



“ONE SWALLOW DOES NOT MAKE A SUMMER” . . . OR DOES IT?

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

The quotation provided in the title is by Aristotle (384 BC–322 BC); in full, it reads: “One swallow does not make a summer, neither does one fine day; similarly one day or brief time of happiness does not make a person entirely happy.” The part of the statement regarding happiness is arguable, however if in the first part of the quote, “seizure” is substituted for “swallow” and “epilepsy” for “a summer,” one immediately recognizes an old conundrum of both clinical and basic epilepsy research. Much has been written about the “seizures beget seizures” aspect of epileptogenesis, and at last check, the controversies still abound (1). However, there is another, albeit less frequently addressed, potentially damaging outcome to the brain caused by a single seizure, which is a change in the brain’s capacity for plasticity at a later time, particularly if the seizure occurs early in development when the brain can best be described as a “plasticity machine” (2). This “metaplasticity” (3), caused by the lone seizure during a critical developmental period, may underlie deficiencies in cognitive function later on in life—which is where the paper by Cornejo et al. weighs in.

In order to induce a single episode of neonatal seizures, the authors subcutaneously administered kainic acid (1–2 mg/kg) to postnatal day 7 (P7) rat pups, a treatment that resulted in

“discontinuous behavioral and electrical seizure activity lasting up to 3 hours.” It is important to consider whether this type of activity is indeed a “single” seizure or whether it is full-blown status epilepticus. Based on the relatively low (3%) mortality rate in their model and the relatively short duration of ictal bursts (<10 min), the authors argue that the term status epilepticus does not apply to their model. However, in the absence of electrical recordings during the seizures, the authors’ claim remains largely unsubstantiated. It is not easy to define status epilepticus in neonates, however “half of the time spent in seizure” is one of the accepted criteria (4,5). In the present study, ictal events of less than 10 min were separated by interictal periods of 5–10 min. Using an average of 7.5 min for each the ictal and interictal periods, during the approximately 3-h period, the total number of ictal events must have been at least 12, equaling about 50% of the total time spent in seizure activity. Considering that in human neonates seizures can go on without any overt physical signs (4,5), behavioral observation alone may not be sufficient to document seizure activity in a neonatal seizure model. The absence of electrical recordings from the brains of the treated animals is one omission in the study. It also would have been extremely useful to know what type of electrical activity was present in the brains of the experimental subjects during the 2–3 months following the neonatal seizures, when the animals were tested in various memory tasks.

Interestingly, the neonatal seizure group performed quite well on water maze memory tasks. Compared with controls,

there were no differences in their performance on the Morris water maze (MWM), a test of spatial memory. To address subtle changes in episodic-like memory deficits, which can still be present in spite of a normal performance on the MWM, the 2-trial radial arm water maze (RAWM) and the 4-trial RAWM were administered successively to the control and experimental groups. These tests were able to distinguish slight differences between the two groups. On the 2-trial RAWM, the neonatal seizure group performed marginally worse than the controls on the second trial, carried out 4 h after the first. On the 4-trial RAWM, there were slightly more errors produced by the neonatal seizure group than the controls on the first trial, but these animals' learning curve on subsequent trials was indistinguishable from controls. Unfortunately, because of the lack of recordings during seizure induction, no correlations can be drawn between the severity of the neonatal seizures and the amount of memory deficits found in the experimental group. A recent longitudinal study in humans emphasizes the lack of correlation between benign partial epilepsy in infancy and cognitive deficits later on in life (6). Only 4 of 39 children had mild cognitive deficits when assessed about 10 years after the seizures (1 of the 4 patients also was diagnosed with tuberous sclerosis and another with Asperger syndrome).

What is surprising in the Cornejo et al. study is the number of electrophysiological and biochemical alterations found in the neonatal seizure group after more than 2 months. In spite of a normal spine density on hippocampal CA1 pyramidal cells, unaltered presynaptic facilitation of glutamate release, and stable synaptic input–output function, long-term potentiation (induced by a 100 Hz train delivered for 1 s) was depressed and long-term depression (induced by 900 paired pulses delivered at 1 Hz at 50-millisecond intervals) was enhanced at P60 in animals that experienced seizures on P7. Large changes in the neonatal seizure group were demonstrated by biochemical studies. A significant, 52% increase was seen in the intracellular pool of the GluR1 subunit of the AMPA receptors; however, this pool constitutes only a small (5–10%) fraction of the total GluR1 subunits, which are mostly located on the plasma membrane. Since the total amount of GluR1 did not change, the membrane fraction must have been reduced by only about 5% in the neonatal seizure group, which is probably why no changes were detected in the synaptic events in electrophysiological studies. Of the NMDA receptor subunits, only the NR2A showed a decrease (28%) in the membrane fraction of the experimental group—a finding that might explain the reduced long-term potentiation found in the animals that experienced neonatal seizures. The scaffolding protein PSD-95 (located at the postsynaptic side of excitatory synapses and known to interact with several receptors, protein kinases, and other scaffold proteins)

displayed the largest change among the postsynaptic proteins examined (a 43% increase in the neonatal seizure group). The authors interpret this finding as an increased amount of PSD-95 per synapse, arguing that the number of spines was unaltered. However, their interpretation may not necessarily be the case, as PSD-95 also is abundant at nonspiny synapses, for example, on inhibitory interneurons. An increased excitatory drive onto GABAergic cells secondary to enhanced levels of PSD-95 at excitatory synapses on interneurons may explain the increased paired-pulse inhibition found in a previous extensive study of cognitive, cellular, and synaptic effects of early life seizures (7). Although not considered in the Cornejo et al. study, inhibitory synaptic plasticity may have been altered in more than one way. At P7 in rats, most GABAergic activity is excitatory, because the combined reversal potential for $\text{Cl}^-/\text{HCO}_3^-$ is still depolarizing compared to action potential threshold, which is unlike in human newborns (8). Thus, the model of seizures in P7 rat pups may not correspond particularly well to human and primate neonates, who are born with a hyperpolarizing GABA reversal potential (2,9).

Cornejo et al. did not examine the mechanisms leading to the described alterations or provide any evidence of whether the behavioral, biochemical, and electrophysiological changes following the neonatal seizures are reactive or proactive. Do these changes occur to dampen excitability in order to prevent further seizures or are they simply necessary consequences of a temporary perturbation of excitability in an immature brain? Clearly, many more studies, using both animal models as well as humans, are needed to answer these questions, and to unequivocally determine whether a single swallow can indeed make summer (2). After all, Aristotle was also fond of saying: “It is the mark of an educated mind to be able to entertain a thought without accepting it.”

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