in ASD patient frontal cerebral cortex compared to healthy controls (Li et al., 2009). Increased Th1 type cytokines, which are typically pro-inflammatory and pro-injury inducing (e.g. IL-6, TNFα and IFNγ), are increased, whilst Th2, reparative/counteracting inflammation cytokines (e.g. IL-4, IL-10 and IL-5) are unchanged resulting in an increased Th1/Th2 ratio in ASD patients. Another study showed an exacerbated immune response in ASD patients (Jyonouchi et al., 2001); peripheral blood mononuclear cells (PBMCs) taken from ASD patients displayed two-fold higher cytokine expression basally compared to controls, and this difference was further increased after LPS stimulation, a classical immune trigger. TNFα was the primary cytokine found to be increased at baseline conditions in ASD samples compared to controls, and increased further after LPS stimulation. Interestingly, when the TNFα levels were compared to unaffected siblings, the difference was still significant yet not so prominent as the comparison to unrelated individuals, suggesting some genetic contribution. In conclusion, the data illustrate that heightened response of immune cells determines the ASD pathology.

Finally, mast cell (MC) activation is a key process found present in ASD patients contributing to neuroinflammation. It is proposed that MC located in the hypothalamus can be activated via triggers such as stress, which is strongly associated with ASD, and lead to changes in blood-brain barrier (BBB) integrity (Theoharides & Zhang, 2011). The hormone neurotensin, which activates MCs, is increased in ASD patients. Activation of MCs then leads to pre-formed TNFα and other MC mediators (e.g. IL-6, vascular endothelial growth factor and mitochondrial DNA) being carried to target endothelial cells and comprising the BBB tight junctions along with other cytokines to exert pro-inflammatory effects driving BBB breakdown. Additional cross-talk between glia and MCs can lead to further immune activation, such as activation of the complement pathway, further promoting neuroinflammation.

II) Inflammatory processes in epilepsy

Like ASD, neuroinflammation is also present in epilepsy as shown by several key studies (reviewed by (Vezzani et al., 2011; Vezzani et al., 2016)). Significant increases in pro-inflammatory cytokines, including TNFα, IL-1β and IL-6, have been found in human temporal lobe epilepsy (TLE) brain samples (Vezzani & Granata, 2005). These cytokines are known to be proconvulsant *in vivo* (Kalueff et al., 2004) and *in vitro* (Chiavegato et al., 2014). IL-1β expression was also ectopically localized to astrocytes in TLE patients with hippocampal sclerosis (HS), which is not observed in patients without HS suggesting relation to the neurodegeneration (Ravizza et al., 2008). Astrocytic end-feet projecting around endothelial cells expressing IL-1β provide the potential for neuroinflammation to affect BBB integrity.

Furthermore, it has been shown in several experimental models, that antagonizing the pro-inflammatory cytokines can be anticonvulsant. Similarly to ASD brains, increased HMBG1 expression is present in the hippocampus of TLE patients (Maroso et al., 2010) and children with febrile seizures (Choi et al., 2011), triggering IL-1/TLR4 signaling cascades driving inflammation and resultant increased excitability. Accordingly, when the endogenous antagonist, IL-1 receptor antagonist (IL-1ra), is applied to the brain or expressed selectively in astrocytes (Vezzani et al., 2000), seizures are inhibited. Antagonists of TLR4 and HMGB1 are also effective at controlling seizures in preclinical models (Maroso et al., 2010).

Further examples of inhibiting cytokine signaling have also proved to be anticonvulsant including modulation of the complement activation pathway. Inhibition of a key pro-inflammatory complement factor, C5a, was shown to be anticonvulsant in several acute and chronic models of epilepsy potentially due to inhibition of downstream cytokine upregulation and signaling (i.e. TNFα. IL-1β) (Benson et al., 2015). Neuroprotective outcomes following status epilepticus were observed following inhibition of C5a signaling, an effect attributed to reduction of TNFα production (Benson et al., 2015) and increases in IL-4 (Benson et al., 2017). Finally, the BBB integrity is compromised following complement activation resulting in increases in peripheral inflammatory immune cell migration (Benson et al., 2017).

III) Common inflammatory pathologies in ASD and epilepsy

Approximately in 30% of childhood ASD patients with epilepsy, the seizures are typically difficult to control adequately with current anticonvulsant drugs (Tuchman et al., 2010a). Shared inflammatory pathologies occurring in both ASD and epilepsy are numerous. More specifically, increased HMBG1, compromised BBB integrity, microglial activation and stress-induced inflammation are key players in both pathologies. The neuroinflammation in both diseases significantly contributes to brain excitability and to the severity or progression of both syndromes, as well as more obviously increase seizure incidence/occurrence. A delicate equilibrium between excitatory and inhibitory neurotransmitters, glutamate and GABA respectively, is key to maintaining homeostasis by maintaining the E-I balance and preventing unwanted and uncontrollable neuronal firing manifesting in seizures, which are observed in both conditions and discussed in details below. Regarding the neuroinflammation, it is known to affect the E-I balance in multiple ways. Astrogliosis, detected in samples from both epilepsy and ASD postmortem brain tissue, leads to impairments in glutamate uptake by astrocytes resulting in excessive extracellular glutamate stores, which drives the glutamate receptor activation (Tian et al., 2005). Further contributing to this rise in extracellular glutamate are the microglial cells, which when activated also expel glutamate (Hu et al., 2000). Increased levels of pro-inflammatory cytokine IL-6, measured in both conditions, worsens this state of heightened excitability as it stimulates excitatory synapse formation in conjunction with impairing inhibitory synapse formation (Nelson & Valakh, 2015; Nelson et al., 2012). The pro-inflammatory cytokine TNFα also increased in both ASD and epilepsy brains works via an NFκB mediated mechanism to repress expression of excitatory amino acid transporter 2 (EAAT2) reducing the glutamate uptake (Sitcheran et al., 2005) and reduces the strength of inhibitory signaling between neurons (Pribiag & Stellwagen, 2013).

Neuronal coordination hypothesis: between etiology and phenotype

The common presence of epileptiform activity and ASD symptoms in various syndromes can be interpreted in two ways: 1) epileptiform activity causes ASD symptoms and/or 2) both are manifestations of a similar underlying cause. In the following section, we will discuss both possibilities separately. However, it is highly probable that they are non-exclusive. Although, epileptiform events may originate from the same etiological origin as the autistic symptoms, they may aggravate the behavioral phenotype.

I) Is Epileptiform activity responsible for ASD symptoms?

The co-existence of seizures and interictal discharges in ASD patients is well documented (Spence & Schneider, 2009). It has been proposed that the EEG abnormalities may pose a causal role in the behavioral disturbances observed in ASD patients. In syndromes such as Rett, Fragile X, Angleman or Prader-Willi and other childhood epileptic encephalopathies, both epileptiform manifestations and ASD phenotypes are commonly observed together. In these syndromes, the onset of behavioral deficits often coincides with the apparition of the first epileptiform manifestations, leading to the hypothesis that seizures and/or epileptiform discharges during critical periods of development contribute, at least in part, to the behavioral deficits.

This hypothesis is indeed supported by animal model studies. When epilepsy is induced by prolonged status epilepticus, be it via chemical, hyperthermia or hypoxia/ischemia, a wide range of modifications is observed at different levels of organization. Specifically, prolonged SE has been shown to induce inflammation, epigenetic changes, cell metabolism, ion channels and receptors, rewiring or aberrant wiring, cell death, apoptosis and neurogenesis. In turn, affected networks become prone to seizures and, less able to sustain normal cognitive function (Lenck-Santini & Scott, 2015). It is important to note that induction protocols in animal models are extremely severe compared to what happens in real life. When prolonged SE occurs it is because of pre-existing conditions, trauma or brain infection that can also directly affect brain function, making it difficult to disentangle the role of etiology *versus* SE. In addition, the correlation between seizure frequency or severity and the extent of cognitive or behavioral impairment is not always straightforward, particularly in the context of epileptic encephalopathies (Nabbout et al., 2013). Finally, seizure suppression, particularly in epileptic encephalopathies, is associated with little to no improvement of the functional aspects. Therefore, it is likely that etiology, i.e. the underlying cause of epilepsy, may also contribute directly to cognitive/behavioral impairment in syndromes with epilepsy.

Apart from seizures, interictal EEG abnormalities are also believed to play an important role in behavioral comorbidities. Indeed, data show that both in humans and animal models (Holmes & Lenck-Santini, 2006; Kleen et al., 2010; Kleen et al., 2013) interictal abnormalities (IAs) can alter sensory, memory and higher cognitive functions such as verbal recognition. IAs interference with cognitive processes is transient, in the sense that the impact is limited to the ongoing process supported by the structure where IAs occur or propagate. However, IAs impact could be dramatic in situations where attention is critical such as when operating machines, driving or while at school. In addition, there are conditions where IAs are particularly frequent during sleep, a process known to play a fundamental role in learning and memory. This is the case of conditions such as continuous spike and wave discharges during slow wave sleep or Landau-Kleffner syndrome where during sleep a large majority of the patient EEG is occupied by IAs. These patients suffer from severe cognitive impairment and commonly have ASD. To help patients with large number of IAs and cognitive impairment although no or only few seizures, attempts have been made to suppress IAs by administration of anticonvulsant drugs with the hope to restore cognitive function. Unfortunately, although few cases have been successful, the efficacy of such approach has not been confirmed and some treatments have actually a negative effect on cognition (Wirrell et al., 2008). Although it is recognized that IAs have a significant impact on cognitive/behavioral function, the limited and inconclusive efficacy of different anticonvulsant drugs in restoring cognitive/behavioral function (see (Frye et al., 2013) for review) suggests that another mechanism is at play. The following section will aim at identifying such mechanism.

II) Is there a common mechanism?

a) E-I balance hypothesis—A hallmark of epilepsy research is the concept of E-I balance. This term is based on the assumption that normal brain function depends on the perfect balance between excitatory and inhibitory inputs to principal cells. Too much excitation or too little inhibition leads to hyperexcitability of the network, in turn leading to

seizures. So far, this notion has been critical to further our understanding of epileptogenesis, ictogenesis and treatment design. Indeed, in a large number of epilepsy syndromes, the apparition of seizures can be explained either by a loss of inhibition, for instance by alterations of GABAergic receptors or interneuron function, or by excessive excitation when there is gain of function of excitatory receptors or increase in excitatory pathways. In accordance with the E-I balance hypothesis, drugs that increase inhibition, such as GABAergic agonists, or decrease excitation, such as sodium or calcium channels blockers are highly efficient in regulating seizures. In this regard, the E-I balance concept is critical as it provides a functional framework with clearly identified benefits, namely suppression of seizures.

In the mindset that seizures result from an alteration of E-I balance, we could logically propose that the mere presence of seizures in ASD is an indication of E-I imbalance in ASD. This hypothesis has been proposed for a more than a decade (Brooks-Kayal, 2011; Nelson & Valakh, 2015; Rubenstein & Merzenich, 2003). Loss of inhibition (via alteration of GABAergic synaptic transmission, interneuron dysfunction and abnormal migration) has been documented in ASD patients. Particularly, there is a decrease of GABA levels in various cortical regions of ASD patients, including the frontal cortex (Puts et al., 2017) where reduced $GABA_A$ receptor binding has been observed (Zurcher et al., 2015). Furthermore, recent post-mortem studies in patients show a reduction of parvalbumin interneurons in the medial prefrontal cortex (Ariza et al., 2018). On the top of loss of inhibition, gain of excitation (via an increased principal cell excitability of glutamatergic transmission) has also been identified in ASD (Nelson & Valakh, 2015). Characteristic findings in ASD syndromes, such as abnormal micro-column organization (Casanova, 2006; Frye et al., 2016), changes in metabolic pathways (Huber et al., 2015; Kwon et al., 2006), homeostatic plasticity (Nelson & Valakh, 2015) or alterations of glial function (Lioy et al., 2011) are also known to affect E-I balance. Finally, various genetic abnormalities, known to alter E-I balance have been identified in both syndrome families (Lee et al., 2017). These include SCN1A (Weiss et al., 2003) GRIN2A mutations (Lesca et al., 2012), PTEN (Elia et al., 2012; Orrico et al., 2009) and others.

A logical consequence of the role of E-I imbalance hypothesis in ASD and epilepsy is that anticonvulsant drugs should restore cognitive/behavioral comorbidities in such syndromes. This strategy has indeed proven successful in animal models. For instance *Scn1a* +/− mice show improvement of social and memory deficits after clonazepam treatment (Han et al., 2012) and juvenile diazepam treatment of the BTBR mouse model of autism prevented the emergence sensory integration deficits in this model. Unfortunately, in humans, anticonvulsant drugs have a limited to no efficacy in restoring cognitive and behavioral function in ASD (Belsito et al., 2001; Frye et al., 2013; Hellings et al., 2005). This is also the case for epilepsy syndromes. For instance, although clonazepam, a common Dravet syndrome treatment (Shi et al., 2016) restores cognitive function in mouse models (Han et al., 2012), it fails to do so in humans. Therefore, targeting E-I balance, at least using traditional anticonvulsant drugs, fails to treat cognitive disturbances (see (Frye et al., 2013) for review). This suggests that the mechanisms involved in cognitive dysfunction observed in ASD and epilepsy may be more complex than the seizures or E-I balance alteration. To

help identify potential mechanisms let us reconsider the E-I balance hypothesis in light of recent theories.

b) Role of etiology: interneurons and information processing—One important caveat with the E-I balance concept is that the term “balance” does not necessary mean static equilibrium. In the eye of early neuroscientists, brain systems were considered as systems prone to remain in an equilibrium state that only fluctuates when disturbed by external or internal (excitatory) inputs and quickly return back to a quiescent state. However, recent advances in fundamental research demonstrate that this is not the case and that, even in the resting state, brain structures are constantly active and that information in the brain is being processed via specific rhythms. Brain rhythms, by coordinating the firing of neurons, enable the transmission of information across and within structures (Fries, 2015). In addition, they are believed to allow the segregation of pyramidal cells into functional groups, called cell assemblies that convey meaningful information (Buzsaki, 2010). Importantly, GABAergic innervation plays a fundamental role in these processes. GABAergic inhibition provides, via perisomatic inhibition, a pace making activity for the oscillations (Bartos et al., 2007). It has been also proposed that such rhythmical silencing of neurons during oscillations creates short time windows, in which information is being segmented in “chunks” that can be transmitted and interpreted efficiently (Buzsaki, 2010). On the other hand, GABA inhibition is also critical for driving the flow of information within the dendritic microcircuits. In the hippocampus for example, each interneuron subtype projects to a specific part of the pyramidal cell dendritic arborization and is active at specific phase of ongoing oscillations, hereby routing and filtering excitatory flow to the cell (Klausberger & Somogyi, 2008; Kullmann & Lamsa, 2011; Leao et al., 2012; Varga et al., 2012).

The GABAergic innervation is altered in ASD. In addition, brain rhythms and coordination of oscillations across structures are also affected (reviewed by (Kessler et al., 2016; Simon & Wallace, 2016)). Indeed, there is now accumulating evidence that ASD patients show reduced gamma (30–80Hz) and alpha (8–14Hz) power and phase synchrony when presented with visual or auditory stimuli (Buard et al., 2013; Edgar et al., 2015; Grice et al., 2001; Milne et al., 2009; Rojas et al., 2008; Stroganova et al., 2012; Sun et al., 2012). Recent work from Vakorin and colleagues (Vakorin et al., 2017) shows that the maturation profile of oscillatory power and network synchrony during resting state are abnormal in children and adolescent with ASD. Although the link between GABAergic alterations, altered rhythms and behavioral deficits in ASD has not yet been completely established, it is likely that, the E-I imbalance observed in ASD induces alterations of brain rhythms, and a poor coordination within and across networks. The resulting deficits in functional connectivity may be responsible for the sensory, perceptual, attention and social disturbances, the characteristics of ASD (Simon & Wallace, 2016).

Similarly to what has been documented in ASD, a significant number of epilepsy syndromes are caused by alterations of GABAergic function. It is highly plausible that the information processes mediated by GABA activity are also directly affected in these syndromes. A classic illustration of this idea is provided by SCN1A mutations in the context of Dravet syndrome: The SCN1A gene codes for the voltage gated sodium channel Nav1.1, that is involved in the generation and propagation of action potentials in the axonal initial segment

and along the axon. While SCN1A mutations are observed in various epilepsy syndromes such as generalized epilepsy with febrile seizures plus (GEFS+), it is only when mutations cause a loss of function of the protein that epileptic encephalopathy is observed. Loss of function of Nav1.1 prevents neurons to fire at high frequencies, yet such mutations paradoxically cause epilepsy. This phenomenon is explained by the fact that Nav1.1 is preferentially highly expressed in GABAergic interneurons expressing parvalbumin (Ogiwara et al., 2007; Yu et al., 2006). Therefore, Nav1.1 down regulation preferentially alters inhibitory function, explaining the seizure phenotype. As for other epileptic encephalopathies, cognitive impairment in Dravet syndrome was for a long time thought to be caused by seizures and ongoing epileptiform activity during infancy. However, there is no correlation between seizure frequency or severity and the extent of cognitive impairment in this syndrome. Moreover, the antiseizure treatments such as clobazam fail to improve cognitive function. Note finally that SCN1A mutations have been observed in ASD patients, suggesting that etiology could directly, in addition to seizures, cause behavioral and cognitive impairment.

The main difficulty in understanding the mechanisms of cognitive impairment in epilepsy is to dissociate the impact of etiology from the impact of seizures. To overcome this difficulty, we developed a targeted approach, in which the etiological alteration is restricted to a specific portion of the brain. This can be readily achieved using RNA interference, a technique enabling to down regulate the expression of a specific gene *in vivo*. Using either lipofectamine or viral vectors injected into the target structure, we managed to down-regulate the expression of *scn1a* in adult rats.

To determine the impact of Nav1.1 deficit on neuronal function independently of seizures, we (Bender et al., 2013; 2016) conducted two sets of experiments. In both experiments, *scn1a* expression was down regulated in a restricted brain region to avoid seizures and in adult rats to prevent developmental disruptions. Our goal was to identify the direct physiological and behavioral consequences of *scn1a* down regulation and avoid the confounding effect of seizures. In the first series of experiments (Bender et al., 2013), we targeted Nav1.1 expression in the medial septum/diagonal band of Broca (MSDB), a structure controlling theta oscillations in the hippocampal formation. Theta oscillations have been shown to play a critical role in learning and memory as well as in organizing cell activity into cell assemblies that represent ongoing trajectories of rodents. We found that reduction of Nav1.1 expression in the MSDB induced a significant decrease of both amplitude and frequency of hippocampal oscillations. Theta frequency decrease was associated with spatial memory deficit in a reaction to spatial change task. Importantly, Nav1.1 down regulation in the MSDB did not cause seizures, therefore demonstrating that cognitive impairment in Dravet syndrome could directly be the result of etiology.

In the second set of experiments using single unit recordings (Bender et al., 2016) we showed that the decrease in hippocampal theta oscillation following MSDB Nav1.1 knock down was likely caused by a selective loss of fast spiking activity, a characteristic of GABAergic neurons in this structure. In addition, the induced decrease in hippocampal theta frequency was correlated with decreased performance in T-maze alternation task. Here again, no seizure was observed.

Finally, there is now accumulating evidence that effective GABA signaling, which is the first one to emerge during development, plays a critical role in orchestrating the maturation of cortical function (see (Le Magueresse & Monyer, 2013) for review). For example, in the primary visual cortex, the initiation of the critical period during which neural circuits are shaped with experience is strongly influenced by the maturation of fast-spiking parvalbumin interneurons (Takesian & Hensch, 2013). It is therefore extremely likely that alterations of GABA signaling during such critical periods of development would have devastating impacts on the establishment and future maturation of cognitive/behavioral processes.

Conclusions

In conclusion, this paper highlights the most important autistic traits commonly tested in models of ASD. These traits should be considered when modeling ASD as a co-morbid functional outcome in models of seizures and epilepsy.

Recent studies implicate novel mechanisms involved in neurodevelopmental diseases such as ASD and epilepsy including the neuroinflammation and deficits in immune system and mechanisms involved in neuronal excitability, timing and network coordination that may directly lead to behavioral disturbances and thus, form a common substrate for epilepsy and ASD.

The clinical impact of neuroinflammation is difficult to interpret and put into action given the diverse types of inflammation observed in both disorders including severity and location within the brain. As discussed above, ASD patients even with severe microglia activation may not present with seizures and *vice versa* (Morgan et al., 2010), similarly not all patients with epilepsy (even the same type, i.e., TLE) may have ongoing neuro-inflammatory processes associated with seizures (Ravizza et al., 2008). Thus, the choice of patients eligible for adjunct anti-inflammatory treatment in both diseases has to be carefully made. Yet, a clear biomarker of neuroinflammation presence in these syndromes still awaits to be established especially given the diverse number of cellular activators and signaling molecules involved in these processes. Nevertheless, clinical trials show promise of adjunct anti-inflammatory and immune system modulating therapies in alleviating of some key symptoms in ASD patients (Chez et al., 2012) as well as in patients with epilepsy targeting the neuroinflammatory signaling seems to show promising directions as a disease modifying therapy (Terrone et al., 2017). Yet, further research has to be done to identify the subpopulation of patients most benefitting by targeting the inflammatory signaling.

Finally, taken together, preclinical studies show that altering interneuron function in the context of an epilepsy syndrome or ASD can also have direct effects on brain rhythms, cognitive function, maturation and ultimately cognitive and behavioral performance. Therefore, designing new treatments targeting these functions, including the anti-inflammatory therapies, and not exclusively seizures will certainly benefit the patients.

Acknowledgments

The work was supported by the UC Davis MIND Institute; NIH grants R21NS084900 (JLS); R01NS092786 (JV), R01NS076763 (PPLS); R01 NS097808 (JLS).

References

Ariza J, Rogers H, Hashemi E, Noctor SC, Martinez-Cerdeno V. The Number of Chandelier and Basket Cells Are Differentially Decreased in Prefrontal Cortex in Autism. Cereb Cortex. 2018; 28:411–420. [PubMed: 28122807]

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Altered T cell responses in children with autism. Brain Behav Immun. 2011; 25:840–849. [PubMed: 20833247]

Association, A.P. Diagnostic and Statistical Manual of Mental Disorders (DSM-5). American Psychiatric Association; 2013.

Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R. Why are autism spectrum conditions more prevalent in males? PLoS biology. 2011; 9:e1001081. [PubMed: 21695109]

Bartos M, Vida I, Jonas P. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. Nat Rev Neurosci. 2007; 8:45–56. [PubMed: 17180162]

Bechard AR, Bliznyuk N, Lewis MH. The development of repetitive motor behaviors in deer mice: Effects of environmental enrichment, repeated testing, and differential mediation by indirect basal ganglia pathway activation. Dev Psychobiol. 2017; 59:390–399. [PubMed: 28181216]

Belsito KM, Law PA, Kirk KS, Landa RJ, Zimmerman AW. Lamotrigine therapy for autistic disorder: a randomized, double-blind, placebo-controlled trial. J Autism Dev Disord. 2001; 31:175–181. [PubMed: 11450816]

Bender AC, Luikart BW, Lenck-Santini PP. Cognitive Deficits Associated with Nav1.1 Alterations: Involvement of Neuronal Firing Dynamics and Oscillations. PLoS One. 2016; 11:e0151538. [PubMed: 26978272]

Bender AC, Natola H, Ndong C, Holmes GL, Scott RC, Lenck-Santini PP. Focal Scn1a knockdown induces cognitive impairment without seizures. Neurobiol Dis. 2013; 54:297–307. [PubMed: 23318929]

Benson MJ, Manzanero S, Borges K. The effects of C5aR1 on leukocyte infiltration following pilocarpine-induced status epilepticus. Epilepsia. 2017; 58:e54–e58. [PubMed: 28225153]

Benson MJ, Thomas NK, Talwar S, Hodson MP, Lynch JW, Woodruff TM, Borges K. A novel anticonvulsant mechanism via inhibition of complement receptor C5ar1 in murine epilepsy models. Neurobiol Dis. 2015; 76:87–97. [PubMed: 25681535]

Besag FM. Current controversies in the relationships between autism and epilepsy. Epilepsy Behav. 2015; 47:143–146. [PubMed: 26091860]

Blundell J, Blaiss CA, Etherton MR, Espinosa F, Tabuchi K, Walz C, Bolliger MF, Sudhof TC, Powell CM. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J Neurosci. 2010; 30:2115–2129. [PubMed: 20147539]

Bozdagi O, Sakurai T, Papapetrou D, Wang X, Dickstein DL, Takahashi N, Kajiwara Y, Yang M, Katz AM, Scattoni ML, Harris MJ, Saxena R, Silverman JL, Crawley JN, Zhou Q, Hof PR, Buxbaum JD. Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. Molecular autism. 2010; 1:15. [PubMed: 21167025]

Brooks-Kayal A. Molecular mechanisms of cognitive and behavioral comorbidities of epilepsy in children. Epilepsia. 2011; 52(Suppl 1):13–20.

Buard I, Rogers SJ, Hepburn S, Kronberg E, Rojas DC. Altered oscillation patterns and connectivity during picture naming in autism. Frontiers in human neuroscience. 2013; 7:742. [PubMed: 24265611]

Buzsaki G. Neural syntax: cell assemblies, synapsembles, and readers. Neuron. 2010; 68:362–385. [PubMed: 21040841]

Casanova MF. Neuropathological and genetic findings in autism: the significance of a putative minicolumnopathy. Neuroscientist. 2006; 12:435–441. [PubMed: 16957005]

Castelhano AS, Ramos FO, Scorza FA, Cysneiros RM. Early life seizures in female rats lead to anxiety-related behavior and abnormal social behavior characterized by reduced motivation to novelty and deficit in social discrimination. Journal of neural transmission. 2015; 122:349–355. [PubMed: 25139483]

Chez M, Low R, Parise C, Donnel T. Safety and observations in a pilot study of lenalidomide for treatment in autism. Autism research and treatment. 2012; 2012:291601. [PubMed: 22997574]

Chiavegato A, Zurolo E, Losi G, Aronica E, Carmignoto G. The inflammatory molecules IL-1beta and HMGB1 can rapidly enhance focal seizure generation in a brain slice model of temporal lobe epilepsy. Frontiers in cellular neuroscience. 2014; 8:155. [PubMed: 24936172]

Choi J, Min HJ, Shin JS. Increased levels of HMGB1 and pro-inflammatory cytokines in children with febrile seizures. Journal of neuroinflammation. 2011; 8:135. [PubMed: 21989210]

Clarke DF, Roberts W, Daraksan M, Dupuis A, McCabe J, Wood H, Snead OC 3rd, Weiss SK. The prevalence of autistic spectrum disorder in children surveyed in a tertiary care epilepsy clinic. Epilepsia. 2005; 46:1970–1977. [PubMed: 16393164]

Copping NA, Berg EL, Foley GM, Schaffler MD, Onaga BL, Buscher N, Silverman JL, Yang M. Touchscreen learning deficits and normal social approach behavior in the Shank3B model of Phelan-McDermid Syndrome and autism. Neuroscience. 2017; 345:155–165. [PubMed: 27189882]

Crawley JN. Translational animal models of autism and neurodevelopmental disorders. Dialogues Clin Neurosci. 2012; 14:293–305. [PubMed: 23226954]

Dhamne SC, Silverman JL, Super CE, Lammers SHT, Hameed MQ, Modi ME, Copping NA, Pride MC, Smith DG, Rotenberg A, Crawley JN, Sahin M. Replicable in vivo physiological and behavioral phenotypes of the Shank3B null mutant mouse model of autism. Molecular autism. 2017; 8:26. [PubMed: 28638591]

Dyer RS, Weldon DA. Blindness-induced hyperactivity in several strains of mice. Physiol Behav. 1975; 15:439–441. [PubMed: 1221451]

Edgar JC, Khan SY, Blaskey L, Chow VY, Rey M, Gaetz W, Cannon KM, Monroe JF, Cornew L, Qasmieh S, Liu S, Welsh JP, Levy SE, Roberts TP. Neuromagnetic oscillations predict evoked-response latency delays and core language deficits in autism spectrum disorders. J Autism Dev Disord. 2015; 45:395–405. [PubMed: 23963591]

Elia M, Amato C, Bottitta M, Grillo L, Calabrese G, Esposito M, Carotenuto M. An atypical patient with Cowden syndrome and PTEN gene mutation presenting with cortical malformation and focal epilepsy. Brain Dev. 2012; 34:873–876. [PubMed: 22469695]

Emanuele E, Boso M, Brondino N, Pietra S, Barale F, Ucelli di Nemi S, Politi P. Increased serum levels of high mobility group box 1 protein in patients with autistic disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2010; 34:681–683. [PubMed: 20302902]

Etherton MR, Blaiss CA, Powell CM, Sudhof TC. Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proc Natl Acad Sci U S A. 2009; 106:17998–18003. [PubMed: 19822762]

Ey E, Yang M, Katz AM, Woldeyohannes L, Silverman JL, Leblond CS, Faure P, Torquet N, Le Sourd AM, Bourgeron T, Crawley JN. Absence of deficits in social behaviors and ultrasonic vocalizations in later generations of mice lacking neuroligin4. Genes Brain Behav. 2012

Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J Neurosci. 2001; 21:8278–8285. [PubMed: 11588199]

Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. Social amnesia in mice lacking the oxytocin gene. Nat Genet. 2000; 25:284–288. [PubMed: 10888874]

Fries P. Rhythms for Cognition: Communication through Coherence. Neuron. 2015; 88:220–235. [PubMed: 26447583]

Frye RE, Casanova MF, Fatemi SH, Folsom TD, Reutiman TJ, Brown GL, Edelson SM, Slattery JC, Adams JB. Neuropathological Mechanisms of Seizures in Autism Spectrum Disorder. Frontiers in neuroscience. 2016; 10:192. [PubMed: 27242398]

Frye RE, Rossignol D, Casanova MF, Brown GL, Martin V, Edelson S, Coben R, Lewine J, Slattery JC, Lau C, Hardy P, Fatemi SH, Folsom TD, Macfabe D, Adams JB. A review of traditional and novel treatments for seizures in autism spectrum disorder: findings from a systematic review and expert panel. Frontiers in public health. 2013; 1:31. [PubMed: 24350200]

Grice SJ, Spratling MW, Karmiloff-Smith A, Halit H, Csibra G, de Haan M, Johnson MH. Disordered visual processing and oscillatory brain activity in autism and Williams syndrome. Neuroreport. 2001; 12:2697–2700. [PubMed: 11522950]

Halls G, Cooper PJ, Creswell C. Social communication deficits: Specific associations with Social Anxiety Disorder. Journal of affective disorders. 2015; 172:38–42. [PubMed: 25451393]

Hamilton SM, Green JR, Veeraragavan S, Yuva L, McCoy A, Wu Y, Warren J, Little L, Ji D, Cui X, Weinstein E, Paylor R. Fmr1 and Nlgn3 knockout rats: novel tools for investigating autism spectrum disorders. Behav Neurosci. 2014; 128:103–109. [PubMed: 24773431]

Han S, Tai C, Westenbroek RE, Yu FH, Cheah CS, Potter GB, Rubenstein JL, Scheuer T, de la Iglesia HO, Catterall WA. Autistic-like behaviour in Scn1a+/− mice and rescue by enhanced GABA-mediated neurotransmission. Nature. 2012; 489:385–390. [PubMed: 22914087]

Hellings JA, Nickel EJ, Weckbaugh M, McCarter K, Mosier M, Schroeder SR. The overt aggression scale for rating aggression in outpatient youth with autistic disorder: preliminary findings. The Journal of neuropsychiatry and clinical neurosciences. 2005; 17:29–35. [PubMed: 15746480]

Holmes GL, Lenck-Santini PP. Role of interictal epileptiform abnormalities in cognitive impairment. Epilepsy Behav. 2006; 8:504–515. [PubMed: 16540376]

Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC. Cytokine effects on glutamate uptake by human astrocytes. Neuroimmunomodulation. 2000; 7:153–159. [PubMed: 10754403]

Huber KM, Klann E, Costa-Mattioli M, Zukin RS. Dysregulation of Mammalian Target of Rapamycin Signaling in Mouse Models of Autism. J Neurosci. 2015; 35:13836–13842. [PubMed: 26468183]

Jeste SS, Tuchman R. Autism Spectrum Disorder and Epilepsy: Two Sides of the Same Coin? J Child Neurol. 2015; 30:1963–1971. [PubMed: 26374786]

Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. J Neuroimmunol. 2001; 120:170–179. [PubMed: 11694332]

Kalueff AV, Lehtimaki KA, Ylinen A, Honkaniemi J, Peltola J. Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats. Neuroscience Letters. 2004; 365:106–110. [PubMed: 15245788]

Kalynchuk LE. Long-term amygdala kindling in rats as a model for the study of interictal emotionality in temporal lobe epilepsy. Neurosci Biobehav Rev. 2000; 24:691–704. [PubMed: 10974352]

Kazdoba TM, Leach PT, Silverman JL, Crawley JN. Modeling fragile X syndrome in the Fmr1 knockout mouse. Intractable & rare diseases research. 2014; 3:118–133. [PubMed: 25606362]

Kazdoba TM, Leach PT, Yang M, Silverman JL, Solomon M, Crawley JN. Translational Mouse Models of Autism: Advancing Toward Pharmacological Therapeutics. Current topics in behavioral neurosciences. 2016; 28:1–52. [PubMed: 27305922]

Kessler K, Seymour RA, Rippon G. Brain oscillations and connectivity in autism spectrum disorders (ASD): new approaches to methodology, measurement and modelling. Neurosci Biobehav Rev. 2016; 71:601–620. [PubMed: 27720724]

Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science. 2008; 321:53–57. [PubMed: 18599766]

Kleen JK, Scott RC, Holmes GL, Lenck-Santini PP. Hippocampal interictal spikes disrupt cognition in rats. Ann Neurol. 2010; 67:250–257. [PubMed: 20225290]

Kleen JK, Scott RC, Holmes GL, Roberts DW, Rundle MM, Testorf M, Lenck-Santini PP, Jobst BC. Hippocampal interictal epileptiform activity disrupts cognition in humans. Neurology. 2013; 81:18–24. [PubMed: 23685931]

Kulage KM, Smaldone AM, Cohn EG. How will DSM-5 affect autism diagnosis? A systematic literature review and meta-analysis. J Autism Dev Disord. 2014; 44:1918–1932. [PubMed: 24531932]

Kullmann DM, Lamsa KP. Interneurons go plastic. Neuropharmacology. 2011; 60:711. [PubMed: 21296094]

Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF. Pten regulates neuronal arborization and social interaction in mice. Neuron. 2006; 50:377–388. [PubMed: 16675393]

Lai MC, Baron-Cohen S, Buxbaum JD. Understanding autism in the light of sex/gender. Molecular autism. 2015; 6:24. [PubMed: 25973161]

Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet. 2014; 383:896–910. [PubMed: 24074734]

Le Magueresse C, Monyer H. GABAergic interneurons shape the functional maturation of the cortex. Neuron. 2013; 77:388–405. [PubMed: 23395369]

Leao RN, Mikulovic S, Leao KE, Munguba H, Gezelius H, Enjin A, Patra K, Eriksson A, Loew LM, Tort AB, Kullander K. OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons. Nat Neurosci. 2012; 15:1524–1530. [PubMed: 23042082]

Lee BH, Smith T, Paciorkowski AR. Autism spectrum disorder and epilepsy: Disorders with a shared biology. Epilepsy Behav. 2015; 47:191–201. [PubMed: 25900226]

Lee E, Lee J, Kim E. Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders. Biol Psychiatry. 2017; 81:838–847. [PubMed: 27450033]

Lee HJ, Caldwell HK, Macbeth AH, Tolu SG, Young WS 3rd. A conditional knockout mouse line of the oxytocin receptor. Endocrinology. 2008a; 149:3256–3263. [PubMed: 18356275]

Lee HJ, Caldwell HK, Macbeth AH, Young WS 3rd. Behavioural studies using temporal and spatial inactivation of the oxytocin receptor. Prog Brain Res. 2008b; 170:73–77. [PubMed: 18655873]

Lenck-Santini PP, Scott RC. Mechanisms Responsible for Cognitive Impairment in Epilepsy. Cold Spring Harbor perspectives in medicine. 2015:5.

Lesca G, Rudolf G, Labalme A, Hirsch E, Arzimanoglou A, Genton P, Motte J, de Saint Martin A, Valenti MP, Boulay C, De Bellescize J, Keo-Kosal P, Boutry-Kryza N, Edery P, Sanlaville D, Szepetowski P. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. Epilepsia. 2012; 53:1526–1538. [PubMed: 22738016]

Lewis MH, Tanimura Y, Lee LW, Bodfish JW. Animal models of restricted repetitive behavior in autism. Behav Brain Res. 2007; 176:66–74. [PubMed: 16997392]

Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M. Elevated immune response in the brain of autistic patients. J Neuroimmunol. 2009; 207:111–116. [PubMed: 19157572]

Lioy DT, Garg SK, Monaghan CE, Raber J, Foust KD, Kaspar BK, Hirrlinger PG, Kirchhoff F, Bissonnette JM, Ballas N, Mandel G. A role for glia in the progression of Rett's syndrome. Nature. 2011; 475:497–500. [PubMed: 21716289]

Macbeth AH, Edds JS, Young WS 3rd. Housing conditions and stimulus females: a robust social discrimination task for studying male rodent social recognition. Nature protocols. 2009; 4:1574–1581. [PubMed: 19816420]

Maroso M, Balosso S, Ravizza T, Liu J, Aronica E, Iyer AM, Rossetti C, Molteni M, Casalgrandi M, Manfredi AA, Bianchi ME, Vezzani A. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. Nature medicine. 2010; 16:413–419.

Medel-Matus JS, Shin D, Sankar R, Mazarati A. Kindling epileptogenesis and panic-like behavior: Their bidirectional connection and contribution to epilepsy-associated depression. Epilepsy Behav. 2017; 77:33–38. [PubMed: 29107199]

Meltzer A, Van de Water J. The Role of the Immune System in Autism Spectrum Disorder. Neuropsychopharmacology. 2017; 42:284–298. [PubMed: 27534269]

Milne E, Scope A, Pascalis O, Buckley D, Makeig S. Independent component analysis reveals atypical electroencephalographic activity during visual perception in individuals with autism. Biol Psychiatry. 2009; 65:22–30. [PubMed: 18774554]

Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, Courchesne E, Everall IP. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry. 2010; 68:368–376. [PubMed: 20674603]

Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, Barbaro RP, Barbaro JR, Wilson LM, Threadgill DW, Lauder JM, Magnuson TR, Crawley JN. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Behav Brain Res. 2007; 176:4–20. [PubMed: 16971002]

Muehlmann AM, Edington G, Mihalik AC, Buchwald Z, Koppuzha D, Korah M, Lewis MH. Further characterization of repetitive behavior in C58 mice: developmental trajectory and effects of environmental enrichment. Behav Brain Res. 2012; 235:143–149. [PubMed: 22963990]

Nabbout R, Chemaly N, Chipaux M, Barcia G, Bouis C, Dubouch C, Leunen D, Jambaque I, Dulac O, Dellatolas G, Chiron C. Encephalopathy in children with Dravet syndrome is not a pure consequence of epilepsy. Orphanet journal of rare diseases. 2013; 8:176. [PubMed: 24225340]

Nelson SB, Valakh V. Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders. Neuron. 2015; 87:684–698. [PubMed: 26291155]

Nelson TE, Olde Engberink A, Hernandez R, Puro A, Huitron-Resendiz S, Hao C, De Graan PN, Gruol DL. Altered synaptic transmission in the hippocampus of transgenic mice with enhanced central nervous systems expression of interleukin-6. Brain Behav Immun. 2012; 26:959–971. [PubMed: 22609298]

Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. J Neurosci. 2007; 27:5903–5914. [PubMed: 17537961]

Ornoy A, Weinstein-Fudim L, Ergaz Z. Genetic Syndromes, Maternal Diseases and Antenatal Factors Associated with Autism Spectrum Disorders (ASD). Frontiers in neuroscience. 2016; 10:316. [PubMed: 27458336]

Orrico A, Galli L, Buoni S, Orsi A, Vonella G, Sorrentino V. Novel PTEN mutations in neurodevelopmental disorders and macrocephaly. Clinical genetics. 2009; 75:195–198. [PubMed: 18759867]

Park HR, Lee JM, Moon HE, Lee DS, Kim BN, Kim J, Kim DG, Paek SH. A Short Review on the Current Understanding of Autism Spectrum Disorders. Experimental neurobiology. 2016; 25:1–13. [PubMed: 26924928]

Penagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Sonnenblick LI, Gruver R, Almajano J, Bragin A, Golshani P, Trachtenberg JT, Peles E, Geschwind DH. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell. 2011; 147:235–246. [PubMed: 21962519]

Pietropaolo S, Guilleminot A, Martin B, D'Amato FR, Crusio WE. Genetic-background modulation of core and variable autistic-like symptoms in Fmr1 knock-out mice. PLoS One. 2011; 6:e17073. [PubMed: 21364941]

Portmann T, Yang M, Mao R, Panagiotakos G, Ellegood J, Dolen G, Bader PL, Grueter BA, Goold C, Fisher E, Clifford K, Rengarajan P, Kalikhman D, Loureiro D, Saw NL, Zhengqui Z, Miller MA, Lerch JP, Henkelman RM, Shamloo M, Malenka RC, Crawley JN, Dolmetsch RE. Behavioral abnormalities and circuit defects in the basal ganglia of a mouse model of 16p11.2 deletion syndrome. Cell reports. 2014; 7:1077–1092. [PubMed: 24794428]

Pribiag H, Stellwagen D. TNF-alpha downregulates inhibitory neurotransmission through protein phosphatase 1-dependent trafficking of GABA(A) receptors. J Neurosci. 2013; 33:15879–15893. [PubMed: 24089494]

Puts NAJ, Wodka EL, Harris AD, Crocetti D, Tommerdahl M, Mostofsky SH, Edden RAE. Reduced GABA and altered somatosensory function in children with autism spectrum disorder. Autism research: official journal of the International Society for Autism Research. 2017; 10:608–619. [PubMed: 27611990]

Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. Neurobiol Dis. 2008; 29:142–160. [PubMed: 17931873]

Rojas DC, Maharajh K, Teale P, Rogers SJ. Reduced neural synchronization of gamma-band MEG oscillations in first-degree relatives of children with autism. BMC psychiatry. 2008; 8:66. [PubMed: 18673566]

Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2003; 2:255–267. [PubMed: 14606691]

Rynkiewicz A, Schuller B, Marchi E, Piana S, Camurri A, Lassalle A, Baron-Cohen S. An investigation of the 'female camouflage effect' in autism using a computerized ADOS-2 and a test of sex/gender differences. Molecular autism. 2016; 7:10. [PubMed: 26798446]

Samotaeva IS, Birioukova LM, Midzyanovskaya IS, Kuznetsova GD, Bazyan AS, Tuomisto L. Metoprine induced behavioral modifications and brain regional histamine increase in WAG/Rij and Wistar rats. Epilepsy Res. 2012; 101:148–156. [PubMed: 22503455]

Sandin S, Lichtenstein P, Kuja-Halkola R, Hultman C, Larsson H, Reichenberg A. The Heritability of Autism Spectrum Disorder. JAMA. 2017; 318:1182–1184. [PubMed: 28973605]

Scattoni ML, Crawley J, Ricceri L. Ultrasonic vocalizations: a tool for behavioural phenotyping of mouse models of neurodevelopmental disorders. Neurosci Biobehav Rev. 2009; 33:508–515. [PubMed: 18771687]

Scattoni ML, McFarlane HG, Zhodzishsky V, Caldwell HK, Young WS, Ricceri L, Crawley JN. Reduced ultrasonic vocalizations in vasopressin 1b knockout mice. Behav Brain Res. 2008; 187:371–378. [PubMed: 18005969]

Seidenberg M, Pulsipher DT, Hermann B. Association of epilepsy and comorbid conditions. Future neurology. 2009; 4:663–668. [PubMed: 20161538]

Shi XY, Tomonoh Y, Wang WZ, Ishii A, Higurashi N, Kurahashi H, Kaneko S, Hirose S. Epilepsy Genetic Study Group, J. Efficacy of antiepileptic drugs for the treatment of Dravet syndrome with different genotypes. Brain Dev. 2016; 38:40–46. [PubMed: 26183863]

Shorvon S. The concept of symptomatic epilepsy and the complexities of assigning cause in epilepsy. Epilepsy Behav. 2014; 32:1–8. [PubMed: 24463301]

Silverman JL, Pride MC, Hayes JE, Puhger KR, Butler-Struben HM, Baker S, Crawley JN. GABAB Receptor Agonist R-Baclofen Reverses Social Deficits and Reduces Repetitive Behavior in Two Mouse Models of Autism. Neuropsychopharmacology. 2015; 40:2228–2239. [PubMed: 25754761]

Silverman JL, Smith DG, Rizzo SJ, Karras MN, Turner SM, Tolu SS, Bryce DK, Smith DL, Fonseca K, Ring RH, Crawley JN. Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. Science translational medicine. 2012; 4:131ra151.

Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. Nat Rev Neurosci. 2010; 11:490–502. [PubMed: 20559336]

Simon DM, Wallace MT. Dysfunction of sensory oscillations in Autism Spectrum Disorder. Neurosci Biobehav Rev. 2016; 68:848–861. [PubMed: 27451342]

Sitcheran R, Gupta P, Fisher PB, Baldwin AS. Positive and negative regulation of EAAT2 by NF-kappaB: a role for N-myc in TNFalpha-controlled repression. EMBO J. 2005; 24:510–520. [PubMed: 15660126]

Spence SJ, Schneider MT. The role of epilepsy and epileptiform EEGs in autism spectrum disorders. Pediatr Res. 2009; 65:599–606. [PubMed: 19454962]

Spencer CM, Alekseyenko O, Hamilton SM, Thomas AM, Serysheva E, Yuva-Paylor LA, Paylor R. Modifying behavioral phenotypes in Fmr1KO mice: genetic background differences reveal autistic-like responses. Autism research: official journal of the International Society for Autism Research. 2011; 4:40–56. [PubMed: 21268289]

Spencer CM, Alekseyenko O, Serysheva E, Yuva-Paylor LA, Paylor R. Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. Genes Brain Behav. 2005; 4:420–430. [PubMed: 16176388]

Spencer CM, Graham DF, Yuva-Paylor LA, Nelson DL, Paylor R. Social behavior in Fmr1 knockout mice carrying a human FMR1 transgene. Behav Neurosci. 2008; 122:710–715. [PubMed: 18513141]

Spencer CM, Serysheva E, Yuva-Paylor LA, Oostra BA, Nelson DL, Paylor R. Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. Hum Mol Genet. 2006; 15:1984–1994. [PubMed: 16675531]

Strasser L, Downes M, Kung J, Cross JH, De Haan M. Prevalence and risk factors for autism spectrum disorder in epilepsy: a systematic review and meta-analysis. Dev Med Child Neurol. 2018; 60:19–29. [PubMed: 29119560]

Stroganova TA, Orekhova EV, Prokofyev AO, Tsetlin MM, Gratchev VV, Morozov AA, Obukhov YV. High-frequency oscillatory response to illusory contour in typically developing boys and boys with autism spectrum disorders. Cortex. 2012; 48:701–717. [PubMed: 21458787]

Sukoff Rizzo SJ, Silverman JL. Methodological Considerations for Optimizing and Validating Behavioral Assays. Current protocols in mouse biology. 2016; 6:364–379. [PubMed: 27906464]

Sun L, Grutzner C, Bolte S, Wibral M, Tozman T, Schlitt S, Poustka F, Singer W, Freitag CM, Uhlhaas PJ. Impaired gamma-band activity during perceptual organization in adults with autism spectrum disorders: evidence for dysfunctional network activity in frontal-posterior cortices. J Neurosci. 2012; 32:9563–9573. [PubMed: 22787042]

Sundelin HE, Larsson H, Lichtenstein P, Almqvist C, Hultman CM, Tomson T, Ludvigsson JF. Autism and epilepsy: A population-based nationwide cohort study. Neurology. 2016; 87:192–197. [PubMed: 27306624]

Szyndler J, Rok P, Maciejak P, Walkowiak J, Czlonkowska AI, Sienkiewicz-Jarosz H, Wislowska A, Zienowicz M, Lehner M, Bidzinski A, Kostowski W, Plaznik A. Effects of pentylenetetrazol-induced kindling of seizures on rat emotional behavior and brain monoaminergic systems. Pharmacol Biochem Behav. 2002; 73:851–861. [PubMed: 12213531]

Takesian AE, Hensch TK. Balancing plasticity/stability across brain development. Prog Brain Res. 2013; 207:3–34. [PubMed: 24309249]

Terrone G, Salamone A, Vezzani A. Inflammation and Epilepsy: preclinical findings and potential clinical translation. Curr Pharm Des. 2017

Theoharides TC, Zhang B. Neuro-inflammation, blood-brain barrier, seizures and autism. Journal of neuroinflammation. 2011; 8:168. [PubMed: 22129087]

Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology (Berl). 2009; 204:361–373. [PubMed: 19189082]

Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. Nat Med. 2005; 11:973–981. [PubMed: 16116433]

Tiedeken JA, Muha N, Ramsdell JS. A cupric silver histochemical analysis of domoic acid damage to olfactory pathways following status epilepticus in a rat model for chronic recurrent spontaneous seizures and aggressive behavior. Toxicologic pathology. 2013; 41:454–469. [PubMed: 22821366]

Tiedeken JA, Ramsdell JS. Persistent neurological damage associated with spontaneous recurrent seizures and atypical aggressive behavior of domoic acid epileptic disease. Toxicol Sci. 2013; 133:133–143. [PubMed: 23457124]

Tuchman R, Alessandri M, Cuccaro M. Autism spectrum disorders and epilepsy: moving towards a comprehensive approach to treatment. Brain Dev. 2010a; 32:719–730. [PubMed: 20558021]

Tuchman R, Cuccaro M, Alessandri M. Autism and epilepsy: historical perspective. Brain Dev. 2010b; 32:709–718. [PubMed: 20510557]

Tuchman R, Rapin I. Epilepsy in autism. Lancet Neurol. 2002; 1:352–358. [PubMed: 12849396]

Uutela M, Lindholm J, Louhivuori V, Wei H, Louhivuori LM, Pertovaara A, Akerman K, Castren E, Castren ML. Reduction of BDNF expression in Fmr1 knockout mice worsens cognitive deficits but improves hyperactivity and sensorimotor deficits. Genes Brain Behav. 2012; 11:513–523. [PubMed: 22435671]

Vakorin VA, Doesburg SM, Leung RC, Vogan VM, Anagnostou E, Taylor MJ. Developmental changes in neuromagnetic rhythms and network synchrony in autism. Ann Neurol. 2017; 81:199–211. [PubMed: 27977875]

Varga C, Golshani P, Soltesz I. Frequency-invariant temporal ordering of interneuronal discharges during hippocampal oscillations in awake mice. Proc Natl Acad Sci U S A. 2012; 109:E2726–2734. [PubMed: 23010933]

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Annals of neurology. 2005; 57:67–81. [PubMed: 15546155]

Veeraragavan S, Wan YW, Connolly DR, Hamilton SM, Ward CS, Soriano S, Pitcher MR, McGraw CM, Huang SG, Green JR, Yuva LA, Liang AJ, Neul JL, Yasui DH, LaSalle JM, Liu Z, Paylor R, Samaco RC. Loss of MeCP2 in the rat models regression, impaired sociability and transcriptional deficits of Rett syndrome. Hum Mol Genet. 2016; 25:3284–3302. [PubMed: 27365498]

Velíšková, J., Velíšek, L. Behavioral characterization of seizures in rats. In: Pitkanen, A.Buckmaster, P.Galanopoulou, AS., Moshé, SL., editors. Models of seizures and epilepsy. 2. Academic Press; San Diego: 2017. p. 111-124.

Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. Nat Rev Neurol. 2011; 7:31–40. [PubMed: 21135885]

Vezzani A, Fujinami RS, White HS, Preux PM, Blumcke I, Sander JW, Loscher W. Infections, inflammation and epilepsy. Acta Neuropathol. 2016; 131:211–234. [PubMed: 26423537]

Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia. 2005; 46:1724–1743. [PubMed: 16302852]

Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, De Simoni MG, Sperk G, Andell-Jonsson S, Lundkvist J, Iverfeldt K, Bartfai T. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc Natl Acad Sci U S A. 2000; 97:11534–11539. [PubMed: 11016948]

Weiss LA, Escayg A, Kearney JA, Trudeau M, MacDonald BT, Mori M, Reichert J, Buxbaum JD, Meisler MH. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. Mol Psychiatry. 2003; 8:186–194. [PubMed: 12610651]

Wipfler P, Dunn N, Beiki O, Trinka E, Fogdell-Hahn A. The Viral Hypothesis of Mesial Temporal Lobe Epilepsy - Is Human Herpes Virus-6 the Missing Link? A systematic review and meta-analysis. Seizure. 2018; 54:33–40. [PubMed: 29195226]

Wirrell E, Sherman EM, Vanmastrigt R, Hamiwka L. Deterioration in cognitive function in children with benign epilepsy of childhood with central temporal spikes treated with sulthiame. J Child Neurol. 2008; 23:14–21. [PubMed: 18184938]

Wohr M, Silverman JL, Scattoni ML, Turner SM, Harris MJ, Saxena R, Crawley JN. Developmental delays and reduced pup ultrasonic vocalizations but normal sociability in mice lacking the postsynaptic cell adhesion protein neuroligin2. Behav Brain Res. 2013; 251:50–64. [PubMed: 22820233]

Wrenn CC, Harris AP, Saavedra MC, Crawley JN. Social transmission of food preference in mice: methodology and application to galanin-overexpressing transgenic mice. Behav Neurosci. 2003; 117:21–31. [PubMed: 12619904]

Yang M, Bozdagi O, Scattoni ML, Wohr M, Roullet FI, Katz AM, Abrams DN, Kalikhman D, Simon H, Woldeyohannes L, Zhang JY, Harris MJ, Saxena R, Silverman JL, Buxbaum JD, Crawley JN. Reduced excitatory neurotransmission and mild autism-relevant phenotypes in adolescent Shank3 null mutant mice. J Neurosci. 2012; 32:6525–6541. [PubMed: 22573675]

Yang M, Silverman JL, Crawley JN. Automated three-chambered social approach task for mice. Curr Protoc Neurosci. 2011; Chapter 8(Unit 8):26.

Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci. 2006; 9:1142–1149. [PubMed: 16921370]

Zurcher NR, Bhanot A, McDougle CJ, Hooker JM. A systematic review of molecular imaging (PET and SPECT) in autism spectrum disorder: current state and future research opportunities. Neurosci Biobehav Rev. 2015; 52:56–73. [PubMed: 25684726]

Highlights

- Autism spectrum disorder (ASD) is a common comorbidity of epilepsy
- ASD and epilepsy have multifactorial etiology with strong evidence of shared underlying neurological abnormalities
- New clinical benchmarks for ASD pose stricter criteria for animal models and provide a more systematic framework to test autistic traits
- Compromised brain immune response and neuroinflammation contributes to excitation-inhibition (E-I) imbalance in ASD and epilepsy
- E-I imbalance due to the loss of GABAergic function disrupts the brain rhythms and leads to cognitive and behavioral deficits observed in ASD and epilepsy

Table 1

summarizes representative behavioral assays and examples in the genetic models of ASD literature that were used to identify phenotypes using the two core ASD-relevant behavioral domains and possible confounding behaviors relevant in epileptic animal models.

Behavioral Domain	Assay	ASD-relevant core symptoms	References for detailed description of the test	Common seizure behaviors that can mimic ASD traits
Reciprocal social communication	Three-chambered task	Equal or less time spent with the novel mouse and the novel object	(Dhamne et al., 2017; Ey et al., 2012; Moy et al., 2007; Silverman et al., 2012; Wohr et al., 2013; Yang et al., 2011)	Increased/decreased anxiety Depression-like behavior Aggressive behavior Hypo-/hyper-activity Olfactory damage Learning and memory deficits due to neurodegeneration
	Reciprocal dyad interactions	Lack of interest in the partner	(Bozdagi et al., 2010; Dhamne et al., 2017; Silverman et al., 2015)	
	Social recognition	Lack in: 1) two-exposure recognition, 2) habituation-dishabituation, and 3) social discrimination	(Ferguson et al., 2001; Ferguson et al., 2000; Lee et al., 2008a; Lee et al., 2008b; Macbeth et al., 2009)	
	Partition test	Lack of interest in the partner	(Hamilton et al., 2014; Spencer et al., 2005; Spencer et al., 2008; Veeraragavan et al., 2016)	
	Social transmission of food preference	Missing	(Wrenn et al., 2003)	
	Ultrasonic vocalization	Reduction in ultrasonic emissions	(Scattoni et al., 2009; Scattoni et al., 2008; Wohr et al., 2013; Yang et al., 2012)	
Repetitive behaviors, with restricted interests and behavioral inflexibility	Stereotypies	repetitive self-grooming; circling; jumping; back flipping; perseverative wood block chewing	(Bechard et al., 2017; Blundell et al., 2010; Copping et al., 2017; Dhamne et al., 2017; Etherton et al., 2009; Hamilton et al., 2014; Lewis et al., 2007; Muehlmann et al., 2012; Portmann et al., 2014; Silverman et al., 2015; Silverman et al., 2012; Yang et al., 2012)	Often part of seizure behavior
	Marble burying	>50% covered marbles	(Thomas et al., 2009)	Hypo-/hyper-activity following seizures
	Insistence on sameness and lack of cognitive flexibility	Impaired reversal learning in the Morris water maze or T maze	(Moy et al., 2007)	Deficits in spatial discrimination learning due to neurodegeneration