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Early-life status epilepticus induces ectopic granule cells in adult mice dentate gyrus

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ABSTRACT

A large number of aberrant hilar granule cells (GCs) are found in the patients and animal models of adult temporal lobe epilepsy (TLE), and these "ectopic" GCs have synchronous epileptiform bursting with other hippocampal neurons. In this study, we investigated whether early-life status epilepticus (SE) induces hilar ectopic GCs that remain in the adulthood because TLE patients frequently experience seizures in the early childhood when a large number of postnatally born GCs migrate in the hilus. To label newborn GCs, bromodeoxyuridine (BrdU) was injected daily for three consecutive days to C57BL/6J mice at different postnatal days starting at postnatal-0-day-old (P0) (Group1), P7 (Group2), or P35 (Group3). Mice in each group underwent pilocarpine-induced SE at P14. Six months later, to determine whether SE induces ectopic GCs, we plotted the distribution of postnatally born GCs which were immunohistochemically defined as BrdU- and the GC marker Prox1-colabeled cells. We also examined whether SE causes the granule cell layer (GCL) dispersion and/or the mossy fiber (MF) sprouting, other representative pathologies of TLE hippocampus. Only SE-experiencing mice in Group1 had significantly more neonatally born ectopic GCs compared with control mice. Neither control nor SE mice had dispersed GCL. All mice that underwent SE had sprouted MFs in CA3. We conclude that early-life SE disrupts a normal incorporation of GCs born pre-SE but not post-SE, inducing ectopic GCs in the adult hilus. Interestingly, the results also indicate that developmentally earlier born GCs are more responsive to early-life SE in terms of the emergence of ectopic GCs.

Introduction

Epilepsy is a neurological disorder induced by anatomical and physiological aberrations of a set of neurons that generate highly synchronized activity, and almost 1% of the population worldwide suffer from the disease (Elger, 2002; Fisher et al., 2005). One of the most common types of intractable epilepsy is temporal lobe epilepsy (TLE), which is characterized clinically by the progressive development of spontaneous recurrent epileptic seizures originating from temporal lobe foci (Engel, 1998). In TLE, the hippocampus is thought to play a pivotal role in the initiation and propagation of seizures (Wieser et al., 1993). The hippocampus of TLE patients and animal models displays several anatomical malformations, including a severe loss of principle neurons and an aberrant growth of neurites leading to the network reorganizations (Armstrong, 1993; Koyama and Ikegaya, 2004). One of these pathological findings is a dispersion of the granule cell layer (GCL) (Houser, 1990; Armstrong, 1993; Thom et al., 2005). Anatomically, granule cells (GCs) form a tight GCL in the dentate gyrus (DG). In the epileptic hippocampus, on the other hand, ectopic GCs emerge both in the hilus and the molecular layer, resulting in a dispersed GCL. A considerable number of ectopic GCs are induced in the hilus after seizures in animal models of TLE and these ectopic GCs have the synchronous epileptiform bursts with CA3 pyramidal cells (for review, see Scharfman et al., 2007). At least some of the ectopic GCs are born after seizures, and it is also suggested that seizures are associated with the malpositioning of adult-generated GCs (Jessberger et al., 2005, 2007; Walter et al., 2007). These data have clarified that the normal incorporation of newborn GCs into GCL is interrupted by seizures in the adulthood.

In contrast to the adulthood, there have been few studies to date which investigate the effects of the early childhood seizures on the incorporation of neonatally born GCs to GCL. Although GC neurogenesis continues throughout the life, most GCs are born during one week after birth (Schlessinger et al., 1975) then migrate a long distance from the hilus to GCL (Altman and Bayer, 1990), thickening GCL in rodents (Angevine, 1965). In C57BL/6J mice, neonatally born GCs survive up to at least 6 months and numerically dominate GCL in the adulthood (Muramatsu et al., 2007). The migration of newborn GCs during neonatal periods is a far more dynamical journey compared with that of adult-generated GCs born in the restricted narrow juxta-GCL area called the subgranular zone. The effects of seizures on GC neurogenesis are also different between the childhood and the adulthood. For example, early-postnatal seizures have been shown to reduce neurogenesis both in rats (McCabe et al., 2001) and human patients (Mathern et al., 2002), whereas adulthood seizures increase neurogenesis in rodents (Parent et al., 1997; Parent, 2003). Thus, it is likely that the effects of seizures on the incorporation of newborn GCs to GCL are also different between the childhood and the adulthood.

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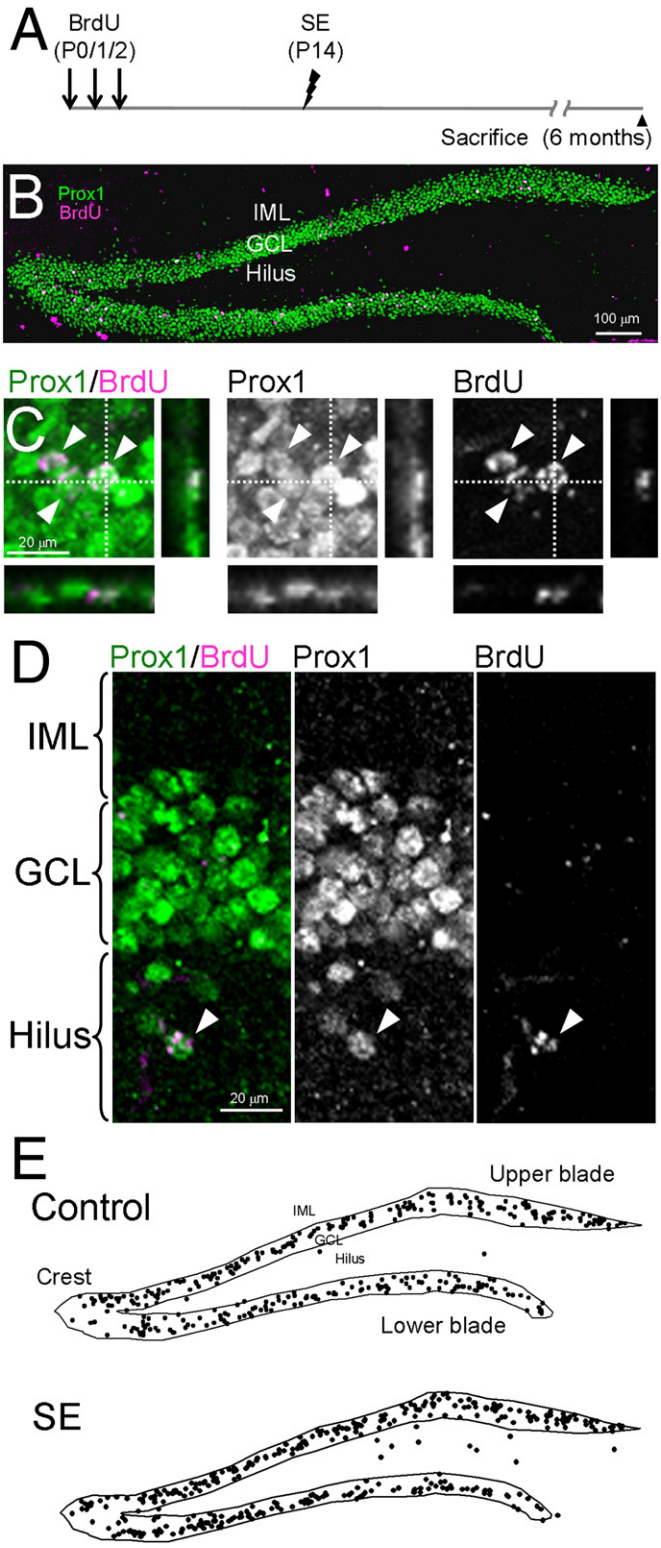
The clinical investigation indicated that TLE often starts as an isolated, prolonged convulsion in early life (Wieser et al., 1993). The retrospective studies suggested a correlation between a history of early childhood convulsions and hippocampal sclerosis (Falconer et al., 1964; Falconer, 1971; Cendes et al., 1993; Harvey et al., 1995, 1997; Van Paesschen et al., 1997). Importantly, Lurton and colleagues have demonstrated that GCL dispersion in human TLE patients is correlated with epileptic events occurring until the first 4 years of life (Lurton et al., 1998). Here we investigated whether early-life status epilepticus (SE), which is characterized by repetitive or prolonged seizures, affects the normal incorporation of newborn GCs to GCL and result in the emergence of ectopic GCs in the adulthood. We have also assessed whether early-life SE results in GCL dispersion and the mossy fiber (MF) sprouting, both of which are the representative abnormalities observed in the hippocampus of TLE patients and their animal models.



Materials and methods

Animals

C57BL/6J mice (SLC, Shizuoka, Japan) were kept under temperature- and humidity-controlled conditions (23±1 °C, 50±10%, respectively). They were weaned at postnatal-35-day-old (P35) then housed in cages separately by their sex with free access to food and water up to 6 months (P180). All experimental procedures conformed to the University of Tokyo guidelines concerning the care and use of animals for minimizing the number of animals used and their suffering.

Bromodeoxyuridine injection

To label dividing cells in early-postnatal days, mice were grouped in three different postnatal days (Group1: P0; Group2: P7; Group3: P35) and received a subcutaneous bromodeoxyuridine (BrdU; Sigma, St. Louis, MO, USA) injection (100 μg/g of body weight) daily for three consecutive days (Group1: P0/1/2; Group2: P7/8/9; Group3: P35/36/37).

Induction of early life status epilepticus

We injected pilocarpine hydrochloride (Sigma; 200 μg/g of body weight) intraperitoneally to P14 mice to induce SE. To reduce peripheral effects of pilocarpine, scopolamine methylbromide (Sigma; 1 μg/g of body weight) was subcutaneously injected 30 min before pilocarpine administration. Control animals were treated equally except that pilocarpine was replaced by saline. The seizure severity was scored every 5 min according to the scale of Lado (Lado et al., 2000). The behavioral seizure scale in pups was summarized by Lado as follows: 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; stage 7, tonus. One hour after pilocarpine injection, mice pups were retuned to their mother in order to keep from decreasing body temperature.

Tissue processing

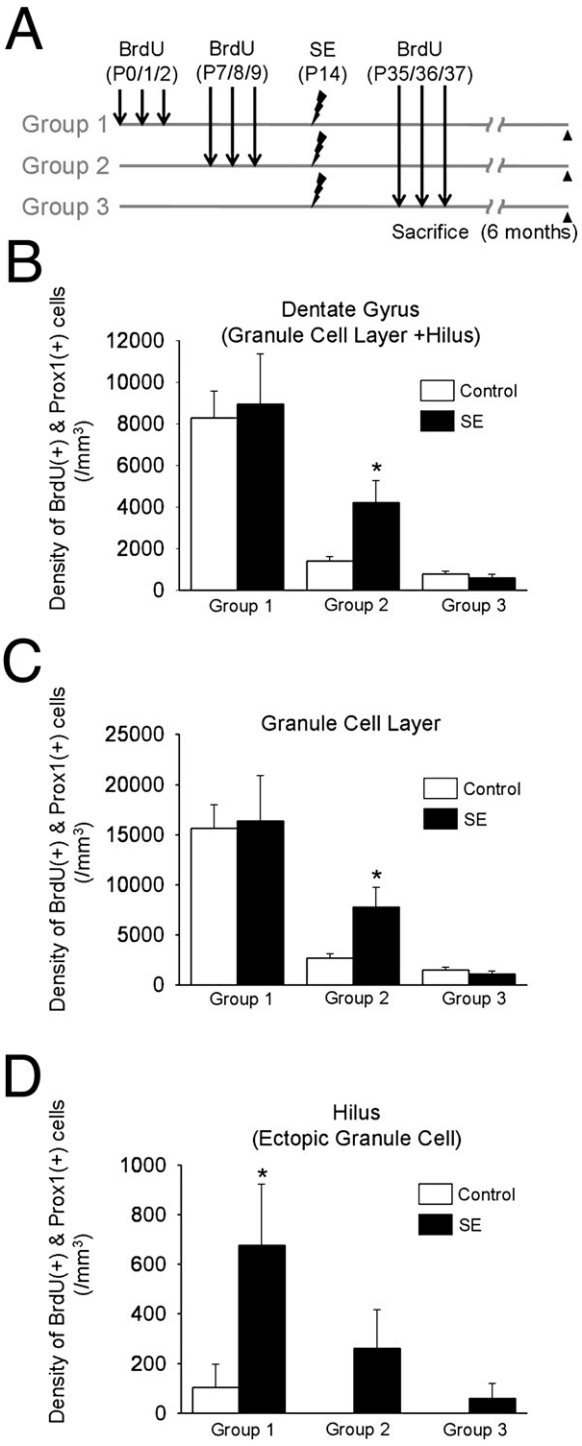
At 6-month-old, the animals were deeply anesthetized and perfused intracardially with phosphate-buffered saline (PBS), followed

Fig. 1. (A) C57BL/6J mice received daily subcutaneous injection of bromodeoxyuridine (BrdU) for three consecutive days from postnatal-0-day-old (P0) to P2 to label dividing cells. Half of the mice in the experimental group (n=13) underwent pilocarpine-induced status epilepticus (SE) and the other half of the mice (n=13) received saline injection (Control) at P14. They were sacrificed at 6-month-old (P180). (B) A confocal image of the dentate gyrus (DG) from a 6-month-old control mouse immunostained for BrdU (magenta) and the granule cell (GC) marker Prox1 (green). The colabelling of BrdU (magenta) and Prox1 (green) was confirmed by confocal three-dimensional reconstruction and the cells double positive for both markers were defined as newborn GCs (C, arrowheads). Note that P0/1/2-born GCs still remained in the granule cell layer (GCL) 6 months after the last BrdU injection (B). (D) P0/1/2-born GCs (arrowheads) were found in the hilus of tissues obtained from animals that had experienced early-life SE. (E) Schematic illustrations of the distribution of neonatally born GCs in Control (n=8 hippocampal tissues from 5 mice) and SE (n=8 hippocampal tissues from 4 mice) DG at 6 months old. Solid circles represent P0/1/2-born GCs. Note that there existed more number of neonatally born ectopic GCs in the hilus of SE-induced animals compared with control mice. One to two representative hippocampal sections/one animal were analyzed (see Materials and methods). IML: the inner molecular layer.

by 4% paraformaldehyde in ice-cold PBS. The coronal series of brain sections (thickness = 30 μm) were prepared with a Leica CM 1800 freezing microtome (Bannockburn, IL, USA) and stored at −80 °C until the following immunohistochemical study and Neo-Timm staining.

Immunohistochemistry

For detecting BrdU-labeled cells, the sections were pretreated with 2 N HCl for 30 min at 37 °C and washed in 0.1 M borate buffer (pH 8.5) for 10 min. They were incubated with 0.25% Triton X-100 and 2% goat serum in PBS at room temperature for an hour and then incubated at 4 °C overnight with mouse monoclonal anti-BrdU antibody (1:1000; Sigma) and rabbit polyclonal antibody against Prox1 (1:5000; Chemicon, Temecula, CA, USA), a marker of GCs (Pleasure et al., 2000; Encinas et al., 2006; Navarro-Quiroga et al., 2006). After extensive washes with PBS, samples were incubated with Alexa 488-labeled anti-rabbit IgG (1:1000; Invitrogen, Gaithersburg, MD, USA) and Alexa 594-labeled anti-mouse IgG (1:1000; Invitrogen). They were imaged with a Bio-Rad MRC-1024 confocal system with a 20× objective (Nikon, Tokyo, Japan) and reconstructed to three-dimensional images using ImageJ to confirm colabelling of BrdU and Prox1 (Fig. 1C). BrdU- and Prox1-colabeled cells were defined as GCs born at the time of BrdU injection (Muramatsu et al., 2007). To investigate the density of BrdU- and Prox1-colabeled cells (Figs. 1, 2), we analyzed one to two coronal sections, which correspond to "section 19" in the Mouse Brain Library (http://www.mbl.org/) (Kempermann et al., 2003; Muramatsu et al., 2007), from one animal. It is because we have previously determined that the density of BrdU- and Prox1-colabeled cells in the section (section 19) represent those of the entire hippocampus in C57BL/6 mice (Muramatsu et al., 2007). In Fig. 2, to calculate the density of BrdU- and Prox1-colabeled cells in GCL, the hilus and DG (defined as "GCL + the hilus" throughout this study), respectively, we divided the cell number in each area by the volume of the corresponding area (GCL: $2.68 \pm 0.08 \times 10^{-3}$; hilus: $2.41 \pm 0.02 \times 10^{-3}$; DG: $5.09 \pm 0.08 \times 10^{-3}$; mean ± SEM mm^3, $n = 7–9$ hippocampal sections from 4–5 mice).



Measurement of a dispersed granule cell layer

The width of GCL in each region of DG (upper blade, lower blade and crest) was measured at interval of 50 μm throughout GCL and averaged (Fig. 3).

Neo-Timm staining

After rinse with phosphate-buffered saline (PBS), brain sections were immersed in 0.4% sodium sulfide solution at 4 °C for 10 min, then fixed with 10% (v/v) formaldehyde for 10 min. After PBS wash, they were dehydrated with 70% and 96% ethanol each twice for 30 min and then dried. To perform silver sulfide (Timm) staining, the slices were incubated with citrate-buffered solution containing 20% Arabic gum, 2.1% $AgNO_3$, and 0.085% hydroquinone in a dark room at 26 °C for 50 min. The slices were washed with distilled water at the end of the reaction. To quantify MF terminals, the images were digitized with a FinePix S1Pro CCD camera (Fuji Photo Film, Tokyo Japan) equipped with an ECLIPSE TE300 microscope (Nikon, Tokyo, Japan) and a 10× objective. The pixel intensity values were measured at 8 bit resolution in circle areas of the dentate hilus, GCL and the inner molecular layer (IML) of DG (diameter = 15 μm) and of the stratum oriens (SO), the stratum pyramidale (SP), and the stratum

Fig. 2. (A) The animals received daily subcutaneous injection of bromodeoxyuridine (BrdU) for three consecutive days starting at postnatal-0-day-old (P0) (Group1, $n = 9$), P7 (Group2, $n = 8$) and P35 (Group3, $n = 9$), respectively. About half of the mice in each group (Group1, $n = 4$; Group2, $n = 4$; Group3, $n = 5$) underwent pilocarpine-induced status epilepticus (SE) and the other half of the mice received saline injection (Control) at P14. They were sacrificed at 6-month-old (P180) for the following immunohistochemical study and BrdU- and Prox1-colabeled cells (BrdU(+) and Prox1(+) cells) were defined as newborn granule cells (GCs) as described in Fig. 1. (B–D) The density of neonatally born GCs in the dentate gyrus (DG) (B), GCL(C) and the hilus (D) were assessed in hippocampal tissues at 6 months in each group. Pilocarpine-induced SE significantly increased the density of postnatally born ectopic GCs in the hilus only in Group1 animals without affecting the whole density of P0/1/2-born GCs in DG. Although postnatally born ectopic GCs were observed in SE-induced mice of Group2 and Group3 but not in control mice, the density of them was not significantly different. In Group2 animals, the significant increase of neonatally born GC density was detected both in DG and GCL. *$p < 0.05$ versus control; Tukey's test after ANOVA. Data are means ± SEM of 7–9 hippocampal sections from 4–5 mice. One to two representative hippocampal sections/one animal were analyzed (see Materials and methods).

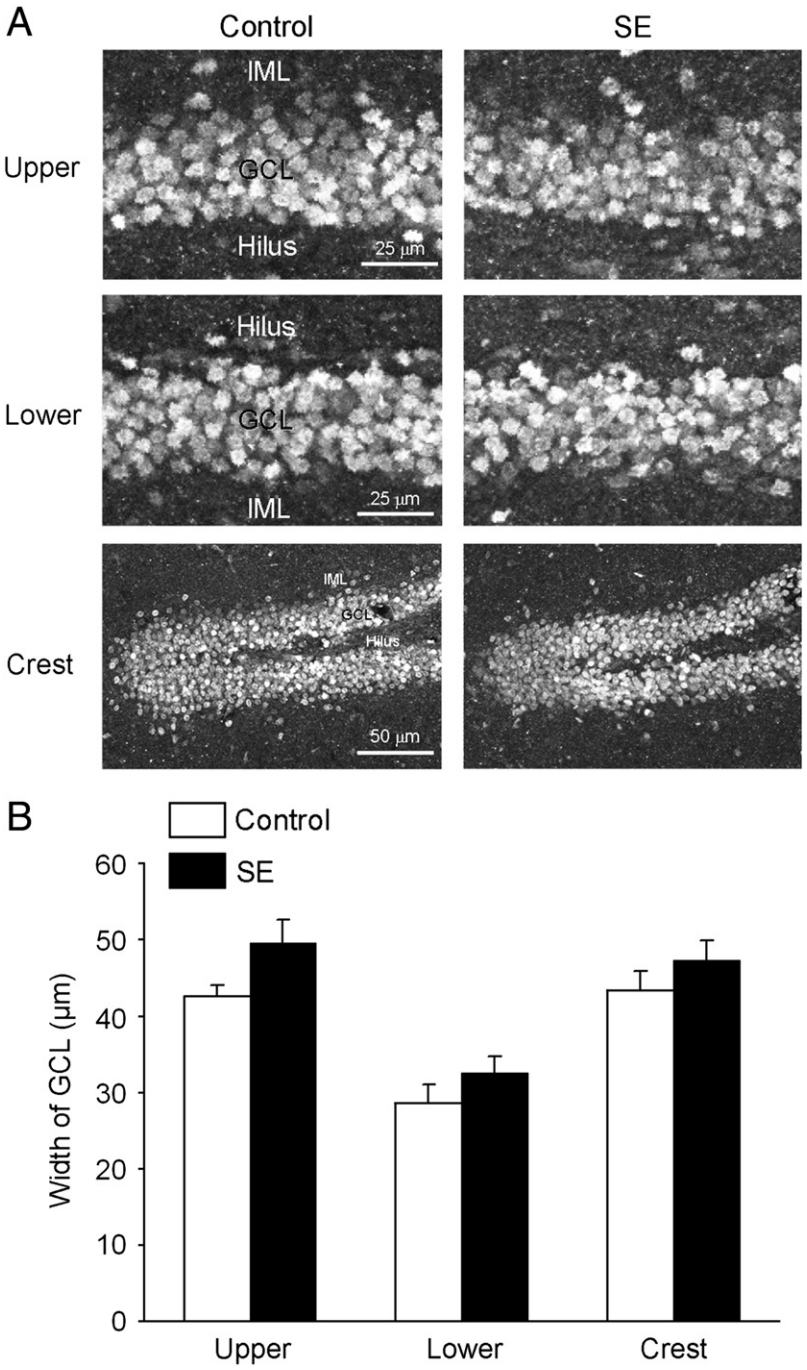


Fig. 3. (A) The hippocampal sections obtained from 6-month-old control mice or animals experiencing pilocarpine-induced status epilepticus (SE) at postnatal-14-day-old (P14) were immunostained for Prox1 and seizure-induced dispersion of the granule cell layer (GCL) was investigated in all area of the dentate gyrus (DG) including the upper blade (Upper), the lower blade (Lower) and the crest of GCL. (B) The width of GCL in each area of the GCL was measured showing no significant difference between control and SE-induced animals. Tukey's test after ANOVA. Data are means ± SEM of each 6 hippocampal sections from control ($n = 3$) and SE ($n = 3$) animals. Two representative hippocampal sections/one animal were analyzed.

lucidum (SL) in CA3 (diameter = 50 μm) and then averaged per substratum (Fig. 4).

Results

Behavioral effects of pilocarpine

The onset of the seizure of C57BL/6J mice was within 10 min (6.38 ± 0.82 min; mean ± SEM, $n = 13$) after pilocarpine injection at P14. The animals stretched their hindlimbs with vocalization, head shaking, asynchronous running movements of the legs and ataxia. All the mice subjected to pilocarpine injection reached stage 3.5–6 of the seizure score (for seizure scoring, see Materials and methods).

In this study, we judged that SE was induced when the seizure severity was maintained over stage 2 at least for 30 min. In these criteria, we found that SE was induced in all the mice that received pilocarpine injection. We monitored mice that received pilocarpine-injection for behavioral seizures at least for 3 h after the injection and the following day, finding no spontaneous seizures. The behavior of both control and pilocarpine-injected mice during the diurnal periods (Cavalheiro et al., 1991; Xiu-Yu et al., 2007) were checked by two observers for 4 h once a week up to 6 months without long-term video-electroencephalogram

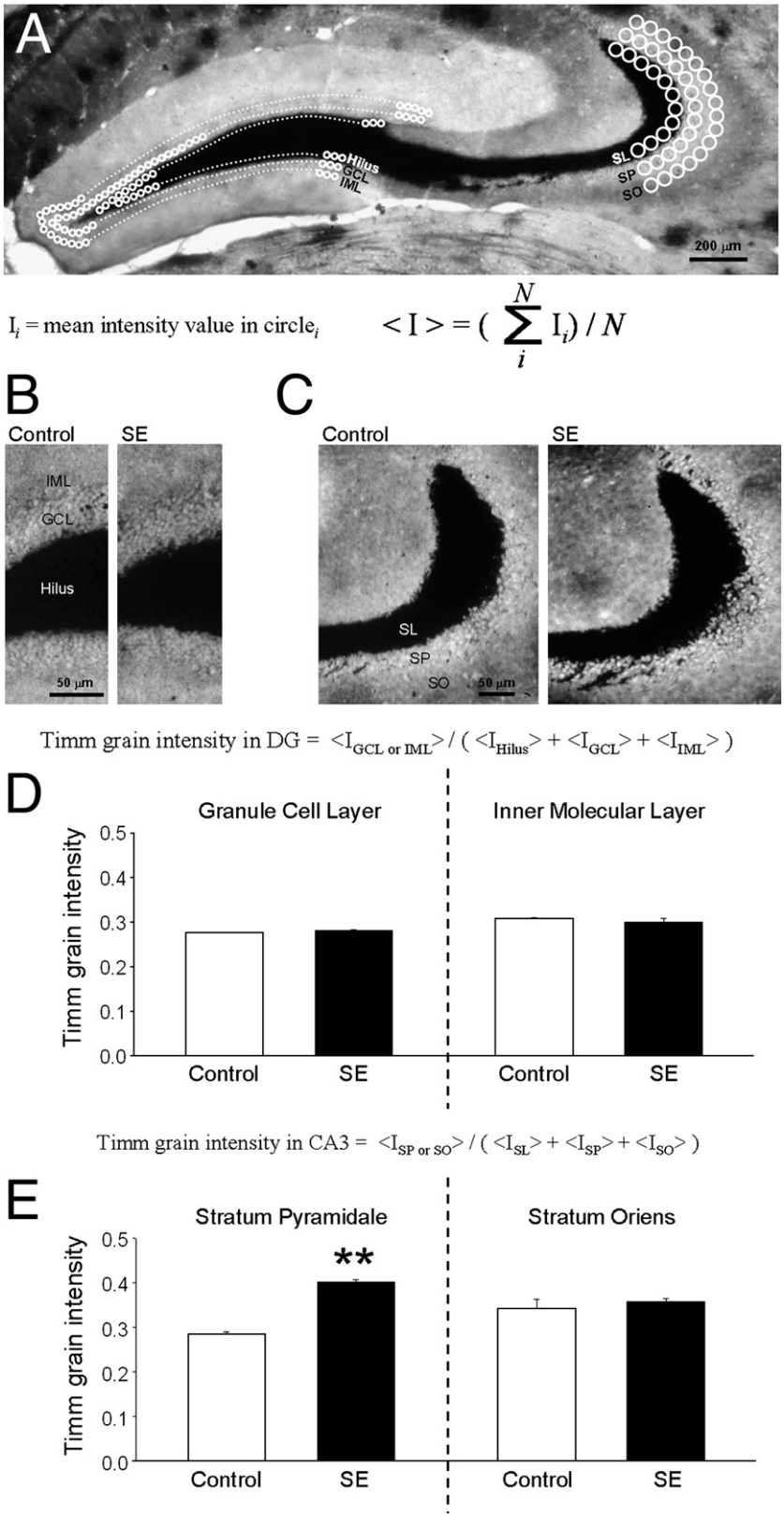


Fig. 4. (A) A representative image of Timm-stained hippocampal section from 6-month-old control mouse. In each subregions including the inner molecular layer (IML), the granule cell layer (GCL) and the hilus of the dentate gyrus (DG) and the stratum oriens (SO), the stratum pyramidale (SP) and the stratum lucidum (SL) of CA3, consecutive circle cursors were put (diameter in DG: 15 μm; diameter in CA3: 50 μm) and the mean signal intensity (I) within the cursors was measured at 8-bit resolution. The I values were averaged for each subregion ($<I_{IML}>$, $<I_{GCL}>$, $<I_{Hilus}>$, $<I_{SO}>$, $<I_{SP}>$, and $<I_{SL}>$). Timm grain intensity was defined as $<I_{GCL}>$ or $<I_{IML}> / (<I_{Hilus}> + <I_{GCL}> + <I_{IML}>)$ in DG (D) and $<I_{SP}>$ or $<I_{SO}> / (<I_{SL}> + <I_{SP}> + <I_{SO}>)$ in CA3 (E). In animals that underwent pilocarpine-induced status epilepticus (SE), the sprouting of mossy fibers were detected in SP but neither in SO (C, E) nor in DG (B, D). $^{**}p < 0.01$ versus control; Tukey's test after ANOVA. Data are mean ± SEM of 30 hippocampal sections from control ($n = 3$) and SE ($n = 3$) animals. Consecutive 10 rostrocaudal coronal sections/one animal were analyzed.

(EEG) monitoring. In this condition, we did not observe spontaneous recurrent seizures which are characterized as forelimb clonus with rearing and falling (Liu et al., 1994). It is well-recognized that pilocarpine-induced SE in adult rodents result in the development of spontaneous recurrent seizures, however, these types of seizure were not induced when pilocarpine was injected to rats at P20 (Liu et al., 1994) or those younger than P18 (Priel et al., 1996). That no spontaneous recurrent seizures occurred in our mice model of early-life SE at P14 is consistent with these previous reports, however, as the observation was done without long-term video-EEG monitoring, we could not completely exclude the existence of spontaneous recurrent seizures, if seizures were rare. Control mice did not display any seizure ($n=13$).

The ectopic granule cells

We addressed whether early-life SE affects the localization of neonatally born GCs in adult DG (Fig. 1). For this purpose, we injected the S-phase marker BrdU daily to the mice from P0 to P2 then induced SE at P14 by injecting pilocarpine to them. The animals were sacrificed at 6-month-old and the distribution of P0/1/2-born GCs (BrdU- and the GC marker Prox1-colabeled cells) were investigated in DG (Figs. 1A–C).

We chose the time points for BrdU injections (P0/1/2) from the reason that GC genesis peaks shortly after birth during the first postnatal week in rats (Schlessinger et al., 1975) and that we have previously shown that overwhelming majority of GCs in DG at 6 months are cells born during P0–2 in C57BL/6J mice (Muramatsu et al., 2007). These data suggest that the effects of early-life SE on the localization of neonatally born GCs in the adulthood could be most prominently detected if we focus on P0/1/2-born GCs but not on the cells born in the later periods.

We chose the time point for the SE-induction at P14 because the long distance migration of GC precursors from the hilus to GCL peaks around P14 in rodents (Altman and Bayer, 1990). From the end of the third postnatal week to the adulthood, newborn GCs are located in the narrow area called the subgranular zone from where GCs migrate a short distance to GCL. These results suggest that P14 was an appropriate time point for examining the effects of early-life SE on the migration of neonatally born GCs and the final localization of them in the adult DG.

We chose the time point for animal scarification at 6-month-old so as to investigate the sequelae induced by early-life SE at the age as old as possible. The time point (6-month-old) was defined according to our previous report which confirmed that neonatally born GCs survive at least for 6 months in C57BL/6J mice (Muramatsu et al., 2007).

Six months after the last injection of BrdU, P0/1/2-born GCs were detected mainly in GCL of control mice (Figs. 1B, E), whereas in DG of SE mice, many P0/1/2-born GCs ectopically existed in the hilus (Figs. 1D, E).

Next to investigate the relationship of the cell birth date and the emergence of ectopic GCs in detail, the three time points of BrdU injection were examined, starting at P0, P7 and P35, respectively (Fig. 2A).

We examined the localization of P7/8/9-born GCs in the adulthood so as to clarify the effects of SE on migrating GC by comparing with the localization of P0/1/2-born GCs. This idea came from the fact that in the early postnatal periods of rodents, there is a day-dependent reduction of the scale in both the number of newborn GCs and the distance they migrate (Schlessinger et al., 1975; Altman and Bayer, 1990; Muramatsu et al., 2007). Thus, in case early-life SE interrupts GC migration and induced ectopic GCs, the density of ectopic GCs in Group2 is expected to be lower than that in Group1.

We examined the localization of P35/36/37-born GCs so as to compare the effects of SE on the migration of GCs born before SE (Group1 and 2) with those born after SE (Group3). The time points P35/36/37 were chosen from the reason that these are the periods when newborn GCs localize in the subgranular zone. Hence, by comparing the localization of P35/36/37-born GCs with those of P0/1/2-born and P7/8/9-born GCs, it is possible to determine whether SE affects the long-distance migration or not to induce ectopic GCs.

In Group1, mice with the history of SE had significantly more P0/1/2-born GCs in the hilus compared with control mice (Fig. 2D), but the density of P0/1/2-born GCs in the whole DG (Fig. 2B) was unchanged, suggesting that normal migration of newborn GCs from the hilus to GCL was interrupted.

In contrast to Group1, there was no statistically significant increase in the density of ectopic GCs in mice experiencing SE compared with control mice both in Group2 and Group3 ($P>0.05$, Student's t-test), even though ectopic GCs were found only in SE mice but not in control mice (Fig. 2D). The significant increase in the density of postnatally born GCs in GCL or DG was detected only in Group2 SE mice (Figs. 2B, C).

These results for the first time indicate that GCs born before early-life SE become ectopic GCs in the adulthood. Further, the results also indicate that early-life SE interrupts the migration of newborn GCs because we found more neonatally born ectopic GCs when BrdU was administrated in the developmentally earlier periods (P0/1/2 vs. P7/8/9) when scale of GC migration is larger.

Granule cell layer dispersion

In contrast to intrahippocampal kainate injection (Suzuki et al., 1995; Bouilleret et al., 1999), there have been no reports that reveal the induction of GCL dispersion, one of the characteristic pathological findings of epileptic patients (Houser, 1990; Armstrong, 1993; Thom et al., 2005), in pilocarpine-induced mouse model of TLE. Here we examined the possibility that the pilocarpine-induced SE in mice at P14 results in a dispersion of GCL because newborn GCs migrate dynamically in the hilus during the early postnatal days and thus the perturbation of their migration might contribute to GCL dispersion. For this purpose, we measured the width of Prox1-immunostained GCL throughout DG at 6 months. As a result, we did not find any significant change in the width in SE-experiencing mice compared with control mice (Fig. 3).

The mossy fiber sprouting

Although we revealed that a single pilocarpine-injection at P14 induced ectopic GCs in mice, there have been no reports using the same condition of early-life SE. Therefore, we determined it was necessary to estimate the severity of SE in our model by comparing with the previous studies from the viewpoint of inducing the pathological changes other than ectopic GCs. For these purposes, we chose MF sprouting because several studies reported that early-life SE results in MF sprouting in rodent models (for review, see Holmes, 2005). Furthermore, as MF sprouting itself is an important epileptogenic aberration observed in adult TLE hippocampus, we determined it was important to ask whether early-life SE results in MF sprouting even after 6 months. Using Timm histochemistry, we also detected sprouted MFs in CA3 but not in IML of the adult hippocampus (Fig. 4). These results suggest that the severity of early-life SE in our model was equivalent to the previous studies at least in the induction of MF sprouting and indicate that even a single early-life SE can result in MF sprouting later in the life.

Discussion

In the present study, we asked whether SE in the early postnatal periods affects the generation of the ectopic GCs, GCL dispersion, and MF sprouting in adult DG. For this purpose, we injected the cholinergic agent pilocarpine to C57BL/6J mice at P14 to induce SE and sacrificed them at 6-month-old for the following immunohistochemistry and Timm histochemistry. The principal findings are that ectopic GCs found in the hilus 6 months after early-life SE were cells born during P0–2. In contrast, GCs born during P7–9 or P35–37 did

not become ectopic GCs. The results that early-life SE induces ectopic GCs are of importance because ectopic GCs in the adult epileptic rats have been revealed to discharge synchronized epileptiform bursting with CA3 pyramidal cells (Scharfman et al., 2000), which also have synchronous bursts with survived excitatory mossy cells (Scharfman et al., 2001), and thus could contribute to the epileptogenesis in the adulthood.

After early-life SE at P14, a number of P0/1/2-born GCs become ectopic GCs in the adulthood (Figs. 1E, 2D). These data suggest that the migration of newborn GCs was interrupted by SE. Although a few ectopic GCs were found in the hilus of control mice (Fig. 1E), it is consistent with the anatomical studies reporting that a small population of GC-like cells were found even in the animals without the history of epileptic seizures (Seress and Pokorny, 1981; Gaarskjaer and Laurberg, 1983; Marti-Subirana et al., 1986).

In contrast to Group1, we did not find statistically significant increase of ectopic GCs in Group2 after SE, although SE mice had more ectopic GCs than control mice in Group2 (Fig. 2D). These results would reflect the day-dependent reduction in the number of newborn GCs (Schlessinger et al., 1975; Muramatsu et al., 2007) that are migrating in the hilus at the time point of seizure induction. As in Group2, no significant increase in the density of ectopic GCs was found in SE mice of Group3. It is probably because that P35–37 is the period when the long-distance GC migration in the hilus is already completed and the neurogenesis takes place only in the subgranular zone (Altman and Bayer, 1990).

Another possible mechanism that might explain the emergence of P0/1/2-born ectopic GCs in the adulthood arises from the viewpoint of the cell survival. There remains the possibility that P0/1/2-born GCs migrate ectopically but are eliminated before the time point of analysis in control mice, whereas in SE mice, some seizure-induced factors enhance the survival of P0/1/2-born GCs only in the hilus. Although we cannot completely exclude these possibilities, it is more reasonable to conclude that SE induced the change in GC localization because the density of P0/1/2-born GCs was increased only in the hilus (Fig. 2D) without changing that of the whole DG (Fig. 2B).

In Group2, we found increased GC neurogenesis both in GCL and DG (Figs. 2B, C), although it has been shown that neurogenesis is reduced after flurothyl-induced neonatal seizures (McCabe et al., 2001). These seemingly paradoxical findings probably result from the different time interval between BrdU-injection and seizure-induction and also from the different effects between SE and recurrent seizures. Indeed, McCabe et al. also reported a transient increase in neurogenesis when the final neonatal seizure was induced immediately after BrdU administration (McCabe et al., 2001). In Group2 mice, we induced SE by pilocarpine administration 5 days after the last BrdU injection (Fig. 2A), whereas McCabe and colleagues induced recurrent brief tonic seizures by repeated flurothyl injections before BrdU injection (McCabe et al., 2001). Changing the expression and/or release of several factors including several growth factors and neurotrophins (Kuhn et al., 1997; Wagner et al., 1999; Åberg et al., 2000; Scharfman et al., 2005), seizures would affect several processes of GC neurogenesis including the proliferation, the survival, the migration, the differentiation and the incorporation to existing neuronal networks. Hence, it is important to consider in which process BrdU-labeled GCs go through at the time point when the seizures are induced. Using mice model of early-life SE in the present study, we expected to discriminate the effects of seizures on the hilar migration of GCs by inducing seizures after BrdU administration (Group1 and 2) from the effects of seizures on the other aspects of neurogenesis such as GC proliferation and survival by inducing seizures before BrdU administration (Group3). Although not examined in the present study, it would be also important to consider the different effects of SE and recurrent seizures because those two conditions would have distinct mechanisms for seizure-induced injury.

We did not find any increase in the density of P35/36/37-born GCs after early-life SE (Fig. 2). These results may reflect the fact that pilocarpine-induced SE at P14 did not induce spontaneous recurrent seizures in the present study.

Although GCL dispersion occurs in pilocarpine-induced adult rat model of TLE (Mello et al., 1993), pilocarpine-induced SE at P14 in mice did not result in the dispersion in the adulthood (Fig. 3). The result indicates that early-life neurogenesis did not contribute to GCL dispersion after early-life SE in our model. It has been also shown in TLE patients (Heinrich et al., 2006) and in a kainate-induced mouse model of TLE (Fahrner et al., 2007) that neurogenesis does not contribute to the development of GCL dispersion.

MF sprouting, another important epileptogenic aberration observed in adult TLE hippocampus, was also detected in CA3 area of our mice model of early-life SE. At least from the viewpoint of inducing MF sprouting, the results confirm that the severity of SE in our model was equivalent to the previous studies which also reported the existence of MF sprouting in the adult rats after early-life SE. For example, when SE was induced by kainate at P15, MF sprouting was detected in CA3 but not in IML (Cross and Cavazos, 2007), consistent with the present study. In lithium-pilocarpine induced rat SE model, Cilio and colleagues revealed that SE at P16 or P20 induced MF sprouting both in CA3 and IML whereas SE at P12 did not result in MF sprouting neither in CA3 nor in IML, indicating age-dependent vulnerability of postnatal hippocampal development to epileptic activities (Cilio et al., 2003). The critical periods and the condition of seizures (i.e. SE and recurrent seizures) would be one cause for inducing MF sprouting in the different areas (Holmes, 2005).

Our study for the first time indicates that early-life SE induces hilar ectopic GCs which are born before SE. Following these findings, two next questions now emerge: 1), How do early-life seizures disrupt normal migration of neonatally born GCs to GCL? and 2), What do neonatally born ectopic GCs do in the adult hippocampus? Further investigations are underway in our laboratory using in vitro system to directly examine the mechanism underlying the migration of early-life seizure experiencing GCs. Giving answers to these questions will lead us to more detailed understanding of the proepileptogenic role of the hippocampus when it developed abnormally because of early-life seizures.

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