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Commentary

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.

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 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

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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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. Postnatal 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