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Morphological and behavioral consequences of recurrent seizures in neonatal rats are associated with glucocorticoid levels

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Abstract: Objective It is well documented that epilepsy can increase neurogenesis in certain brain regions and cause behavioral alternations in patients and different epileptic animal models. A series of experimental studies have demonstrated that neurogenesis is regulated by various factors including glucocorticoid (CORT), which can reduce neurogenesis. Most of studies in animal have been focused on adulthood stage, while the effect of recurrent seizures to immature brain in neonatal period has not been well established. This study was designed to investigate how the recurrent seizures occurred in the neonatal period affected the immature brain and how CORT regulated neurogenesis in immature animals. **Methods** Neonatal rats were subjected to 3 pilocarpine-induced seizures from postnatal day 1 to day 7. Then neurogenesis at different postnatal ages (*i.e.* P8, P12, P22, P50) was observed. Behavioral performance was tested when the rats were mature (P40), and plasma CORT levels following recurrent seizures were simultaneously monitored. **Results** Rats with neonatal seizures had a significant reduction in the number of Bromodeoxyuridine (BrdU) labeled cells in the dentate gyrus compared with the control groups when the animals were euthanized on P8 or P12 ($P < 0.05$); whereas there was no difference between the two groups on P22. Until P50, rats with neonatal seizures had increased number of BrdU-labeled cells compared with the control group ($P < 0.05$). In Morris water maze task, pilocarpine-treated rats were significantly slower than the control rats at the first and second day, and there were no differences at other days. In probe trial, there was no significant difference in time spent in the goal quadrant between the two groups. Endocrine studies showed a correlation between the number of BrdU positive cells and the CORT level. Sustained increase in circulating CORT levels was observed following neonatal seizures on P8 and P12. **Conclusion** Neonatal recurrent seizures can biphasely modulate neurogenesis over different time windows with a down-regulation at early time and up-regulation afterwards, cause persistent deficits in cognitive functions of adults, and increase the circulating CORT levels. CORT levels are related with the morphological and behavioral consequences of recurrent seizures.

Keywords: epilepsy; development; cell proliferation; learning; memory; glucocorticoid

1 Introduction

Seizures occur more frequently in the neonatal period than at any other time in life. In addition to the increased risk for epilepsy in children, seizures during early development stage may be more detrimental than those occurring during adulthood[1]. In mature rats, animal studies have confirmed that single episode of status epilepticus induced by pilocarpine[2], kainic acid[3] and kindling[4] can increase cell proliferation in the subgranular zone of the dentate gyrus, cause neuronal loss in the hippocampus, and lead to aberrant sprouting of mossy fibers in the pyramidal cell region of CA3 and the supragranular zone of dentate gyrus. In immature rat, previous researches[5-6] have manifested that a prolonged seizure resulted in more neurogenesis but less cell loss and sprouting than it did in adult. However, other studies have demonstrated that neonatal seizures resulted in reduced neurogenesis[7-8]. So, it is controversy regarding the consequences of neonatal seizures.

Neurogenesis in the adult brain is now well established since its first report 40 years ago[9]. Two regions, the subventricular zone and the dentate gyrus of the hippocampus,

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Article ID: 1673-7067(2007)02-0083-09
CLC number: R742.1
Document code: A
Received date: 2006-07-31

are known to produce new neurons throughout life[10-11]. The hippocampus is a structure involved in epileptogenesis and learning[12], and such functions have raised the possibility that new-born neurons may play crucial roles in epileptogenesis and learning[13].

Neurogenesis is not constant. It is regulated by many factors such as seizure[14], exercise[15], neural injuries[16], alcohol[17], stress[18], etc. Many reports[19,20] have demonstrated that granule cell progenitors were numerous in rats during the first postnatal week, a time when adrenal steroid levels were low. The levels of adrenal-derived steroids glucocorticoids (CORT, cortisol in human or corticosterone in rodents) appear to regulate the rate of neurogenesis. CORT enter the brain through the blood brain barrier, binding to two types of CORT receptors—kidney mineralocorticoid receptor and glucocorticoid receptor—which are expressed prominently in the hippocampus. It is well known that CORT suppresses neurogenesis and impairs hippocampus-dependent learning in adult animals, but there is little information about the effect of CORT on the neurogenesis and behavior in immature rats, especially those experienced recurrent seizures ones. In the current study, we assessed neurogenesis and behavior in the immature rats that had neonatal seizures at different time points with emphasis on the relationship among CORT, neurogenesis and behavior. The results described here would deepen our understanding about the consequences of neonatal seizures and the reasons responsible for it.

2 Materials and methods

2.1 Animals and housing Neonatal Wistar rats were obtained from the Experimental Animal Center of Shandong University. The birth date was designated as postnatal day 0 (P0). Animals were housed with their littermates with *ad libitum* accessed to food and water till weaning on P21, when they were group-housed in the plastic cages with a standard 12/12 h light/dark cycle (light on 8:00 am to 8:00 pm). The attempts were made to minimize the number of animals used. In all experiments, littermates were randomly assigned to either the experimental or the control groups. Experimental or control animals remained together with their mother until weaning.

2.2 Induction of seizure Pilocarpine (Sigma, St. Louis, MO, USA) was dissolved in distilled water at a concentration of 100 mg/mL and administered intraperitoneally (i.p.) to the experimental neonatal rats on P2, P4 and P7 at the dosage of 350 mg/kg. All animals received scopolamine (1 mg/kg, i.p.) 30 min before administration of pilocarpine to limit peripheral cholinergic effects. Animals were monitored throughout seizure induction, and seizure severity was assessed according to the behavioral seizure scale summarized by Lado *et al*.[21]: stage 0, behavioral arrest; stage 1, mouth clonus; stage 2, head bobbing; stage 3, unilateral forelimb clonus; stage 3.5, alternating forelimb clonus; stage 4, bilateral forelimb clonus with rearing; stage 5, bilateral forelimb clonus with rearing and falling over; stage 6, wild running and jumping with vocalization; and stage 7, tonus. If animals did not show obvious signs of seizure 30 min after pilocarpine administration, an additional dosage of 10 mg/kg pilocarpine was administered to the rat every 10 min until onset of seizure. After acute seizure, the activities of animals such as the onset of spontaneous seizure or death from seizure were observed between 8:00 am and 8:00 pm. The decision to record only the activities during diurnal period was based on the previous reports that spontaneously recurring seizures were most prevalent in the light period in the pilocarpine model[22]. Control rats were given an equal volume of saline.

2.3 BrdU staining BrdU (Sigma), a thymidine analog that incorporated into dividing cells during DNA synthetic phase (S-phase) of the cell cycle, was dissolved in 0.9% NaCl and sterile-filtered. Rats received only once BrdU injection (100 mg/kg, i.p.). A single BrdU injection paradigm was chosen to ensure that the population of labeled cells would come from the same time frame, and thus all newborn cells were in the same developmental phase.

2.4 Experimental procedures Sixty-four male rats [experimental (exp), $n = 40$ rats; control (con), $n = 24$ rats] were used for time course study of neurogenesis and behavioral change in immature rats. Five rats of the experimental group died after three seizures, thirty-two rats were selected from the live rats of the experimental group for the further study. None of the rats in the control group died. Experimental rats were stochastically divided into four groups (groupI, groupII, groupIII, groupIV, $n = 8$ rats for each group) and received BrdU injection on P7, P11, P21 and P49 respectively (each time point, $n = 6$ rats for con group). The animals were euthanized 36 h after BrdU injection. The blood samples were taken via tail bleeding method before euthanasia. The groupIV and its control group were tested for spatial memory by Morris water maze since P40.

2.5 Immunohistochemistry for BrdU Animals were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) 36 h after BrdU injection, then perfused transcardially with 100-200 mL of heparinized saline, followed by 100–200 mL of 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4). After perfusion, rats were put in 4 °C refrigerator overnight. The following day, the brains were removed and placed in 20% sucrose overnight. The sections were sliced at 20 μm through the entire dentate gyrus with an oscillating tissue slicer in cryostat and mounted on a gelatin-coated slide for BrdU labeling.

For DNA denaturation, sections were incubated in the 4 mol/L HCl for 30 min at 37 °C, then in 0.1% trypsin for 10 min at 37 °C. After washing in the 0.01 mol/L PBS, the sections were incubated with a primary antibody to BrdU (mouse monoclonal, 1:500, Sigma) overnight at 4 °C. The sections were washed again with PBS for three times, then incubated with a secondary antibody conjugated to fluorescein isothiocyanate (FITC, goat anti-mouse IgG, 1:80, Beijing Zhongshan, China) for 60 min at 37 °C. Finally, the sections were washed with PBS for several times and coverslipped with glycerol:PBS (1:9). Fluorescence was visualized with Olympus BX51 fluorescence microscope.

2.6 Blood sampling All blood samples were collected between 9:00 am and 10:00 am before the rats were euthanized. After collection, the blood samples (1 mL) were immediately transferred to EDTA tubes. Samples were then centrifuged at 4 °C at 3 000 r/min for 10 min. The plasma fraction was extracted and stored at –80 °C until assayed for CORT concentration by RIA kit (Diagnostics Products Co., Los Angeles, CA, USA) following the manufacture's instruction.

2.7 Morris water maze On P40, rats of the groupIV and control group were tested in the water maze for 4 d. A stainless-steel circular swimming pool (117 cm in diameter, and 50 cm high) was filled with water to a depth of 25 cm. Two hundred milliliters of evaporated milk was added to make the water opaque and to prevent the platform from being visualized. Four points on the rim of the pool were designated as north (N), south (S), east (E), and west (W), thus dividing the pool into four quadrants (NW, NE, SE, SW). An 8 cm × 8 cm plexiglass platform, onto which the rat could escape, was positioned in the center of one of the quadrants, 1 cm below the water surface.

One day before testing, each rat was placed in the pool for 60 s without the platform present. This free swim enabled the rats to become habituated to the training environment. On day 1–4, rats were trained for 24 trials (6 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 among N, E, S and W in a quasi-random order for the 24 trials, so that the rat was unable to predict the platform location from the point that it was placed into the pool. The latency from immersion into the pool to escape onto the platform was recorded for each trial. If a rat did not find the platform in 120 s, it was manually placed on the platform for a 30-s rest. On day 5, the platform was removed. Rats were allowed 60 s of free swimming. The time spent in the quadrant, where the platform was previously located, was measured (probe trial), which was considered to assess memory for platform location.

2.8 Cell counting and data analysis At least 10 sections per animal were analyzed in an area encompassing the entire granule cell layer and extending approximately one to two cell layers width into the hilus. For computer-assisted counting, images were magnified 100-fold. And images were captured digitally by a monitor and analyzed using an image analysis system (Image Pro; Media Cybergenics, Silver Spring, MD, USA). Significant differences between the control and experimental groups for the BrdU-labeling studies were determined by one-way and two-way analyses of variance (ANOVA). The Person product moment correlation coefficient (r) was calculated for comparing the numbers of BrdU labeled cells with endogeneous CORT levels. Other between-group comparisons were made using Student's t test. SPSS13.0 software for the Windows was used for statistical treatment. Significance level was taken as $P < 0.05$.

3 Results

3.1 Behavioral effects of the seizures The experimental rats had different manifestations at different seizure times. At 5-15 min after the first pilocarpine injection, neonatal rats showed hyperactivity, vigorous lateral head shaking followed by squealing, agitation, crawling, bilateral but asynchronous running movements of the legs, ataxia, and intermittent extension of the hindlimbs; the mean latent time to onset of seizure was (13.4±2.3) min. At 5–15 min after the second pilocarpine injection, rats showed mechanical chewing, nodding, tonic extension of both the forelimbs and hindlimbs; the mean latent time was (10.3±1.5) min, 2 rats died. At 5–10 min after the third pilocarpine injection,

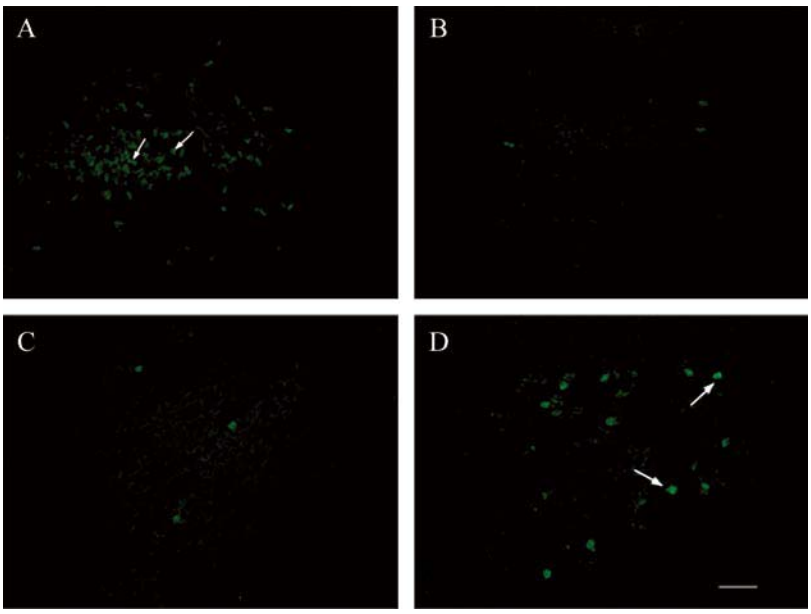

Fig. 1 Effects of recurrent pilocarpine-induced seizures on the neurogenesis of dentate gyrus in the rats with recurrent seizures (B, D) and the controls (A, C). A, B: Suppression of cell proliferation by recurrent pilocarpine-induced seizures on P8; C, D: increased neurogenesis occurred in the rats with recurrent seizures on P50. The arrows in A point toward the new born cells in the hilar of hippocampus. The arrows in D point toward the new born cells in the dentate gyrus. Scale bar, 50 μm.

rats showed peripheral choline reaction at first, then showed mechanical chewing, nodding, facial spasm, squealing, labored breathing, and some rats progressed to wild running followed by tonic extension of both the forelimbs and hindlimbs and then clonic activity. All rats reached 3.5–7 stage, and mean latent time was (7.1±1.2) min. Three rats died from seizures. Mortality rate in the control rats was 0. Rats with recurrent seizures had a mortality rate of 5/40 (12.5%). In all experimental groups, rats that died typically demonstrated wild running followed by a prolonged tonic period with apnea. Rats did not show spontaneous seizure during 42 d after the third seizure. None of the controls showed seizure activities.

3.2 BrdU labeling The number of BrdU-labeled cells in the control group decreased in an age-dependent manner. Statistical analysis confirmed significant differences in the

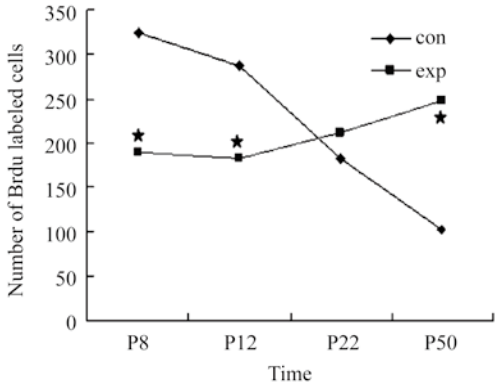

Fig. 2 Number of postnatal BrdU-labeled cells in the dentate gyrus of the experimental rats and the age-matched controls. In the control rats the number of BrdU-labeled cells decreased in an age-dependent pattern. In experimental rats the number of BrdU-labeled cells decreased at early time point (P8, P12) and increased at later time point (P22, P50). Con, control; exp, experimental. ★ $P < 0.05$ *vs* control.

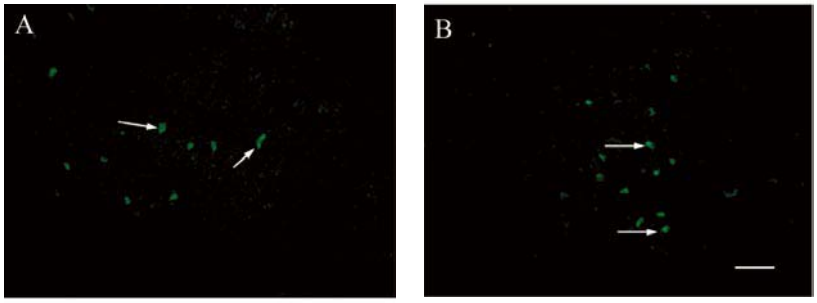

Fig. 3 Examples of BrdU-labeled sections of the subiculum (A) and the entorhinal cortex (B) in the rats after recurrent seizures. The arrows point toward the new born cells. Scale bar, 50 μm.

mean values of BrdU-labeled cells among different ages in the control group ($F = 22.36$, $P < 0.01$). In the control rats, BrdU-labeled cells were small in size, highly abundant, and randomly dispersed throughout the hilus and dorsal and ventral blades of the dentate gyrus on P8 (Fig. 1A). Two-way ANOVA revealed that the mean values of BrdU-labeled cells in control and experimental group were significantly different after allowing for difference in the time ($F = 6.07$, $P < 0.05$). BrdU-labeled cells in the dentate gyrus decreased significantly in the experimental group compared with the matched controls on P8 (Fig. 1B) and P12 (Fig. 2) ($P < 0.05$); while there was no difference between the experimental and control groups on P22 ($P > 0.05$). On the contrary, BrdU-labeled cells that became larger and well organized in the granule cell layer (GCL) and hilus increased significantly in the experimental group compared with the matched controls on P50 (Fig. 1D, $P < 0.05$). In addition, in the experimental group of P22 and P50, BrdU-labeled cells were also located in subiculum (Fig. 3A) and entorhinal cortex (Fig. 3B).

3.3 CORT measurements Plasma CORT levels were measured at different time points after the last seizure. In the control animals, basal CORT levels were low on P8, increased slightly on P12, then increased sharply on P22, and declined on P50 (Fig. 4). CORT plasma levels were negatively correlated with the number of BrdU-labeled cells as a function of age ($r = –0.85$, $P < 0.05$) (Fig. 5). CORT levels remained significantly higher than the baseline 1 d and 5 d after the third seizure (Fig. 4). On P22 and P50, the CORT level of the experimental group was similar to that of the control. Elevated levels of CORT after recurrent perinatal seizures on P8 or P12 were significantly correlated with the decline of the number of BrdU-labeled cells ($r = –0.68$, $P < 0.05$) (Fig. 6). Therefore, the postictal elevations of CORT level in total circulating plasma were associated with the suppression of cell proliferation induced by the earlier neonatal seizures.

3.4 Morris water maze All rats learnt to find platform. When comparing the performance of the rats on the Morris water maze during all four testing days, the rats with recurrent seizures in neonatal period were slower to reach platform than the control rats. But only at the first and second day, there were differences between the experimental group and the control (Tab. 1). In probe trial there was no significant difference in time spent in the goal quadrant between the experimental group and the control (Fig. 7).

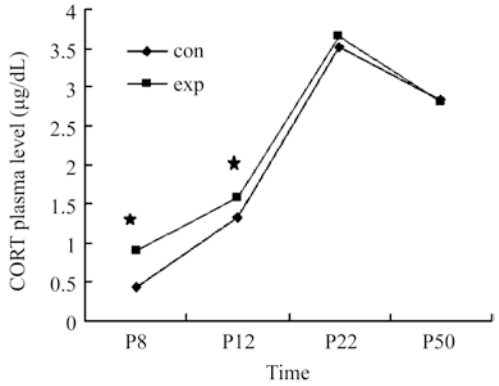

Fig. 4 Plasma glucocorticoid (CORT) levels in the experimental groups after recurrent seizures and the age-matched controls. In the control rats, plasma CORT levels elevated as a function of age, sharp rise occurred on P22. In the experimental rats, CORT levels elevated after recurrent seizures and acute increases were sustained for a prolonged period until P12. con, control; exp, experimental. ★$P < 0.05$ *vs* control.

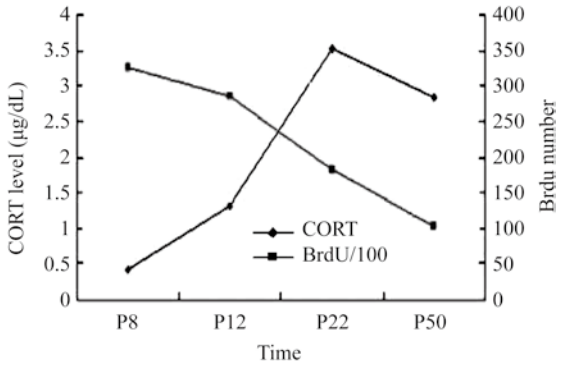

Fig. 5 Number of BrdU-labeled cells and plasma CORT levels (μg/dL) in the control rats. The number of BrdU-labeled cells negatively correlated with plasma CORT levels as a function of age ($r = –0.85$, $P < 0.05$).

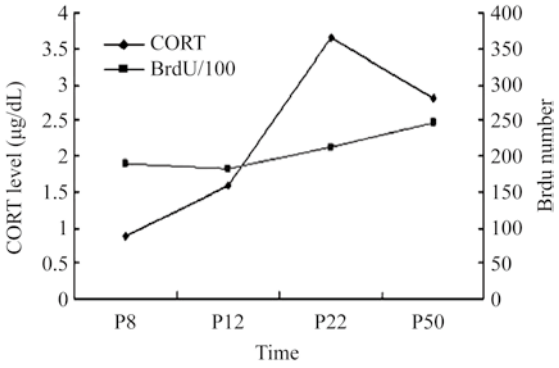

Fig. 6 Number of BrdU-labeled cells and plasma CORT levels (μg/dL) in the experimental rats. The CORT levels after recurrent perinatal seizures on P8 or P12 were negatively correlated with the number of BrdU-labeled cells significantly ($r = –0.68$, $P < 0.05$). The number of BrdU-labeled cells at later time point (P22 and P50) was not significantly correlated with CORT levels.

Tab. 1 Mean latencies across trials in the Morris water maze task

	Mean latency (mean ± SD, s)		*t*	*P*
	exp (n = 8)	con (n = 6)		
1 d	56.8±9.3	42.2±5.8	3.379	0.005
2 d	40.3±6.4	29.3±6.2	3.236	0.007
3 d	29.9±5.3	27.8±4.3	0.813	0.432
4 d	24.8±5.2	23.6±6.7	0.387	0.705

exp: experimental; con: control

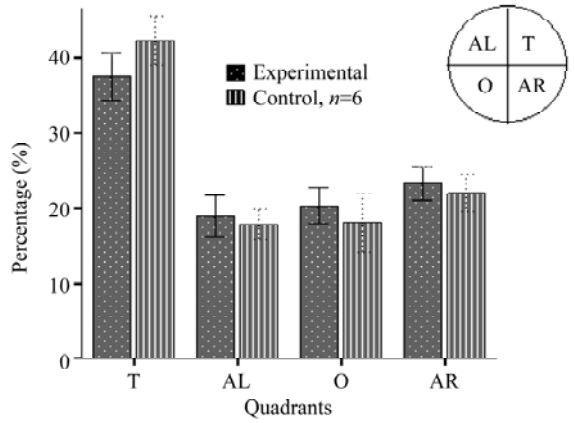

Fig. 7 Effect of pilocarpine-induced seizures on the probe trials of Morris water maze performance. Each graph shows the mean percentage of total time swimming in each quadrant for control and experimental rats. T, target; AL, adjacent left; O, opposite; AR, adjacent right.

4 Discussion

This study systematically evaluated effect of recurrent seizure on neurogenesis and plasma CORT level in neonatal rats at different time points, as well as the hippocampal-dependent learning when the rats matured. Since dead rats can not be perfused, the pathology change was not analyzed. The principal findings of this study include: (1) neurogenesis of normal rat changed in an age-dependent manner, *i.e.*, new born cells decreased while aging; (2) recurrent seizures in neonatal period resulted in a subsequent decrease in neurogenesis in the dentate gyrus and hilus of hippocampus at early time points (P8 and P12), whereas resulted in an increase at later time point (P50); (3) the elevation of CORT levels remained for a long time after the earlier perinatal seizures; and (4) Morris water maze showed that the hippocampal-dependent learning was injured in rats with earlier perinatal seizures.

Dentate granule cells appear to be particularly vulnerable to postnatal insults, because the majorities of them are generated after birth[10]. Dentate granule cells begin to migrate into dentate gyrus on embryonic day 17, and by embryanic day 22 the dentate gyrus is present throughout the hippocampus[10]. It is now well established that the neurogenesis of dentate granule cell continues to adulthood, although the rate of neurogenesis declines significantly with the aging of the animal[23-24]. The progressive thickening of the granule cell layer of the dentate gyrus after birth is attributable to accumulation of neurons proliferating along subgranular layer.

The reduction of newly formed cells in the dentate gyrus and hilus was observed on 1 d and 5 d after the last seizure. The decreased neurogenesis seemed not to result from loss of neurons, because our previous study[25] has confirmed that recurrent seizures in neonatal rats did not cause obvious loss of neurons. The mechanism by which recurrent seizures resulted in reduced neurogenesis in our animals may be due to the elevated CORT level because there is an established inverse relationship between the endogenous surge of circulating CORT level and neurogenesis. Gould *et al*.[20] found that increased CORT level significantly decreased the cell birth in the dentate gyrus in the developing brain of rat. Similarly, adrenalectomy performed in adult animals stimulated progenitors and their progeny[26]. Previous study found that[7] the rats that had single or 5 flurothyl seizures on P4 had no difference in the number of BrdU-labeled cells comparing with the matched controls; while in the rats that had 10, 15, and 20 flurothyl seizures, BrdU-labeled cells decreased. The reason may be that cumulatively increased CORT was insufficient to induce reduction in BrdU cell count until more than five seizures were induced. Of course, CORT is not the only cause. Seizures in rat pups also trigger a cascade of glutamate. Some studies[27] have demonstrated that treating rat pups with NMDA receptor antagonist during the first postnatal week may increase the density of ^{3}H-thymidine-labeled cells in the dentate granule cell layer. However, all above studies can not directly explain why recurrent neonatal seizures resulted in an increase in dentate gyrus neurogenesis at later time point, and further studies are warranted for this open topic.

In our study, the CORT levels of experimental groups decreased on P22, similar to that of the control on P50. Interestingly, neurogenesis of experimental rats also slightly

increased on P22 and significantly increased on P50, but all above can not explain why the neurogenesis increased during this period, because CORT levels of the experimental groups were not lower than those of the controls. There must be some other mechanisms. One possible reason for the increased neurogenesis at later time point may be hypoxia. Just as we have demonstrated here, rat pups suffered from brief hypoxia during the tonic phase of the seizure. As such, seizure-induced hypoxia *per sue* over the first postnatal week may affect the neurogenesis. Kee *et al*.[28] found that ischemia resulted from hypoxia of cerebral hemisphere resulted in an increase in neurogenesis of dentate granule cells in adult rats. Furthermore, there may be a multitude of other biochemical changes occurring during the seizure process, which could alter the rate of neurogenesis. For example, several studies have demonstrated that neurotrophins are increased in some specific brain regions after seizures by using immunohistochemistry, *in situ* hybrizition, or biochemical assays. Kornblum *et al*.[29] found that seizures induced by lithium-pilocarpine or KA resulted in dramatic elevations of brain-derived neurotrophic factor mRNA in immature rats. It is well known that neurotrophins are important in the regulation of neurogenesis and may play a role in stimulating neurogenesis and increasing the survival rate of new born cells at later time points.

Additionally, we used the Morris water maze, a well-validated test of spatial learning and memory, to measure the hippocampal integrity. In this task, the rats with recurrent perinatal seizures spent more time on searching platform location than the controls did. The result adds to the accumulating evidence that seizures during early brain development could result in lifelong behavioral and cognitive deficits. That result may be caused by the elevated CORT levels induced by recurrent seizures, for a large body of literatures suggest that excess corticosterone is detrimental to hippocampal functions such as working and spatial memories[30-31]. Recently, it was confirmed that high levels of CORT could regulate all stages of neurogenesis. Elevated levels of CORT suppress the proliferation of progenitor cells in the dentate gyrus, reduce the survival of newly-formed cells[32], and inhibit the differentiation of the newly-formed cells into mature neurons[33]. Because new neurons in the hippocampus may be responsible for the formation of new memory[34], the detrimental effect of CORT on neurogenesis may account for the behavioral deficits which were observed in this study. Furthermore, many researchers[35-36] observed that elevated CORT can induce atrophy of the apical dendrites of hippocampal CA3 pyramidal neurons that appeared to retract their dendrite, which might have an impact on the total number of dendritic synapses. Impaired synapses would disrupt synapsin function. However, synapses and synapsin[37] play important role in hippocampus-dependent learning and memory. Therefore, the impairment of synapses and synapsin induced by elevated CORT may be another cause of the behavioral deficits. Besides, the rats exposed to neonatal seizures gained less weight than the control group, which may affect their performance in Morris water maze.

High CORT level was detrimental, and acute deprivation of plasma CORT (adrenalectomy) in adult rats evoked a burst of granule cell neurogenesis. Nevertheless, complete elimination of these hormones (by stopping hormone supplementation) was also detrimental[38]. Removal of CORT by adrenalectomy not only impairs hippocampal-dependent learning, but also causes dramatic cell death in the granule cell layer. These data indicate that the relationship between the CORT level and the granule cell neurogenesis is not simply inverse. Chronic modulation of CORT level commencing early in life evokes additional, adaptive, and compensatory mechanisms that contribute to the regulation of granule cell proliferation. Moreover, cortical hormone is well widely used to treat infantile spasm although the mechanisms remain unknown, which indicates that the postnatal limbic-hypothalamic-pituitary-adrenal axis (LHPA) in the immature rodent is different from the adult[39], and the relationship between cortical hormone and epileptogenesis is more complicated in immature rodent than in adult. So we should make more efforts to study the precise function of CORT in immature rats in the future.

In conclusion, we provided evidence that recurrent seizures in neonatal period resulted in a decrease in neurogenesis at early time point (P8 and P12), whereas resulted in an increase in neurogenesis at later time point (P50); and elevated CORT levels remained for a prolonged time after the earlier perinatal seizures. Morris water maze showed that hippocampal-dependent learning was injured in rats with earlier perinatal seizures. The differences between humans and animals in the brain development and sensitivity to the corticosteroid receptor make it difficult to extrapolate the results of the current study to humans

directly. Therefore, further investigation will involve gathering more information about clinical experiments, to regulate the CORT to the normal level and to examine whether the normal level CORT will diminish the adverse effect of earlier perinatal seizures in the brain.

Acknowledgments: This work was supported by the grant from the Doctoral Research Fund of Science and Technology Department of Shandong Province, China (No. 2004BS02015) . We thank Chun-Xi Liu, Ping Liu for their technical assistance with data collection and care of the animals during the study as well as Yan-Ling Wang for editorial assistance.

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新生期大鼠反复痫性发作的形态学及行为学结果与糖皮质激素水平升高有关

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摘要：目的 探讨新生期大鼠反复痫性发作后的形态学，行为学以及糖皮质激素水平的变化。**方法** 64只出生后一天的Wistar大鼠随机分为惊厥组40只和对照组24只。惊厥组的新生鼠在出生后1天(P1)、4天(P4)、7天(P7)给予腹腔注射匹罗卡品，制备新生鼠癫痫模型；对照组的新生鼠腹腔注射生理盐水。惊厥组分别在第3次致痫后在即刻(Ⅰ组)、第4天(Ⅱ组)、第14天(Ⅲ组)、第42天(Ⅳ组)四个时间点处死，各时间点设相应对照组，处死前36 h惊厥组和对照组的大鼠腹腔注射BrdU。所有大鼠处死前均取血检测糖皮质激素。第Ⅳ组从P40开始进行Morris水迷宫试验。**结果** 新生鼠3次发作后即刻和第4天与相应日龄对照组相比，齿状回BrdU阳性细胞数明显减少($P < 0.05$)，而癫痫发作后14天和42天BrdU阳性细胞数增加，但发作后14天差异无统计学意义($P > 0.05$)。在4天的Morris水迷宫试验中，匹罗卡品处理组大鼠到达平台的时间均长于对照组，但是只有第1天和第2天有统计学意义($P < 0.05$)。检测结果表明高水平的糖皮质激素一直持续到发作后第4天，糖皮质激素水平与BrdU阳性细胞数呈负相关。**结论** 新生大鼠反复痫性发作会造成早期神经发生减少，而后期神经发生增加；造成大鼠成年后认知功能缺陷；造成糖皮质激素水平增高，这与痫性大鼠形态学和行为学方面的改变有关。

关键词：癫痫；发育；神经发生；Morris水迷宫；糖皮质激素