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Seizures in the Developing Brain Cause Adverse Long-term Effects on Spatial Learning and Anxiety

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Summary: *Purpose*: Seizures in the developing brain cause less macroscopic structural damage than do seizures in adulthood, but accumulating evidence shows that seizures early in life can be associated with persistent behavioral and cognitive impairments. We previously showed that long-term spatial memory in the eight-arm radial-arm maze was impaired in rats that experienced a single episode of kainic acid (KA)-induced status epilepticus during early development (postnatal days (P) 1–14). Here we extend those findings by using a set of behavioral paradigms that are sensitive to additional aspects of learning and behavior.

Methods: On P1, P7, P14, or P24, rats underwent status epilepticus induced by intraperitoneal injections of age-specific doses of KA. In adulthood (P90–P100), the behavioral performance of these rats was compared with that of control rats that did not receive KA. A modified version of the radial-arm maze was used to assess short-term spatial memory; the Morris water maze was used to evaluate long-term spatial memory and retrieval; and the elevated plus maze was used to determine anxiety.

Results: Compared with controls, rats with KA seizures at each tested age had impaired *short-term* spatial memory in the radial-arm maze (longer latency to criterion and more reference errors), deficient *long-term* spatial learning and retrieval in the water maze (longer escape latencies and memory for platform location), and a greater degree of anxiety in the elevated plus maze (greater time spent in open arms).

Conclusions: These findings provide additional support for the concept that seizures early in life may be followed by life-long impairment of certain cognitive and behavioral functions. These results may have clinical implications, favoring early and aggressive control of seizures during development. **Key Words:** Seizure—Development—Behavioral testing—Water maze—Kainic acid.

Epilepsy is a common neurologic disorder that occurs much more frequently in children than in adults (1). Many data support the idea that prolonged or frequent seizures in young animals and patients lead to later cognitive deficits, which can often be subtle [e.g., (2–11)]. In both laboratory animals and humans, the brain's susceptibility to seizures is increased during specific developmental epochs, yet the behavioral consequences of seizures tend to be less pronounced when seizures occur early in life than in adulthood. Reasons for these age dependencies are not fully understood but are clearly complex (12–14).

To investigate the long-term consequences of epilepsy early in life, several animal models have been developed (3,6,7,10,13,15). Most of these models use chemoconvulsants to induce acute seizures, mimicking status epilepticus. Later, a variety of tests assessing behavioral and cognitive function, seizure susceptibility, and histologic evidence of neuronal damage is applied. For example, kainic acid (KA) has been used to investigate the behavioral consequences of status epilepticus at various ages. In adult animals, KA causes an epilepsy syndrome similar to human temporal lobe epilepsy, with mesial temporal sclerosis, spontaneous seizures, and significant deficits in learning and memory (16). In developing animals compared with adults, however, the long-term effects of KA-induced status epilepticus are less severe in terms of macroscopic structural damage and behavioral impairments (17–21). When administered intraperitoneally at ages younger than about postnatal day (P) 20 in rats, KA does not cause significant hippocampal cell loss or recurrent seizures, and synaptic reorganization (mossy fiber sprouting) is less pronounced than that in adults, even if the seizures are more severe in the pups (19). These observations are not restricted to the KA model; seizures induced by kindling, pilocarpine, and flurothyl are likewise not associated with significant synaptic reorganization, as assessed by mossy fiber sprouting (22,23). Nevertheless, seizure-induced behavioral impairments may occur in later life in response to status epilepticus during early development (24–26).

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Depending on the method of seizure induction, dose, age of administration, and behavioral testing, investigators have found varied effects of early seizures on subsequent learning and behavior (25–29). We recently provided additional evidence for long-term adverse cognitive changes after KA-induced status epilepticus in early development (30). KA seizures were evoked during early postnatal development (P1–P24). A seizure between P1 and P14 was associated with life-long impairment in spatial learning and memory in the radial-arm maze. These rats also exhibited impaired induction of hippocampal long-term potentiation (LTP). Seizure-induced alteration of synaptic plasticity also was manifested as a long-term reduction in susceptibility to kindled seizure development and enhanced paired-pulse inhibition in the dentate gyrus (30). Such chronic behavioral effects of early seizures on hippocampal-based memory demonstrate the influence of activity-dependent and seizure-induced plasticity during development on subsequent cognitive function in adulthood. Together, these findings suggest that seizures early in development are not as harmless as was once thought (24).

The present study addressed the behavioral effects of KA-induced status epilepticus at various stages of hippocampal development (31), focusing on whether early-life seizures differentially alter several hippocampal-dependent behavioral and cognitive processes, including short- and long-term spatial learning and memory, anxiety, and exploratory activity.

MATERIALS AND METHODS

In these experiments, seizures were induced by the systemic administration of KA on one of the following postnatal days: P1, P7, P14, or P24. Rats were allowed to reach adulthood (>P90) and were then evaluated with behavioral tests. Separate groups of rats were used for the radial-arm maze, water maze, and elevated plus maze/open-field tests. All procedures involving animals were approved by the Research Animal Care Committee of the University of Wisconsin.

Administration of kainic acid

KA was dissolved in saline and administered intraperitoneally to male rats at dosages of 1 to 2 mg/kg (P1, P7), 2 to 4 mg/kg (P14), and 8 to 10 mg/kg (P24) according to previous studies, which demonstrated that these doses reliably induce electrographic and behavioral seizures at these ages (20,30). Male littermates, either uninjected or injected with 0.9% (wt/vol) NaCl, served as controls. Seizures were observed and their characteristics noted over a 4- to 8-h period. Both sham-treated controls and animals that received KA were then returned to their home cages, in pairs (P24) or with their dams (P $\leq$14).

Radial-arm maze testing

To assess the short- and long-term effects of early postnatal seizures on spatial memory in adulthood, subsets of the adult rats that experienced seizures on P1, P7, P14, or P24 were evaluated in a modified eight-arm radial maze beginning on P90 to P100. Before the experiment was started, the rats were calorically restricted until they lost 15% of their body weight, to ensure adequate motivation in this appetitive task (32). Before the experiment, the rats were put into the radial-arm maze to habituate for 5 min, to learn to navigate the apparatus and find and eat food pellets (bait) hidden in recesses at the ends of four arms of the maze. Arm entry was defined as placement of all four feet into an arm of the maze.

The test consisted of six trials, 30 min apart, for a total testing time of $\sim$3 h. Small food pellets were placed at the end of the same arms of the radial-arm maze that were baited during the habituation trial. In addition, visual clues (large geometric shapes) were attached at the entrance of each arm in which a pellet was located, to assist the rats in learning the task. The rat was placed at the center of the radial-arm maze and allowed to explore the maze until the four baits were consumed. The time to consume the four pellets (latency), total number of entries into the arms, and number entries into unbaited arms (reference errors) were recorded. The same four arms were baited each trial.

Water maze

A different subset of rats was tested in a modified water maze (33), designed to assess visual spatial learning (acquisition) and memory (retention). A circular steel tank (117-cm diameter) was filled with water (26 $\pm$ 1°C) to a depth of 25 cm. The water was made opaque by addition of $\sim$100 ml evaporated milk. The room was illuminated by overhead lights, and visual cues around the room (furniture, shelves) were kept constant from day to day. Four points on the perimeter of the pool were designated north (N), east (E), south (S) and west (W), thus dividing the pool into four quadrants (NW, NE, SE, SW). An 8 $\times$ 8-cm Plexiglas platform, onto which the rat could escape, was positioned in the center of one of the quadrants, 1 cm below the water surface. Because the opaque water precluded visual determination of platform position, rats had to use other means to locate it (i.e., distant visual cues).

On day 1 of training, each rat was placed in the pool for 60 s without the platform present; this free swim enabled the rat to become habituated to the training environment. On days 2 to 5, rats were trained for 24 trials (six trials per day) to locate and escape onto the submerged platform. For each rat, the quadrant in which the platform was located remained constant, but the point of immersion into the pool varied between N, E, S, and W in a quasirandom order for the 24 trials, so that the rat would not be able to

predict the platform location from the point at which it was placed into the pool. The latency from immersion into the pool to escape onto the platform was recorded for each trial by one observer, while another observer manually mapped the route taken by the rat to reach the platform. On mounting the platform, the rat was given a 30-s rest, after which the next trial commenced. If the rat did not find the platform in 120 s, it was manually placed on the platform for a 30-s rest.

Four hours after the final training trial on day 5, the spatial bias in the rats' search pattern was tested to assess memory for platform location ("probe trial"). The probe test assessed the hypothesis that control rats would spend more time searching in the quadrant in which the platform was located than would KA-treated rats. The platform was removed from the pool, and the rat was placed back into the pool at the point opposite the previous platform location. The rat was allowed 60 s of free swimming, and its swim route was recorded manually. The distance swum in each quadrant was calculated by tracing the swim path by using a digital curvimeter. These swimming distances are proportional to the time spent in each quadrant, because swimming speed was equivalent in each group.

Open-field and elevated plus maze

In another subset of rats that was not assessed on the radial-arm maze or water maze, locomotor activity and exploratory behavior were tested by using an open-field apparatus. The purpose of this test was to determine whether the control and experimental groups differed in baseline locomotor activity. The open-field test board consisted of a wooden box 60 × 60 × 35 cm, with four holes of 4 cm in diameter symmetrically spaced on the floor (34). The floor of the box was divided into nine equal-sized squares, allowing an observer to count the number of squares visited during a given period of time. For testing, adult rats (P90–P100) were placed on the board for 5 min. The number of head dips into the holes (exploration), squares visited (locomotor activity), and rearings were recorded.

After the open-field test, anxiety was tested by using the elevated plus maze. The elevated plus maze consisted of two open arms 50 × 10 cm at right angles to two covered (closed) arms, 50 × 40 × 10 cm. The maze was elevated to a height of 50 cm above the ground, forming an aversive stimulus to animals in the open arms, as described previously (35,36). The animal was placed in the center of the elevated plus maze with its head facing an open arm. Animals were tested for 5 min. Entry into a particular arm was defined as the placement of all four feet into that arm. The relative time spent in open versus closed arms is a measure of anxiety, with anxious rats preferring closed arms. Anxiolytic drugs increase the time spent in open arms, whereas anxiogenic drugs increase the time spent in closed arms (35,37).

Statistical analysis

Radial-arm maze, water maze, open-field maze, and elevated plus maze data were analyzed with analysis of variance, by using either analysis of variance (ANOVA) or the Kruskal–Wallis test on ranks, depending on whether the data were normally distributed (thereby generating variance ratios of F or H, respectively). For ANOVA, either one or two factors were used, with repeated measures when appropriate. For post hoc testing, we used the Student–Newman–Keuls test or Bonferroni test. Significance was defined as $p < 0.05$ for all comparisons.

RESULTS

In KA-treated rats of all ages, status epilepticus developed, with a sequence of clinical features that differed somewhat by age (20,30). P1 and P7 rats initially became immobile with loss of limb tone and ataxia. They then exhibited intermittent hyperactivity with rhythmic "bicycling" movements of all extremities, opisthotonic arching, and tonic limb extension. Previous studies with implanted hippocampal electrodes showed that such clinical signs represent electrographic ictal activity (30). In older rats (P14, P24), KA-induced seizures consisted of initial immobility and staring. At P14, repetitive scratching movements and rudimentary wet dog shakes (WDSs) were seen. At P24, WDSs were prominent, followed by facial clonus, masticatory automatisms, and increased salivation; tonic–clonic movements of one or more limbs, rearing, and falling then ensued. At all ages, seizures (status epilepticus) persisted intermittently for 2 to 6 h. The status epilepticus was not terminated pharmacologically. Later, spontaneous seizures were not monitored specifically, but none was observed during routine handling and behavioral testing.

Radial-arm maze

The performance of rats in the radial-arm maze was judged by the latency to find and consume the four baits, the total number of arm entries, and the number of arm entry "errors." Entering an arm without bait present is referred to as a reference memory error, whereas entering an arm in which the bait had already been consumed is referred to as a working memory error. The number of total arm entries is a reflection of anxiety; a more anxious rat would tend to remain stationary and explore the maze less (fewer arm entries). Therefore each of these criteria measures a different aspect of radial-arm maze performance.

All groups of rats learned to find and consume the four baits in each trial (Fig. 1). As a function of trial number, the latency to reach criterion (find and eat all four baits) was significantly longer in rats that received KA (Fig. 1). By post hoc testing, differences were significant when comparing the first trial with the fifth and sixth trials, for each age group. It took significantly more time for the

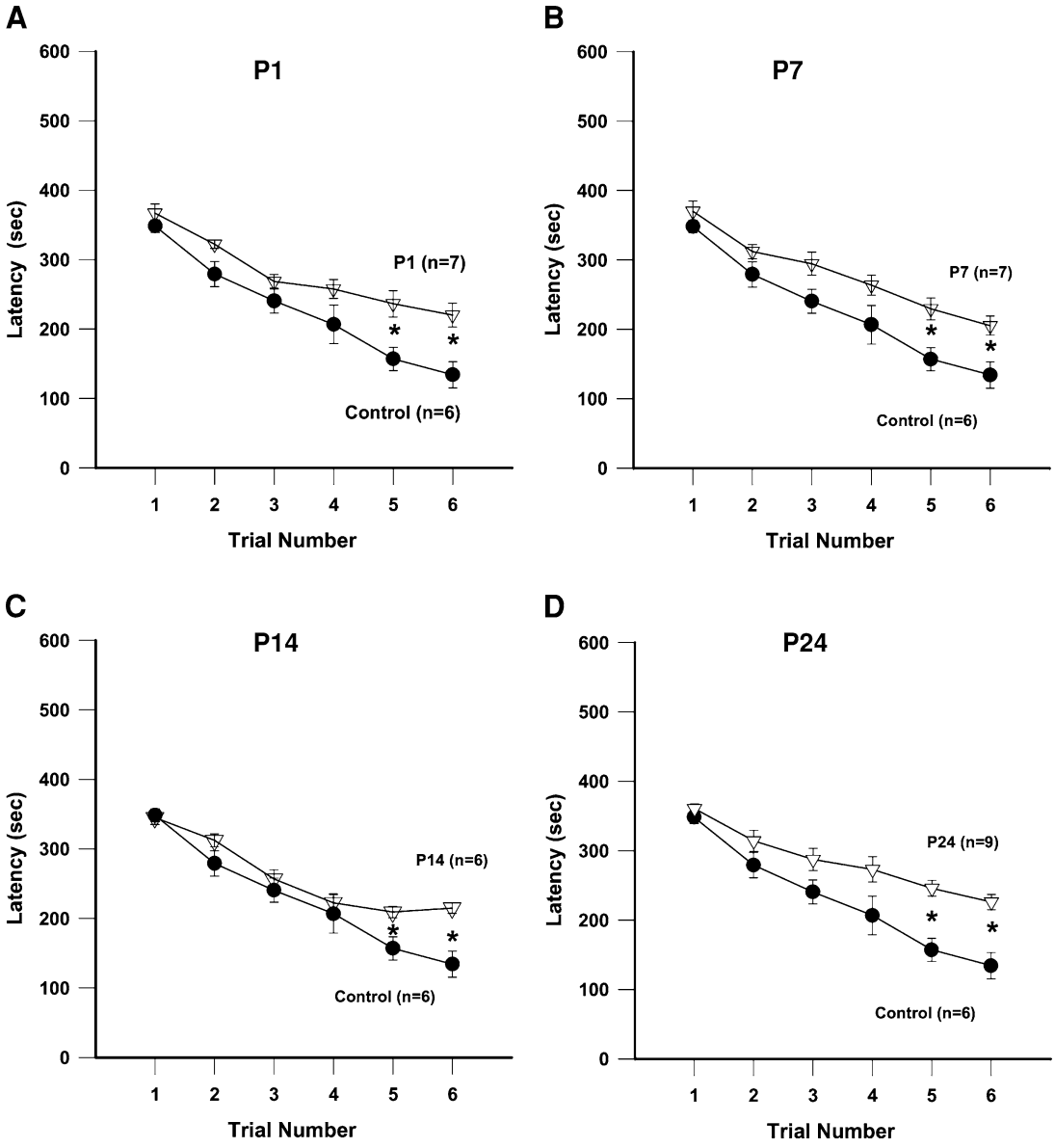

FIG. 1. Radial-arm maze performance: latencies to criterion. Rats underwent kainic acid (KA)–induced status epilepticus at various postnatal ages [P1 **(A)**, P7 **(B)**, P14 **(C)**, P24 **(D)**] and were tested in the radial-arm maze as adults (P90–P100). The age-matched controls received saline rather than KA. Testing consisted of six trials on a single day. The latency to criterion (time to find four baits) is plotted against trial number. In each age group, significant differences become apparent between experimentals and controls by the fifth trials (*). See text and Table 1. In each plot, *solid circles* are controls and *open triangles* are experimentals (KA-treated).

KA-treated P1, P7, P14, and P24 rats to find the baits than for the controls.

In addition, in each age group, fewer reference errors (entries into unbaited arms) were committed over time (i.e., with consecutive trials; Fig. 2). Post hoc analysis showed that, for each age group, by the third or fourth trial, a significant difference existed between controls and experimentals in terms of the total number of reference errors, with the animals that experienced seizures committing more errors (Fig. 2).

Previous studies using the radial-arm maze showed that anxious rats are less active and avoid exploring arms of the maze (38,39). Therefore to rule out an effect of anxiety on radial-arm maze performance between control and experimental groups, the *total* number of arm entries (baited, unbaited, previously baited) was compared (Fig. 3). On the first trial, no difference was found between controls and any KA-treated group as to the total number of arm entries ($p < 0.05$; Fig. 3, First Trial). Therefore anxiety was not a factor limiting initial arm exploration. By the sixth trial, controls and each KA-treated group made significantly fewer total arm entries compared with the first trial (i.e., they each learned the task, as described earlier). However, on the sixth trial, the control animals had

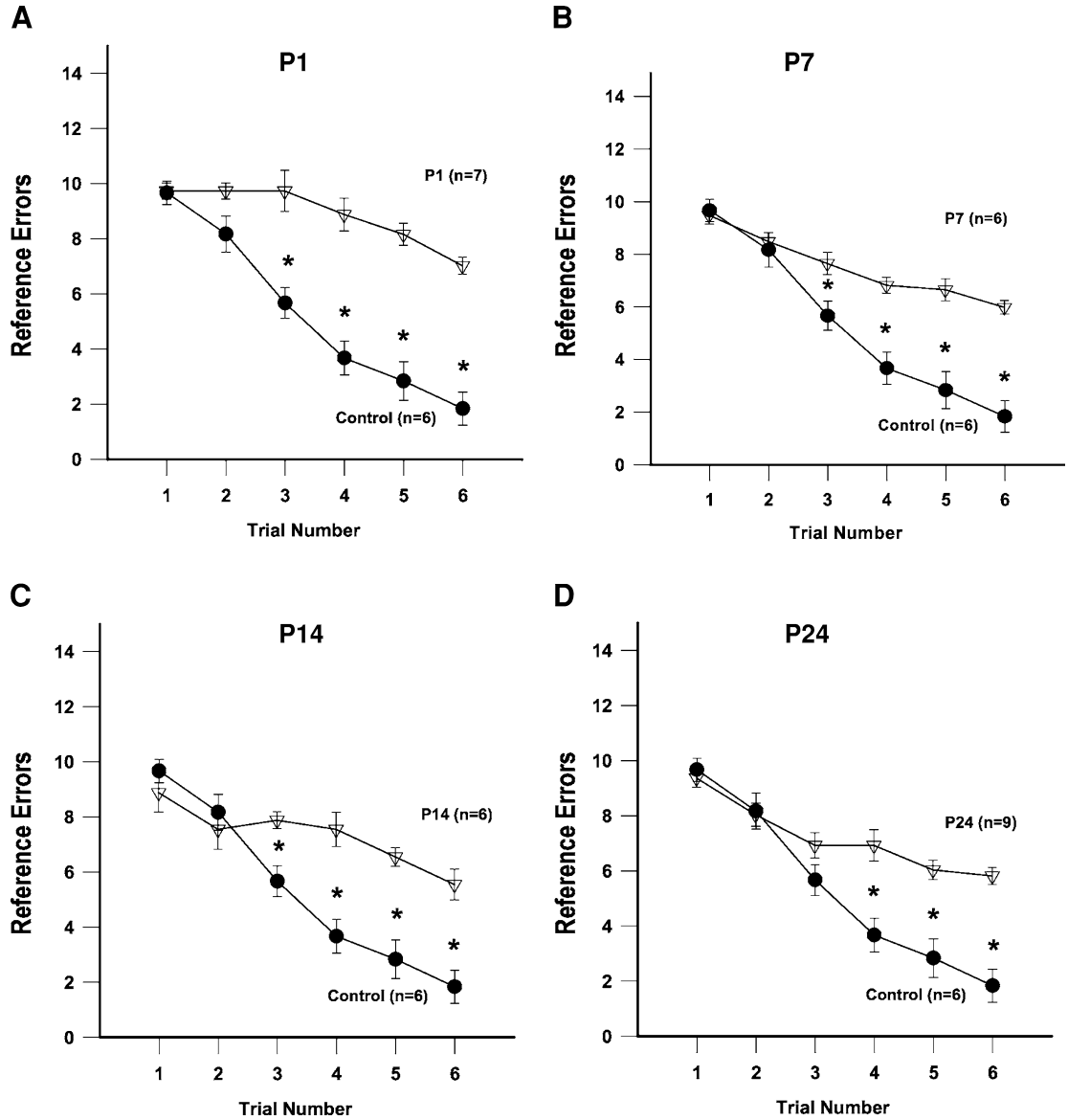

FIG. 2. Radial-arm maze performance: total number of reference errors (entries into unbaited arms). Rats underwent kainic acid (KA)–induced status epilepticus at various postnatal ages [P1 **(A)**, P7 **(B)**, P14 **(C)**, P24 **(D)**] and were tested in the radial-arm maze as adults (P90–P100). The age-matched controls received saline rather than KA. Testing consisted of six trials on a single day. The number of reference errors is plotted against trial number. Significantly more reference errors were committed by the treated animals on the third through sixth trials at ages P1 **(A)**, P7 **(B)**, and P14 **(C)**, and on the fourth through sixth trials at P24 **(D)**. See text and Table 1. In each plot, *solid circles* are controls and *open triangles* are experimentals (KA-treated).

significantly fewer total arm entries than each KA-treated group ($p < 0.05$; Fig. 3, Sixth Trial). These findings suggest that differences in total arm entries from the first trial to the sixth trial cannot be explained by a difference in anxiety between the groups.

Water maze

During the habituation trial, the rats swam randomly around the pool, with no preference for a particular quadrant. The acquisition of place learning is shown in Fig. 4A, in which escape latencies are plotted against day of training. All rats learned to find the hidden platform and escape onto it, as indicated by the progressive decrease in mean escape latency over the 4 training days in each group. The escape latencies for the experimental groups that received KA on P1, P7, or P24 differed significantly from untreated controls (by experimental groups: $F = 14.9$, $p < 0.0001$; by days of testing, $F = 42.0$, $p < 0.0001$; Group × Days interaction, $F = 4.5$, $p = 0.0009$). By post hoc testing, the performance of the P7 and P24 KA-treated groups differed significantly from controls, whereas P1 KA-treated rats performed similar to controls. Although the performance of the rats that received KA on P7 reached control values by testing day 4, their overall learning was much

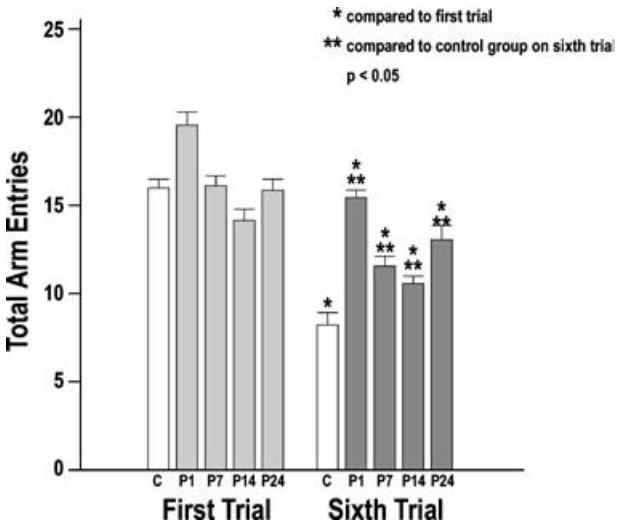

FIG. 3. Radial-arm maze performance: total number of entries into baited, unbaited, and previously baited arms. The total number of arm entries is shown for the first and sixth trials in controls and all KA-treated age groups. The number of arm entries is proportional to the level of anxiety. On the sixth trial, control rats and each age group of rats treated with KA had significantly fewer arm entries compared with the first trial (*); these data indicate that all rats learned the task and required fewer entries as a function of trial number. On the sixth trial, each KA-treated group had significantly more arm entries than controls (**); these data indicate that anxiety differences cannot explain radial-arm maze learning differences between control and seizure groups.

slower than controls, as evidenced by results on testing days 1, 2, and 3, each of which differed significantly from controls on post hoc testing.

On the probe test (Fig. 4B), the distance spent swimming in the target quadrant (proportional to the dwell time spent in that quadrant) differed between controls and each experimental age group (P1, P7, P24; $F = 6.4$; $p < 0.002$ by one-way ANOVA with post hoc Student–Newman–Keuls test). In Fig. 4C, the probe test results for the P24 KA group is compared with those of controls. For controls, the distance swum in the target quadrant differed from that in each other quadrant ($F = 26.0$; $p < 0.0001$), whereas for rats treated with KA on P24, swimming distances did not differ between quadrants ($F = 0.97$; $p = 0.42$). Similar results were found with the P1 and P7 groups (data not shown). Therefore each KA-treated group showed deficits in spatial bias by spending equivalent time in all quadrants rather than dwelling in the target quadrant.

Elevated plus maze

Anxiety scores were lower (indicating *greater* anxiety levels) in all age groups (P1, P7, P14, P24) among rats that had experienced KA–induced status epilepticus compared with the controls that did not receive KA (Fig. 5A; $F = 26.7$; $p < 0.0001$; post hoc Bonferroni test, $p < 0.05$). These findings demonstrate that increased anxiety existed in all experimental groups, and they spent less time in the open arms, compared with the controls.

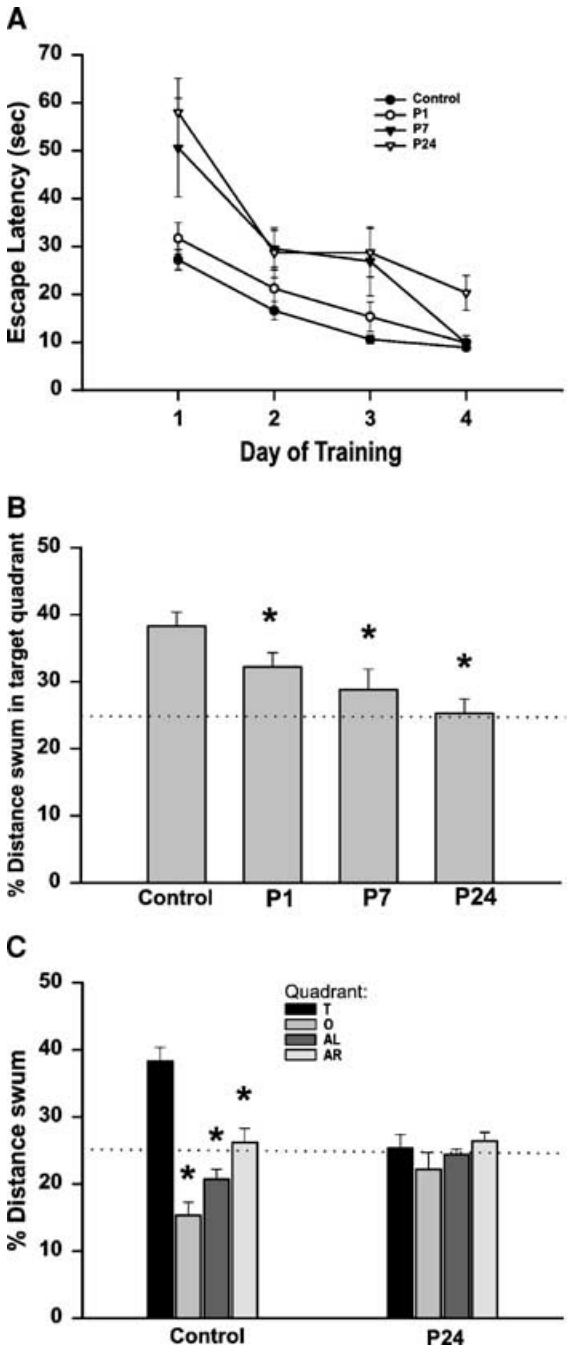

FIG. 4. Water maze testing. **A:** Acquisition learning of platform location, plotting escape latency versus day of testing for controls (*filled circles*) and rats treated with kainic acid (KA) at various postnatal ages (P1, *open circles*; P7, *filled triangles*; P24, *open triangles*). All groups learned to find and escape onto the platform over the four testing days, but learning was significantly slower in KA-treated rats. **B:** Probe test. After the final acquisition trial, the platform was removed, and the rats were placed back in the pool for 60 s. The distance (proportional to time) swum in each quadrant was calculated. Compared with control rats, each KA-treated group spent significantly less time in the target quadrant (*), where the platform was previously located. **C:** Probe test. The percentage distance in each quadrant is shown for controls and rats treated with KA on P24. Control rats swam significantly longer in the target quadrant, whereas P24-treated rats spent similar time in each of the four quadrants. Similar results were found for the P1 and P7 groups (data not shown).

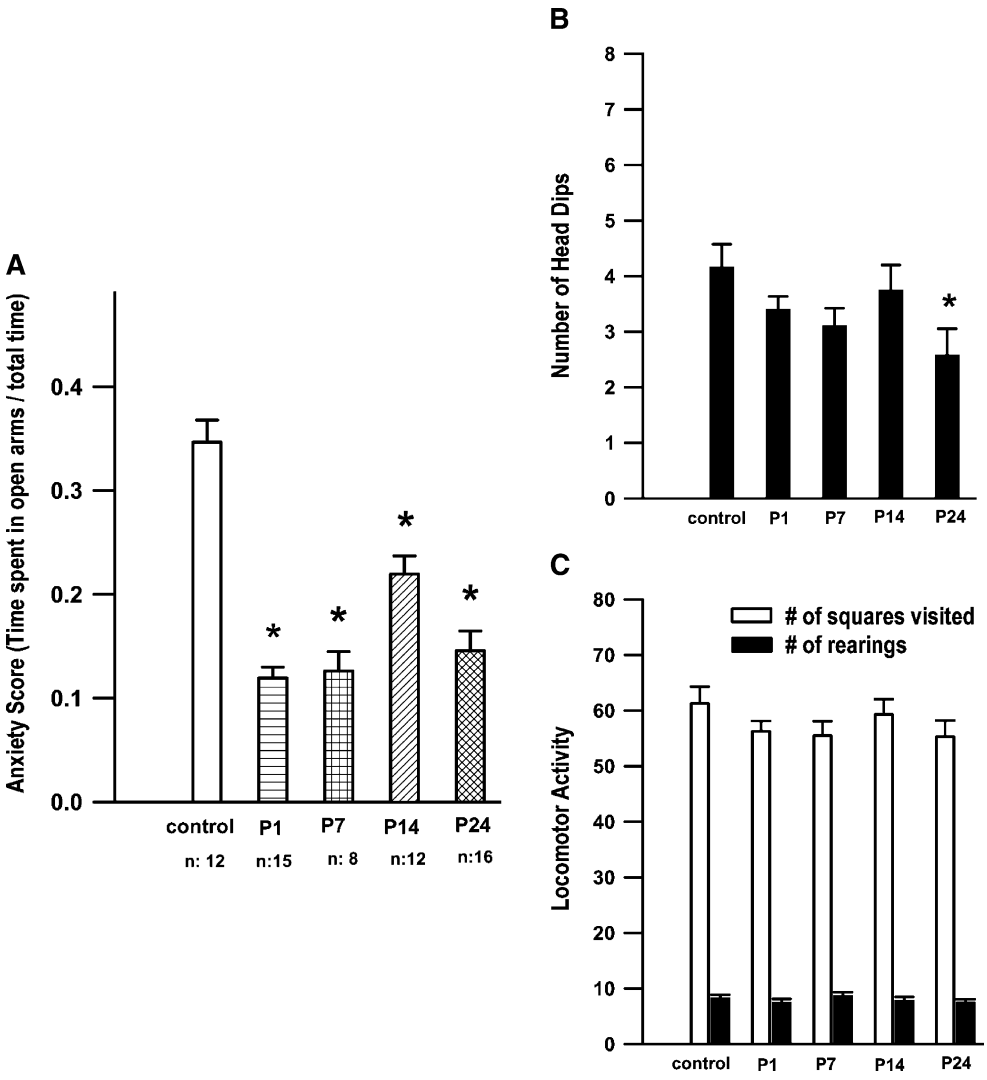

FIG. 5. Elevated plus maze and open-field testing. **A:** Anxiety scores (time spent in open arms as a proportion of total maze time) is plotted for controls and rats treated with kainic acid (KA) at various ages (P1–P24). All KA-treated groups were significantly more anxious than were controls. The number of rats (n) in each group is indicated below each bar. **B:** Exploratory behavior in the open field. Number of head dips into holes in the floor of the open field box is plotted for controls and rats treated with KA at various ages (P1–P24). Only the P24-treated group differed from controls. **C:** Locomotor activity in the open field. The number of squares visited (*open bars*) and number of rears (*filled bars*) are plotted for controls and rats treated with KA at various ages (P1–P24). None of the KA-treated groups differed from controls. The results in **B** and **C** show that the anxiety differences seen in **A** were not due to inherent differences in locomotor activity.

On open-field testing, no difference was seen in exploratory activity (number of head dippings) or locomotor activity (number of squares visited and rearings) between age groups or between controls and rats that had experienced KA seizures (Fig. 5B and C). Therefore the differences in anxiety scores between the control and experimental groups were not due to an alteration of locomotor or exploratory activity and may therefore be a consequence associated with the seizure history.

DISCUSSION

These results add to the accumulating evidence that seizures during early brain development can result in lifelong behavioral and cognitive deficits. In this study, status epilepticus induced by the glutamate receptor agonist KA early in life was associated with significant learning and memory deficits and persistent anxiety in adulthood. As summarized in Table 1, KA seizures on P1, P7, P14, and P24 (radial-arm maze) or P7 and P24 (water maze) were associated with both short- and long-term impairments in spatial learning and memory, respectively, both of which are hippocampus-dependent functions. The finding that controls and rats that received KA on P1 did not differ on water maze testing, whereas P1 rats demonstrated impaired radial-arm maze learning, suggests that the latter test may be more sensitive for seizure-induced behavioral dysfunction. Furthermore, rats that experienced status epilepticus at these early ages exhibited heightened levels of anxiety as adults (elevated plus maze),

TABLE 1. *Summary of results*

Test	Function tested	Result/interpretation
Radial-arm maze	Short-term spatial learning and memory	Animals with KA seizures early in life (P1, P7, P14, P24): – took longer to learn food location – made more reference errors (entries into unbaited arms) – no difference was found in performance as a function of age at which seizure occurred.
Water maze	Long-term spatial learning and memory	Animals with KA seizures early in life (P1, P7, P24): – took longer to learn platform location (except P1) – spent less time swimming in target quadrant (probe test) – deficits varied inversely with age at the time of status epilepticus: P24 > P7 > P1.
Elevated plus maze	Anxiety	Animals with KA seizures early in life (P1, P7, P14, P24):- – were more anxious (preferred closed arms).
Open-field test	Locomotion, exploration	Animals with KA seizures early in life (P1, P7, P14, P24): – exhibited no difference from controls in head dips, squares visited, or number of rears (therefore anxiety differences were not explainable on basis of differences in locomotion).

KA, kainic acid.

again correlating with abnormal hippocampal/limbic system function. The lack of major hippocampal structural damage in other studies using similar protocols (18,19,30) suggests that seizures early in life can have long-term cognitive consequences despite the absence of overt cellular damage. Although some synaptic reorganization may occur after multiple postnatal seizures (40), its functional consequences are unclear and may differ by age. It is already known that KA–induced status epilepticus in adult rats causes a marked disruption of spatial learning and memory, far greater than the changes seen in developing rats (21,41).

These experiments confirm and extend those of Lynch et al. (30). In that study, a different version of the radial-arm maze was used, with the protocol requiring that rats learn the location of the food pellet over several days of testing, until a criterion was reached (consuming all food pellets in the first five arm entries). The task was considered "learned" once the animal achieved five consecutive criterion performances. Here, we used a modified radial-arm maze protocol, in which all learning and testing occurred over multiple trials on a single day. In this abbreviated training condition as well, KA seizures early in development were associated with impaired learning of the task and more reference errors compared with those in controls. However, compared with the previous data (30), our results in the radial-arm maze had no relation to age. That is, the latency to criterion, number of reference errors, and total errors were similar, regardless of the age at which status epilepticus occurred. Similarly, in the elevated plus maze, anxiety levels were increased to a similar degree in each KA-treated group. Therefore the fact that status epilepticus occurred had a greater impact than the age at which it occurred.

In this study, we used an additional measure of hippocampal integrity, the water maze, a well-validated test of spatial learning and memory (33,42,43). In this task, rats that were treated with KA on P7 or P24 learned the platform location slower than did controls, with a rank order of performance (worst to best): P24 > P7 > P1, controls. Therefore performance varied inversely with the age at which KA status epilepticus occurred. On the probe (spatial bias) component of the water maze, rats are allowed to swim freely without a platform present, to assess their preference (memory) for platform location in the target quadrant. Each KA-treated group (P1, P7, P24) spent significantly less time swimming in the target quadrant than controls, suggesting that the seizure groups had poorer memory of platform location.

Compared with previous studies using the Morris water maze (21), the current study detected more evidence for memory impairment as a consequence of seizures at P5, P10, and P20. The reasons for these discrepancies are uncertain, but accumulating evidence indicates that behavioral and learning deficits occur as a consequence of early-life status epilepticus. The persistence of increased anxiety from early-life status epilepticus suggests that affect is influenced as well as cognition.

Prolonged seizures caused by other etiologies [e.g., corticotropin-releasing hormone (44)] early in development also are associated with later impairment of cognitive function such as water-maze learning, and the deficits appear to be progressive over time (6). Lithium-pilocarpine status epilepticus at P16 or P20, but not at P12, was associated with cognitive impairment in the water maze in early adulthood (P55) (11).

In addition to prolonged seizures early in life leading to later cognitive abnormalities, even brief, recurrent seizures at young ages also can be detrimental (4,8,45,46). Recurrent pentylenetetrazol (PTZ) seizures in early development (P10–P14) caused significant spatial deficits in the water maze when the animals were tested on P35 and P60 (10).

The choice of model and assessment tool is critical, because different results have been obtained with different methods. The limitations of rodents as an experimental

model for epilepsy were discussed recently (47,48). Furthermore, detailed knowledge of the chosen behavioral test is crucial before attributing a cognitive deficit to an experimental paradigm (25,42,43,49).

In summary, on multiple measures of hippocampus-based cognitive function, rats that experienced KA seizures during early development had persistent deficits as adults: radial-arm maze, long-term version (30), radial-arm maze, abbreviated version (present study); and water-maze acquisition learning and spatial memory (present study). These results suggest that seizures disrupt some aspect of hippocampal function during the early, vulnerable "critical period" of brain development, with deficits observable long afterward (in adulthood). Early-life KA seizures reduced long-term potentiation, increased paired-pulse inhibition in the dentate gyrus, and reduced the susceptibility to kindled seizures as adults (30). The increased inhibition was thought to be related to the loss of plasticity caused by the excessive neuronal activity of the seizure. Therefore the dentate gyrus appears to be a site of long-term cellular alterations induced by abnormal patterns of postnatal neural activity. It is likely that other hippocampal and extrahippocampal areas also are involved in these maladaptive plastic changes after early-life seizures. Indeed, each of the behavioral tests we used reflected more than just hippocampal function; rather, each also requires integration of several neural systems (31,50,51).

Together, these studies demonstrate that disruption of normal neural activity by seizures during early postnatal development produces deficits in a variety of cognitive measures and behaviors in adulthood. As such damage occurs even before the full maturation of hippocampal circuits (52), it is apparent that subsequent developmental events are not sufficient to overcome the adverse effects of early postnatal seizures. As substantial evidence indicates that status epilepticus in the developing brain does not produce the overt macroscopic damage seen in adults, the observation of long-term behavioral and memory deficits in these experiments supports the view that the term *seizure-induced damage* also should include adverse functional consequences. These observations may have clinical implications for cognitive and memory dysfunction associated with epilepsy during development.

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REFERENCES

1. Hauser W, Hersdorffer D. *Epilepsy: frequency, causes and consequences*. New York: Demos, 1990.
2. Farwell JR, Dodrill CB, Batzel LW. Neuropsychological abilities of children with epilepsy. *Epilepsia* 1985;26:395–400.
3. Holmes GL, Gaiarsa J-L, Chevassus-Au-Louis N, et al. Consequences of neonatal seizures in the rat: morphological and behavioral effects. *Ann Neurol* 1998;44:845–57.
4. Huang L, Cilio MR, Silveira DC, et al. Long-term effects of neonatal seizures: a behavioral, electrophysiological and histological study. *Dev Brain Res* 1999;118:99–107.
5. Neyens LG, Aldenkamp AP, Meinardi HM. Prospective follow-up of intellectual development in children with a recent onset of epilepsy. *Epilepsy Res* 1999;34:85-90.
6. Brunson KL, Khan N, Eghbal-Ahmadi M, et al. Corticotropin (ACTH) acts directly on amygdala neurons to down-regulate corticotropin-releasing hormone gene expression. *Ann Neurol* 2001;49:304–12.
7. Lee CL, Hannay J, Hrachovy R, et al. Spatial learning deficits without hippocampal neuronal loss in a model of early-onset epilepsy. *Neuroscience* 2001;107:71–84.
8. de Rogalski-Landrot I, Minokoshi M, Silveira DC, et al. Recurrent neonatal seizures: relationship of pathology to the electroencephalogram and cognition. *Dev Brain Res* 2001;129:27–38.
9. Austin JK, Dunn DW. Progressive behavioral changes in children with epilepsy. *Prog Brain Res* 2002;135:419–27.
10. Huang L-T, Yang SN, Liou C-W, et al. Pentylenetetrazole-induced recurrent seizures in rat pups: time course on spatial learning and long-term effects. *Epilepsia* 2002;43:567–73.
11. Cilio MR, Sogawa Y, Cha B-H, et al. Long-term effects of status epilepticus in the immature brain are specific for age and model. *Epilepsia* 2003;44:518–28.
12. Stafstrom CE, Lynch M, Sutula TP. Consequences of epilepsy in the developing brain: implications for surgical management. *Semin Pediatr Neurol* 2000;7:147–57.
13. Sanchez RM, Jensen FE. Maturational aspects of epilepsy mechanisms and consequences for the immature brain. *Epilepsia* 2001;42:577–85.
14. Lado FA, Laureta EC, Moshe SL. Seizure-induced hippocampal damage in the mature and immature brain. *Epileptic Disord* 2002;4:83–97.
15. Sankar R, Shin DH, Liu H, et al. Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J Neurosci* 1998;18:8382–93.
16. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* 1985;14:375–403.
17. Nitecka L, Tremblay E, Charton G, et al. Maturation of kainic acid seizure-brain damage syndrome in the rat, II: histopathological sequelae. *Neuroscience* 1984;13:1073–94.
18. Albala BJ, Moshe SL, Okada R. Kainic acid induced seizures: a developmental study. *Dev Brain Res* 1984;13:139–48.
19. Sperber EF, Haas KZ, Stanton PK, et al. Resistance of the immature hippocampus to seizure-induced synaptic reorganization. *Dev Brain Res* 1991;60:88–93.
20. Stafstrom CE, Thompson JL, Holmes GL. Kainic acid seizures in the developing brain: status epilepticus and spontaneous recurrent seizures. *Dev Brain Res* 1992;65:227–36.
21. Stafstrom CE, Chronopoulos A, Thurber S, et al. Age-dependent cognitive and behavioral deficits after kainic acid seizures. *Epilepsia* 1993;34:420–35.
22. Sperber EF, Haas KZ, Romero MT, et al. Flurothyl status epilepticus in developing rats: behavioral, electrographic, histological, and electrophysiological studies. *Dev Brain Res* 1999;116:59–68.
23. Haas KZ, Sperber EF, Opanashuk LA, et al. Resistance of the immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. *Hippocampus* 2001;11:615–25.
24. Holmes GL, Ben-Ari Y. Seizures in the developing brain: perhaps not so benign after all. *Neuron* 1998;21:1231–4.
25. Stafstrom CE. Assessing the behavioral and cognitive effects of seizures on the developing brain. *Prog Brain Res* 2002;135:377–90.
26. Rutten A, van Albada M, Silveira DC, et al. Memory impairment following status epilepticus in immature rats: time-course and environmental effects. *Eur J Neurosci* 2002;16:501–13.
27. DeFeo MR, Mecarelli O, Palladini G, et al. Long-term effects of early status epilepticus on the acquisition of conditioned avoidance behavior in rats. *Epilepsia* 1986;27:476–82.

28. Koh S, Storey TW, Santos TC, et al. Early-life seizures in rats increase susceptibility to seizure-induced brain injury in adulthood. *Neurology* 1999;53:915–21.
29. Zhang X, Cui S-S, Wallace AE, et al. Relations between brain pathology and temporal lobe epilepsy. *J Neurosci* 2002;22:6052–61.
30. Lynch M, Sayin U, Bownds J, et al. Long-term consequences of early postnatal seizures on hippocampal learning and plasticity. *Eur J Neurosci* 2000;12:2252–64.
31. Bast T, Feldon J. Hippocampal modulation of sensorimotor processes. *Prog Neurobiol* 2003;70:319–45.
32. Jaffard R, Bontempi B, Menzaghi F. Theoretical and practical considerations for the evaluation of learning and memory in mice. In: Buccafusco JJ, ed. *Methods of behavioral analysis in neuroscience*. Boca Raton, FL: CRC Press, 2001:295–323.
33. Morris RGM, Garrud P, Rawlins JNP, et al. Place navigation impaired in rats with hippocampal lesions. *Nature* 1982;297:681–3.
34. File SE, Wardill A. Validity of head dipping as a measure of exploration in a modified hole board. *Psychopharmacolgia* 1975;44:47–51.
35. Pellow S, Chopin P, File SE, et al. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 1985;14:149–67.
36. Sayin U, Purali N, Ozkan T, et al. Vigabatrin has an anxiolytic effect in the elevated plus maze test of anxiety. *Pharmacol Biochem Behav* 1992;43:529–35.
37. File SE. New strategies in the search for anxiolytics. *Drug Deliv* 1990;5:195–201.
38. Marczynski TJ, Urbancic M. Animal models of chronic anxiety and "fearlessness." *Brain Res Bull* 1988;21:483–90.
39. Park CR, Campbell AM, Diamond DM. Chronic psychosocial stress impairs learning and memory and increases sensitivity to yohimbine in adult rats. *Biol Psychiatry* 2001;50:994–1004.
40. Holmes GL, Sarkisian M, Ben-Ari Y, et al. Mossy fiber sprouting after recurrent seizures during early development in rats. *J Comp Neurol* 1999;404:537–53.
41. Letty S, Lerner-Natoli M, Rondouin G. Differential impairments of spatial memory and social behavior in two models of limbic epilepsy. *Epilepsia* 1995;36:973–82.
42. Terry AV. Spatial navigation (water maze) tasks. In: Buccafusco JJ, ed. *Methods of behavioral analysis in neuroscience*. Boca Raton, FL: CRC Press, 2001:153–66.
43. D'Hooge R, Deyn PPD. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev* 2001;36:60–90.
44. Baram TZ, Schultz L. Corticotropin-releasing hormone is a rapid and potent convulsant in the infant rat. *Dev Brain Res* 1991;61:97–101.
45. Kotloski R, Lynch M, Lauersdorf S, et al. Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. *Prog Brain Res* 2002;135:95–110.
46. Velisek L, Moshe SL. Effects of brief seizures during development. *Prog Brain Res* 2002;135:355–64.
47. Cole AJ, Koh S, Zheng Y. Are seizures harmful? What can we learn from animal models? *Prog Brain Res* 2002;135:13–23.
48. Holmes GL, Khazipov R, Ben-Ari Y. Seizure-induced damage in the developing brain: relevance of experimental models. *Prog Brain Res* 2002;135:321–34.
49. Levin ED. Use of the radial-arm maze to assess learning and memory in rodents. In: Buccafusco JJ, ed. *Methods of behavioral analysis in neuroscience*. Boca Raton, FL: CRC Press, 2001:189–99.
50. McDonald RJ, White NM. Hippocampal and nonhippocampal contributions to place learning in rats. *Behav Neurosci* 1995;109:579–93.
51. Gerlai RT, McNamara A, Williams S, et al. Hippocampal dysfunction and behavioral deficit in the water maze in mice: an unresolved issue? *Brain Res Bull* 2002;57:3–9.
52. Ribak CE, Navetta MS. An immature mossy fiber innervation of hilar neurons may explain their resistance to kainate-induced cell death in 15-day old rats. *Dev Brain Res* 1994;79:47–62.