EPILEPSY SPECTRUM DISORDER

Molecular mechanisms of cognitive and behavioral comorbidities of epilepsy in children

Amy Brooks-Kayal

Departments of Pediatrics and Neurology, Division of Pediatric Neurology, University of Colorado Denver School of Medicine, Aurora, Colorado, U.S.A.

SUMMARY

Intellectual and developmental disabilities (IDDs) such as autistic spectrum disorders (ASDs) and epilepsies are heterogeneous disorders that have diverse etiologies and pathophysiologies. The high rate of co-occurrence of these disorders, however, suggests potentially shared underlying mechanisms. A number of well-known genetic disorders share epilepsy, intellectual disability, and autism as prominent phenotypic features, including tuberous sclerosis complex, Rett syndrome, and fragile X syndrome. In addition, mutations of several genes involved in neurodevelopment, including *ARX*, *DCX*, neuroligins, and neuropilin 2 have been identified in children with epilepsy, IDDs, ASDs, or a combination of thereof. Finally, in animal models, early life seizures can result in cellular and molecular changes that could contribute to learning and behavioral disabilities. Increased understanding of the common genetic, molecular, and cellular mechanisms of IDDs, ASDs, and epilepsy may provide insight into their underlying pathophysiology and elucidate new therapeutic approaches for these conditions.

KEY WORDS: Epilepsy, Intellectual disability, Autism, Synaptic plasticity, Hippocampus.

Approximately 30% of children with epilepsy have autism and/or intellectual or developmental disabilities (Tuchman et al., 2009). What might explain the increased association of IDDs (intellectual and developmental disabilities), ASDs (autistic spectrum disorders), and epilepsy? All three could result from the same pathophysiologic mechanisms. For example, it has been proposed that IDDs, ASDs, and epilepsy can be understood as disorders of synaptic plasticity resulting in a developmental imbalance of excitation and inhibition (Fig. 1). This may occur in genetic conditions that result in abnormal excitability and disrupted synaptic plasticity in the developing brain such as fragile X syndrome (FXS), Rett syndrome (RTT), cyclin-dependent kinase-like 5 (*CDKL5*) mutations, tuberous sclerosis complex (TSC), neuroligin mutations, and ''interneuronopathies'' resulting from aristaless-related homeobox, X-linked (*ARX*) and neuropilin 2 (*NRP2*) gene mutations, all of which include ASDs, IDDs, and epilepsy. In addition, the process of epilepsy development (i.e. epileptogenesis) and/or spontaneous seizures themselves may result in maladaptive synaptic plasticity producing imbalances of excitation and inhibition that contribute to learning and behavioral difficulties. Abnormalities in synaptic plasticity can arrive from alterations in receptors, signaling molecules, or neurotropins, many of which are known to be altered after early life seizures and in genetic conditions known to be associated with both ASDs and epilepsy (Fig. 1).

Synaptic plasticity describes the process whereby synapses get strengthened by experience or practice. When synapses are activated, depolarization mediated by α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors allows release of magnesium blockade and calcium entry through N-methyl-D-aspartate (NMDA) receptors. This triggers calcium-dependent activation of kinases and other signaling pathways resulting in enhanced gene transcription and trafficking of receptors that result in faster and stronger synaptic connections. This is known as long-term potentiation (LTP) and is thought be the cellular basis of learning. Synaptic plasticity depends on a variety of proteins whose genes are disrupted in genetic conditions associated with autism and epilepsy. These include CDKL5 in West syndrome, methyl-CpG binding protein 2 (MeCP2) in Rett syndrome,

Address correspondence to Amy Brooks-Kayal, The Children's Hospital, 13123 E 16th Ave., B155, Aurora, CO 80045, U.S.A. E-mail: Brooks-Kayal.Amy@tchden.org
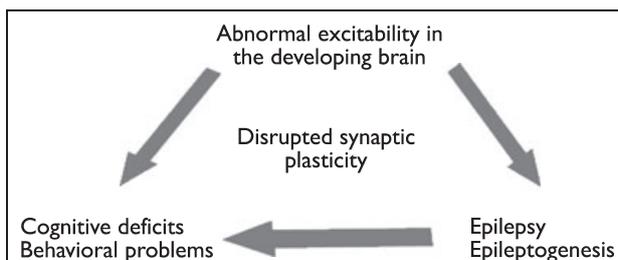
Figure 1.
There is evidence that epilepsy and disorders of learning and behavior can all arise from abnormal excitability and disrupted synaptic plasticity in the developing brain. This abnormal plasticity can result from genetic conditions. In addition, epilepsy development (epileptogenesis) and/or seizures during early postnatal development may alter synaptic plasticity and contribute to learning and behavioral disorders. Abnormalities in synaptic plasticity can arise from alterations in receptors, signaling molecules, or neurotrophins. Many of these molecules are known to be altered by early life seizures and genetic conditions associated with IDDs, ASDs, and epilepsy.
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fragile X mental retardation protein (FMRP) in fragile X mental retardation syndrome, mammalian target of rapamycin (mTOR) in tuberous sclerosis, and reelin in lissencephaly.

FRAGILE X SYNDROME

Fragile X syndrome (FXS) is the most frequent form of inherited mental retardation and often presents with IDDs, ASDs, and epilepsy. The hallmark of FXS pathology is the hyperabundance of dendritic spines with a long, thin, and otherwise immature morphology (Irwin et al., 2000; Grossman et al., 2006). Fragile X results from an expanded triplet repeat in the *FMR1* gene. *Fmr1* knockout mice (an animal model for FXS) exhibit a similar excess of long, thin spines (Comery et al., 1997) and display altered learning and behavior, greater susceptibility to seizures, and altered synaptic plasticity (Penagarikano et al., 2007). The *FMR1* gene is located on the X chromosome (Bassell & Warren, 2008), and expanded CGG repeats within the 5′-untranslated region of *FMR1* result in FXS (normal length of ~30 triplets, 55–200 triplets in premutations in FXS carriers, 200–800 triplets in affected FXS individuals). The *FMR1* gene codes for the FMRP, which is an mRNA-binding protein abundant in the brain that binds to and regulates 4% of brain mRNA, including many RNAs important for synaptic plasticity. FMRP regulates mRNA transport and local protein synthesis in neuronal dendrites and spines, which is important for spine

development and synaptic plasticity. In the absence of FMRP, excess and dysregulated mRNA translation leads to altered synaptic function and loss of protein synthesis–dependent plasticity. FMRP is also involved in axonal development, synapse formation, and the development and wiring of neuronal circuits (Feng et al., 1997; Laggerbauer et al., 2001; Li et al., 2001; Zalfa et al., 2003; Antar et al., 2004; Lu et al., 2004; Muddashetty et al., 2007; Bassell & Warren, 2008; Gibson et al., 2008).

FMRP regulates metabotropic glutamate receptor (mGluR)–induced long-term depression (LTD), and in the absence of FMRP, there is excessive AMPA receptor (AMPAR) internalization and exaggerated LTD, leading to impaired synaptic excitatory function ["The mGluR theory of fragile X"; (Bear et al., 2004)]. Stimulation of group1 mGluRs activates protein phosphatase 2-A (PP2A) and dephosphorylates FMRP, allowing rapid translation of FMRP-associated mRNAs that lead to excessive AMPA receptor internalization, disrupted synaptic function, and exaggerated LTD. This is known as "the mGluR theory of fragile X," and although it adds to our understanding of intellectual impairment in fragile X syndrome, it does not seem to readily explain the central nervous system (CNS) hyperexcitability and resultant epilepsy associated with this disorder. Recent studies on group I mGluR–mediated epileptogenesis, however, have begun to reveal a possible connection (Hagerman & Stafstrom, 2009). Bianchi et al. provided compelling evidence that a voltage-gated inward current, ImGluR(V), is the cellular basis for the epileptogenic behavior induced by activation of the mGluR5 receptor (Chuang et al., 2000, 2001, 2005; Bianchi et al., 2009). Evidence for a connection between the absence of *Fmr1* and epileptogenesis was further supported by a study of neocortical circuits in FMRP knockout mice that found an increased intrinsic excitability in excitatory neurons from the knockout (Gibson et al., 2008). In addition, dysregulation of glutamatergic neurons in FXS can disrupt the normal actions of inhibitory γ-aminobutyric acid (GABA)ergic neurons, and result in downregulation of GABA receptor subunits (El Idrissi et al., 2005; D'Hulst et al., 2006; Gantois et al., 2006) and altered expression of a number of enzymes involved in the metabolism of GABA (D'Hulst et al., 2009), which could also contribute to hyperexcitability and epilepsy in the FXS.

TUBEROUS SCLEROSIS COMPLEX

Tuberous sclerosis complex (TSC) is a neurocutaneous syndrome characterized by benign tumors, early onset epilepsy, intellectual disability, and autism. TSC results from mutations of hamartin or tuberin (encoded by *TSC1* and *TSC2* genes), which together inhibit the phosphatidyl inositol 3-kinase (PI3) signaling pathway, involving the mTOR and a cascade of other downstream

kinases and translational factors that stimulate protein translation, cell growth, and proliferation. Mutations of hamartin or tuberin in TSC lead to hyperactivation of the mTOR, and downstream signaling pathways result in increased cell growth, proliferation, and abnormal gene expression. Exact mechanisms of epilepsy, IDDs, and ASDs in TSC are still unknown, but alterations in trafficking of AMPARs, and in expression of specific glutamate and GABA$_A$ receptor subunits and decreases in the glutamate transporter GLT-1 may all contribute to imbalances of excitation and inhibition (White et al., 2001; Wong et al., 2003).

RETT SYNDROME

Rett syndrome (RTT) is a postnatal progressive neurodevelopmental disorder that manifests in girls during early childhood. Symptoms appear over stages beginning at 6–18 months and include loss of acquired speech, social skills, purposeful use of the hands, and motor skills. Patients typically go on to develop profound cognitive impairment, anxiety, seizures, and a host of autonomic abnormalities (Chahrour et al., 2008). RTT is caused by mutations in the gene encoding methyl-CpG binding protein 2 (MeCP2), a transcriptional regulator involved in chromatin remodeling and the modulation of RNA splicing. In resting neurons, MeCP2 regulates gene expression by binding to methylated CpG dinucleotides and recruiting histone deacetylase (HDAC) co-repressor complexes and chromatin remodeling proteins. This most commonly leads to chromatin compaction, making the promoter inaccessible to the transcriptional machinery and transcriptional repression (Chen et al., 2003), although recent evidence suggests that MeCP2 can also act as a transcriptional activator for some genes (Chahrour et al., 2008). Neuronal activity induces MeCP2 phosphorylation, dissociation of MeCP2 and the corepressor complex from promoters, and target gene expression. In RTT, the absence of MeCP2 causes a loss of activity-dependent changes in gene expression that may disrupt synaptic plasticity (Chahrour & Zoghbi, 2007). MeCP2 loss has been shown to induce changes in expression of thousands of genes (Chahrour et al., 2008), although the precise mechanism by which loss of MeCP2 results in epilepsy, IDDs, or ASDs remains uncertain. Recent evidence suggests that alterations in cortical glutamatergic synaptic responses and excitatory connectivity resulting in a relative excess of inhibition compared to excitation may play an important role (Dani et al., 2005; Chao et al., 2007; Dani & Nelson, 2009).

NEUROLIGIN/NEUREXIN MUTATIONS

Neuroligins and neurexins are proteins crucial for aligning and activating both excitatory and inhibitory synapses during development. Mutations in a number of these genes, along with the associated Shank3 scaffolding protein, have been implicated in autism.

An altered balance between excitatory synapses and inhibitory synapses could affect cognition and social behavior as well as contribute to epilepsy. Mutations in neuroligin (NL)-1, -3, and -4 have been identified in human patients with ASDs (Jamain et al., 2003; Durand et al., 2007). The Arg451 → Cys451 substitution in NL-3 found in patients with ADSs results in enhanced inhibitory transmission and impaired social behavior in knockin mice (Tabuchi et al., 2007). Overexpression of the mutant NL-1 found in ASD patients depressed the number of excitatory synapses and excitatory synaptic strength and resulted in abnormal social behavior in mice (Chubykin et al., 2007).

INTERNEURONOPATHIES

ARX

Developmental abnormalities resulting in reduced numbers of cortical and hippocampal interneuron subtypes have been reported to cause severe early life epilepsies, IDDs, and autism. In humans, mutations of the aristaless-related homeobox, X-linked (*ARX*) gene result in several clinical syndromes, all of which are associated with intellectual disability, ASDs, and early life seizures, most often infantile spasms. In animal models, *ARX* knockouts have reduced interneuron cell types and a variety of seizure types (absence, myoclonic, generalized tonic–clonic) beginning in early life (Marsh et al., 2009).

Neuropilin 2 mutations

Neuropilin 2 (NRP2) is a receptor for the axon guidance mediator semaphorin 3F. Polymorphisms of the *NRP2* gene have been associated with autism (Wu et al., 2007). *NRP2*-deficient mice have shorter seizure latency after chemoconvulsants, develop spontaneous seizures, have reductions in specific subsets of hippocampal interneurons (parvalbumin and neuropeptide Y), and reduced dendritic length and complexity on CA1 pyramidal neurons (Gant et al., 2009).

In conclusion, a number of different genetic mutations can result in IDDs, ASDs, and epilepsy. Many of these mutations cause abnormalities of synaptic plasticity that result in imbalances in excitation and inhibition in the developing brain. In addition to genetic abnormalities that disrupt synaptic plasticity, seizures and epilepsy development (epileptogenesis) in early life may impact synaptic plasticity and potentially contribute to ASDs and intellectual disability. What are the changes resulting from epileptogenesis and/or seizures in the developing brain that may alter synaptic plasticity and contribute to IDDs and ASDs?

Effects of Seizures and Epileptogenesis on the Developing Brain

Epileptogenesis is a process that proceeds over months to years in humans, and over days to weeks in rodent models. After an initial precipitating event such as a prolonged febrile seizure or head trauma, there are processes that occur very rapidly including ion channel activation, post-translational changes, and immediate early genes. Next, over a period of days to weeks, there are transcriptional events, neuronal death, and inflammation. Sprouting, network reorganization, neurogenesis, and gliosis occur over the ensuing weeks, months, and years. These processes may lead to the development of the first spontaneous seizures, and then be recapitulated with each seizure, resulting in perpetuation or progression of epilepsy. Changes associated with epileptogenesis and seizures occur simultaneously with, and may disrupt, normal activity dependent developmental processes in the brain including synaptic pruning, dendritic and axonal refinement, and receptor and ion channel maturations (Fig. 2). These effects may occur independently of and in addition to genetic disruptions of synaptic plasticity discussed earlier (Fig. 2).

There is substantial evidence from animal models that early life seizures induced by a variety of methods includ-ing chemoconvulsants, tetanus toxin, corticotropin-releasing hormone (CRH), and hyperthermia/fever can all result in later cognitive dysfunction in a subset of animals (Liu et al., 1995; Lynch et al., 2000; Brunson et al., 2001a; de Rogalski Landrot et al., 2001; Lee et al., 2001; Kubova et al., 2004; Sayin et al., 2004; Cornejo et al., 2007; Dube et al., 2009). Until recently, no specific biomarkers were available that could predict the cognitive outcome after early life seizures. In a recent study, animals that experienced prolonged febrile seizures (PFS) in early life underwent MRI imaging after seizures and then testing of spatial learning and memory in adulthood (Dube et al., 2009). Half of animals exposed to PFS were found to have T_2-signal abnormalities in hippocampus, and the spatial learning and memory performances in rats that experienced PFS and also had T_2 MRI changes were impaired compared to the rats that had prolonged febrile seizures with no T_2 changes, thus identifying MRI imaging as a possible biomarker for future hippocampal learning and memory dysfunction after early life seizures.

There are many potential effects of seizures and epileptogenesis in the developing brain on synaptic plasticity that could explain the observed effects on cognition and behavior. These include a number of cellular and molecular changes that have been shown to occur during developmental epileptogenesis, including cellular changes such as altered neurogenesis and reduced place cell function,
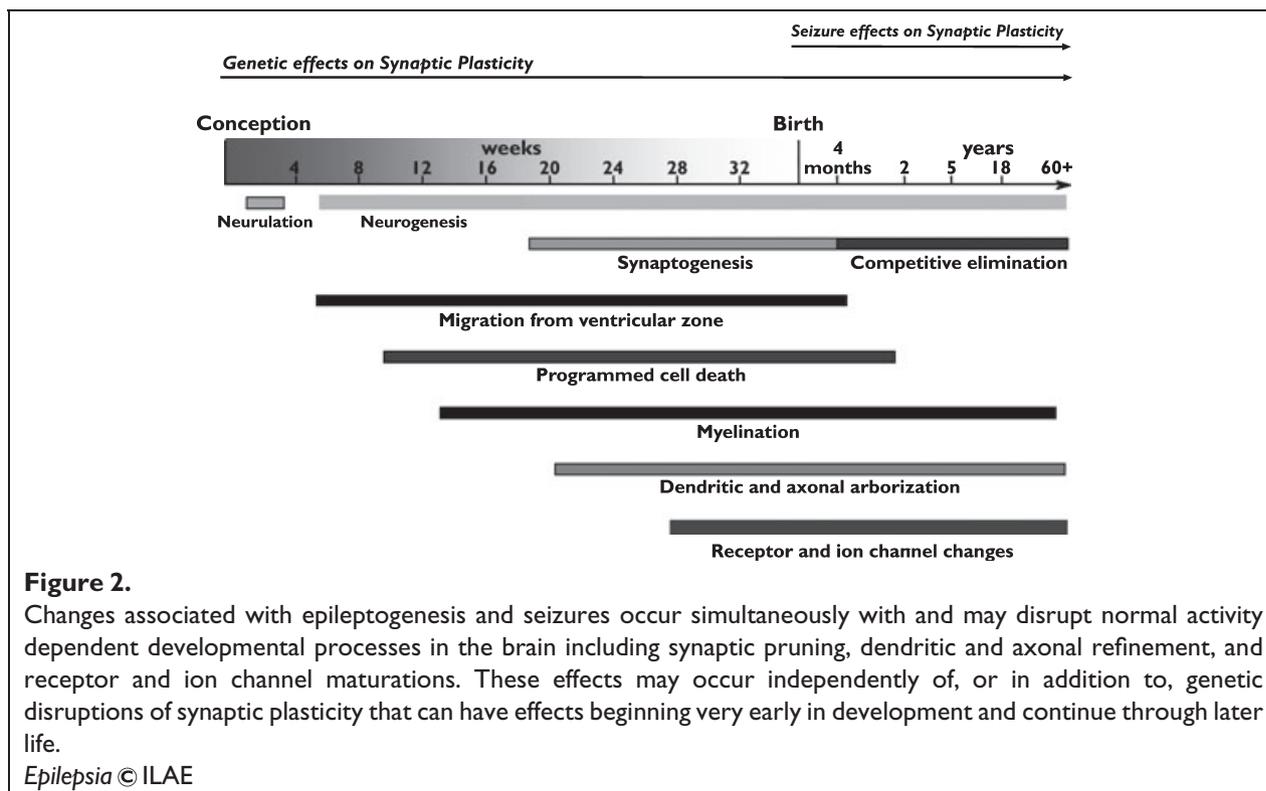

Figure 2.
Changes associated with epileptogenesis and seizures occur simultaneously with and may disrupt normal activity dependent developmental processes in the brain including synaptic pruning, dendritic and axonal refinement, and receptor and ion channel maturations. These effects may occur independently of, or in addition to, genetic disruptions of synaptic plasticity that can have effects beginning very early in development and continue through later life.
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molecular changes resulting in altered inhibitory and excitatory neurotransmission, and changes in regulatory and neuromodulatory pathways. Dentate granule cell neurogenesis is critical for learning, and both enhanced and reduced neurogenesis have been reported after early life seizures, depending on age and model (McCabe et al., 2001; Porter et al., 2004). The location of an animal in his environment is coded by the activity of a category of pyramidal neurons called place cells, a subset of CA1 cells that fire preferentially at particular locations (firing fields), and this encoding is critical for the formation of long-term spatial memory. Recent work examining place-cell recordings in CA1 of rats demonstrated that those rats exposed to early life febrile seizures (EPFS) that had significant hippocampal MRI changes, had impaired place-cell function (including reduced coherence and stability) that correlated with spatial memory deficits on behavioral testing (Dube et al., 2009).

Emerging evidence suggests that early life seizures can alter the function of neurotransmitter systems and intrinsic neuronal properties that are critical for learning and memory. GABA is the main inhibitory neurotransmitter in the brain and $GABA_A$ receptors mediate most fast synaptic inhibition. Changes in inhibitory neurotransmission are known to affect learning. Enhancement of $GABA_A$ receptor function with benzodiazepines disrupts LTP and memory formation (del Cerro et al., 1992; Seabrook et al., 1997) and $GABA_A$ receptor α subunits have been shown to be key regulators of "critical periods" for cortical plasticity (Fagiolini et al., 2004) and hippocampal-dependent spatial learning (Rudolph & Mohler, 2004). Evidence exists for enhanced inhibition after early life seizures that could impair these cognitive processes. On a circuit level, increased paired-pulse inhibition in hippocampus has been seen after both hyperthermic and kainate-induced seizures in the postnatal period (Sutula et al., 1992). At the cellular/molecular level, early life lithium and pilocarpine–induced seizures produce an increase in $GABA_A$ receptor expression and a selective increase in α1 subunit in the hippocampal dentate gyrus, both immediately and when the animals reach adulthood (Zhang et al., 2004; Raol et al., 2006). These alterations are associated with functional changes including enhanced type I benzodiazepine augmentation of the receptor (Zhang et al., 2004). This is in contrast to α1 subunit expression decrease seen in adult rats following pilocarpine-induced seizures (Brooks-Kayal et al., 1998). These findings suggest that the effects of seizures on expression of $GABA_A$ receptor subunits are age-dependent and that increased $GABA_A$ receptor expression and resulting enhanced inhibition could contribute to cognitive deficits following early life seizures. Although $GABA_A$ receptor activation in mature neurons results in membrane hyperpolarization and inhibition of cell firing, due to developmental changes in the chloride gradient, in immature neurons, $GABA_A$ receptor activa-

tion results in membrane depolarization and is thus excitatory (Cherubini et al., 1990). Depolarizing GABA currents are critical for Ca^{2+}-dependent developmental processes including neuronal proliferation, migration, targeting, and synaptogenesis (LoTurco et al., 1995; Owens et al., 1996; Ben-Ari et al., 1997; Leinekugal et al., 1997). Early life seizures have recently been shown to accelerate the switch of GABA currents from depolarizing to hyperpolarizing in hippocampal CA1 neurons, and have been associated with spatial learning deficits (Galanopoulou, 2008).

Changes in excitatory neurotransmission may also contribute to learning and behavioral differences after early life seizures. Glutamate is the primary excitatory neurotransmitter in brain and its activity is mediated by a variety of receptor subtypes including NMDA and non-NMDA (AMPA and kainate) ionotropic receptors and metabotropic receptors. Excitatory signaling through both the AMPA and NMDA receptors are critical for different types of LTP and hippocampal learning (Mongillo et al., 2003; Riedel et al., 2003; Yasuda et al., 2003; Schmitt et al., 2005), and mutant mice lacking subtypes of AMPA or NMDA receptors have impaired learning (Nakazawa et al., 2002; Mead & Stephens, 2003). Deficits in excitatory synaptic density and in excitatory signaling through both AMPA and NMDA receptors have been found after early life seizures. Early life hypoxia-induced seizures have been shown to result in rapid posttranscriptional effects on AMPA receptor subunit (GluR) phosphorylation, including increased phosphorylation of sites in both GluR1 and GluR2 that result in enhanced excitatory synaptic currents due to increased conductance and membrane insertion of Ca-permeable GluR1-containing receptors associated with GluR1 phosphorylation and reduced membrane insertion of Ca-impermeable GluR2-containing receptors (Rakhade et al., 2008). Tetanus toxin–induced seizures in the postnatal period produce long-term effects including a 30% decrease in dendritic spine density on hippocampal CA3 neurons (Jiang et al., 1998) and a 30–40% decrease in NMDA receptor NR1, NR2A, and NR2B subunit proteins in hippocampus (Lee et al., 2001; Swann, 2004). Acute and chronic decreases in AMPA receptor GluR2-subunit expression has been shown after postnatal hypoxia-induced seizures (Sanchez et al., 2001) and lithium-pilocarpine–induced seizures (Zhang et al., 2004). Even a single seizure induced with kainic acid at P7 can result in impairments in spatial memory and impaired CA1 LTP and enhanced LTD (Cornejo et al., 2007). This altered synaptic plasticity is associated with decreases in NMDA receptor NR2A expression and AMPA receptor subunit GluR1 surface expression, and it increases in the excitatory synaptic anchoring protein PSD-95 (Cornejo et al., 2007).

In addition to effects of early seizures on neurotransmitter receptor systems, these seizures also affect a number of

molecules that are essential for intrinsic neuronal function. Prolonged postnatal febrile (hyperthermic) seizures produce a profound, long-lasting enhancement of intrinsic hyperpolarization activated membrane current, Ih, (Chen et al., 2001) due to a decrease in hyperpolarization activated, cyclic nucleotide-gated channel-1 (HCN1) mRNA and simultaneous enhancement of HCN2 mRNA expression in hippocampal CA1 neurons (Brewster et al., 2002, 2005). These changes are associated with persistent limbic hyperexcitability and a 35% incidence of spontaneous seizures in adulthood (Dube et al., 2000, 2006; Chen et al., 2001). This suggests that a variety of receptors and important cell signaling proteins may be permanently altered following early life seizures.

Changes in neuromodulatory pathways may also contribute to learning and behavioral differences after early life seizures. cAMP response element binding protein (CREB) is a key mediator of stimulus-induced changes in gene expression that underlie plasticity of the nervous system, and phosphorylation of CREB is required for LTP, learning, and memory (Lonze & Ginty, 2002). CREB phosphorylation with learning has been shown to be diminished after repetitive febrile seizures in animals (Chang et al., 2003). Corticotropin-releasing hormone (CRH) is a neuromodulatory peptide released from hippocampal interneurons in response to stress. Early life seizures have been shown to enhance hippocampal CRH mRNA expression in adulthood (Brunson et al., 2001b), and excessive CRH (Chen et al., 2004) and early life stress (Brunson et al., 2005) have been shown to lead to reductions in dendritic length and arborization as well as progressive cognitive deficits.

Conclusions

Early life seizures may produce a variety of cellular and molecular changes in hippocampus that may contribute to the enhanced risk of IDDs and ASDs in patients with early life seizures and epilepsy. Abnormalities of synaptic plasticity resulting in imbalances of excitatory and inhibitory neurotransmission resulting either from genetic mutations or effects of early life seizures may provide a common mechanism of epilepsy, IDDs, and ASDs, and may provide a basis for understanding of the frequent co-occurrence of these disorders. Although this concept of a shared pathophysiology can provide a broad framework in which to begin to understand the frequent association of epilepsy, intellectual disability, and autism, many questions remain unanswered. Why are epilepsy, ASDs, and IDDs sometimes comorbid, but not always? In what specific brain regions, pathways, and cell types and at what point in development do impairments of synaptic plasticity occur, and does the spatial and temporal profile in part determine the specific phenotype? Do identified changes in synaptic plasticity and the cellular and molecular mechanisms that produce them represent new therapeutic targets for treatment of these conditions, and if so what is the developmental window in which these treatments might be effective? Continued research is essential to begin to address these and other gaps in our understanding of the neurobiologic basis of the complex association between epilepsy and comorbid learning and behavioral disorders.

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Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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