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Short review

Epileptogenesis in immature rats following recurrent status epilepticus

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Abstract

Strong evidences link status epilepticus (SE) in childhood with the later development of epilepsy. Pilocarpine-induced SE in developing rats leads to late appearance of spontaneous epileptic seizures only when SE is induced after the 18th day of life. We examined the possibility that 3 consecutive episodes of pilocarpine-induced SE on postnatal days 7, 8 and 9 could induce behavioral, electrographic and histological epileptic changes in adult life. The animals also underwent behavioral tests (inhibitory step-down avoidance, skinner box, rota-rod, open field and elevated plus-maze). EEG recordings made at the age of 30, 60 and 90 days showed the occurrence of several episodes of spikes and/or polyspikes appearing simultaneously in hippocampus and cortex. Only three isolated spontaneous seizures were observed during the whole period of observation (120 days). The long-term effects of three consecutive episodes of SE include increased spontaneous exploratory activity, learning impairment, and reduced anxiety when tested on P60. Our findings provide evidence for EEG changes and cognitive deficits in adult life following recurrent SE on postnatal days 7–9.

Keywords: Immature brain; Pilocarpine; Learning; Memory; Repeated *status epilepticus*; Epileptogenesis

Contents

1. Introduction

Status epilepticus (SE) occurs mainly in the early years of life [1], a period in which SE and other epileptic events can be more detrimental to further brain development [2].

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One question frequently raised is whether patients with one episode of SE are more prone to manifest further episodes. There are only a few data that would allow the clinician to identify children at risk of recurrent SE [3], although some studies show that 10–18% of children with SE presented at least one additional episode [4,5].

Retrospective studies indicate a correlation between temporal lobe epilepsy in adult patients and the occurrence of seizure and SE in their childhood [6–8]. According to current knowledge, some factors such as fever [9] and hypoxia [10,11] among several others, contribute to neonatal seizures, and have long-term consequences on brain development [12]. Studies on seizures during childhood suggest a low risk of recurrence following a single unprovoked episode [13] or following SE without an antecedent injury [14,15]. In contrast, neurologically abnormal children account for almost all pediatric cases of multiple episodes of SE [3].

Some authors have also observed that seizures beginning in early childhood are associated with a higher risk of intellectual impairment as compared with cases of late childhood onset [16–19].

Experimental studies in both in vivo and in vitro animal models have shown that the immature limbic system is a particularly frequent site of epileptogenesis during a critical period in postnatal life [20–23]. Studies on focal seizure models have found that there is an increased susceptibility to focal seizures during the second and third postnatal week in the rat [24]. Such increased epileptogenicity has been also described in amygdala kindling [25], hippocampal kindling [26,27] and hippocampal electrical stimulation [28].

We have recently shown that chronic seizures follows pilocarpine-induced SE in rats only if the drug is administered after the 18th day of life [29].

SE indeed produces hippocampal lesions, which in turn generate chronic epilepsy. In the immature brain, however, several types of prolonged experimental seizures fail to produce structural lesions or generate chronic epilepsy. Kainic acid (KA)-induced SE causes increased seizure susceptibility in mature rats, but has no appreciable effects in rats undergoing KA-induced SE at ages ≤ 15 days [30–32]. In adult rats, KA seizures cause severe neuronal loss in hippocampal fields CA1 and CA3, and in dentate hilus, as well as synaptic reorganization in the form of mossy fiber sprouting. In contrast, no structural changes are noted in immature rat pups after KA SE [32]. Similar results were observed in the pilocarpine model of epilepsy [29,33–35].

The effect of SE on subsequent long-term behavior was also studied in immature brain. Some recent works showed long-term deficits in memory, learning, and behavior in mature rats after KA-induced SE [30,36] or long-term adverse effects on cognition in pubescent and mature rats after continuous hippocampal stimulation but not in immature animals [37].

The experimental model of chronic epilepsy, using the cholinergic muscarinic agonist pilocarpine as the convulsant agent, shows acute effects on adult rodents characterized by long-lasting limbic SE associated to sustained electrographic discharges in limbic structures. After a single application of pilocarpine, SE lasts from 6 to 24 h. Animals surviving the so-called acute period proceed to a latent ''seizure free'' period with an apparently normal behavior [35]. This silent period lasts 4–44 days and ends with the occurrence of the first spontaneous seizure, which characterize the chronic period. This model has been successfully utilized in adult rats [33], mice [38,39] and developing rats [29,34] to experimentally reproduce the long-term behavioral, EEG, and histologic features of temporal lobe epilepsy.

In accordance with these observations, the present study was designed to analyze the possibility that consecutive episodes of pilocarpine-induced SE in the immature brain (postnatal days 7, 8 and 9) would determine epileptic changes in the adult brain. We also evaluated the effects of such early consecutive episodes of SE on some memory and learning tests (inhibitory step-down avoidance and skinner box), locomotor activity (rota-rod), exploratory activity (open field) and anxiety (elevated plus-maze) of the adult animal.

2. Material and methods

2.1. Animals

Developing Wistar male rats aged P7–9 (postnatal days-old) ($N = 45$) were used in this study. The colony room had a temperature of 21 ± 2°C with a 12-h light/dark schedule. The rats were bred in our laboratories and the date of birth was considered day 0. The pups were housed with their mother in individual cages until weaning at day 21. The animals were selected randomly for the experimental (SE) or saline (control) groups. SE was induced through the administration of pilocarpine (Sigma, St. Louis, MO) in the dose of 380 mg/kg, i.p., at all ages (P7–9), preceded 30 min by scopolamine methyl-nitrate (Sigma; 1 mg/kg, s.c.) to limit peripheral cholinergic effects [33] during three consecutive days. Control rats P7–9 ($N = 15$), were injected with the same volume of 0.9% saline instead of pilocarpine preceded 30 min by scopolamine methyl-nitrate.

The latency (time elapsed from pilocarpine injection to the first behavioral epileptic manifestations), duration, and severity of SE (presence of tonic seizures) were observed.

Following the SE period, the surviving animals were video-monitored for spontaneous seizures around the clock during the following 120 days. Infrared emitting light were used for video recording of animal activity during the dark periods.

2.2. Surgical techniques and recording

At 30, 40, 60 and 90 days of age, animals were anesthetized under sodium pentobarbital and stereotaxically implanted with twisted bipolar electrodes of nichrome wires (100 μm), positioned in the dorsal hippocampus according to the stereotaxic atlas of Swanson [40] to monitor EEG changes. Three stainless steel screws were attached to the skull for surface recordings: one in the frontal bone served as an electrical ground and the two others in the frontal region were used for EEG recording.

Three days after surgery the EEG was recorded for 60 min once a day between 1000 and 1200 h for 30 consecutive days.

2.3. Histological analysis

The animals were processed for histological analysis 120 days after pilocarpine administration. Sections cut 40 μm thick were stained according to neo-Timm and/or Nissl methods. The neo-Timm method is a modification of traditional Timm's method in order to increase the specific staining of zinc and thereby produce a more distinct visualization of mossy fiber system [41].

2.4. Behavioral tests

Two other experimental (SE) and control (saline) groups were studied at P60–90 to verify the effects of three consecutive episodes of early SE (P7, 8 and 9) on memory and learning performance (inhibitory step-down avoidance and skinner box), locomotor activity (rota-rod), exploratory activity (open field) and anxiety (elevated plus-maze). Experiments were carried out between 0900 and 1100 h. All apparatus were carefully cleaned with 0.1% acetic acid after each individual test.

The *step-down inhibitory avoidance* task was carried out in a $50 \times 25 \times 25$ cm illuminated box, with a floor consisting of a series of 1-mm caliber parallel bronze bars. The left half of the grid was covered with a 24-cm-wide, 24-cm-deep and 5-cm-high platform. In the training session, animals were placed on the platform and their latencies to step down onto the grid were measured. Immediately upon placing their four paws on the grid, a 0.2-mA, 60 Hz, 2 s shock was delivered. In test sessions, animals avoided the shock by staying on the platform. The procedure was carried out for a period of 5 min. Test latencies were taken as a measure of retention [42].

The *elevated plus-maze* consisted of two open arms, 50×10 cm and two enclosed arms, $50 \times 10 \times 40$ cm with an open roof, arranged in such a way that the two arms of each type were opposite each other. The maze was elevated to a height of 50 cm. The results indicated were taken by two different observers sitting in the same room. Rats were placed individually in the center of the maze, the platform center, with their head inside one of the closed arms. The following behavioral measures were taken for 5 min: the number of entries into open and closed arms and the time spent on open and closed arms and the total number of arm entries. The procedure of the test has been described in detail by Pellow and File [43].

The *open field test* was used to evaluate the behavioral responses of rats to a novel environment [44,45]. A circular open field (1 meter diameter) lit with a 60-W bulb above the field was used to measure activity in an unfamiliar environment. The floor of the open field was divided into 12 parts and the number of parts crossed during a 5-min period was quantified as locomotor activity. In addition, the number of rearings on hind feet and number of defecations were counted during this period.

The *Skinner box* consisted of $25 \times 16 \times 20$ cm. The animals were maintained for 23 h of water restriction before testing and were trained to press a bar to obtain water. When the animal pressed the bar, water (200 μl) was delivered associated to a cue noise. The sessions lasted 30 min and ended when animals pressed the bar and drank for five consecutive times. After each session animals were allowed to drink water for a period of 1 h.

The motor performance was evaluated using a *rota-rod test* (Model 7700, Ugo Basile). All animals were tested for their ability to remain for 5 min on the rotating bar at the speed of 16 rpm. After 24 h, the animals were again tested for motor ability. Each animal was individually given a minimum of three trials to complete the task. The higher value was considered for statistical analysis. Latency to fall off the rotating rod was noted for each trial, with 5 min maximum to termination of the trials.

2.5. Statistical analysis

Comparison of behavioral data obtained from animals subjected to three episodes of SE and control animals were analyzed using the non parametric Mann–Whitney test.

3. Results

3.1. Pilocarpine-induced SE

A few minutes after pilocarpine administration, all animals presented continuous scratching, strong body tremor, mastication, clonic movements of forelimbs and head bobbing culminating in SE. Although only one animal (out of 30) presented tonic convulsions during SE during the first SE episode at P7, this number increased to 3 and 12 rats in the second and third SE episodes (P8 and P9), respectively.

The mean latency for the first motor signs following pilocarpine administration on P7 was 108 ± 27.37 s and

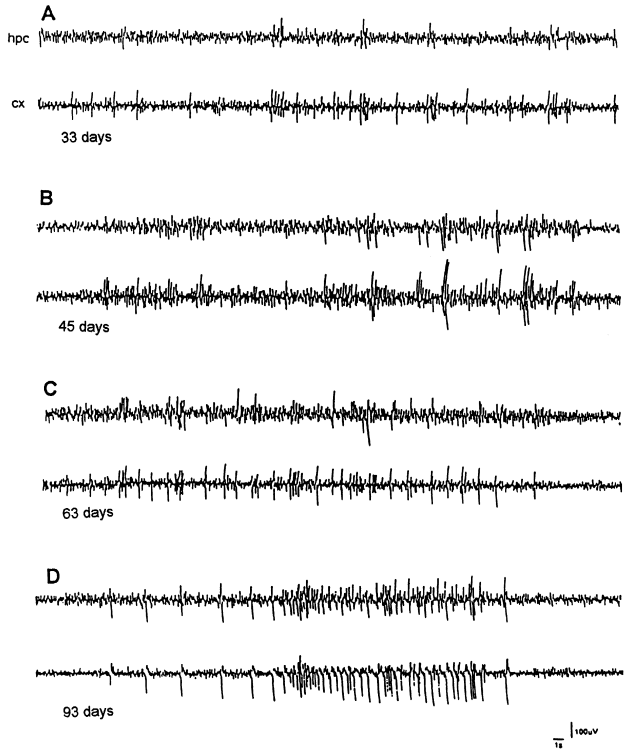

Fig. 1. Electrographic activity registered at P33, P45, P63 and P93 in animals subjected to three SE episodes during development. Notice the presence of spiking and/or polyspiking activity registered both at hippocampal (hpc) and neocortical (cx) leads.

increased progressively until the third injection on P9 (324 ± 24.97 s). The mean duration of SE increased from the first to the second SE episode and did not change from the second to the third treatment session. In this context, it is important to notice that those animals with tonic convulsions present SE of longer duration in the three sessions. Only one animal died during the three SE sessions. Surviving animals did not show, with rare exceptions, any behavioral manifestation that could be directly associated to epileptic activity during the whole period of observation (120 days). Despite continuous video monitoring of all animals, a single episode of spontaneous clonic seizures could be observed in only three specific animals that had shown one or more tonic convulsions during the SE sessions. These seizures were similar to stage 5 kindled seizures and lasted less than 1 min.

3.2. Electrographic effects

All surviving animals EEG-recorded on P33, P45, P63 and P93 exhibited some degree of electrographical alterations both in the hippocampus and neocortex (Fig. 1). These EEG changes were mostly subclinical, i.e., they could not be correlated to any particular behavioral change indicative of seizure activity and were characterized by high-amplitude spiking or polyspiking activity that appeared mainly during behavioral arrest. This epileptiform activity was more intense and longer in those animals that presented tonic convulsions during the SE sessions. The severity of the electrographic epileptiform features increased with age, i.e., they were longer and more pronounced when recording was carried out in older animals. As aforementioned, in rare cases (3/29), an electrographic seizure was recorded during the occurrence of the spontaneous clonic seizure (Fig. 2). These three spontaneous seizures occurred when these animals were older than 60 days.

3.3. Histologic analysis

Microscopic examination of the brains of rats subjected to three consecutive episodes of SE showed no gross anatomic changes. Stained sections showed no gliosis, and areas believed to be involved in seizure-related injury,

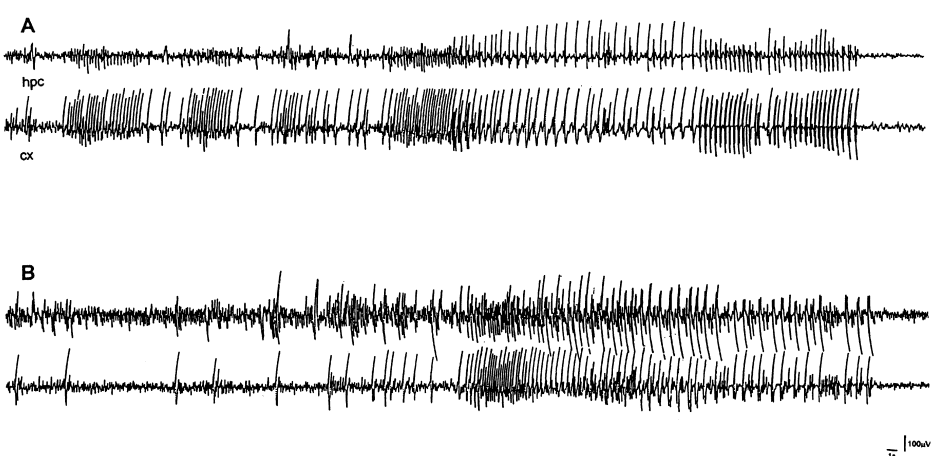

Fig. 2. Two electrographic seizures registered in different animals ((A) P98 and (B) P112) after pilocarpine-induced status epilepticus at P7–9. Notice the presence of fast high-voltage spiking activity followed by polyspikes in the hippocampal (hpc) and cortical (cx) recordings.

such as the amygdala, hippocampus and dentate gyrus were intact. This observation was also true for the three animals that showed single spontaneous seizures. Similarly, Timm stained sections did not reveal the presence of mossy fiber sprouting in the supragranular layer even in those three animals.

3.4. Behavioral tests

3.4.1. Rota-rod test

In order to check the general locomotor performance of experimental ($n = 10$) and control ($n = 10$) rats before other behavioural studies, each rat underwent a 5-min rota-rod test. No significant differences were observed between the groups.

3.4.2. Skinner box test

All saline treated animals ($n = 10$) learned to press the bar for water. Five animals learned to obtain water in the second day of experiment and the remaining in the third day of experiment. In contrast, all experimental animals ($n = 10$) failed to learn to obtain water by pressing the bar during three consecutive sessions.

3.4.3. Step-down inhibitory avoidance

A significant difference between the experimental ($n = 12$) and control ($n = 10$) groups was observed in the initial two sessions of this behavioral test. While control animals presented retention times of 287 ± 16.1 and 299 ± 0.8 s, respectively, in the first and second sessions, animals subjected to three consecutive SE episodes in early life showed times of 9.8 ± 6.0 and 49.6 ± 84.1 s ($p < 0.05$), respectively. However, in the third session, no difference was noted between the experimental and control groups (225 ± 10 and 300 s, respectively) (Fig. 3).

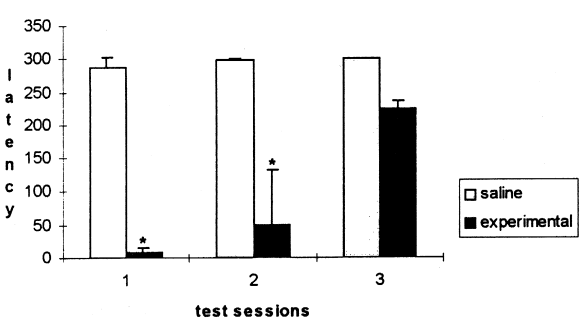

Fig. 3. Step-down latencies in control and experimental rats in test sessions of inhibitory avoidance. *Significant difference from control (saline) group during the first and second test session ($p < 0.05$). Data are presented as mean ± S.E.M.

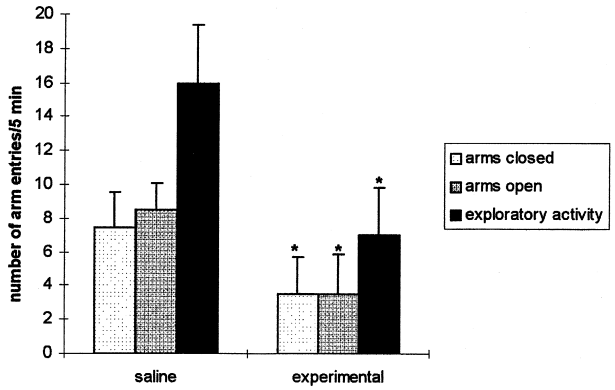

Fig. 4. Number of open and closed arm entries and exploratory activity measured by total entries into each of the arms in the elevated plus-maze in control and experimental rats. *Significant difference from control (saline) group ($p < 0.05$). Data are presented as mean ± S.E.M.

3.4.4. Elevated plus-maze test

The total time spent by the control group (46 ± 16.5 s, $n = 8$) in the open arms of the elevated plus-maze apparatus was significantly lower when compared to the experimental group (100 ± 39.8 s, $n = 11$) ($p < 0.05$). The experimental group spent significantly longer time (195 ± 41.7 s) in closed arms than the control group (231 ± 19.4) ($p < 0.05$). A higher number of entries in both closed (7.5 ± 2.1) and open (8.5 ± 1.6) arms was observed in saline treated rats when compared to the experimental group (3.5 ± 2.2 and 3.5 ± 2.4, respectively) ($p < 0.05$) (Fig. 4) and the exploratory activity measured as total entries into each of the arms was significantly higher in the control group (Fig. 5).

3.4.5. Open field behavior

The number of rearings (12 ± 6.9 vs. 20 ± 4.5) or defecation bolus (5.7 ± 2.4 vs. 4.0 ± 2.7) of experimental ($n = 12$) and control ($n = 10$) groups during the 5-min observation period in the open field box did not differ significantly. On the other hand, experimental animals

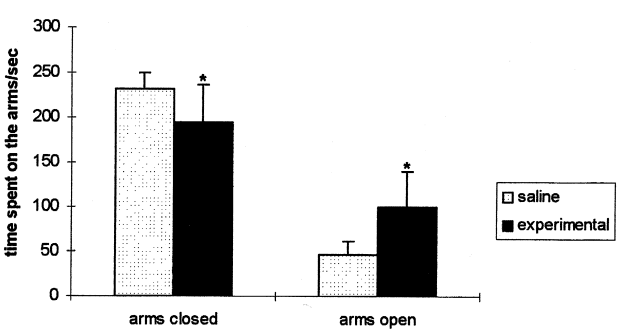

Fig. 5. Mean time (s) spent in the closed and open arms in the elevated plus-maze. *Significant difference from saline group ($p < 0.05$). Data are presented as mean ± S.E.M.

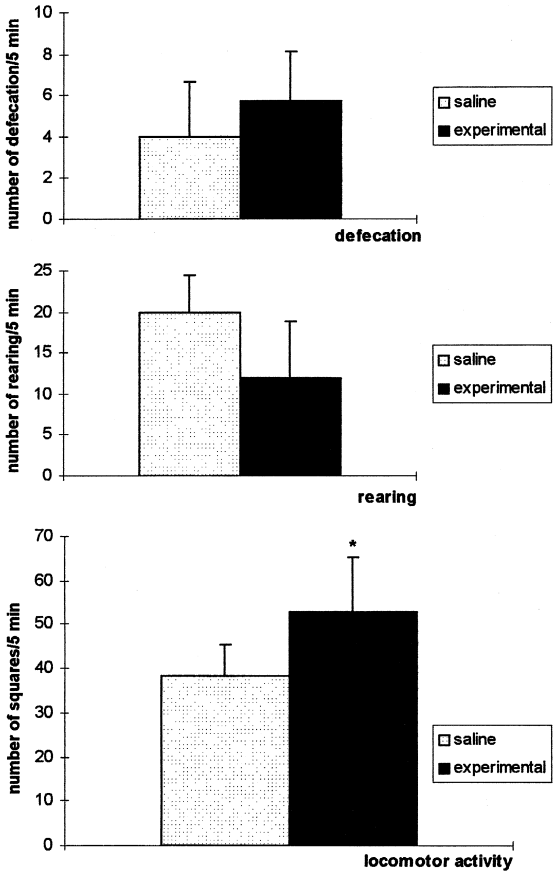

Fig. 6. Defecation (upper graph), rearing (middle graph) and locomotor activity (lower graph) in open field shown from saline and experimental rats. Data are presented as mean ± S.E.M. * Significant difference from saline group ($p < 0.05$, locomotor activity).

showed higher locomotor activity (53 ± 12.4 squares) when compared to the control group (38.3 ± 7.1 squares) ($p < 0.05$) (Fig. 6).

4. Discussion

In this study, we examined the hypothesis that three consecutive episodes of pilocarpine-induced SE in immature animals could lead to epileptic or some other behavioral changes later in life. Despite the absence of gross anatomical changes and chronic epilepsy our results showed that this treatment in young rats (P7–9) can induce important electrographic and behavioral changes that persist into adult life.

While many studies have analyzed the long-term effects of a single prolonged seizure in the immature brain, few investigations have been done on the long-term effects of recurrent SE episodes during development [46]. One study examined the effects of multiple administrations of the convulsant KA in immature rats (P20–26), using four injections of convulsive doses of KA at 2-day intervals and found no pathological changes in the hippocampus [47]. Although we analyzed the effect of three consecutive administrations of pilocarpine in younger animals (P7–9), our histological data are similar to those observed by Sarkisian et al. [47], suggesting an increased resistance of the developing brain to the SE-induced cell loss in contrast to the adult brain [34].

Experimental studies have shown discordant results in relation to hippocampal damage following experimentally-induced SE depending on the experimental design and the age of the animals. Albala et al. [30] found neuronal cell loss in the hippocampus, amygdala and pyriform cortex only in pubescent and adult rats, but not in pups (P15–18) subjected to systemic KA administration. Similar results were also reported by other authors who did not find any hippocampal lesion following severe flurothyl induced convulsions in 4-day-old [48] or 15-day-old [49] rat pups. As previously observed [34], the morphological analysis of developing rat brains following pilocarpine-induced SE shows a remarkable age-dependent brain susceptibility to epilepsy-related cell death and, as similarly reported by others [29,34], the third week of postnatal life appears to be critical for the occurrence of the epilepsy-related damage in the brain of animals treated with pilocarpine.

All animals injected with pilocarpine developed SE in the three consecutive sessions with increasing duration and severity. In addition, the number of animals exhibiting tonic convulsions increased progressively until the third SE session. This result is contrary to those observed by Sarkisian et al. [47], who observed a progressive decrease in seizure severity and an increase in latency-to-seizure onset with each of four subsequent KA injection in immature rats (P20–26). Based on these results, the authors suggested that KA could act differentially than some other convulsants (such as cholinomimetic drugs) which could produce a ''kindling effect'' [47], as we observed in our experiments.

In a preliminary work [29], we observed that spontaneous recurrent seizures following pilocarpine-induced SE were manifested if SE was induced after the 18th day of life. Contrary to what we expected, only three over 30 animals presented a single spontaneous seizure during the following 120 days of observation.

It has been shown that oxygen deprivation is acutely epileptogenic in the immature brain [10,11]. Jensen et al. [10] using a model of global hypoxia in rodents at different ages demonstrated that only rat pups aged postnatal days 10–12 exhibited seizure activity. Moreover, rats exposed to hypoxia at P10, but not at younger or older ages, exhibited increased seizure susceptibility in adulthood [11]. Other studies exposing rat pups to hypoxia or hypoxic–ischemic insults reported no change in susceptibility later in life to either flurothyl-induced seizures or to changes in kindled seizures [50,51]. In contrast, Chiba [52] and Matsumoto [53] found that rat pups exposed to anoxia showed decreased seizure thresholds to pentylenetetrazol (PTZ) when the animals were challenged at various older ages. In

spite of the fact that hypoxia is the primary insult in these studies, the results of these investigations showed the probability that seizure-induced hypoxia may be a mechanism by which seizure can injure the developing brain. Although most of the animals in this study did not develop spontaneous seizures later in life, they presented significant changes in hippocampal and cortical recordings with episodes of complex spiking activity. Since these changes were more pronounced in animals with tonic convulsions during the episodes of SE, one possibility to explain the later development of the epileptic characteristics is these rats is the occurrence of SE associated hypoxia. In fact, Mathern et al. [54] observed that adult rats exposed to KA-induced SE and then a hypoxic insult present increased hippocampal neuron loss and grater supragranular mossy fiber sprouting when compared to animals subjected to KA-induced SE alone. Although developing rats do not show evident neuronal damage and mossy fiber sprouting, the SE-associated hypoxia may lead to important plastic changes in critical periods of brain maturation that can become apparent through a variety of epileptic manifestations.

The results of this study showed no deficits in the general locomotor performance in both experimental and control groups tested before the behavioral studies as analyzed by rota-rod. On the other hand, we observed learning impairment and increased spontaneous exploratory activity in other specific behavioral tests in adult animals subjected to multiple pilocarpine-induced SE in early life.

Some authors [36,55] have demonstrated that the immature brain is less vulnerable to the long-term cognitive deficits following a single seizure than the mature brain. Lui et al. [55], analyzing the long-term behavior in immature animals following a single pilocarpine injection, showed that the deficits of learning tests are age dependent. In this study, animals receiving pilocarpine at P20 and P45 showed deficits in the water maze test. However, when they were compared to each other, a significant impairment was observed in the P45 group. Stafstrom et al. [36] found behavioral and cognitive deficits in KA-treated P20, P30 and P60 rats, but not at P5 and P10, when tested at P80. These results could be explained by plasticity processes. It is well known that this effect is most intense during development and is believed to decline with aging [56,57].

The results presented here showed an impairment of learning (Skinner box, step-down avoidance) after repetitive SE episodes when tested on P60–70. Therefore, multiple seizure episodes in the immature brain could lead to long-term cognitive deficits. The ability of young neurons to grow and make new synaptic contacts may facilitate aberrant connections following SE [58], and determine behavioral or cognitive changes.

Studies of SE in children demonstrated a low risk for subsequent seizures in the absence of acute neurologic insults [14,15]. Such findings support the view that seizures during early human development do not cause excitotoxic hippocampal injury, adult hippocampal sclerosis, and temporal lobe epilepsy. In spite of these data, our findings show that repetitive SE episodes in developing rats induce electrographic epileptic features and cognitive disorders that are not accompanied by gross neuronal damage. Considering that the susceptibility of young animals to chronic epilepsy following SE is age-related, as demonstrated by several studies, further research in different phases of early brain development is necessary to elucidate which plastic changes (neuronal reorganization, changes in specific receptor subunit composition, etc.) could be responsible for the epileptogenesis in developing animals.

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References

[1] W.A. Hauser, D.C. Hesdorffer, Epilepsy: Frequency, Causes and Consequences, Demos, New York, 1990.
[2] C.G. Wasterlain, Y. Shirasaka, Seizures, brain damage and brain development, Brain Dev. 16 (1994) 279–285.
[3] S. Shinnar, J. Maytal, L. Krasnoff, S.L. Moshe, Recurrent status epilepticus in children, Ann. Neurol. 31 (1992) 598–604.
[4] J. Aicardi, J.J. Chevrie, Convulsive status epilepticus in infants and in children: a study of 239 cases, Epilepsia 11 (1970) 187–197.
[5] G.B. Cavazzuti, P. Ferrari, M. Lalla, Follow-up study of 482 cases with convulsive disorders in the first years of life, Dev. Med. Child Neurol. 26 (1984) 425–437.
[6] M.A. Falconer, Genetic and related etiologic factors in temporal lobe epilepsy. A review, Epilepsia 12 (1971) 13–21.
[7] J.H. Margerison, J.A.N. Corsellis, Epilepsy and the temporal lobes: a clinical, electroencephalographic and neuropathological study of the brain in epilepsy with particular reference to the temporal lobes, Brain 89 (1966) 499–530.
[8] H.J. Sagar, J.M. Oxbury, Hippocampal neuron loss in temporal lobe epilepsy: correlation with early childhood convulsions, Ann. Neurol. 22 (1987) 334–340.
[9] A.T. Berg, S. Shinnar, E.D. Shapiro, M.E. Salomon, E.F. Crain, A. Hauser, Risk factors for a first febrile seizure: a matched case-control study, Epilepsia 36 (4) (1995) 334–341.
[10] F.E. Jensen, C.D. Applegate, D. Holtzman, T.R. Belin, J.L. Burchfiel, Epileptogenic effect of hypoxia in the immature rodent brain, Ann. Neurol. 29 (6) (1991) 629–637.
[11] F.E. Jensen, G.L. Holmes, C.T. Lombroso, H.K. Blume, I.R. Firkusny, Age-dependent changes in long-term seizure susceptibility and behavior after hypoxia in rats, Epilepsia 33 (6) (1992) 971–980.
[12] C.G. Wasterlain, D.G. Fujikawa, L. Penix, R. Sankar, Pathophysiological mechanisms of brain damage from status epilepticus, Epilepsia 34 (suppl. 1) (1993) S37–S53.
[13] S. Shinnar, A.T. Berg, S.L. Moshé, M. Petix, J. Maytal, H. Kang, E.S. Goldensohn, W.A. Hauser, Risk of seizure recurrence following a first unprovoked seizure in childhood: a prospective study, Pediatrics 85 (6) (1990) 1076–1085.
[14] D.W. Dunn, Status epilepticus in children: etiology, clinical features, and outcome, J. Neurol. Child. 3 (1988) 167–173.
[15] J. Maytal, S. Shinnar, S.L. Moshè, L.A. Alvarez, The low morbidity

and mortality of status epilepticus in children, Pediatrics 83 (1989) 323–331.
[16] J.J. Chevrie, J. Aicardi, Childhood epileptic encephalopathy with slow spike-wave. A statistical study of 80 cases, Epilepsia 13 (1972) 259–271.
[17] S. Dikmen, C.G. Matthews, J.P. Harley, Effect of early versus late onset of major motor epilepsy upon cognitive–intellectual performance, Epilepsia 16 (1975) 73–81.
[18] S. Dikmen, C.G. Matthews, J.P. Harley, Effect of early versus late onset of major motor epilepsy upon cognitive–intellectual performance: further considerations, Epilepsia 18 (1977) 31–36.
[19] J.A. Wada, M. Sato, M.E. Corcoran, Persistent seizure susceptibility and recurrent spontaneous seizures in kindled cats, Epilepsia 15 (1974) 465–478.
[20] S.L. Moshe, Epileptogenesis and the immature brain, Epilepsia 28 (suppl 1) (1987) S3–S15.
[21] S.L. Moshe, E.F. Sperber, B.J. Albala, Kindling as a model of epilepsy in developing animals, in: F. Morrel (ed.), Kindling and Synaptic Plasticity. The legacy of Graham Goddar, Birkhauser Boston, 1991, pp. 177–194.
[22] J.W. Swann, K.L. Simith, C.M. Gomez, R.J. Brady, The ontogeny of hippocampal local circuits and focal epileptogenesis, in: J. Engel Jr., C. Wasterlain, E.A. Cavalheiro, U. Heinemann, G. Avanzini (Eds.), Molecular Neurobiology of Epilepsy (Epilepsy Res. Suppl.9), 1992, pp. 115–126.
[23] J.W. Swann, K.L. Smith, R.J. Brady, M.G. Pierson, Neurophysiological studies of alterations in seizure susceptibility during brain development, in: P. Schwartzkroin (Ed.), Epilepsy. Models, Mechanisms, and Concepts, Cambridge Univ. Press, 1993, pp. 209–243.
[24] A. Gottieb, I. Keydor, H.T. Epstein, Rodent brain growth stages. An analytical review, Biol. Neonate 32 (1977) 166–167.
[25] S.L. Moshe, The effects of age on the kindling phenomenon, Dev. Psychobiol. 14 (1981) 75–81.
[26] K.Z. Haas, E.F. Sperber, S.L. Moshe, Kindling in developing animals: expression of severe seizures and enhanced development of bilateral foci, Dev. Brain Res. 56 (1990) 275–280.
[27] H.B. Michelson, J.M. Williamson, E.W. Lothman, Ontogeny of kindling: the acquisition of kindled responses at different ages with rapidly recurring hippocampal seizures, Epilepsia 30 (1989) 672.
[28] L. Velísek, P. Marés, Increased epileptogenesis in the immature hippocampus, Exp. Brain Res. Ser. 20 (1991) 183–185.
[29] M.R. Priel, N.F. Santos, E.A. Cavalheiro, Development aspects of the pilocarpine model of epilepsy, Epilepsy Res. 26 (1996) 115–121.
[30] B.J. Albala, S.L. Moshe, R. Okada, Kainic-acid induced seizures: a developmental study, Dev. Brain Res. 13 (1984) 139–148.
[31] L. Nitecka, E. Tremblay, G. Charton, J.P. Bouillot, M.L. Berger, Y. Ben-Ari, Maturation of kainic acid seizure-brain damage syndrome in the rat: II. Histopathological sequelae, Neuroscience 13 (1984) 1073–1094.
[32] E.F. Sperber, K.Z. Haas, P.K. Stanton, S.L. Moshe, Resistance of the immature hippocampus to seizure-induced synaptic reorganization, Dev. Brain Res. 60 (1991) 88–93.
[33] W.A. Turski, E.A. Cavalheiro, M. Schwarz, S.J. Czucwar, Z. Kleinrok, L. Turski, Limbic seizures produced by pilocarpine in rats: behavioral, electroencephalographic and neuropathological study, Behav. Brain Res. 9 (1983) 315–335.
[34] E.A. Cavalheiro, F.S. Delrio, W.A. Turski, L.S. Calderazzo-Filho, Z.A. Bortolotto, L. Turski, The susceptibility of rats to pilocarpine-induced seizures is age-dependent, Dev. Brain Res. 37 (1987) 43–58.
[35] E.A. Cavalheiro, J.P. Leite, Z.A. Bortolotto, C. Turski, C. Ikonomidou, L. Turski, Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures, Epilepsia 32 (1991) 778–782.
[36] C.E. Stafstrom, G.L. Holmes, A. Chronopoulos, S. Thurber, J.L. Thompson, Age-dependent cognitive and behavioral deficits following kainic acid-induced seizures, Epilepsia 34 (1993) 420–432.
[37] S. Thurber, A. Chronopoulos, C.E. Stafstrom, G.L. Holmes, Behavioral effects of continuous hippocampal stimulation in the developing rat, Dev. Brain Dev. 68 (1992) 35–40.
[38] W. Turski, E.A. Cavalheiro, Z.A. Bortolotto, L.M. Mello, M. Schwarz, L. Turski, Seizures produced by pilocarpine in mice: a behavioral, electroencephalographic and morphological analysis, Brain Res. 321 (1984) 237–253.
[39] E.A. Cavalheiro, N.F. Santos, M.R. Priel, The pilocarpine model of epilepsy in mice, Epilepsia 37 (1996) 1015–1019.
[40] L.W. Swanson, Brain Maps: Structure of the Rat Brain, Elsevier, Amsterdam, 1992.
[41] T.I. Babb, W.R. Kupfer, J.K. Pretorius, P.H. Crandall, M.F. Levesque, Synaptic recurrent excitation of granule cells by mossy fibers in human epileptic hippocampus, Neuroscience 42 (1991) 351–363.
[42] A.C. Netto, M. Maltchik, β-Endorphin enhancement of retrieval in a three-session paradigm, Behav. Neural Biol. 54 (1990) 97–104.
[43] S. Pellow, S.E. File, Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat, Pharmacol. Biochem. Behav. 24 (1985) 525–529.
[44] R.N. Walsh, R.A. Cummins, The open-field test: a critical review, Psychol. Bull. 83 (1976) 482–504.
[45] M. Vergnes, C. Marescaux, A. Boehrer, A. Depaulis, Are rats with genetic absence epilepsy behavioral impaired, Epilepsy Res. 9 (1991) 97–104.
[46] G.L. Holmes, J.L. Thompson, Effects of serial of administration of Kainic acid on the developing brain, Neuropharmacology 27 (1988) 209–212.
[47] M.R. Sarkisian, P. Tandon, Z. Liu, Y. Yang, A. Hori, G.L. Holmes, C.E. Stafstrom, Multiple kainic acid seizures in the immature and adult brain: ictal manifestations and long-term effects on learning and memory, Epilepsia 38 (11) (1997) 1157–1166.
[48] C.G. Wasterlain, Effects of neonatal status epilepticus on rat brain development, Neurology 26 (1975) 975–986.
[49] E.F. Sperber, K.Z. Haas, S.L. Moshé, Developmental aspects of status epilepticus, Int. Pediatr. 7 (1992) 213–222.
[50] S.L. Moshe, B.J. Albala, Perinatal hypoxia and subsequent development of seizures, Physiol. Behav. 35 (1985) 819–823.
[51] G.L. Holmes, D.A. Weber, Effects of hypoxic–ischemic encephalopathies on indling in the immature brain, Exp. Neurol. 90 (1985) 194–203.
[52] S. Chiba, Long-term effect of postnatal hypoxia on the seizure susceptibility in rats, Life Sci. 37 (1985) 1597–1604.
[53] M. Matsumoto, The effects of perinatal hypoxia on pentylenetetrazol-induced seizures in developing rats, Life Sci. 46 (1990) 787–1792.
[54] G.W. Mathern, G. Price, C. Rosales, J.K. Pretorius, A. Lozada, D. Mendoza, Anoxia during kainate status epilepticus shortens behavioral convulsions but generates hippocampal neuron loss and supragranular mossy fiber sprouting, Epilepsy Res. 30 (1998) 133–151.
[55] Z. Lui, A. Gatt, S.J. Werner, M.A. Mikati, G.L. Holmes, Long-term behavioral deficits following pilocarpine seizures in immature rats, Epilepsy Res. 19 (1994) 191–204.
[56] L. de Toledo-Morrell, Y. Geinisman, F. Morrell, Individual differences in hippocampal synaptic plasticity as a function of aging: behavioural electrophysiological and morphological evidence, in: T.L. Petit, G.O. Ivy (Eds.), Neural Plasticity: A Lifespan Approach, Alan R. Liss, New York, 1988, pp. 283–328.
[57] D.G. Jones, Synaptic trends and synaptic remodelling in the developing and mature neocortex, in: T.L. Petit, G.O. Ivy (Eds.), Neural Plasticity: A Lifespan Approach, Alan R. Liss, New York, 1988, pp. 21–42.
[58] P.A. Schwartzkroin, Plasticity and repair in the immature central nervous system, in: P.A. Schwartzkroin, S.L. Moshé, J.L. Noebels, J.W. Swann (Eds.), Brain Developmental and Epilepsy, Oxford Univ. Press, New York, 1995, pp. 34–65.