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STATUS EPILEPTICUS-RELATED HIPPOCAMPAL INJURY IN THE IMMATURE RAT BRAIN

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Status epilepticus (SE)-related neuronal degeneration and glial activation in different regions of the developing rat hippocampus were investigated in an age- and time-dependent manner. Wistar rat pups of postnatal day (P) 7, 15, and 21 were injected i.p. with lithium+pilocarpine to induce SE or saline to make controls. Rats were sacrificed at 2, 12, 24 h, 3 days (d) and 7 d after SE induction. Neurodegeneration in the hippocampus was assessed by Fluoro-Jade B staining. The expressions of the astrocyte marker (GFAP) and microglia marker (Iba-1) were evaluated by immunohistochemistry. In P7 rats, there was no neuronal damage at any time points in SE. Two hours after SE induction, the number of degenerating neurons in the hippocampus significantly increased in the *CA1* region of P15 rats and in both *CA1* and *CA3* regions of P21 rats. Degenerating neurons in the dentate gyrus appeared at 24 h after SE in P15 and P21 rats. In P7 rats, there was no up-regulation of GFAP- or Iba-1-positive cells in SE. The expression of GFAP was dramatically elevated at 12 h in the *CA1* and *CA3* regions of P15 rats. The number of GFAP-positive cells did not increase in the dentate gyrus until 24 h after SE induction in P15 rats. In P21 SE rats, the mentioned index increased in the *CA1*, *CA3*, and dentate gyrus at 2 h. The number of Iba-1-positive cells increased significantly in the *CA1*, *CA3*, and dentate gyrus at 12 h in P15 rats and as early as at 2 h in P21 rats. These findings suggest that SE-related neuronal damage and glial activation in the immature brain are, in general, less intense than in the adult one, and the development of these processes in different structures of the hippocampus demonstrates significant temporal and spatial specificity.

Keywords: pilocarpine, epilepsy, hippocampus, gilal fibrillary acidic protein (GFAP), Iba-1.

INTRODUCTION

Status epilepticus (SE) is a life-threatening emergency inducing high mortality. After successful rescue, permanent brain damage and the risk for the epilepsy development are two main concerns. Neuronal death and glial activation are characteristic pathological changes after SE in the adult brain. Neuronal excitation during SE triggers glial activation via various extracellular signals such as glutamate, ATP, and IL-1в [1]. Activated glial cells might contribute to neuronal damage by releasing inflammatory factors. An immature brain is rather prone to seizure activity. It is, however, less vulnerable to irreversible seizure-induced damage, which indicates that the respective pathological changes are age-dependent.

In our study, we observed pathological changes at different time points in the immature rat hippocampus after induction of the SE, to find the manner in which neuronal degeneration and glial activation occur in the developing brain.

METHODS

Animals. Male Wistar rats of postnatal day (P) 7, P15, and P21 were purchased from the Shandong University. P7 and P15 rat pups were housed with their dams until weaning or sacrificed. P21 rats were maintained in quiet uncrowded facilities and given unlimited access to food and water. All rats were kept in a room at constant temperature (23°C) and humidity (60%) on a 12-h light schedule. Rats in each age group

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were divided into the SE and control groups.

Lithium+Pilocapine-Induced SE Model. All Wistar rats of the SE group were injected intraperitoneally (i.p.) with 127 mg/kg lithium chloride. After 18 to 24 h, P7, P15, and P21 rats were administered i.p. with pilocarpine at dosages of 120, 80, and 60 mg/kg, respectively. Atropine (1 mg/kg) was i.p. injected 30 min before pilocarpine. Animals of the control group were given with the same volumes of saline. Seizure activity was graded, as previously described, according to the following manifestations [2]: Mouth and facial movements corresponded to level I, head nodding, to level II, forelimb clonus, to level III, rearing with forelimb clonus, to level IV, and rearing and falling with forelimb clonus, to level V. Rats that exhibited grade-IV and V seizures were included in the experiment. Seizures could be terminated with 400 mg/kg i.p chloral hydrate. The pilocarpine dosages used led to approximately 20% mortality in rats of all groups. Finally, 50 rats were included in the SE group, and 25 rats were included in the control group. Both groups were further divided into subgroups and sacrificed at 2, 6, and 24 hours and 3 or 7 days after the SE induction.

Tissue Preparation. Rats of both groups were perfused transcardially with chilled PBS followed by 4% paraformaldehyde. The brains were taken off, manually dissected from the calvarium, and immersed in 4% paraformaldehyde for 24 h at 4°C before paraffin embedding. Embedded brains were sectioned coronally with a microtome into 5 mm-thick sections and collected on gelatin-coated microscope slides.

Fluoro Jade B Stain. The slides were first immersed in a solution containing 1% sodium hydroxide in 80% alcohol (20 ml of 5% NaOH added to 80 ml absolute alcohol) for 5 min. This was followed by a 2 min-long-stay in 70% alcohol and 2 min in distilled water. The slides were then transferred to 0.06% potassium permanganate for 10 min and rinsed in distilled water for 2 min. After 20 min in 0.0004% Fluoro Jade B staining solution, the slides were rinsed for 1 min in each of three distilled-water washes and then placed on a slide warmer set at approximately 50°C, until they were fully dry. Finally, the tissue was examined using an epifluorescent microscope with blue (450-490 nm) excitation light.

Immunohistochemistry. Hippocampal sections were incubated with diluted mouse anti-GFAP antibody (1:100; Millipore Corporation, USA) or mouse anti-Iba1 antibody (1:100; Epitomics Inc., USA) overnight at 4°C. Subsequently, they were exposed to HRP-conjugated anti-mouse antibody (1:200; ZSGB-BIO, China). After treatment with DAB (ZSGB-BIO, China), the slides were counterstained with hematoxylin and mounted with permanent mounting medium. Immunohistochemical staining was observed under a light microscope. An image-Pro Plus 6.0 set was used for quantitative analysis of GFAP- and Iba-1-positive cells.

Statistical Analysis. Numerical values were expressed as means ± s.d. for each group; SPSS 11.0 was used for all statistical analyses. The *t*-test was performed to compare the SE and control groups for Fluoro Jade B and immunohistochemistry; P values less than 0.05 were considered statistically significant.

RESULTS

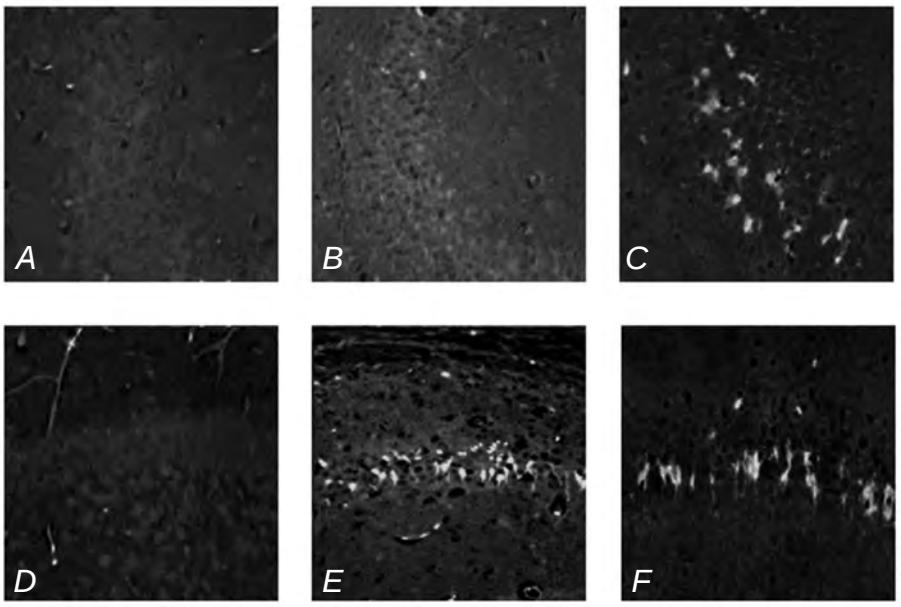
Fluoro Jade B. In the SE P7 age group, there were no positive cells at all time points. In the SE P15 group, there were some positive cells in the *CA1* zone at 2 h, 12 h, 24 h, 3d, and 7 d. No positive cells were seen in the *CA3* region in the P15 group at any time interval. There were positive cells in the dentate gyrus at 24 h, 3 d, and 7 d in P15 rats in the above status. In the SE P21 group, there were positive cells in the *CA1* and *CA3* zones at 2 h, 12 h, 24 h, 3 d, and 7 d. In SE P21 rats, positive cells were observed in the dentate gyrus only at 24 h, 3 d, and 7 d (Fig. 1; Table 1).

Immunohistochemistry. There was no significant difference in the number of GFAP-positive cells between the control and SE groups at all five time intervals in P7 rats. In SE P15 rats, the numbers of such cells in the *CA1* and *CA3* regions were significantly greater at 12 h, 24 h, 3 d, and 7 d after SE, while this index was higher in the dentate gyrus at 24 h, 3 d and 7 d. In the SE P21 group, GFAP-positive cells were more numerous at all time points in the *CA1*, *CA3*, and dentate gyrus (Fig. 2; Table 2).

In the P7 group, the number of Iba-1-positive cells did not differ significantly between control and SE groups at any time. Iba-1-positive cells were more numerous at 12 h, 24 h, 3 d, and 7 d in the *CA1*, *CA3* and dentate gyrus in the SE P15 group. The respective values were greater in P21 SE than in the control at 2 h, 12 h, 24 h, 3 d, and 7 d in the *CA1*, *CA3*, and dentate gyrus (Fig. 3; Table 3).

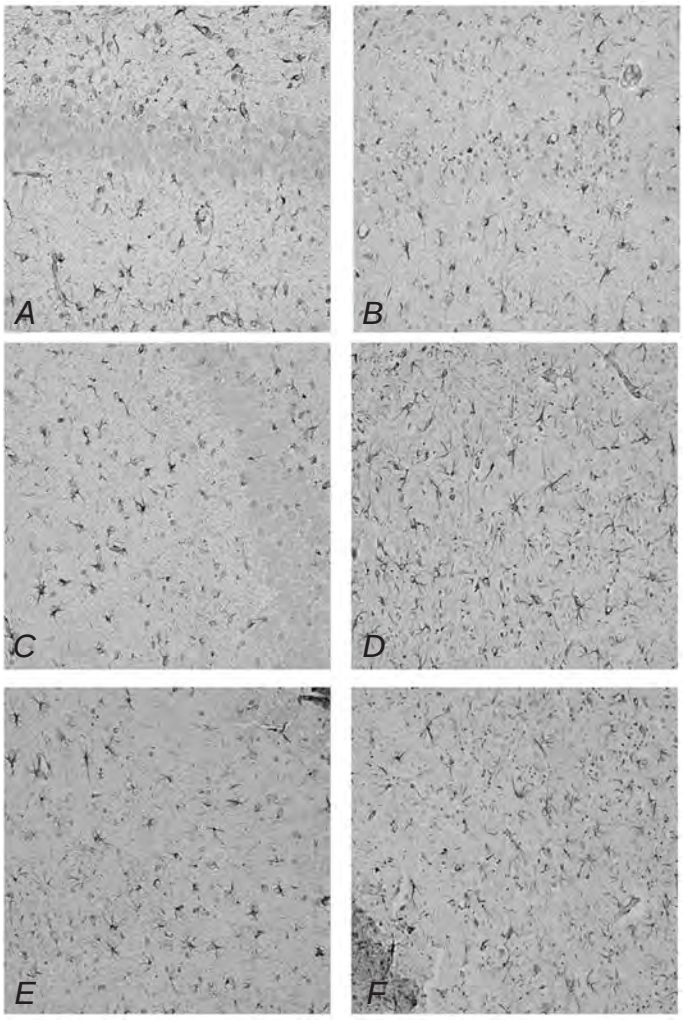
DISCUSSION

Available reports concerning the effects of *status epilepticus* on the immature brain were focused on cer-



F i g. 1. Fluoro Jade B staining at 24 h after SE. A) CA3 region in P7 rats, B) CA3 region in P15 rats, C) CA3 region in P21 rats, D) CA1 region in P7 rats, E) CA1 region in P15 rats, F) CA1 region in P21 rats.

Р и с. 1. Результати забарвлення гіпокампа Fluoro Jade B через 24 год після індукції епілептичного стану.



F i g. 2. GFAP staining in the hippocampus at 3 d after SE in P15 rats. A) CA3 region, control, B) CA3 region, group SE, C) CA1 region, control, D) CA1 region, group SE, E) dentate gyrus, control, F) dentate gyrus, group SE.

Р и с. 2. Результати імуногістохімічного забарвлення гіпокампа щодо GFAP через три доби після індукції епілептичного статусу у П15-щурят.

tain specific aspects such as recurrent seizures, EEG, neuronal death, and mossy fiber sprouting. It should be mentioned that studies of neuronal death and glial activation in this state are still patchy. In our experiments, we found that there was no SE-related neuronal damage or glial activation in the hippocampus of P7 rats. Degenerating neurons in the hippocampus appeared at 2 h after SE induction in P15 and P21 rats. Astrocytes and microglia were activated at 12 h after SE in P15 animals and at 2 h in P21 rats.

Results of Fluoro Jade B staining suggest that the neonatal brain is more resistant to SE-related neurodegeneration; significant changes could only be detected after postnatal day 15. There are several possible mechanisms that could explain different vulnerability to SE within different developmental stages. *Status epilepticus* produces neuronal damage mostly by excessive activation of glutamate receptors. Functional studies in the rat indicated that the glutamate receptor density appears to peak around P10 [3]. Thus, increased expression of functional glutamate receptors is likely to play a major role in increased vulnerability of the brain only after two weeks of life. Immediate early gene

TABLE 1. Density of Fluoro-Jade B-positive cells in the hippocampus in P15 and P21 rats

Т а б л и ц я 1. Щільність клітин, позитивних щодо Fluoro-Jade B, у гіпокампі щурят віком P15 та P21

	P15					P21				
Time after induction of the SE	2 h	12 h	24 h	3 d	7 d	2 h	12 h	24 h	3 d	7 d
CA1	7±2	14±3	17±4	18±3	19±4	12±2	19±4	20±6	22±5	23±6
CA3	Neg	Neg	Neg	Neg	Neg	11±1	14±3	18±4	19±5	21±3
DG	Neg	Neg	8±1	7±2	11±2	Neg	Neg	10±2	12±4	15±3

Footnotes. Numbers of positive cells (means ± s.d.) per 0.25 mm^2 area are shown. Neg corresponds to the absence of positive cells

TABLE 2. Density of GFAP-positive cells in rats of different ages

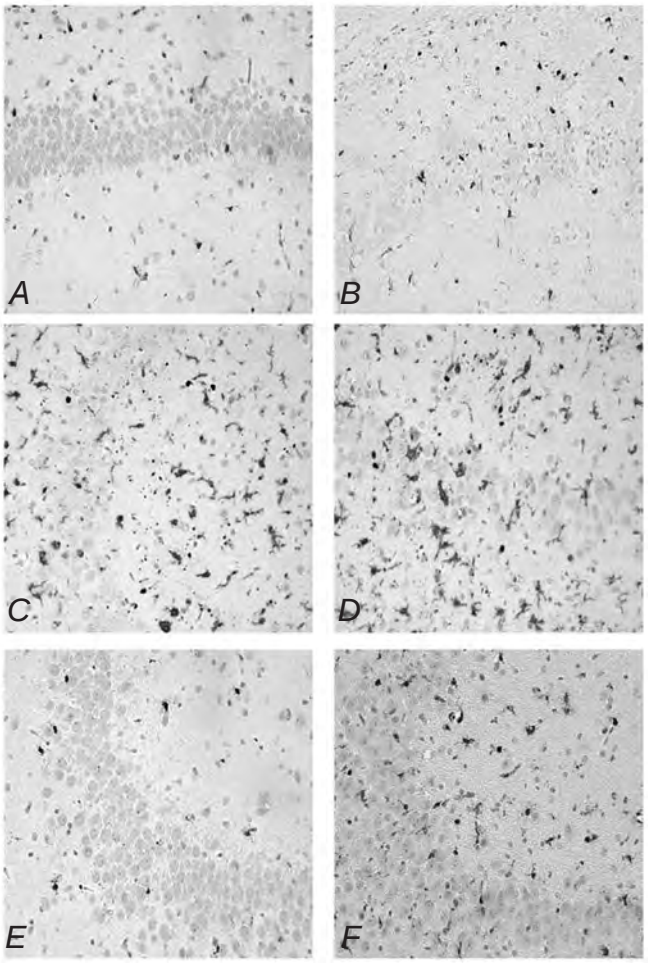
Т а б л и ц я 2. Щільність клітин, позитивних щодо GFAP, у щурят різного віку

Age	Region	Group	2 h	12 h	24 h	3 d	7 d
P7	*CA1*	Contr.	24.4±2.9	23.8±5.1	26.4±4.2	24.8±2.6	27.3±4.0
		SE	25.3±3.5	24.25±3.0	25.6±3.9	25.0±5.2	26.2±3.5
	CA3	Contr.	22.0±2.7	22.7±3.6	23.4±3.1	25.7±5.1	24.9±4.3
		SE	20.8±3.1	23.4±5.6	22.5±5.0	26.2±3.5	25.9±3.0
	DG	Contr.	27.8±2.3	29.5±4.6	30.6±5.0	34.7±3.7	35.0±3.3
		SE	26.8±2.2	31.1±3.7	32.3±4.5	35.7±4.1	32.3±3.0
P15	*CA1*	Contr.	27.4±4.1	28.6±3.4	28.1±4.3	29.6±4.4	30.1±4.2
		SE	29.7±4.3	37.6±2.6*	39.1±3.9*	40.3±3.0*	45.8±3.5*
	CA3	Contr.	25.2±4.5	26.8±3.7	27.6±3.8	29.6±3.3	30.5±4.0
		SE	24.5±3.0	34.5±3.5*	36.4±3.6*	44.3±4.2*	42.3±4.1*
	DG	Contr.	34.4±3.0	32.3±3.8	35.9±4.7	33.1±3.5	33.6±3.8
		SE	32.4±2.9	35.3±2.8	44.2±4.5*	49.8±3.1*	55.3±4.7*
P21	*CA1*	Contr.	29.1±4.0	30.0±3.6	31.2±3.5	32.8±4.0	35.6±4.2
		SE	36.5±4.2*	36.0±3.2*	39.2±4.5*	49.7±5.6*	40.3±4.0*
	CA3	Contr.	31.4±4.9	33.6±4.1	32.3±3.9	35.7±3.6	34.7±4.3
		SE	37.6±5.3*	39.5±3.5*	42.9±3.6*	47.6±5.1*	49.6±3.6*
	DG	Contr.	34.8±2.7	36.8±4.0	33.6±2.9	35.1±3.2	34.9±4.6
		SE	45.6±5.0*	44.2±4.3*	47.1±4.2*	46.4±5.5*	49.9±4.7*

Footnotes. Contr. and SE are controls and *status epilepticus* groups, respectively. Difference is significant compared with the control group ($P < 0.05$) in a specific region in each age group. Other designations are similar to those in Table 1.

responses could be determinants of neuronal survival; such responses in the immature and mature brains are dissimilar. For example, there is no *c-fos* transcription in the neonatal hippocampal and cortical structures after kainic acid (KA)-induced SE. At the same time, *c-fos* induction could be detected after postnatal day 13 [4]. Greater GABA synthesis [5] and less mitochondrial oxidative stress [6] under SE conditions in the immature brain are also possible mechanisms providing higher resistance of the immature brain to SE.

May be, a more important finding is that neurons in different regions of the hippocampus respond in a dissimilar manner to excitation signals. Neurons in the *CA1* and dentate gyrus degenerated after SE in both P15 and P21 rats, whereas the *CA3* region underwent neurodegeneration only in P21 rats. Intrinsic characteristics of hippocampal neurons could explain this age-dependent and region-specific mode of changes. Vulnerable neurons have higher expression of the genes related to stress. The *CA1* neurons in organotypic cultures maintained *in vitro* have a higher level of oxidative activity under baseline conditions when compared with the *CA3* neurons [7]. As was reported, extracellular potassium regulation was less effective in the *CA1* compared to the *CA3* region in immature rabbits after repetitive stimulation [8]; this is why the *CA1* region



F i g. 3. Iba-1 staining in the hippocampus at 3 d after SE in P15 rats. A) CA3 region, control, B) CA3 region, group SE, C) CA1 region, control, D) CA1 region, group SE, E) dentate gyrus, control, F) dentate gyrus, group SE.

Р и с. 3. Результати імуногістохімічного забарвлення гіпокампа щодо Iba-1 через три доби після індукції епілептичного статусу у П15-щурят.

is more vulnerable to such excitation. The neuronal excitability reaches the level typical of mature animals in the *CA1* region after postnatal day 14, while inhibitory processes did not reach such level even several weeks later [9]. The development of synaptic inhibition lag in the *CA1* compared to the *CA3* can explain preferential *CA1* damage in 2-week-old animals [10].

Not only neuronal degeneration but also glial activation are age-dependent. Microglia and astrocytes in the rat hippocampus were not activated until two weeks after birth. These gliocytes types were simultaneously activated at 12 h in P15 rats and at 2 h in P21 rats after SE induction, which means that microglia and astrocytes are involved in the pathological process in SE practically simultaneously. The precise mechanism of seizures-related glial activation remains unknown. After neuronal excitation, an increased extracellular K^+ concentration and various signals (such as glutamate, ATP, and calcitonin gene-related peptide) are possible factors triggering microglial activation [1, 11-13]. IL-1в, produced mainly by activated microglia,

TABLE 3. Density of Iba-1-positive cells rats of different ages

Т а б л и ц я 3. Щільність клітин, позитивних щодо Iba-1, у щурят різного віку

Age	Region	Group	2 h	12 h	24 h	3 d	7 d
P7	*CA1*	Contr.	7.8 ± 1.2	8.1 ± 1.3	9.2 ± 1.7	13.4 ± 2.1	16.9 ± 3.2
		SE	8.4 ± 1.8	8.2 ± 1.6	10.0 ± 2.7	15.2 ± 2.5	14.5 ± 1.9
	CA3	Contr.	8.7 ± 1.7	8.4 ± 1.4	10.0 ± 2.1	13.1 ± 2.3	13.6 ± 2.0
		SE	6.8 ± 2.0	7.9 ± 1.6	11.2 ± 2.8	13.8 ± 2.8	14.8 ± 2.1
	DG	Contr.	4.4 ± 0.9	6.9 ± 0.8	7.3 ± 1.1	9.7 ± 1.6	11.3 ± 1.5
		SE	5.1 ± 1.3	5.2 ± 1.0	7.7 ± 2.0	11.4 ± 2.0	10.0 ± 1.4
P15	*CA1*	Contr.	18.5 ± 2.6	23.8 ± 2.9	24.5 ± 3.0	29.4 ± 3.2	32.8 ± 3.9
		SE	19.4 ± 3.2	28.2 ± 3.3*	36.7 ± 3.7*	39.7 ± 4.2*	44.2 ± 4.6*
	CA3	Contr.	15.3 ± 3.1	18.7 ± 3.4	19.6 ± 4.0	22.6 ± 3.4	27.4 ± 2.7
		SE	16.7 ± 3.6	24.2 ± 2.9*	26.4 ± 3.0*	33.4 ± 2.4*	38.8 ± 4.0*
	DG	Contr.	11.7 ± 1.8	12.6 ± 1.4	18.7 ± 2.1	22.9 ± 2.4	27.3 ± 1.8*
		SE	13.1 ± 2.6	19.2 ± 3.3*	25.2 ± 3.4*	32.6 ± 2.6*	37.0 ± 4.8*
P21	*CA1*	Contr.	31.5 ± 2.4	35.6 ± 3.2	36.8 ± 3.5	37.4 ± 4.1	40.9 ± 4.6
		SE	39.0 ± 3.0*	46.3 ± 2.9*	49.1 ± 3.1*	53.9 ± 3.9*	58.1 ± 5.0*
	CA3	Contr.	25.5 ± 1.8	24.3 ± 2.1	26.7 ± 2.4	33.7 ± 3.5	34.3 ± 3.7
		SE	37.2 ± 3.7*	39.7 ± 3.8*	38.1 ± 2.3*	51.3 ± 3.0*	55.7 ± 3.6*
	DG	Contr.	28.9 ± 2.1	29.3 ± 2.6	35.5 ± 3.7	36.1 ± 4.2	39.4 ± 3.6
		SE	37.8 ± 2.4*	37.3 ± 2.6*	40.2 ± 2.1*	55.0±2.5*	59.6 ± 4.2*

Footnote. Designations are similar to those in Table 2.

is one of the most powerful inducers of reactive astrogliosis [14]. Swann et al. [10] reported that, after penicillin-induced epileptogenesis, the extracellular K^+ concentration in the *CA3* region of hippocampal slices taken from 9- to 16-day-old rats is higher than that in more mature rats (30-35 days old). This finding seems to be contradictory to our observation of quiescent glial cells in P7 rats. Oxidative substances from degenerating neurons are, however, also strong stimulators of glial activation, and this explains well the inactivated status of glial cells in the immature brain.

Furthermore, glial activation showed a region-specific pattern at different time points after SE. In our experiments, astrocytes were activated at 12 h in the *CA1* and *CA3* regions and later (at 24 h) in the dentate gyrus of P15 rats. Astrocytes in all three regions in P21 rats were activated 2 h after SE induction. Microglial cells were activated in the *CA1*, *CA3*, and dentate gyrus at 12 h after SE in P15 rats and at 2 h after such influence in P21 rats. Whether glia activation leads to neuronal death, or neuronal death results in long-term glial activation after SE, has not been clarified in previous studies. In our research, neuronal degeneration was detected earlier than glial activation in the *CA1* region in P15 rats, while glial cells were activated earlier than neuronal injury developed in the dentate gyrus in P21 rats. Microglia and astrocytes were all activated in the *CA3* region at 12 h after SE in P15 rats, but no degenerating neurons were observed at any time point in this region. According to this time-sequence analysis, we suppose that the cause/effect relationship between neuronal death and glial activation depends on the region specificity, time point, and developmental stage. In addition, glial activation without neuronal death in the *CA3* region in P15 rats suggests that there must be some factor other than glial activation precipitating neuronal degeneration.

Thus, pathological changes after SE in the immature brain differ from those in the adult brain. Neuronal degeneration and glial activation in the hippocampus demonstrate age, time, and regional specificity. Detailed observations in our research probably can provide some help for further studies of the pathological processes in the immature brain under SE conditions.

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ІНДУКОВАНІ ЕПІЛЕПТИЧНИМ СТАТУСОМ УШКОДЖЕННЯ ГІПОКАМПА В НЕЗРІЛОМУ МОЗКУ ЩУРІВ

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Р е з ю м е

Досліджували залежність дегенерації нейронів та активації глії, індукованих епілептичним статусом (ЕС) у різних ділянках гіпокампа щурів від віку тварини і часу після розвитку ЕС, що розвивається. Щурятам лінії Вістар (постнатальні дні П7, П15 та П21) внутрішньоочеревинно ін'єкували сіль літію+пілокарпін для індукції ЕС або фізіологічний розчин для створення контролю. Щурят піддавали евтаназії через 2, 12, 24 год та через три або сім діб після індукції ЕС. Нейродегенерацію в гіпокампі виявляли за допомогою забарвлення Флуоро-Джейд Б. Рівні експресії маркера астроцитів GFAP та маркера мікроглії Iba-1 оцінювали з використанням імуногістохімічних методів. У П7-щурят в ЕС будь-якого ушкодження нейронів на усіх відрізках часу не виявлялося. Через 2 год після індукції ЕС кількість дегенеруючих нейронів у гіпокампі щурят П15 істотно підвищувалася в зоні *CA1*, а у П12-щурят це відбувалося в зонах *CA1* і *CA3*. У щурят П15 та П21 дегенеруючі нейрони в зубчастій звивині з'являлися через 24 год. У щурят П7 не спостерігалося збільшення кількості GFAP- або Iba-1-позитивних клітин, зумовленого ЕС. Експресія GFAP у щурят П15 дуже сильно зростала в зонах *CA1* та *CA3* через 12 год. У той же час у таких щурят не відмічалося збільшення імунореактивності цього білка в зубчастій звивині. У щурят П21 даний індекс збільшувався в зонах *CA1*, *CA3* та зубчастій звивині вже через 2 год після індукції ЕС. Кількість Iba-1-позитивних клітин у трьох згаданих регіонах істотно збільшувалась у щурят П15 через 12, а у тварин П21 – вже через 2 год. Ці результати вказують на те, що пов'язані з ЕС ушкодження нейронів та активація глії в незрілому мозку в цілому є менш інтенсивними, ніж у дорослому, а розвиток даних процесів у гіпокампі є специфічним у просторовому та часовому аспектах.

REFERENCES

1. K. Biber, H. Neumann, K. Inoue, and H. W. Boddeke, "Neuronal 'on' and 'off' signals control microglia," *Trends Neurosci*., **30**, No. 11, 596-602 (2007).
2. R. J. Racine, "Modification of seizure activity by electrical stimulation. II. Motor seizure," *Electroencephalogr. Clin. Neurophysiol.,* **32**, No. 3, 281-294 (1972).
3. F. E. Jensen, H. Blume, S. Alvarado, et al., "NBQX blocks acute and late epileptogenic effects of perinatal hypoxia," *Epilepsia*, **36**, No. 10, 966-972 (1995).
4. S. S. Schreiber, G. Tocco, I. Najm, et al., "Absence of *c-fos* induction in neonatal rat brain after seizures," *Neurosci. Lett.*, **136**, No. 1, 31-35 (1992).
5. R. Sankar, D. H. Shin, and C. G. Wasterlain, "GABA metabolism during *status epilepticus* in the developing rat brain," *Dev. Brain Res.*, **98**, No. 1, 60-64 (1997).
6. M. Patel and Q. Y. Li, "Age dependence of seizure-induced oxidative stress," *Neuroscience*, **118**, No. 2, 431-437 (2003).
7. X. Wang, A. Zaidi, R. Pal, et al., "Genomic and biochemical approaches in the discovery of mechanisms for selective neuronal vulnerability to oxidative stress," *BMC Neurosci.*, **19**, doi: 10.1186/1471-2202-10-12 (2009).
8. M. M. Haglund and P. A. Schwartzkroin, "Role of Na-K pump potassium regulation and IPSPs in seizures and spreading depression in immature rabbit hippocampal slices," *J. Neurophysiol*., **63**, No. 2, 225-239 (1990).
9. H. B. Michelson and E. W. Lothman, "An *in vivo* electrophysiological study of the ontogeny of excitatory and inhibitory processes in the rat hippocampus," *Dev. Brain Res.*, **47**, No. 1, 113-122 (1989).
10. J. W. Swann, R. J. Brady, and D. L. Martin, "Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus," *Neuroscience*, **28**, No. 3, 551-561 (1989).
11. J. Priller, C. A. Haas, M. Reddington, and G. W. Kreutzberg, "Calcitonin gene-related peptide and ATP induce immediate early gene expression in cultured rat microglial cells," *Glia*, **15**, No. 4, 447-457 (1995).
12. Y. Taniwaki, M. Kato, T. Araki, and T. Kobayashi, "Microglial activation by epileptic activities through the propagation pathway of kainic acid-induced hippocampal seizures in the rat," *Neurosci. Lett.*, **217**, No. 1, 29-32 (1996).
13. W. J. Streit, S. A. Walter, and N. A. Pennell, "Reactive microgliosis," *Prog. Neurobiol.*, **57**, No. 6, 563-581 (1999).
14. M. Oprica, C. Eriksson, and M. Schultzberg, "Inflammatory mechanisms associated with brain damage induced by kainic acid with special reference to the interleukin-1 system," *J. Cell Mol. Med*., **7**, No. 2, 127-140 (2003).