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Commentary

Commentary on Hewapathirane et al. (*in vivo* imaging of seizure activity in a novel developmental seizure model) seizure-induced brain damage: From tadpoles to children

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Epilepsy, a condition characterized by repeated, unprovoked seizures, is unfortunately quite common, affecting approximately 1% of the population (Hauser, 1994), Epilepsy is most common in the first year of life and approximately 75% of epilepsy begins during childhood. The underlying mechanisms responsible for this increased excitability during this period of life are not completely understood but are clearly age-dependent. During the early postnatal period, at a time when the immature brain is highly susceptible to seizures (Jensen et al., 1992; Jensen and Baram, 2000; Khazipov et al., 2004), GABA exerts paradoxical excitatory action, indicating that in the young brain enhanced excitability is due to the excitatory rather than inhibitory actions of GABA (Dzhala and Staley, 2003; Khazipov et al., 2004). The lack of an efficient time-locked inhibition, the delayed maturation of postsynaptic GABA(B)-mediated currents and the high input resistance of small and densely packed neurons also facilitates the generation of action potentials and synchronized activities (Gaiarsa et al., 1995; McLean et al., 1996). As the brain matures, enhanced excitatory neurotransmission may play an additional role in the propensity for the immature brain to seize as there is an overabundance of excitatory receptors (AMPA and NMDA) (Insel et al., 1990; McDonald et al., 1990; Miller et al., 1990; Brennan et al., 1997). With further maturation, axonal collaterals and attendant synapses regress (Swann et al., 1990, 1991).

While seizures are common in children there is controversy regarding whether seizures result in neurological sequelae. It is known that neonatal seizures are often associated with long-term neurological consequences including post-neonatal epilepsy, behavioral problems, and mental retardation (Scher et al., 1989, 1993; Brunquell et al., 2002). While it is widely recognized that the etiology of the seizures is a primary determinant of outcome, there remains controversy regarding whether the occurrence of seizures themselves in the young children contribute to the poor outcome (Mizrahi and Clancy, 2000). This is a difficult problem to study in children because of the multitude of etiologies responsible for seizures and variations in seizure type, duration, and frequency. Because of the complexities in humans, investigators have tried to address the question of whether or not seizures cause brain damage through use of animal models (Holmes and Ben-Ari, 2001).

It is known that in the adult animal, prolonged seizures status epilepticus causes widespread neuronal cell loss, particularly in the hippocampus and entorhinal cortex (Nadler, 1981; Ben-Ari, 1985). In addition to cell death, prolonged seizures in the adult brain leads to synaptic reorganization with aberrant growth (sprouting) of granule cell axons (the so-called mossy fibers) in the supragranular zone of the fascia and infrapyramidale region of CA3 (Represa et al., 1987, 1994, Sutula et al., 1988). Young rodents are far less vulnerable to cell loss following a prolonged seizure than mature animals (Albala et al., 1984; Berger et al., 1984; Nitecka et al., 1984; Tremblay et al., 1984; Holmes et al., 1988; Sankar et al., 1998, 2000). However, one concern in extrapolating these results to the human is that the presence or absence of cell loss with seizures in the young brain could be species specific (Thompson and Wasterlain, 1997).

A major advance in the study of the effects of seizures in the developing brain is now provided by Hewapathirane et al. (2008) who studied seizures in the transparent albino *Xenopus laevis* tadpole. The tadpole has been widely used for *in vivo* imaging of neuronal growth and synaptogenesis and is an ideal model for imaging of populations of neurons within the intact developing brain. By providing paralytic agents and immersion of the tadpole in agar the investigators were able to perform extracellular electrophysiological recordings in the intact brain.

As with other species, the tadpole has behavioral seizures when administered chemoconvulsants (pentylenetetrazol, kainic acid, bicuculline, picrotoxin, 4-aminopyridine, and pilocarpine) in the swimming water, as evidenced by alterations in their swimming pattern. Electrophysiological recordings further showed that these convulsants resulted in clear ictal epileptiform discharges, confirming that the tadpoles were

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having seizures. The work in tadpoles is reminiscent of similar studies in zebrafish larvae (*Danio rerio*) in which pentylenetetrazol induces a stereotyped and concentration-dependent sequence of behavioral changes culminating in clonus-like convulsions and ictal and interictal-like electrographic discharges (Baraban et al., 2005).

In the tadpoles fluorescence imaging of neuronal calcium dynamics during ictal events showed repetitive waves of abnormal, synchronous, high amplitude calcium spikes which progressed across the tectum, moving in a rostral-to-caudal direction. The number of neurons recruited into these synchronized waves of activity increased with duration of PTZ exposure. Imaging intracellular calcium fluxes is a powerful technique. Rapid and transient elevations of calcium within cellular microdomains have an important role in the regulation of many signal transduction pathways. There is also emerging evidence that recurrent mitochondrial load of calcium ions during seizures contributes to the pathophysiology of epilepsy (Kovacs et al., 2005). Fluorescence imaging could also provide a powerful tool in understanding the dynamics on neuronal synchronization during seizures (Timofeev et al., 2004).

The tadpole model had many features of rodent and primate seizure models: i) ictal behavioral and electroencephalographic activity occur with a number of chemoconvulsants; ii) a dose response to the seizure-inducing agents with more severe behavioral changes with increasing dose of the chemoconvulsants iii) elimination of the seizures with antiepileptic drugs; and iv) propagation of calcium spikes reflecting depolarization that spreads in neuronal structures during the seizure. The potential for this model is substantial since it allows exquisite visual examination of seizure generation and spread at a macroscopic level *in vivo*. The model also appears ideal for studying mechanisms of synchrony of epileptiform discharges. Finally, it would appear to be a useful model for rapid throughput of putative antiepileptic drugs.

The tadpole model described by Hewapathirane et al. (2008) provides further proof that organisms not normally considered for epilepsy research can provide valuable insights into the pathophysiological processes responsible for the seizures. As reviewed by Baraban (2007), invertebrates such as nematodes (*Caenorhabditis elegance*) (Locke et al., 2006) and fruit flies (*Drosophila melanogaster*) and simple invertebrates, such as zebrafish (Baraban et al., 2005), have been widely evaluated in neurobiology. The homologs of many human genetic disease loci show selective expression in the tissues of these invertebrates and simple vertebrates that are analogous to the affected human tissues, providing a useful filter for potential candidate genes (Chintapalli et al., 2007). In addition to their value in genetic studies, these simple models can be quite useful in studying pharmacology and electrophysiology. Furthermore, these organisms are inexpensive and easy to maintain, making them ideal for high-throughput studies.

A key feature of the Hewapathirane et al. (2008) study was the observation that that seizures in the tadpoles which typically began within 10–20 minutes of drug exposure and lasted for several hours resulted in no substantial cell death, using cellular propidiumiodide incorporation *in vivo* and or TUNEL labeling techniques. Whether prolonged seizures in the adult frog would have had a different effect was not addressed in this study. Presumably, prolonged seizures in frogs, as in many other species, would lead to cell death.

The lack of cell loss, despite long seizures, is quite consistent with a vast amount of data from immature rats (reviewed in Holmes and Ben-Ari, 2001; Haut et al., 2004; Holmes, 2005; Ben-Ari and Holmes, 2006). The relative resistance of the immature brain to seizure-induced damage is paralleled by resistance to other insults. Immature hippocampal neurons are also less sensitive to anoxic insults responding to synaptic stimuli in a fully anoxic environment for longer durations than adult ones (Cherubini et al., 1989). The immature brain appears to be less vulnerable to the toxic effects of glutamate than the mature brain likely due the lower amount of Ca^{2+} entry into immature brain cells during seizures (Bickler et al., 1993; Marks et al., 1996; Liu et al., 1996). Additional reasons for reduced cell death in young animals is a smaller density of active synapses, lower energy consumption, and in general, the relative immaturity of biochemical cascades that lead to cell death following insults. Additional "protective" factors likely include high levels of brain-derived neurotrophic factor in the newborn brain which have been found to be neuroprotective (Tandon et al., 1999), reduced proinflammatory cytokines associated with seizures in young rats (Rizzi et al., 2003), and better maintenance of GABA synthesis in the face of seizures in the immature rather than the mature brain (Sankar et al., 1997). Neonatal seizures are also associated with lower oxcidative stress than adult seizures (Patel and Li, 2003). High levels of the mitochondrial uncoupling protein (UCP2) which are basally increased in neonatal brain by the fat-rich diet of maternal milk appear to reduce the formation of reactive oxygen species and protect the mitochondrial from seizure-induced damage (Sullivan et al., 2003).

This is not to say that seizures in the immature brain are totally harmless. There is evidence that seizures in the developing animal could result in neurological sequelae through means other than cell loss (Raol et al., 2003). During the construction of developing cortical networks there is a sequential shift from an ensemble of immature cells with little or no organized communication devices to an active network composed of neurons with thousands of active synapses. This shift is mediated by a series of sequences that includes intrinsic programs and extrinsic factors. Seizures, through activity-dependent mechanisms, likely modify these sequences leading to persistent deleterious sequelae (Ben-Ari and Holmes, 2006).

A variety of changes in receptors and transporters have been found after seizures in young rats (Zhang et al., 2004a,b; Cornejo et al., 2007). For example, in young rats GluR2 expression and protein levels are decreased in the dentate gyrus following lithium/pilocarpine induced seizures (Zhang et al., 2004b) and in the neocortex and hippocampus following hypoxia-induced seizures (Sanchez et al., 2001). The excitatory amino acid carrier 1 (EAAC1) protein is increased in the dentate gyrus following seizures (Zhang et al., 2004b). Seizures in young rats result in increases in the α1 subunit expression of the GABA(A) receptor (Zhang et al., 2004a). Interestingly, these changes in the α1 subunit were opposite to those seen in adult rats subjected to SE (Brooks-Kayal et al., 1999;Coulter, 1999). Recurrent seizures in rat pups have also be shown induces a decrease in the total amount of NR2A and an increase in the primary sub-synaptic scaffold, PSD-95, causing impaired CA1 hippocampal long-term potentiation, enhanced long-term depression, and impaired learning in a hippocampal-dependent radial arm water maze task without inducing mossy fiber sprouting or altering dendritic spine density (Cornejo et al., 2007).

The tadpole model used by Hewapathirane et al. (2008) provides exciting opportunities for furthering our understanding of the pathophysiological mechanisms responsible for seizures. Combining electrophysiology, behavioral, and dynamic calcium imaging provides a powerful technique for increasing our understanding of how paroxysmal excitatory begin, spread, and end in the developing brain. In addition, questions about the sequelae of seizures can be addressed in this model. How is connectivity of neuronal networks modified by recurrent seizures? If seizures induce aberrant connectivity can it be aborted or modified with therapy? While there are questions about the consequences of seizures that need to be answered, the tadpole model provides the means the move the field forward.

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