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Early life seizures: Evidence for chronic deficits linked to autism and intellectual disability across species and models

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Abstract

Recent work in *Exp Neurol* by Lugo et al. (2014b) demonstrated chronic alterations in sociability, learning and memory following multiple early life seizures (ELS) in a mouse model. This work adds to the growing body of evidence supporting the detrimental nature of ELS on the developing brain to contribute to aspects of an autistic phenotype with intellectual disability. Review of the face validity of behavioral testing and the construct validity of the models used informs the predictive ability and thus the utility of these models to translate underlying molecular and cellular mechanisms into future human studies.

Keywords

Early life seizure; Autism; Intellectual disability; Animal model; Social; Learning; Memory; Anxiety; Communication

Introduction

Recent work by Lugo et al

Lugo et al. (2014b) queried whether early life seizures (ELS) result in autistic features and cognitive dysfunction. While this is not a novel query, they sought to both re-address this question and pose it in a mouse model to open the field to easier genetic manipulation. They induced 15 flurothyl (FL) seizures from post-natal days 7 to 11 in C57BL/6 mice. Mice were then probed for long term behavioral changes in a battery of tests related to a phenotype with symptoms consistent with autism spectrum disorder (ASD) and intellectual disability (ID). Their results provide evidence to support the hypothesis that ELS do indeed produce

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behavioral correlates of this phenotype, namely, deficits in socialization (ASD) and learning and memory (ID). Comparatively, no changes were detected in anxiety or repetitive behaviors. These experiments provide additional evidence for a causal relationship between ELS and ASD/ID. Importantly, this work demonstrates that the ELS–ASD/ID relationship extends to mice, as previously reported in rats (Bernard et al., 2013; Castelhano et al., 2013; Lippman-Bell et al., 2013; Moreira et al., 2011; Sayin et al., 2004; Talos et al., 2012; Waltereit et al., 2011). This cross species validation not only adds robustness to the theory that ELS may lead to ASD/ID, but also raises questions regarding other aspects of the ELS–ASD/ID phenotype and how, mechanistically, they are expressed in mice. Specifically, it is unclear if all models of ELS in both species lead to similar phenotypes by the same mechanism(s). However, the discovery of an ELS–ASD/ID relationship in mice will also allow future exploration of the effects of ELS on various genetically manipulated mice.

Early work: ELS are benign, or are they?

For decades it was thought that early life seizures in rodents were not harmful (i.e., while the seizures may be more severe, they do not have substantial long term consequences) compared to similar seizures in adults (Albala et al., 1984; Ben-Ari et al., 1984; Haas et al., 2001; Nitecka et al., 1984; Sperber et al., 1991, 1999; Stafstrom et al., 1992, 1993; Tremblay et al., 1984; Yang et al., 1998). This was likely due to the discrepancy between seizures in immature rats and those in adult rats. Seizures, specifically limbic seizures, in adult rodents result in widespread hippocampal cell loss, axonal reorganization and pronounced behavioral deficits (Ben-Ari, 1985; Ben-Ari and Represa, 1990; Ben-Ari et al., 1986; Stafstrom et al., 1992). Gross morphological changes were not observed following ELS, making them appear comparatively benign (Nitecka et al., 1984), although some cognitive sequelae were reported following many (Burke, 2003) ELS (Holmes and Ben-Ari, 1998). Thus the potential causal relationship between ELS and any phenotype did not receive significant attention until recently. This has led to debate in the literature (Holmes, 1997; Holmes et al., 1998) as clinically, it was identified several decades ago that there is a relationship between ELS and ASD/ID. More recently, a debate about cause versus correlation has arisen in the experimental literature with the backdrop of a proposed correlative relationship between ELS and ASD/ID that had been identified many decades ago (Brooks-Kayal, 2010; Creak and Pampiglione, 1969; Schain and Yannet, 1960).

Approximately 3/1000 infants suffer from neonatal seizures as a result of a variety of insults including stroke and hypoxia. Many develop learning disabilities thought to be mediated in part (Rennie and Boylan, 2003) or even worsened (Ballantyne et al., 2008; van Rooij et al., 2007) by the seizures themselves (Glass et al., 2009). Severe neonatal seizures, or status epilepticus (SE), both correlate (McBride et al., 2000) and have been independently associated with an adverse developmental outcome (Miller et al., 2002; Ortibus et al., 1996). While this may be a proxy for other brain injuries, the odds-ratio of autism is 3-fold higher in pre-term infants with seizures (Buchmayer et al., 2009). In some epileptic syndromes, the impact on development can be catastrophic such that regression frequently occurs (Arzimanoglou et al., 2004); children who have ELS in the first year of life are more likely to develop ASD (Saemundsen et al., 2007a). Delay in treatment of typically benign epileptic syndromes is associated with learning disabilities (Aldenkamp et al., 1999; Croona et al.,

1999; Metz-Lutz et al., 1999; Vanderlinden and Lagae, 2004). Overall, it is thought that a majority of children with epilepsy may have some degree of learning difficulty including ID (reviewed in Arzimanoglou et al., 2004). The additional cost of educating learning disabled children can be 2–5 times that of their peers (Burke, 2003). However, the question of causation between ELS and ASD/ID remains unresolved (Berg and Plioplys, 2012). Lack of effective treatments of early life seizures has prevented these correlational clinical observations from moving forward.

Given the clinical evidence that there is a relationship between ELS and ASD/ID, researchers have begun to investigate this issue in animal models. While initial studies using acute seizures reported minimal long term consequences (such as morphological damage), later studies using multiple or more severe seizures demonstrated anatomical changes (Holmes et al., 1998) often correlating with abnormal behavioral (de Rogalski Landrot et al., 2001) and altered seizure threshold (Lynch et al., 2000). More recent studies using single, mild seizures demonstrate long term behavioral and physiological changes (Bernard et al., 2013, 2014; Cornejo et al., 2007, 2008; Lynch et al., 2000; Sayin et al., 2004) without anatomical abnormalities. Rodent models of ELS are reviewed to explore their construct validity (i.e., relationship of triggering mechanisms to human disease) and then examined for evidence of the development of the ASD/ID behavioral phenotype following ELS. These findings are discussed with respect to several categories of behavior that are related to the core features of ASD (face validity, i.e., relationships to human phenotypes): repetitive behaviors, social interaction and communication. While changes in learning and memory and anxiety are not viewed as the core features of ASD, ID, anxiety and other co-morbidities typically cluster with ASD, especially when clinically linked with epilepsy (Berg and Plioplys, 2012). Thus, it is important to discuss these linked behaviors as well. Understanding the mechanisms entertained from recent studies will inform the predictive ability of these models to translate into human studies.

Models of ELS

Rodent models used to mimic ELS vary substantially (Stafstrom, 2002). This is advantageous as clinically ELS are also heterogeneous, and multiple models may ultimately be necessary to properly model the entire spectrum of ELS. Variety in ELS models also tests the robustness of findings. However this variety can be problematic, as comparisons among various models may reveal specific differences that may be model specific resulting in a potential loss of impact. In fact, differences among various ELS models should be anticipated as many aspects of these models (induction, species and strain) differ and heterogeneous outcomes are observed following clinical ELS. Understanding the differences among rodent models will yield insight into long-term outcomes following clinical ELS, including predicting outcomes based on the exact nature (cause, developmental stage and duration) of the seizure. Some ELS models induce multiple seizures versus single, acute seizures. Generally, morphological damage is more pronounced in the multiple seizure models (Holmes et al., 1998); however not all models are assessed for morphological changes with the same rigor. Post-natal days (P)7–10 (range depending on species) in rats is roughly equivalent to the neonatal period in humans (Dobbing and Sands, 1979; Talos et al., 2006); therefore most models of ELS induce seizures on or around this developmental time

point. We reviewed common methods that have explored behavioral outcomes later in development.

Kainate

Kainate (KA), a fixed glutamate analog, simulates clinical conditions resulting in glutamatergic over-excitation, as may occur in hypoxia/ischemia or other metabolic or genetic derangements (Traynelis et al., 2010). Most commonly ELS are induced using a single subcutaneous injection of KA (2 mg/kg) at P7 in Sprague-Dawley (SD) rats (KA-P7), equivalent to human neonatal seizures clinically (Arzimanoglou et al., 2004), biochemically (Talos et al., 2006) and electro-encephalographically (Dzhala et al., 2005). Studies have not reported any evidence of appreciable hippocampal cell loss due to ELS induced by KA (Lynch et al., 2000; Nitecka et al., 1984; Wirrell et al., 2001). KA activation (measured using the 2-deoxyglucose autoradiographic method) of the brain at P7 is limited to the hippocampus and lateral septum (Tremblay et al., 1984). Tonic/clonic seizures are reliably induced in nearly all animals, behavioral seizure activity is limited to 2–3 h and animal loss is less than 3%. Morphological changes (cell loss, axonal sprouting) are not detected in this model and spontaneous recurrent seizures (SRS) do not occur (Bernard et al., 2013; Cornejo et al., 2007; Stafstrom et al., 1992, 1993; Yang et al., 1998). Lower doses (1 mg/kg) produce similar results in Wistar rats (Moreira et al., 2011). KA induced seizures on P1, P7, P14 or P21 in SD rats did not cause overt histological damage; however later in life, ELS animals developed generalized kindled seizures at a significantly slower rate than age matched controls (Lynch et al., 2000; Sayin et al., 2004). One KA seizure per day on P16–20 or P20–24 was not associated with cell loss (Sarkisian et al., 1997; Tandon et al., 2002). Others have reported different mortality rates after KA seizures at P7 (3 mg/kg, 17% mortality); surviving animals received a second KA seizure at P14 (4 mg/kg, no mortality) (Waltereit et al., 2011).

Flurothyl

Flurothyl (FL), an inhaled GABA receptor antagonist (Alder, 1975; Eger et al., 2002; Gerstin et al., 2003), is used to address clinical scenarios where an excitatory/inhibitory imbalance may occur. This model involves inducing several brief ELS per day for several days during development. The number of ELS can vary greatly between studies, some inducing as few as 6 seizures in total (Bo et al., 2004) and others more than 100 (Karnam et al., 2009b). Post-natal day of ELS induction also varies between models. Lugo et al. used 3 daily FL seizures on P7–11 in C57BL/6 mice. Morphological changes, mortality and SRS were not assessed, thus it is not clear how closely it may resemble rat models. In SD rats, 7–8 daily FL induced seizures on P1–10 (75 total seizures) did not result in SRS (Kleen et al., 2011). 100+ seizures in SD rats during the first two weeks of life resulted in 22% mortality, but no hippocampal cell loss or behavioral SRS (no EEG confirmation) were observed (Karnam et al., 2009b). No cell loss occurred following 50 seizures on P0–10 or P15–25 (Karnam et al., 2009a). However, 55 FL seizures in SD rats during the first 12 days of life caused aberrant mossy fiber sprouting (MFS) in CA3 but not in the dentate gyrus (DG) (de Rogalski Landrot et al., 2001). Multiple FL ELS also reduced dendritic and spine complexity in CA1 (Casanova et al., 2014) and reduced neurogenesis (McCabe et al., 2001).

Daily single seizures on P6–12 (strain not indicated) have been employed to investigate later behavior with no mortality; morphology was not investigated (Bo et al., 2004).

Pilocarpine

Pilocarpine (PILO), a muscarinic acetylcholine receptor agonist, given intraperitoneally and sometimes used in conjunction with lithium, induces probably the most severe (continuous and prolonged) early life seizure. Like the FL models, variability in usage is observed with the PILO model. Dosing is reported to vary greatly, with some studies using as little as 60 mg/kg and others using as much as 380 mg/kg (dos Santos et al., 2000; Rutten et al., 2002). Depending on age at the time of administration, PILO ELS can incur significant mortality and substantial morphological damage (Cilio et al., 2003). PILO (380 mg/kg) induced ELS in P9 Wistar rats with high mortality (37%) (PILO-P9) (Castelhano et al., 2013). PILO (200 mg/kg) on P20 was reported to induce no morphological damage (Liu et al., 1994), while others report substantial cell loss and/or axonal reorganization and significant mortality (up to 28%) (Castelhano et al., 2013; Cilio et al., 2003; Rutten et al., 2002; Sankar et al., 1998, 2000). While SRS have not been reported, abnormal EEGs (frequent sharp waves and spikes) have been observed after lithium–PILO on P20 (Rutten et al., 2002). Following consecutive PILO (380 mg/kg) ELS on P7, P8, and P9 all rats demonstrated abnormal EEGs later in life, some with SRS but without detected cell loss, gliosis or MFS; mortality rate was not reported (dos Santos et al., 2000). Others have found cell loss without MFS following lithium–PILO ELS at P14 (Wu et al., 2001). This same dose on P20 (60 mg/kg) resulted in substantial morphological damage including MFS (Liu et al., 2003). Lithium–PILO ELS on P12 has resulted in SRS with abnormal EEGs (Kubova et al., 2004). In this study the authors also reported significant deficits in weight gain during development. Thus both SRS and deficits in weight gain could have each contributed to the behavioral deficits reported in various ELS models. These potentially confounding variables and the problems they produce highlight the importance of monitoring all aspects of development following ELS, including dam–pup interactions, as well as monitoring for SRS.

Hyperthermia model

The hyperthermia model (HT) induces a febrile seizure typically at P11 in Long–Evans (LE) or SD rats by increasing body and brain temperature using a warmed air stream (Baram et al., 1997; Dube et al., 2009a). Hyperthermia is maintained for 30 min, induces 20 min of seizures and can result in long term morphological changes, such as MFS in SD rats (Bender et al., 2003) or not (Chang et al., 2003) (strain not indicated). Initially it was believed that HT animals do not develop SRS (Dube et al., 2000); however prolonged monitoring has recently demonstrated that following prolonged febrile seizures, as many as 30% of HT animals develop brief SRS (Dube et al., 2009a, 2012).

Hypoxia

The global hypoxia (GH) ELS model is typically induced at P10 in LE rats (Jensen et al., 1991, 1992; Talos et al., 2012). While rat pups are exposed to graded global hypoxia for 15 min, 95% of pups experience tonic–clonic seizures, automatisms followed by head and limb movements, and myoclonic jerks; 75% have electrographic abnormalities that persist for 24 h, including seizures. Most (60%) rat pups have electrographic abnormalities, including

seizures 24–48 h following the initial hypoxic insult (Jensen et al., 1991). This model has later rare and brief SRS, MFS and CA1 cell loss (Lippman-Bell et al., 2013; Mikati et al., 2005; Rakhade et al., 2011). When only considering the seizures that occur after cessation of hypoxia as clinically relevant, this model has significant construct validity since hypoxia is the most common cause of seizures in the neonatal population. As with PILO, the occurrence of SRS, MFS and cell loss confounds the causal relationship of GH–ELS to later behavioral phenotypes.

Summary

We have reviewed the most common models; other models exist. Each seeks to address different questions. However, whether or not a model results in SRS, cell loss or synaptic reorganization is important as these can each impact long-term behavioral consequences. Unfortunately the assessments conducted on morphological changes and SRS vary between labs and across models. In adult models of SE, cell loss and synaptic reorganization correlate with abnormal behavioral outcome, while SRS may not (reviewed in Lenck-Santini, 2013). However, each phenomenon must be investigated as it becomes impossible to determine if behavioral consequences are a result of ELS, or if they are consequence(s) of the other phenomena.

Behavioral phenotypes following ELS

In the following sections we will review various behavioral perturbations following ELS. Unless otherwise stated, all seizures occur during development and testing occurs in the adult. While we attempt to draw general conclusions within and across models, it should be noted that much variability exists in the field (even within labs) with respect to the exact day of ELS(s) and/or the age at which behavior was assessed in the adult. It is likely that these variations have influenced the results.

Social behavior

Deficits in social communication and interaction are a core feature of autism (DSM-5) (American Psychiatric Association., 2014). The social approach task is the most commonly used test to assess social behavior in rodents. The task was developed for the mouse, but has been modified for use in the rat (Bambini-Junior et al., 2011; Bernard et al., 2013). In a three chambered testing apparatus, preference for a novel rodent versus a novel object is measured. This version is thought to identify abnormalities reflective of altered socialization (Yang et al., 2011) and is referred to as social approach (SA). Modifications of this task that demonstrate preferences for a novel rodent versus a familiar rodent may reflect abnormalities in social memory and not necessarily socialization (Yang et al., 2011). This is referred to as social novelty, social preference or social partition (SP). Nevertheless, abnormalities in distinguishing novel versus familiar have been seen in mice genetically altered with human mutations associated with autism (Lugo et al., 2014a; Spencer et al., 2005).

Deficits in SA and/or SP are reported in ELS models (Bernard et al., 2013; Castelhano et al., 2013; Lugo et al., 2014b; Talos et al., 2012; Waltereit et al., 2011). However interpretation

of SA results can often differ. A conservative interpretation limits “reduction in socialization” to no preference for the novel rodent as measured by time in the chamber housing the novel rodent (Yang et al., 2011). According to this strict interpretation, none of the ELS models display reduced SA (they all demonstrate preference for the novel rat chamber over the novel object chamber) (Bernard et al., 2013; Lugo et al., 2014b). Since rats are more socially complex (Baker, 2011; Chiappa et al., 1979; Dolgin, 2010; Iannaccone and Jacob, 2009; Jacob, 1999), other groups have used a more liberal interpretation of SA. For example following KA-P7, ELS rats spent significantly less time in the novel rat chamber compared to controls. However ELS rats still spent significantly more time in the novel rat chamber versus the novel object chamber (Bernard et al., 2013). It was concluded that while ELS rats still appeared to be social, they were less social than their control littermates; SP was not investigated (Bernard et al., 2013). This more liberal interpretation of differences in this task may more accurately reflect the wide range of severity of abnormal socialization in the clinical population. Assessment of performance in these tasks should take into account the fact that ASD and associated social deficits are not all or none. Degrees of socialization are present in the clinical population, which argues that these occur in rodent models as well.

Lugo et al. found no changes in SA (total time in chambers; their Fig. 2B), but do report that FL-ELS mice spend significantly less time sniffing the novel mouse versus the novel object while in those chambers (their Fig. 2CD). Further, they found reduced SP (their Fig. 1). Following GH–ELS (Lippman-Bell et al., 2013; Talos et al., 2012) there were no changes in SA, but reduced SP. It is unclear if and how SA and SP each relate to an autistic phenotype versus other aspects of learning and memory function.

Communication

Embedded within the DSM-5 criteria for ASD is the use of language and communication. This has not been explored in ELS models. To our knowledge, no published studies exist that have explicitly explored communication, though this is likely embedded within social behaviors. Communication has been extensively studied in mouse genetic models of autism (Crawley, 2012; Wohr, 2014; Wohr and Scattoni, 2013; Wohr and Schwarting, 2013) and this work has now begun in rat genetic models (Engineer et al., 2014a, b). Translation of these assays from the mouse to the rat is a priority as rats are the preferred rodent species to measure higher order cognitive tasks (Baker, 2011; Chiappa et al., 1979; Dolgin, 2010; Ianaccone and Jacob, 2009; Jacob, 1999).

Repetitive behavior

A core feature of autism is restricted or repetitive behavior, interests or activities (DSM-5) (American Psychiatric Association., 2014). Lugo et al. report no change in repetitive behaviors following FL-ELS in either the marble burying or hole poke tasks. However, increased grooming is reported in the PILO-P9 model (Castelhano et al., 2013) and we have found reduced marble burying following KA-P7 ELS (unpublished). Higher order or complex repetitive behaviors and compulsions (Lewis et al., 2007) have been rarely assessed due to their time intensive nature. In the FL P1–10 model, impaired behavioral flexibility

has been reported (Kleen et al., 2011), reminiscent of the behavioral inflexibility observed in clinical ASD.

Anxiety

Elevated anxiety is commonly associated with clinical ASD (Ozsivadjian and Knott, 2011; Simonoff et al., 2008). Many mouse models of autism display increased anxiety (Brodkin, 2007; Chahrour and Zoghbi, 2007; Kwon et al., 2006). In the KA-P7 ELS model, anxiety was not altered in the elevated plus maze (EPM) or open field test (OFT) (Cornejo et al., 2008; Stafstrom et al., 1993). Others also reported no change in OFT or EPM after KA-P7 (Cognato et al., 2010, 2011) or OFT after GH (Lippman-Bell et al., 2013; Mikati et al., 2005; Talos et al., 2012). Similarly Lugo et al. reported no change in anxiety in the FL P7-11 model, as measured using EPM; however hyperactivity was noted in the OFT. Other studies reported increased anxiety in multiple ELS models (KA, PILO), as measured using EPM (Castelhano et al., 2013; dos Santos et al., 2000; Moreira et al., 2011; Sayin et al., 2004).

Learning and memory

Deficits in learning and memory as a proxy for ID have been consistently analyzed following ELS. Outcome varies depending on the ELS protocol. Multiple brief seizures induced by FL during the first post-natal weeks are consistently reported to result in spatial learning deficits later in life in the Morris water maze (MWM), typically with acquisition (learning) (Bo et al., 2004; Chang et al., 2003; de Rogalski Landrot et al., 2001; Holmes et al., 1998; Huang et al., 1999; Karnam et al., 2009a, b; Lugo et al., 2014b; Mikati et al., 2005; Neill et al., 1996; Ni et al., 2012).

KA-P7 ELS resulted in deficits in acquisition of the MWM (Cornejo et al., 2008), but recall (memory) was intact. In another study, KA at P1, P7, P14 or P24 resulted in deficits in MWM acquisition and impaired recall (Sayin et al., 2004). However others did not find any deficits in the MWM (Stafstrom et al., 1993) even after multiple KA ELS (Sarkisian et al., 1997; Stafstrom et al., 1993; Tandon et al., 2002; Waltereit et al., 2011). When challenged with more difficult spatial memory tasks, impairments in hippocampal dependent long-term (Sayin et al., 2004) and short term (Cornejo et al., 2007) memory were detected with radial arm mazes. KA P7 ELS also impaired spatial learning in the Y maze but only in older (>P90) rats (Cognato et al., 2010, 2011). KA P7 ELS also impaired inhibitory avoidance (Moreira et al., 2011). KA ELS did not result in detectable reductions in object or place recognition memory or contextual fear conditioned memory after single (KA-P7) (Cornejo et al., 2008) or multiple KA ELS (Waltereit et al., 2011). Spatial learning deficits in the MWM are also reported following PILO ELS; these deficits have been correlated with changes in hippocampal place cell physiology (Liu et al., 2003; Rutten et al., 2002; Wu et al., 2001). Similarly, spatial learning deficits in the MWM have been correlated with changes in hippocampal place cell physiology following HT on P11 (Dube et al., 2009b). Spatial learning deficits are also reported in the MWM after GH on P10; however recall has not been probed (Mikati et al., 2005).

Molecular mechanisms

As noted, SRS and chronic morphological changes (primarily investigated in the hippocampus) following ELS can be seen in some models, generally in older pups at the time of ELS. Long term deficits could be solely or in part due to SRS, morphological changes or overt damage. However several models report long term behavioral deficits in the absence of SRS or morphological changes. Long term changes in synaptic plasticity that correlate with behavioral deficits have been found in the absence of morphological damage in the hippocampus (Bernard et al., 2013, 2014; Cornejo et al., 2007; Karnam et al., 2009a; Lynch et al., 2000) and cortex (Hernan et al., 2013; Isaeva et al., 2013). Reduced levels of hippocampal long term potentiation (LTP) (Chang et al., 2005; Cornejo et al., 2007; Feng et al., 2003; Karnam et al., 2009a; Lynch et al., 2000) and increased metabotropic glutamate receptor (mGluR) long term depression (LTD) (Bernard et al., 2013, 2014) are contrasted to increased cortical LTP (Isaeva et al., 2013). Increased mGluR LTD and related biochemical abnormalities correlated (Bernard et al., 2013, 2014) with those found in a well-established genetic model of ASD, the Fragile X Mental Retardation Protein (FMRP) knock-out (KO) mouse (Bear et al., 2004; Hou et al., 2006; Koekkoek et al., 2005; Volk et al., 2007) and provided mechanistic links between ELS and ASD/ID. Increased mGluR LTD in combination with decreased LTP underlying the social deficits in the acute KA ELS model is in agreement with the theory that autism is the result of aberrant synaptic homeostasis (Bourgeron, 2009; Kelleher and Bear, 2008). Consistent with abnormal synaptic homeostasis, overgrowth (in contrast to cell loss) of the medial prefrontal cortex has been found in association with ASD features after ELS (Kleen et al., 2011).

Many chronic molecular changes have been detected following ELS that correlated with ID/ASD phenotypes. These include alterations in GluA1 (Cornejo et al., 2007), GluA2 (Zhang et al., 2004), GluN2A (Chang et al., 2005; Cornejo et al., 2007; Swann et al., 2007), PSD95 (Cornejo et al., 2007; Swann et al., 2007), EAAC1 (Zhang et al., 2004), CREB (Chang et al., 2003), neuroligin 3, CaMKII and ERK (Bernard et al., 2013) to list a few. Altered HCN channel expression and function after HT ELS correlates with later SRS in this model (Dube et al., 2009a; Noam et al., 2011) but also could contribute to ASD/ID. Transcriptome profiling following ELS has also been pursued (Friedman et al., 2013; Theilhaber et al., 2013). It is likely that several of these alterations in cellular mechanisms contribute to the ASD/ID phenotype, while others are reactionary changes in response to ELS, SRS, ID and/or ASD and do not contribute to the phenotype. Too little has been discussed and explored regarding gender, especially since ASD primarily affects males (reviewed in Lintas and Persico, 2009). Most studies have focused primarily on males. Critically, females respond differently than males to ELS specifically regarding GABAergic reversal potentials (Galanopoulou, 2008) and this may be a factor that places males at greater risk of developing ASD (Tyzio et al., 2014). Determining which role(s) each molecular target plays is crucial for determining molecularly based therapeutics.

Commentary

While co-morbidities associated with epilepsy are widely acknowledged (reviewed in Brooks-Kayal et al., 2013), examination of the causal relationship between ELS and

ASD/ID is still in its infancy. Many questions about the current models remain. Ultimately, how will these findings translate to clinical situations to demonstrate predictive ability? A primary question concerns the robustness of the findings among various rodent strains and ELS models. For example, does ASD/ID triggered by either FL-ELS in C57BL/6 mice (Lugo et al., 2014b), FL-ELS in SD rats (Kleen et al., 2011), KA-P7 ELS in SD rats (Bernard et al., 2013) or GH in LE rats (Lippman-Bell et al., 2013) hold true for all rodent strains and models? What constitutes a complete ASD phenotype in the rodent (Yang et al., 2011)? Are the mechanisms of initiation (Rakhade et al., 2008, 2012; Zhou et al., 2011) and maintenance of ASD/ID similar? What is the role of SRS? Are certain strains genetically predisposed to be susceptible to long term consequences following ELS? A similar hypothesis could be proposed for humans as certain individuals may have no perceivable long term consequences following ELS, while others may be genetically predisposed to develop ASD following ELS. Only a portion of clinical ELS are associated with an autistic phenotype (Saemundsen et al., 2007a, b). Thus we should not expect all of our ELS models to develop a complete autistic phenotype, as this would contradict the clinical situation. Indeed, understanding why some ELS models develop ASD/ID, while others do not is another key to understanding the potential causal relationship between ELS and ASD/ID. Thus, the pursuit of “negative” data might actually be informative. The contribution of prior predisposing conditions to ASD cannot be excluded in the clinical setting and as such, animal studies may be important to dissociate predisposition to both seizures and ASD from causative effects of ELS to ASD.

Finding aspects of ASD/ID phenotypes in both mice and rats following different types of ELS demonstrates that these findings are robust and reproducible. However, completely defining each ELS phenotype requires that key gaps be addressed. There is an abundance of knowledge on long term learning and memory deficits following ELS, assessments of socialization and repetitive behaviors are emerging, while virtually no information exists on deficits in communication. Substantial unpublished evidence may exist demonstrating no changes in behavioral correlates of ASD/ID following ELS, emphasizing our previous point that negative data would be helpful to the wider research community in this field. Each model requires the tedious reexamination of cell loss, synaptic reorganization and SRS with continuous and prolonged video EEG. Further, synaptic correlates of behavior determined through measurements of synaptic function are also required. When possible, these abnormalities in synaptic function should be tied to signaling pathways that can be targeted to develop translational therapeutics that address long-term consequences. This has suggested that glutamate receptor antagonists acutely may prevent long-term consequences (Cha et al., 2002; Lippman-Bell et al., 2013; Mikati et al., 1999) while targeting mGluR-mediated signaling chronically may ameliorate behavioral abnormalities (Bernard et al., 2013, 2014). Targeting HCN channels may prevent SRS (Dube et al., 2009a; Noam et al., 2011) and may also affect the behavioral phenotype.

Lugo et al. point out that mice models are advantageous due to the availability of many genetic KO models with which to assess the impact of ELS on different genotypes. This is balanced by rats being the preferred species to model higher order cognitive and social tasks to avoid missed nuances in behavior (Baker, 2011; Chiappa et al., 1979; Dolgin, 2010; Iannacone and Jacob, 2009; Jacob, 1999). It is currently unclear if rat KO models may be

useful in this regard though they are undoubtedly more expensive. The role played by genetic differences in background strains must also be considered; significant differences exist between rodent strains in response to KA induced seizures with respect to seizure intensity, mortality and hippocampal pathology (McKhann et al., 2003; McLin and Steward, 2006; Schauwecker, 2011).

Lugo et al. raised additional points to benefit future ELS research. Prior work has predominantly focused on limbic regions, often solely the hippocampus. Occasionally the amygdala or other portions of the limbic system are assessed as well. However other brain regions, especially the cortex, may play a role (Hernan et al., 2013; Isaeva et al., 2013; Kleen et al., 2011), particularly in ASD phenotypes. Due to the lack of obvious morphological damage following ELS in many cases, identifying other important brain regions is a significant challenge.

As the causes of ELS, ID and ASD are each heterogeneous, a singular cause and effect may not apply to all cases of ELS-ASD/ID. Determining that a causative relationship between ELS-ASD/ID exists in a subset of the ASD population would be a significant impact for future therapies. Due to the heterogeneity of ASD/ID, it is likely that some forms of ASD/ID may have ELS that are causative or contributory, while other forms of ASD/ID have a history of ELS that are simply correlative. The precise developmental time point of the ELS, duration of the seizure, underlying cause and genetics of the subject may significantly impact the long term consequences of ELS. This stresses the importance of multiple animal models, as many different models may be required to accurately model all instances in which an ELS may result in ASD/ID. Not all models may demonstrate similar long term changes as not every ELS may be clinically linked to ASD/ID.

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Abbreviations

ASD	autism spectrum disorder
DG	dentate gyrus
ELS	early life seizures
EPM	elevated plus maze
FL	flurothyl
FMRP	fragile X mental retardation protein
GH	global hypoxia
HT	hyperthermia
ID	intellectual disability

KA	kainic acid
KO	knock out
LE	Long–Evans
LTD	long term depression
LTP	long term potentiation
MFS	mossy fiber sprouting
mGluR	metabotropic glutamate receptor
MWM	Morris water maze
OFT	open field test
PILO	pilocarpine
P	post-natal day
SA	social approach
SD	Sprague–Dawley
SE	status epilepticus
SP	social partition
SRS	spontaneous recurrent seizures

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