J Pharmacol Sci **113**, 14 – 22 (2010)

Journal of Pharmacological Sciences

Forum Minireview

Novel Etiological and Therapeutic Strategies for Neurodiseases: Mechanisms and Consequences of Febrile Seizures: Lessons From Animal Models

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Received October 2, 2009; Accepted December 21, 2009

Abstract. Febrile seizures (FS) are the most common type of convulsive events in infancy and childhood. Genetic and environmental elements have been suggested to contribute to FS. FS can be divided into simple and complex types, the former being benign, whereas it is controversial whether complex FS have an association with the development of temporal lobe epilepsy (TLE) in later life. In the hippocampus of TLE patients, several structural and functional alterations take place that render the region an epileptic foci. Thus, it is important to clarify the cellular and molecular changes in the hippocampus after FS and to determine whether they are epileptogenic. To achieve this goal, human studies are too limited because the sample tissues are only available from adult patients in the advanced and drug-resistant stages of the disease, masking the underlying etiology. These facts have inspired researchers to take advantage of well-established animal models of FS to answer the following questions: 1) How does hyperthermia induce FS? 2) Do FS induce neuroanatomical changes? 3) Do FS induce neurophysiological changes? 4) Do FS affect the behavior in later life? Here we introduce and discuss accumulating reports to answer these questions.

Keywords: childhood seizure, epilepsy, hippocampus, dentate gyrus, granule cell, neurodisease

1. Introduction

Febrile seizures (FS), which take place during fever (typically greater than 38.5°C), are the most common type of seizures in infants and young children after age 1 month, mostly occurring between 6 months and 5 years of age, peaking at 16 – 18 months of age (1 – 4). Overall, 2% – 14% of the world's population have FS (3, 5 – 10).

FS can be classified into two types: about 60% – 70% of FS being "simple" and 30% – 40% being "complex" (6, 11). Simple FS consist of a generalized tonic-clonic seizure without focal neurological features, lasting less than 10 (1, 12) or 15 min (7), without a recurrence within 24 h. Complex FS are characterized by a prolonged seizure duration lasting 15 min or more, focal neurological features, or seizure recurrence within 24 h or within the same febrile illness (1, 6, 12). Simple FS are not followed by epilepsy in most cases (13), whereas there is controversy regarding the long-term sequelae of complex FS. Most prospective epidemiological studies have failed to find strong associations between complex FS and adult temporal lobe epilepsy (TLE) (6, 7, 10, 14, 15), although Annegers et al. suggested an increased incidence of TLE after complex FS (1). Furthermore, retrospective studies have linked a history of complex FS and subsequent TLE in as many as 40% of adult patients (16 – 19). The hippocampi of TLE patients are characterized by several pathological changes such as the dispersion of the granule cell layer (20, 21), sprouting of hippocampal mossy fibers (22, 23), and hippocampal sclerosis, including selective neuronal loss and reactive gliosis in Ammon's horn (24); each of these features is likely to be involved in the initiation and propagation of the epileptic activity of hippocampal neurons. The relationship between the development of these pathological changes and early-life FS

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Published online in J-STAGE on April 16, 2010 (in advance)
doi: 10.1254/jphs.09R19FM

have been a topic of discussion; however, the cellular and molecular approaches necessary to dissect this relationship could not be adequately applied to resected, and often fixed, human tissues.

The biological basis of FS also remains largely unclear. Although a genetic predisposition to FS is widely suggested, both sporadic and familial cases of the seizures have been reported (25), indicating a considerable contribution of environmental factors to the induction of FS. Of these environmental factors, the immature brain (age-specificity) and fever are obviously indispensable. However, the limitations of human studies have made it difficult to clarify the mechanisms involved.

These unsolved questions inspired researchers to develop animal models of FS that enable the direct examination of the potential mechanisms behind the generation of FS and the consequences of FS. The results obtained from these FS models have attracted the attention of not only clinicians but also neurobiologists from a wide range of backgrounds.

2. Animal models of FS

Reliable rodent models of complex FS have been developed. Hyperthermia is induced by several methods in postnatal rodents to evoke seizures, such as the hair dryer model (26 – 29), heated chamber model (30, 31), hot water model [which might also be categorized as a model for hot-water epilepsy (32 – 34)], microwave model (35), and lipopolysaccharide model (36).

Here, we will briefly introduce the characteristics of the widely-used "hair dryer model", which was developed and refined by Baram and coworkers (for details, see refs. 37 and 38). Postnatal days 10 – 11 (P10–11) rats (mainly Sprague-Dawley rats) or P14–15 mice (mainly 129/Sv or C57BL mice) are used because the developmental stage of hippocampal formation likely corresponds to those in human infancy and young childhood when they are most susceptible to FS. Furthermore, the threshold temperature to evoke seizure at these ages is comparable to those observed in human complex FS. Although the gender is not clearly described sometimes, a single sex is used in most cases. No significant gender difference in the average seizure duration was reported in this model (male: 7.86 ± 0.81 min and female: 10 ± 1.54 min) (39).

The animals are placed in a glass jar and heated by a hair dryer. The prolonged experimental FS (eFS) are generated by maintaining hyperthermia (40°C – 42°C) for 30 min, by measuring the rectal temperature every 2 min. Importantly, hyperthermic controls using the injection of a short-acting barbiturate prior to the induction of hyperthermia (pentobarbital, i.p.; refs. 27, 40, 41) should be performed to distinguish whether any of the observed consequences of the eFS are due to the seizures or the hyperthermia. The sequence at eFS onset consisting of an acute sudden arrest of hyperthermia-induced hyperactivity, such as running, followed by oral automatism (biting and chewing) is typical for seizures of limbic origin. Indeed, electroencephalographic (EEG) recordings from the basal amygdala, dorsal hippocampus, and frontoparietal cortex of freely moving rat and mouse pups suggested that the hippocampal and amygdaloid EEG spike waves and trains, whose onset coincides with the behavioral arrest and oral automatisms, precede those recorded in the cortex (26, 27, 41, 42). The oral automatisms are often followed by forelimb clonic movements. Later in the seizures, tonic body flexion is observed in rats but not in mice.

In the hair dryer model, long-term hyperexcitability of the hippocampal network following eFS has been confirmed by both in vitro and in vivo experiments (27). Firstly, in all of the adult rats that had experienced eFS (10 – 11 weeks following eFS), administration of low-dose kainate (5 mg/kg, i.p.) induced hippocampal EEG seizures and associated behavioral seizures, most of which (8 of 11 rats) progressed to status epileptics (SE), whereas only 25% (2 of 8) of normothermic controls and 16.6% (1 of 6) of hyperthermic controls exhibited EEG or behavioral seizures, none of them developing into SE. These in vivo data were further supported by in vitro electrophysiological experiments: after the stimulation of Schaeffer collaterals in entorhino-hippocampal slices prepared one week after eFS, the recurrent, spontaneous, and self-sustaining field discharges, with their amplitude and frequency progressively increasing, were recorded in the CA1 pyramidal cell layer. These phenomena were not observed in slices from control animals. Thus, these results indicate that early-life eFS reduced the seizure threshold to the convulsant challenge in adulthood.

In addition, the presence in later-life (around 3 months of age) of limbic epilepsy in a significant proportion of rats after eFS in the hair dryer model have been revealed by chronic video monitoring of spontaneous behavioral seizures with concurrent hippocampal and cortical EEGs (40).

Using a heated air stream (not by a hair dryer) on P26–29 rats to induce eFS, the behavioral and EEG activity has been shown to resemble those observed in a 5-year-7-month-old girl who had an initial FS at 7 months and subsequently suffered from occasional seizures associated with fever (43).

To date, the strain-dependent differences in warm air stream–induced eFS susceptibility have been reported in mice (29) but not in rats.

Most of the data introduced in this review were ob-

tained using the hair dryer model or its modified version; however, in those instances when other models were used, we will provide the necessary information.

Other than FS models, there also exist several animal models for early-life seizures such as hypoxia-induced seizures that are induced by lower inhaled–oxygen concentration (44 – 46), recurrent generalized tonic-clonic seizures induced by inhalation of flurothyl (47 – 49), both absence and generalized tonic-clonic seizures induced by pentylenetetrazol injection (50 – 52), status epilepticus induced by the injection of kainic acid (53 – 55) or pilocarpine (56, 57), and recurrent generalized tonic-clonic seizure induced by electroshock (58, 59). To find the methods and features of these seizure models in detail, the readers are strongly recommended to refer to the excellent reviews by Sanchez and Jensen (60) and Zhao and Holmes (61).

3. How does hyperthermia induce eFS?

Although it has been suggested that fever itself does not cause FS mainly because antipyretics did not reduce the risk of FS (62, 63), animal studies have revealed the molecular links between fever and FS. Among them, the role of interleukin-1β (IL-1β), a proinflammatory cytokine, has been well examined. The threshold temperature for the generation of eFS was significantly higher in transgenic mice deficient in the type I receptor for IL-1β (*IL-1R1*$^{-/-}$ mice; 42.4 ± 0.3°C) than both wild-type 129/Sv (41.3 ± 0.2°C) and C57BL mice (39.7 ± 0.2°C) (64). The seizure threshold of wild-type 129/Sv but not *IL-1R1*$^{-/-}$ mice was decreased by the intracerebroventricular (i.c.v.) infusion of IL-1β (5 ng). Furthermore, infusion of a high dose of IL-1β (116 ng) to normothermic wild-type 129/Sv mice resulted in limbic behavioral seizures confirmed by simultaneous hippocampal EEG recordings that showed prolonged spike trains. These data suggest that activation of IL-1R by endogenous IL-1β underlies the fever-induced neuronal hyperexcitability that leads to the generation of eFS.

Using a "heated chamber model" in which the body temperature of the rat pups is raised by an infrared lamp (30), with the temperature increase being much slower compared to the hair dryer model, Schuchmann et al. have found that hyperthermia-induced respiratory alkalosis precipitates eFS (31). In P8–11 rats, the behavioral seizures occurred about 30 – 40 min after the induction of hyperthermia when the frequency of breathing increased from its control level of 163 ± 14 to 254 ± 44 breaths/min. The hyperthermia-induced increase in breathing rate was also reproduced in the hair dryer model (65). The thermal tachypnea observed in a heated chamber was closely paralleled by a rise in the intracortical pH by a maximum of 0.27 ± 0.04 units from its control level of 7.22 ± 0.096. An increase in the cortical pH of 0.29 ± 0.05 units by intraperitoneal (i.p.) injections of bicarbonate also caused seizures whose behavioral characteristics resembled those induced by hyperthermia, suggesting that the hyperthermia-induced brain alkalosis triggered eFS. Importantly, exposure of the rats to 5% CO_2 in the chamber blocked the hippocampal and cortical electrographic ictal activity and the associated behavioral activity. The electrographic ictal activity was abolished within approximately 15 – 25 s without affecting the body temperature during hyperthermia. Thus, although there remain some problems to be solved, the application of CO_2-enriched air could be a novel therapeutic strategy for the treatment of FS (66).

In vitro studies directly examined the effect of hyperthermia on the neuronal excitability. In the hippocampal slices obtained from P2–38 rats, the effect of the temperature elevation on the stimulation-evoked extracellular field potential in the CA1 region was investigated. When the temperature of the recording chamber was elevated from its control level of 35°C – 36°C to >38°C, an increase in the amplitude and duration of the field response was recorded and it continued for not less than 15 min even after the temperature was returned to the control level (67). In addition, temperature elevation caused epileptiform activity of neurons such as spontaneous multiple population spikes and long-lasting ictal-like discharges. These effects were most frequently observed in slices from P13–20 rats and was not observed in slices from <P4 or >P28 rats, suggesting the existence of age-dependent factors caused the differential neuronal responses to hyperthermia. The age-dependency of the hyperthermia-induced hippocampal excitability was also found in the in vivo electrophysiological studies: hyperthermia induced with a heating pad resulted in a decrease of paired-pulse inhibition in CA1, which was more prominent in P15–17 rat pups than in adult rats (68).

The involvement of $GABA_A$ receptor–governed gamma oscillations have been identified using P17–29 rat slices as a cellular mechanism that underlies the temperature elevation–induced population spikes (69). Studies using P11–17 rat slices have suggested that temperature elevation induced the attenuation of inhibitory neurotransmission in CA1 pyramidal neurons, including both the reduction of GABA release from pre-synaptic terminals and the decreased post-synaptic function of $GABA_A$ receptors (70, 71). The reduction of GABAergic transmission would rapidly contribute to hippocampal hyperexcitability after hyperthermia, although it should be noted that the GABA content significantly increased in several regions of the brain, including the hippocampus, 2 – 6 h after ultra-red light–induced eFS in P16 rats,

which returned to the control level within 24 h after the seizures (72).

4. Do eFS induce neuroanatomical changes?

Causal relationships between childhood FS and later hippocampal atrophy or sclerosis in TLE patients have been controversial. Several retrospective studies have found considerable relationships between the history of FS and sclerosis (73 – 75) or a reduction in the volumes of the hippocampus and amygdala (16); whereas prospective studies, including recent magnetic resonance imaging (MRI) studies, have failed to confirm these observations (13, 76, 77). These contradictions would, in part, come from the inevitable characteristics of human studies such as the diversity in the frequency of seizures (both febrile and afebrile), the history of medication for retrospective studies, and the period of follow-up research in prospective studies.

These difficulties associated with human studies can be controlled by using the experimental models of FS. These models have revealed several reliable findings regarding neuronal loss, neurogenesis, and alterations in dendritic and axonal morphogenesis after eFS.

Silver staining revealed that eFS (P10) resulted in a number of neurons with shrunken dendrites in the central nucleus of the amygdala and the hippocampal CA1 and CA3 pyramidal cell layers within 24 h, and these 'injured' neurons survived for at least two weeks (78). These alterations were not followed by significant DNA fragmentation, and no significant neuronal cell loss was found in these regions by four weeks. Thus it could be possible that the surviving neurons with malformed dendrites disrupted the balance of the excitatory and inhibitory inputs to these neurons, leading to the hyperexcitability of the focal networks. However, in another study, biocytin-mediated tracing after whole-cell recording at one week following eFS (P10) failed to find differences between control and eFS slices in the dendritic arborization of CA1 pyramidal cells (28).

No significant loss of pyramidal cells in the CA1 and CA3 region, GluR2/3-immunoreactive mossy cells in the hilus, or GAD67 mRNA–positive interneurons in these regions was found by histological studies at 3 months after eFS, indicating that FS did not induce delayed, chronic hippocampal cell loss (79). There was, however, another study that reported a reduced density of parvalbumin-immunopositive interneurons in the hilus at 5, 7, and 11 weeks after a mercury vapor lamp–induced eFS at P11 (80).

To examine the effects of eFS on granule cell neurogenesis, Bender et al. performed a single injection of the S-phase marker 5-bromo-2′-deoxyuridine (BrdU) to rat pups either 3, 7, or 28 days after eFS (P10–11) and perfused them 48 h later. The number of BrdU-positive nuclei in the granule cell layer was not significantly different compared to control animals (79). Lemmens et al. found 25% more BrdU-positive cells (BrdU was injected twice daily from P11–16) in the dentate gyrus of eFS (P10)–induced male but not female animals at P66 compared to age-matched normothermic controls (39). The authors reported that the gender difference also affected the survival of newborn cells in the dentate gyrus, showing that the survival of BrdU-positive cells at P66 (% of P17) was significantly higher in eFS-induced female (Control: 44% vs. eFS: 53%, $P < 0.05$) but not male rats (Control: 20% vs. eFS: 23%, not significant). The factors that regulate the effects of gender difference on these phenomena remain to be elucidated.

A recent study has reported that eFS (P10) decreased the population of P11–16-born cells that survive and differentiate into excitatory amino acid transporter 3 (EAAT3), which eliminates glutamate from the synaptic cleft, –positive neurons from the control level of 23% to 14% ($P < 0.01$) in the granule cell layer at P66; implying an influence of eFS on the excitability of the regional network (81).

Taken together, these results suggest that eFS, in part, affects the survival (but there exists a gender difference) and differentiation but not proliferation of newborn cells in the dentate gyrus. Whether these 'newborn' cells are dentate granule cells should be further determined with more specific markers for granule cells such as prospero-related homeobox 1 (Prox1).

The contribution of eFS to the induction of the sprouting of hippocampal mossy fibers has also been examined. Mossy fiber sprouting is an important pathology frequently observed in the TLE hippocampus and its relation to hyperexcitability of the dentate gyrus has been suggested (82). Significantly increased Timm-positive puncta, which indicate the zinc-containing pre-synaptic terminals of mossy fibers, were found in the granule cell layer and the molecular layer in 3-month-old rats that had experienced eFS induced at P10–11 (79). The bundled dense sprouting in the molecular layer was also reported at 11 weeks after mercury vapor lamp–induced eFS at P11, which was later followed by spontaneous recurrent seizures (80). Although the cellular mechanisms underlying the eFS-induced sprouting remain unknown, brain-derived neurotrophic factor (BDNF), which is sufficient and necessary to induce the sprouting in vitro (83), could be a strong candidate because BDNF mRNA has been shown to increase after hot water–induced FS at P21 (34).

5. Do eFS induce neurophysiological changes?

Long-lasting modulation of neuronal network functions after eFS have been reported. One week after eFS at P10, whole-cell patch clamp recordings from CA1 pyramidal cells in brain slices revealed that the amplitude of evoked inhibitory postsynaptic currents (IPSCs) was significantly increased compared to the control (28). The authors further showed that the frequency of miniature IPSCs (mIPSCs) was nearly doubled without any change in the mIPSC amplitude and kinetics in eFS-experienced rats, indicating that the potentiation of the inhibitory transmission had a presynaptic locus. Finally, it was pharmacologically revealed that the activation of protein kinase A (PKA) underlies the potentiation of the inhibitory responses.

Considering these results, it could be expected that the long-lasting potentiation of inhibitory transmission would decrease the susceptibility for seizure; however, increased inhibition was associated with a persistent decrease of seizure threshold after eFS such as the reduction in seizure threshold in response to chemical convulsants in vivo and electrical stimulation in vitro (27).

As a mechanism underlying this paradox, the contribution of the 'molecular inhibition excitation converter' has been clearly demonstrated (28). The converter is the hyperpolarization-activated depolarizing current I_h, which is generated by hyperpolarization-activated cyclic nucleotide–gated (HCN) channels. In CA1 pyramidal cells in brain slices, the membrane potential for the half-maximal activation (V_{50}) of I_h was significantly shifted towards the depolarizing direction by 3.3 mV and by 5.9 mV after 1 and 9 weeks, respectively, following eFS at P10. The significantly larger I_h in cells from eFS rats than control rats was also shown. Importantly, in pyramidal cells from eFS rats, a short train of inhibitory postsynaptic potentials (IPSPs) (6 IPSPs at 50 Hz), which resembled those that occur spontaneously during the θ rhythm in vivo, but not single IPSPs, triggered post-inhibitory rebound depolarization and firing, phenomena abolished by the selective HCN blocker ZD-7288. Recently, from both whole-cell dendritic recordings and computer models of CA1 pyramidal cells, Dyhrfjeld-Johnsen et al. have further confirmed the upregulation of dendritic I_h in eFS rats leading to persistent dendritic hyperexcitability, primarily due to increased I_h–induced depolarization of the resting membrane potential (84). Thus, it is likely that the conjunction of the potentiation of inhibitory inputs and the frequency-dependent modified I_h–mediated events generated persistent hyperexcitable foci in the hippocampus following FS.

Another solution to the above paradox has been suggested by the demonstration of persistent potentiation of the depolarization-induced suppression of inhibition (DSI) in CA1 pyramidal cells 1 – 5 weeks after eFS at P10 (85). Depolarization of CA1 pyramidal cells resulted in a transient depression of spontaneous IPSCs (sIPSCs), and this DSI was significantly increased both in magnitude and duration in eFS rat slices. DSI in both control and eFS slices were abolished in the presence of SR141716A, a cannabinoid type 1 (CB1)-receptor antagonist, indicating the involvement of endocannabinoid signaling. Further pharmacological approaches on the evoked IPSCs (eIPSCs) suggested the activation of presynaptic CB1 receptors by the retrograde release of endocannabinoids from pyramidal cells. Potentiation of the endocannabinoid-mediated retrograde signaling after eFS was explained by using several qualitative and quantitative methods that showed the increased number of CB1 receptors in the presynaptic terminals of cholecystokinin (CCK)-positive interneurons, without any significant change in the concentration of endocannabinoids between the hippocampi of control and eFS animals.

Thereafter, the same group succeeded in preventing both the potentiation of DSI and the increase in CB1 receptor number by the i.p. injection of SR141716A 1 h before eFS induction (86). By blocking CB1 receptors in vivo, the authors further prevented eFS-induced long-lasting hippocampal excitability in vitro and in vivo: both the electro-stimulation–induced self-sustaining population activity of neurons in vitro (1 week after eFS) and the decreased seizure threshold in response to the injection of kainate in vivo (6 weeks after eFS) were blocked.

It is especially noteworthy that dentate granule cells, which do not normally show DSI in control rats in contrast to CA1 pyramidal cells, exhibited DSI after eFS via CB1-receptor activation (85). The dentate gyrus has been believed to function as a high-resistance gate that blocks the invasion of epileptiform activity from the entorhinal cortex to the hippocampus (87 – 89) via the static characteristics of the granule cells including strong tonic and phasic GABA inhibition by surrounding inhibitory interneurons (90). Thus, it is possible that the dentate DSI and the mossy fiber sprouting following eFS cause the malfunction of this gate and bring about the hyperexcitation of the hippocampus. This idea was supported by EEG recordings that showed synchronized spiking and long-lasting abnormal discharges in the dentate gyrus 11 weeks after mercury vapor lamp–induced eFS at P11 (80).

6. Do FS affect the behavior in later life?

Epidemiologic studies of FS outcomes in humans have reported no association between early-life FS and global cognitive dysfunctions in later life. A FS cohort in the United States demonstrated that the intelligence and performance at school of 7-year-old children with experience of FS was not different from their unaffected siblings (91); a cohort study in the United Kingdom (92) found that 10-year-old FS-children did not differ in academic progress, intellect, or behavior compared to children without FS.

These studies, however, did not specifically investigate whether FS induced memory dysfunction. In another population-based study that examined the effects of FS on working memory, Chang et al. revealed that 87 school-aged children with FS performed significantly better than 87 age-matched control children in learning, consolidation, memory retrieval, and delayed recognition (93); however, it should be noted that those children with an onset of FS before 1 year of age had deficits in these properties, suggesting an age-dependent vulnerability to FS.

Several animal studies have investigated the molecular mechanisms underlying the relationship between early-life FS and future hippocampal-dependent memory dysfunction. In an infrared lamp–induced eFS model, it was shown that a single eFS at P10, P15, or P20, but not P5 resulted in impaired inhibitory avoidance responses at P50–60 (94). Repeated episodes of brief hyperthermia (10 min with a rectal temperature of 40°C – 43°C)–induced seizures during P10–12 resulted in long-term memory deficits in both the Morris water maze (starting from P36) and the inhibitory avoidance task at P45; although the study did not model prolonged FS but brief and recurrent FS (95). Phosphorylation of cAMP response-element binding (CREB) protein was found to be significantly reduced in the hippocampus of these repeated-eFS rats by western blotting compared to the control. Furthermore, the authors showed that administration of rolipram, which activates the cAMP–CREB signaling pathway, reversed the long-term memory deficits via enhanced CREB phosphorylation. The repeated-FS also caused long-lasting deficits in synaptic plasticity in CA1 pyramidal cells and reduced tyrosine phosphorylation of the NMDA-receptor subunit NR2A (96). Recently, an abnormal firing rate and poor stability of hippocampal CA1 place cells have been suggested as a basis for the deficits in eFS-induced hippocampal–dependent memory (97).

To understand the effect of FS on future memory dysfunction, a combination of larger-scale human studies and animal studies targeting the age-dependent effects of FS and the underlying cellular and molecular mechanisms are necessary. Furthermore, the development of noninvasive methods such as MRI (97) to detect the future effects of FS on hippocampal malfunction is required.

7. Conclusions

Animal models have not only uncovered the mechanisms underlying the FS-induced neuroanatomical and neurofunctional changes, which are difficult to assess using human tissue samples, they have also suggested that prolonged FS affects later-life outcomes such as memory dysfunction.

Importantly, in almost all of the studies, only a single episode of childhood eFS caused the later effects observed in the adulthood brain. This fact suggests the possible existence of common upstream factors that are directly activated by hyperthermia at the single cell level and subsequently regulate cellular and molecular changes. The use of eFS will be essential to clarify these factors and their relationship to epileptogenesis and to prevent the development of TLE.

Similarly to human studies, animal studies have indicated that FS do not result in a severe loss of primary neurons both in Ammon's horn and the dentate gyrus; however, it remains largely unknown whether the surviving dentate granule cells possess normal morphological, anatomical, and functional properties. Considering that the dentate gyrus serves as a 'fail safe' gate, it is of interest to investigate whether and how the properties of the region are modulated by early-life FS. From this viewpoint, utilizing several techniques including time-lapse imaging of cultured neurons prepared from eFS rats, we have been examining whether eFS induce hilar ectopic granule cells in the adult brain, whose existence are associated with the epileptiform bursting of hippocampal neurons (98). As we have previously demonstrated that an overwhelming majority of dentate granule cells in the adult brain are born during the two weeks after birth and migrate towards the granule cell layer (99), we hypothesized that eFS during this period would disturb this developmental process. This hypothesis is supported by our previous study showing that P0–2-born granule cells become ectopic granule cells in 6-month-old mice brains of pilocarpine-induced SE at P14 in mice (100).

Finally, although results from eFS should be carefully treated when applied to human FS, the accumulating data from well-established experimental animal models have provided invaluable information for not only clinicians but also for developmental and functional neurobiologists.

Acknowledgments

We thank Mr. Junya Ichikawa for discussions on the manuscript. This work was supported in part by a Grant-in-Aid for Young Scientists (B) (No.21790059) from The Ministry of Education, Culture, Sports, Science, and Technology.

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