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A single pentylenetetrazole-induced clonic-tonic seizure episode is accompanied by a slowly developing cognitive decline in rats

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ABSTRACT

According to different studies, between 5% and 10% of people suffer a single isolated seizure episode at some time in their life. However, little is known about the effects of a single seizure episode on cognitive function, and clinical investigations of this issue are not easy to perform. In this situation, animal models may be a reasonable choice. The aim of our study was to follow the time course of delayed effects of generalized clonic-tonic convulsions on learning and memory functions in rats. A clonic-tonic seizure episode was induced by a single i.p. injection of pentylenetetrazole (70 mg/kg). Different behavioral tests were performed between days 10 and 100 after the convulsant administration. A single seizure episode resulted in a gradual decline in short-term memory function as assessed by novel object recognition and social recognition tests. The seizure episode induced a quick increase in hippocampal cell proliferation; however, the excessive newly generated cells seemed to be eliminated by the time of obvious cognitive impairment. These observations are indicative of a slowly developing and long-lasting influence of a single seizure episode on cognitive function. A rather long time period between the seizure episode and the manifestations of cognitive decline provides a window for a possible therapeutic intervention, and an elaboration of such "post-conditioning" treatments may be a promising opportunity to prevent subsequent mental impairments in patients.

1. Introduction

According to different studies, between 5% and 10% of people suffer a single isolated seizure episode at some time in their life. Generalized tonic-clonic seizures may occur in people of any age and, if occurring repeatedly, be a part of a chronic condition — epilepsy. Cognitive deficits are a well-known complication that often accompanies chronic epilepsy in humans [5,6]. Recently, it was shown that memory impairments arise in children suffering from idiopathic generalized epilepsy with rare convulsions and without any detectable cortical pathology [14]. Similarly, impaired functions of learning and memory were reported in different animal models of acute and chronic seizures [36]. The information on possible cognitive impairments in patients with new-onset epilepsy is limited [54], and clinical studies of this issue are methodologically difficult to perform.

Animal models provide a good opportunity to investigate cognitive functions after a single seizure episode, while the data of such experiments may have a predictive clinical value. Impaired functions of learning and memory were demonstrated in mature rodents after severe seizures in models of pilocarpine- or kainate-induced status epilepticus or pentylenetetrazole (PTZ) kindling; this cognitive decline was accompanied by neurodegeneration and neuronal loss [4,34,50]. Cognitive deficits after seizures could be easily attributed to neuronal damage and loss that develop as a result of excitotoxicity. However, there is some evidence on cognitive dysfunction that appears even in the absence of evident neuronal death. Recently, Assaf and colleagues [2] have demonstrated a mild cognitive decline in adult mice 3–5 weeks after a single episode of PTZ-induced status epilepticus, a model of temporal seizures apparently not influencing neuronal density in the hippocampus or temporal cortex [28,29,45,52]. The delayed manifestations of cognitive impairment after seizures that were reported in their article suggest that they develop independent of neuronal degeneration or death that, if they occurred, would take place earlier. However, it still remains unknown whether the observation made by Assaf et al. [2] is a general phenomenon typical for other animals as well. Also, the time of cognitive decline appearance and its time course remain to be investigated in detail.

Obviously, a long-lasting effect of mild seizure activity on brain function suggests a contribution of structural reorganization of some

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kind. It is known that structural plasticity is attributed to adult hippocampal formation to a greater extent than to any other regions of the brain. Besides the remodeling of dendritic and axonal branches and synaptic reorganization, plasticity in the hippocampus is also maintained by ongoing neurogenesis in the dentate gyrus (DG) throughout the entire life of the animal (at least in rodents and humans) [39]. The proliferation and maturation of neural precursors in the DG are processes that are highly sensitive to a variety of factors ranging from learning and physical exercise to brain insults and seizures (see Reference [1] for a review). In animals, seizure activity was shown to increase the level of adult neurogenesis. However, there is a large body of evidence showing that seizure-induced neurogenesis is characterized by a variety of pathological features: altered polarity of newborn granule neurons, mossy fiber sprouting, ectopic migration into the dentate hilus instead of the granule cell layer, abnormal pattern of electric activity, etc. (for a review see Reference [56]). These findings assume that new granule cells born in the DG after seizures may impair the processing of information within the hippocampal neuronal circuits and thus deteriorate hippocampus-dependent functions even in the absence of evident neuronal death. This point of view was supported by the findings of Jessberger et al. [26] who demonstrated the prevention of cognitive damage if hippocampal neurogenesis was suppressed after seizures. Later, the same group demonstrated partially improved performance in several hippocampal-dependent tasks after transient genetic ablation of neurogenesis in rats [25]. Taken together, these data imply the participation of seizure-induced aberrant neurogenesis in the development of cognitive impairments after seizures.

According to this assumption, we aimed to investigate the following issues: i) to check whether a single episode of PTZ-induced generalized clonic-tonic seizures is accompanied by cognitive decline in rats, ii) to evaluate the temporal pattern of developing cognitive deficits in rats using different memory paradigms, and iii) to trace the impact of PTZ-induced convulsions on the proliferation and survival of neural precursor cells in the DG.

2. Materials and methods

2.1. Animals

Adult (2 months old at the beginning of the experiment) male Wistar rats (n = 62) weighing 200 ± 15 g were used in the study. The animals were housed in standard laboratory conditions (12-h light–dark cycle, controlled temperature) with free access to food and water. All animals were handled for at least four days prior to experiments in order to minimize stress influence on experimental results. Experiments were performed in accordance with the European Communities Council Directive (86/609/EEC) for the care and use of animals for experimental procedures and approved by the institutional ethical committee. All efforts were made to minimize animal suffering.

2.2. Assessment of seizures

All rats were randomly divided into two groups: control group (n = 32) and experimental group (n = 30). Experimental animals received one intraperitoneal injection of the chemoconvulsant pentylenetetrazole (PTZ, 70 mg/kg, dissolved in isotonic saline) to stimulate seizures. The animals of the control group received isotonic saline solution instead. Immediately after PTZ injection, the rats were placed in isolated Plexiglas cages and observed for 20 min. The intensity of the convulsions was rated according to a 5-point scale: stage 0 — no response, stage 1 — ear and vibrissae tremor, stage 2 — convulsive waves along the body axis, stage 3 — myoclonic seizures with rearing, stage 4 — clonic-tonic seizures with loss of posture (falling), and stage 5 — generalized tonic extension [12].

2.3. Behavioral tests

Starting from day 10 after PTZ (n = 22) or saline injection (n = 20), the rats were used in behavioral experiments assessing short-term memory function by means of different paradigms: novel object recognition (NOR) test, social recognition (SR) test, and radial arm maze (RAM) test.

2.3.1. Novel object recognition test

We used a white plastic box (60 × 35 × 20 cm) as an experimental cage. One day before the test, the animals were allowed to freely explore the empty experimental cage for 5 min. On the day of the experiment, each animal was placed in the center of the experimental cage with two identical objects located 10 cm apart from the walls of the cage, and the behavior was observed for 3 min (probe session). Then, the animal was returned back into its home cage for 30 min. During the test session, the animal was again placed into the experimental cage, with one of the objects randomly replaced by a new one, and again allowed to explore the cage and the objects for 3 min. The time spent to explore each object (during both the probe session and the test session) was measured. The index of discrimination between the new and the old objects in the test session was calculated as: $(T_{new} - T_{old}) * 100\% / (T_{new} + T_{old})$.

2.3.2. Social recognition test

The animals were isolated in individual cages for 1 week in order to enhance their social motivation. The experiment consisted of two phases, probe and test, 5-min each, with a 30-min interval between them. During each session, the same juvenile male rat (~1 month) was placed into the home cage, and the time spent by the resident rat to examine the guest was measured. The index of social recognition was counted as the ratio of the time spent in the test (second) session to the time spent in the probe (first) session.

2.3.3. Radial arm maze test

The animals were food deprived one week before the experiment and kept at the level of 80% of their initial body weight during the five weeks of training in a radial arm maze. The maze consisted of a center platform (20 × 20 cm) and eight arms (15 × 50 cm), all made of opaque dark Plexiglas. The maze was elevated 1 m above the floor. Four arms of the maze were baited with a food pellet. The location of the baited arms was randomly chosen for each animal and remained unchanged during the whole experiment. Each animal was allowed to explore the maze for 5 min or until all food pellets were taken. Each animal performed one trial per day and 5 trials per week (Monday–Friday). The mistakes of working memory were counted during each trial as the number of arms visited again after the food pellet was taken and averaged for 5 days (for a week).

2.3.4. Elevated plus maze

To check the possible effect of a single PTZ-induced generalized convulsion on the degree of anxiety and exploratory/locomotor activity, the animals were additionally tested in an elevated plus maze. The Plexiglas maze was elevated 1 m above the floor level. It consisted of four equally spaced 40-cm long arms radiating out from a central square measuring 10 × 10 cm. Two opposing arms were enclosed by 20-cm high opaque walls from all sides except the side adjacent to the central square. Two other arms were exposed; the outer rim of the open arms was guarded by a perimeter border of 1 cm. The animal was placed in the center of the maze facing the open arm, and, within 5 min, the following parameters were recorded: time before leaving the central square (start latency), total time conducted in the closed and open arms, number of entries into the closed and open arms, number of rearings, and number of squares crossed. The maze was cleansed with 70% ethanol between trials.