Invulnerability of the Immature Brain to Seizures: Do Dogmas Have Nine Lives?

Effects of Pentylenetetrazole-Induced Status Epilepticus on Behavior, Emotional Memory, and Learning in Immature Rats

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Status epilepticus (SE) can be harmful to the developing brain. Our knowledge of the emotional and behavioral consequences of generalized SE in developing animals remains limited. Therefore, we investigated the short- and long-term effects of pentylenetetrazole (PTZ)-induced SE on emotional memory and learning and behavioral parameters in immature rats. SE was induced in 16- to 20-day-old rats (P16–P20) using intraperitoneal injections of PTZ ($n = 21$); control rats received saline ($n = 10$). All animals were tested using an elevated T- maze and open-field test 2, 14, 30, and 180 days after SE, to evaluate emotional memory and learning and behavior. Anxiety levels decreased 2 and 14 days after SE, and conditioned learning of PTZ-treated immature rats was better than that of the control rats. These results indicate that a decreased anxiety level facilitates conditioned learning. Behavioral changes are transient, and no emotional memory or learning deficits occur following PTZ-induced SE in immature rats.

COMMENTARY

Like cats, some dogmas seem to have nine lives: as soon as they appear to be losing ground, fresh support for the theory pops up and controversy starts anew. Thus, theories concerning the invulnerability to seizure of the immature brain have been presented, dismissed, and then resurrected. Initially, brain damage associated with seizures was blamed on the cause or the complications of the seizure activity rather than on the seizures themselves. Following studies by Meldrum et al., brain damage was accepted as a direct result of seizure activity in the adult but not in the developing brain (1). Then, evidence appeared that many (but not all) seizure types in the young, if sufficiently severe and prolonged, can produce brain damage, albeit more slowly than in the adult. The studies that found seizure-induced disturbances of brain and behavioral development (2,3) and seizure-induced neuronal injury (4–7) in the immature brain, have received additional support lately (8–14). Now, an interesting new publication is raising questions about the short-lived consensus that seizure activity can produce brain damage in the immature brain.

Erdogan and collaborators subjected rat pups at postnatal day 16–20 (P16–P20) to pentylenetetrazol (PTZ)-induced status epilepticus (SE), which they regard as a model of primarily generalized SE, and studied their behavior after they reached adulthood. They found only transient changes in the open-field test and no learning deficits in the elevated T-maze assessment. The investigators concluded that this seizure type produces no "emotional memory" or learning deficits in immature rats.

Does this study revive the old theory that the immature brain is invulnerable to SE-induced damage and that seizures, even as severe as SE, are benign for the developing brain? Or, does the study simply suggest that the invulnerability is limited to generalized SE but not applicable to focal-onset SE with secondary generalization? Why would generalized SE have different outcomes depending on the site of origin of the seizures? Do these findings portend another decade of controversy on this emotionally charged topic? How does this new evidence measure up against elegant studies showing, for example, that after lithium-pilocarpine–induced SE at P20, hippocampal place cells show permanent functional deficits and spatial memory is impaired (15)?

Age seems unlikely to be the key factor here. At the ages of P12–P20, many studies describe behavioral deficits and neuronal loss after SE. Rat pups (P1–P14) subjected to kainate-induced seizures had long-term impairment in the radial arm maze performance, which is a hippocampus-dependent spatial memory task (10). Rats receiving kainate at P10 were also found to have impairment in righting responses and a prolonged reaction time in an active avoidance task when studied during adolescence (16). Lithium–pilocarpine SE at P12 leads to impaired memory and emotional behavior 3 months later (11).

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Lithium–pilocarpine SE at P16–P20, but not at P12, resulted in impaired Morris water maze performance in adulthood (13). Multiple episodes of pilocarpine SE at P7—P9 were associated with severe cognitive deficits in adulthood (12). Other studies found no deficit in Morris water maze, open-field, or handling tests below age P20 (17). Many recent reports spanning ages P12–P20 found SE-associated neuronal death. Lithium–pilocarpine SE at P12 caused thalamic damage (9). Lithium–pilocarpine SE at ages P15 and beyond caused hippocampal and extrahippocampal neuronal loss in a pattern that was highly species- and age-dependent (4,5,6,7). Thus, it seems likely that some (but not all) types of SE can induce behavioral deficits and neuronal loss in P16–P20 rat pups.

Seizure type seems a more likely explanation than age for the findings in the Erdogan et al. study, since it is known that the outcome of SE is highly model-dependent (18–19). Erdogan and colleagues injected PTZ every 10 minutes intraperitoneally, starting with 40 mg/kg, followed by 20 mg/kg, then by 10 mg/kg every 10 minutes until they induced SE that lasted at least 30 minutes. The total dose of PTZ injected ranged from 60 mg/kg to 100 mg/kg. Strangely enough, other investigators have reported that rat pups kindled with PTZ between P1–P28 (20) showed impaired water maze performance, abnormal emotional responses in the open-field, handling, and forced swim tests, as well as hippocampal neuronal loss with mossy fiber sprouting. These rats had only received 30 mg/kg of PTZ per day (admittedly, on a recurrent basis), in contrast to the much higher doses and more severe seizures of the SE paradigm used by Erdogan et al. Similarly, another study demonstrated that PTZ injected daily from P10–P14 caused a wide variety of cognitive and emotional anomalies as well as neuronal loss (21). Persistent biochemical abnormalities even have been described following seizures induced by a single injection of PTZ (70 mg/kg to 80 mg/kg) in P20 rats (22).

The answer to this paradoxical sparing of function after SE becomes apparent when one looks at the details of the experiment. First, Erdogan et al. selected a seizure duration of at least 30 minutes. However, few studies find behavioral deficits or neuronal loss with seizures lasting less than 30 minutes (6,7,23), unless special techniques are used to detect subtle hippocampal cell loss (24). Thus, the damage may have been mild and hard to detect by a very few behavioral tests done months later. Second and most importantly, PTZ-induced SE at P20 does not elevate metabolic rate in the hippocampus; therefore, in this model, seizures do not involve the hippocampus, and hippocampal damage or deficits of hippocampal function (such as memory) should not be expected. Pereira et al. have carefully studied brain metabolism during PTZ-induced seizures at various ages, and in P21 pups, glucose utilization increased in many brain regions but actually decreased in hippocampus and dentate gyrus (25). There is minimal hippocampal participation in the seizures, as shown by Fos induction (26), but clearly it is too weak to do any damage, since it does not even elevate the glycolytic rate. Therefore, the preservation of memory found by Erdogan et al. provides a good example of the substrate for long-term sequellae of seizures: seizure anatomy can vary with seizure type and results in differences in anatomical vulnerability.

Erdogan et al. also found a decrease in the number of fecal boli in the open-field test after SE as well as an increase in the number of rearings and of squares crossed. The investigators logically interpreted this finding as a decrease in anxiety and concluded that: "no emotional deficits occur[ed]." Yet, arguably, it is not at all obvious how a loss of anxiety induced by generalized SE should be interpreted. Frontal lobotomy is very effective at reducing anxiety, but does lack of anxiety resulting from such a lesion reflect "no . . .deficit"? It would be valuable to better understand the context in which behavioral changes occur and to know their biological substrate before evaluating their significance.

Finally, is it correct to assume, as the authors did, that PTZ-induced SE is a model of generalized SE? Low-dose PTZ historically has been used for evaluating whether drugs are effective against petit mal absence seizures, a form of generalized epilepsy. However, with high-dose PTZ, seizures involve cortex and then brainstem (26), causing tonic extension and with this spread, all pharmacologic specificity is lost (27). Inducing SE requires high-dose PTZ and, as a model, has no specificity for generalized SE.

In summary, Erdogan et al., along with previous studies (25,26), have provided a valuable example of the anatomical basis of selective vulnerability for a very specific seizure type. Their work did not truly test the dogma of the developing brain's invulnerability to seizures or even to generalized seizures. However, it nicely illustrates the formidable complexity of the relationship between seizures and their long-term consequences for brain and behavior.

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