



DOES ONE NEONATAL SEIZURE ALTER SYNAPTIC PLASTICITY AND CAUSE LIFELONG COGNITIVE IMPAIRMENT?

A Single Episode of Neonatal Seizures Permanently Alters Glutamatergic Synapses. Cornejo BJ, Mesches MH, Coultrap S, Browning MD, Benke TA. *Ann Neurol* 2007;61(5):411–426. **OBJECTIVE:** The contribution of seizures to cognitive changes remains controversial. We tested the hypothesis that a single episode of neonatal seizures (sNS) on rat postnatal day (P) 7 permanently impairs hippocampal-dependent function in mature (P60) rats because of long-lasting changes at the synaptic level. **METHODS:** sNS was induced with subcutaneously injected kainate on P7. Learning, memory, mossy fiber sprouting, spine density, hippocampal synaptic plasticity, and glutamate receptor expression and subcellular distribution were measured at P60. **RESULTS:** sNS selectively impaired working memory in a hippocampal-dependent radial arm water-maze task without inducing mossy fiber sprouting or altering spine density. sNS impaired CA1 hippocampal long-term potentiation and enhanced long-term depression. Subcellular fractionation and cross-linking, used to determine whether glutamate receptor trafficking underlies the alterations of memory and synaptic plasticity, demonstrated that sNS induced a selective reduction in the membrane pool of glutamate receptor 1 subunits. sNS induced a decrease in the total amount of *N*-methyl-D-aspartate receptor 2A and an increase in the primary subsynaptic scaffold, PSD-95. **INTERPRETATION:** These molecular consequences are consistent with the alterations in plasticity and memory caused by sNS at the synaptic level. Our data demonstrate the cognitive impact of sNS and associate memory deficits with specific alterations in glutamatergic synaptic function.

COMMENTARY

There is a burgeoning literature on the effects of seizures, incurred at various ages, on subsequent cognitive and behavioral function (1). The goal of these studies is to understand the mechanisms by which seizure-induced cognitive damage occurs and to prevent such sequelae. Since clinical studies cannot unequivocally separate the effects of seizure type, duration, frequency, and etiology on outcome, researchers have turned to animal models, in which many of those variables can be controlled. The notion that seizures early in life cause minimal cognitive deficits is undergoing revision (2,3). It is now recognized that a seizure at any age can exert an adverse effect on subsequent cognition and behavior, across models and seizure induction techniques. Adverse effects are not limited to prolonged seizures; brief, recurrent seizures also carry an increased risk of later cognitive sequelae (4). Current consensus holds that early life seizures disrupt one or more ongoing developmental processes, resulting in long-term detriment (5).

So far, studies have been largely descriptive. A perplexing question has been the mechanism(s) by which early seizures wreak their havoc. Seizure-induced cell death does not seem

to play a major role in the neonatal period. Reorganization of axonal connections (sprouting) occurs in the developing brain, albeit in a different location and pattern than in the mature brain, and is not due to the same initiating mechanism (i.e., neuronal death). Neurogenesis occurs in an age-dependent fashion, but again, the pattern is different than that seen in adult brains after seizures. Alteration of neurotransmitter receptors (e.g., density, stoichiometry, subtypes, sensitivity) likely plays a role in seizure-induced deficits, with both excitatory and inhibitory systems implicated (6,7).

Using correlative physiological, behavioral, cognitive, and molecular techniques, Cornejo et al. describe an informative set of experiments designed to elucidate the mechanism by which neonatal seizures lead to cognitive impairment. Their overall hypothesis was that neonatal seizure-induced alterations in learning and memory are related to altered synaptic plasticity. Each individual seizure was brief (less than 10 minutes), but seizures recurred over periods of up to 3 hours, similar to the pattern seen in many human newborns. When these rats reached adulthood, visuospatial learning and memory were tested using the standard Morris water maze and the 4-trial radial arm water maze, a more challenging test of memory. The authors found that adult rats that had experienced a single episode of seizure in the neonatal period differed from control rats (with no neonatal seizures) in subtle but important ways. Both groups mastered the two mazes similarly, showing that the neonatal

seizure did not impact visuospatial learning, except that the rats with neonatal seizures had more errors on the first day of the 4-trial radial arm water maze test, suggesting a possible subtle learning deficit.

The authors then probed cognitive abilities of the two groups in further detail, using a variation called the 2-trial radial arm water maze, in which rats learn the location of a hidden platform on one trial and then are tested to see if they remember its location 4 hours later in a single additional trial; the test measures episodic-like memory for a single event (8). In this test, rats with neonatal seizures had significantly more errors in finding the platform (showing defective episodic-like memory); they also took longer to find the platform and more often re-entered an incorrect arm (both measuring working memory). The functions assessed by the 2-trial radial arm water maze are highly dependent upon hippocampal NMDA receptors. Therefore, a single episode of neonatal seizure was sufficient to demonstrate robust, lifelong deficiencies in episodic and working memory. The authors found no mossy fiber sprouting or alterations in dendritic spine density or branching to account for the cognitive impairment.

Because of the involvement of glutamate receptors (particularly NMDA subtype) in learning, memory, and synaptic plasticity, the authors then performed extracellular electrophysiology experiments on hippocampal slices using field potential analysis, long-term potentiation (LTP), and long-term depression (LTD). There was no seizure-induced change in paired-pulse facilitation (ruling out a presynaptic effect) or in overall network excitability. However, LTP was decreased and LTD was enhanced after neonatal seizures. Maintenance of the ability to learn visuospatial information, but defective episodic and working memory function, led the authors to hypothesize that differences in glutamate receptor number, type, or distribution could explain the altered neuroplasticity. Hippocampi were fractionated into subregions and subjected to western blot and cross-linking analysis, to examine glutamate receptor expression and distribution. By both methods, intracellular levels of the AMPA receptor subtype GluR1 were increased significantly, while there were no differences in the expression of GluR2/3 or in NMDA receptor subtype 1 (NR1), and the total amount of NR2A was decreased. The increased intracellular GluR1 related to trafficking of the receptor from the membrane pool. An unexpected finding was that PSD-95, a scaffolding protein that interacts with glutamate receptors, was markedly increased after neonatal seizures. Changes in levels of scaffolding proteins may be emerging as a critical factor after seizures though the direction of the change may vary (9). The model used by Cornejo et al.—and expanded upon in an accompanying editorial (10)—proposes how specific receptor alterations could explain the plasticity results.

The permanent nature of all these changes after a single neonatal seizure raises legitimate concern about early seizure effects and their prevention. It is uncertain whether the data described here can be translated into the clinical realm. The experiments of Cornejo et al. are unique in that they present a comprehensive approach, using behavioral, molecular, and electrophysiological techniques, to address the mechanism of cognitive impairment from early life seizures. Methodologically, it would be reassuring to know if the seizures in all rats were similar in frequency, duration, and severity; this information would require electrographic monitoring during kainate treatment, which previously has been demonstrated (11). With such robust effects as a consequence of a single neonatal seizure, it would also be important to know if similar changes occur after multiple seizure episodes or after status epilepticus. The occurrence of spontaneous seizures could influence behavioral performance and plasticity, and these were not reported. If the mechanism proposed here is universal, the results should be verifiable at other ages and in female rats as well. It is also known that GABA_A receptors are chronically altered after neonatal seizures (7) and that altered inhibition has been associated with seizure-induced synaptic plasticity at young ages (12). At the age tested here (P7), GABA is still excitatory (11) and seizures at this age can prevent the transition of GABA's action from excitatory to inhibitory (5,13). These concerns notwithstanding, the authors provide a consistent and exciting model that is one of the first comprehensive explanations for cognitive impairment from early life seizures. There now are additional targets (i.e., glutamate receptor redistribution and trafficking) for further experimental study and eventually, for innovative therapeutic interventions.

by Carl E. Stafstrom MD, PhD

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