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Original Article

Neurogenic function in rats with unilateral hippocampal sclerosis that experienced early-life *status epilepticus*

Mark Dunleavy[1], Clara K Schindler[2], Sachiko Shinoda[2,3], Shane Crilly[1], David C Henshall[1,4]

[1]*Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland;* [2]*Robert S. Dow Neurobiology Laboratories, Legacy Research, Portland, OR, USA;* [3]*Department of Neurosurgery, Mie University School of Medicine, Mie, Tsu, Japan;* [4]*Irish Centre for Fetal and Neonatal Translational Research, Cork, Ireland*

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Abstract: Status epilepticus in the adult brain invariably causes an increase in hippocampal neurogenesis and the appearance of ectopic cells and this has been implicated as a causal factor in epileptogenesis. The effect of status epilepticus on neurogenesis in the developing brain is less well characterized and models of early-life seizures typically do not reproduce the hippocampal damage common to human mesial temporal sclerosis. We recently reported that evoking status epilepticus by intra-amygdala microinjection of kainic acid in post-natal (P) day 10 rats caused substantial acute neuronal death within the ipsilateral hippocampus and rats later developed unilateral hippocampal sclerosis and spontaneous recurrent seizures. Here, we examined the expression of a selection of genes associated with neurogenesis and assessed neurogenic function in this model. Protein levels of several markers of neurogenesis including polysialic acid neural cell adhesion molecule, neuroD and doublecortin were reduced in the hippocampus three days after status epilepticus in P10 rats. In contrast, protein levels of neurogenesis markers were similar to control in rats at P55. Pulse-chase experiments using thymidine analogues suggested there was a reduction in new neurons at 72 h after status epilepticus in P10 rats, whereas numbers of new neurons labelled in epileptic rats at P55 with hippocampal sclerosis were similar to controls. The present study suggests that status epilepticus in the immature brain suppresses neurogenesis but the neurogenic potential is retained in animals that later develop hippocampal sclerosis.

Keywords: Bromodeoxyuridine, epileptogenesis, febrile seizure, granule layer dispersion, neurogenesis, hippocampal sclerosis

Introduction

Prolonged seizures, including febrile status epilepticus, are the most common neurological emergency in childhood [1, 2]. The immature brain is quite resistant to seizure-damage but there remains concern that such seizures are harmful. In particular, that they cause hippocampal sclerosis, the hallmark lesion in patients with mesial temporal lobe epilepsy [2]. Recent neuroimaging studies show that febrile status epilepticus can produce injury to the hippocampus but only a small number of patients will later develop hippocampal sclerosis [2-4]. A majority of animal data show prolonged seizures in immature rodents produce only subtle injury to the brain and these animals do not develop hippocampal sclerosis [5, 6].

Prolonged seizures in the developing brain may contribute to adverse outcomes and epilepsy via mechanisms besides overt cell death. Neurogenesis is critical to the renewal capacity of the brain, learning and memory [7, 8]. Injury to the brain alters neurogenic activity, often producing a surge in the production of new cells. This may aid network repair but some new cells display immature functional properties that may contribute to epileptogenesis [9]. Ectopic cells have also been reported, both in individuals with temporal lobe epilepsy [10, 11], and in experimental models [12, 13].

In adult rodents, seizures invariably cause in an increase in neurogenesis [8, 14]. There have been mixed reports on whether neurogenesis is altered in adult human epilepsy. Indirect measures suggest neurogenesis may be increased

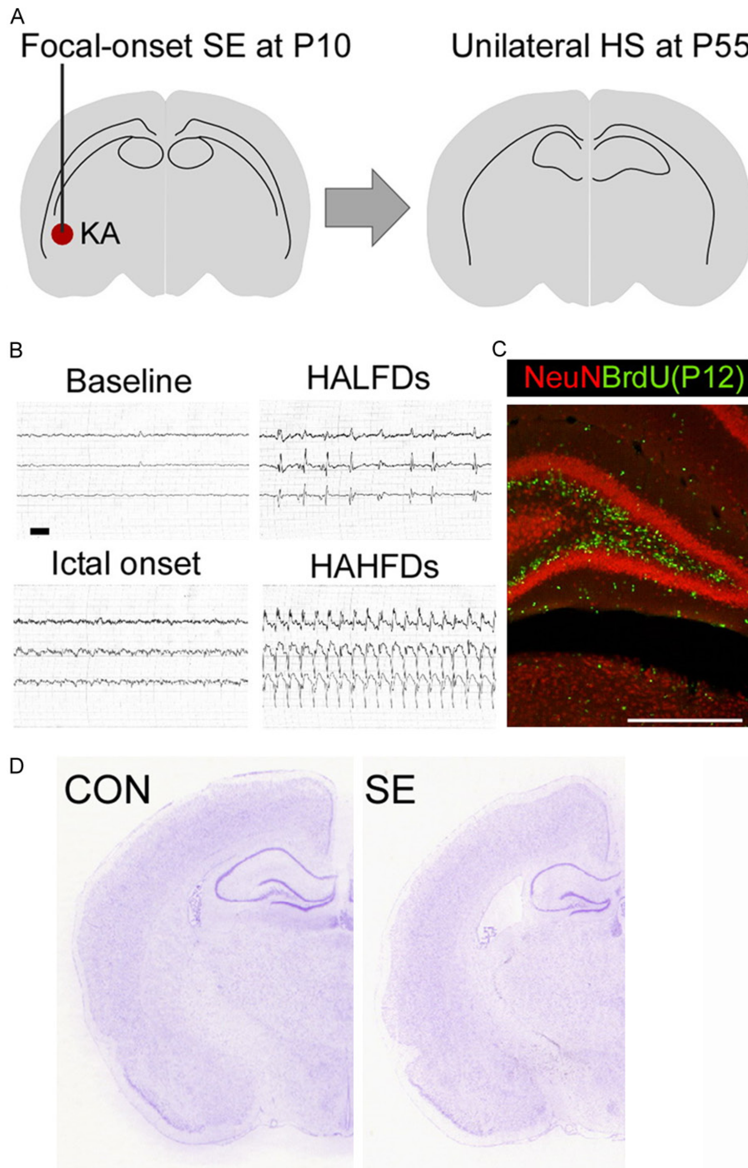

Figure 1. *Characteristics of model of status epilepticus in P10 rat pups by intra-amygdala KA*. A. Cartoon showing the stereotaxic level of injection and the later unilateral sclerosis in the model. B. EEG panels show representative EEG changes during the evolution of status epilepticus in a P10 rat given intra-amygdala KA. The EEG shows high frequency changes that lead to high amplitude low frequency discharges (HALFDs) that later develop into continuous high amplitude high frequency discharges (HAHFDs). Bar, 1 sec. C. Section through a P13 rat hippocampus stained for NeuN and BrdU that received BrdU at P12. Note localisation of new cells within the hilar region. Bar, 0.5 mm. D. Representative nissl-stained ipsilateral hemispheres of male rats at P55 that received either vehicle at P10 (CON) or intra-amygdala KA to induce status epilepticus (SE). Note the unilateral hippocampal sclerosis but relative preservation of brain structures outside the hippocampus in the status epilepticus rat.

[15-17], decreased [18] or unchanged [19, 20]. Seizures in the developing brain show critical age-dependent effects on neurogenesis. It is thought that rats at post-natal day (P) 8 - 10 are equivalent to a human new born or infant, while P11 to P24 spans the period from infancy through to preadolescence [21, 22]. Prolonged seizures in rodents of 2 - 4 weeks of age increase neurogenesis [23-25] whereas seizures in animals younger than this lead to suppression of neurogenesis [26-29]. This may be either a transient or permanent impairment [26]. There is less data on neurogenesis in children with epilepsy, but reports suggest neurogenesis may be suppressed [30].

Since hippocampal sclerosis is not a consequence of early-life seizures in most models it is uncertain whether neurogenic recovery is impaired in animals that later develop hippocampal sclerosis. We recently showed that intra-amygdala microinjection of kainic acid (KA) in P10 rat pups triggered seizures lasting many hours and resulted in significant damage to the ipsilateral hippocampal CA3, CA1 and hilar regions [31, 32]. By P55, rats displayed unilateral hippocampal sclerosis and spontaneous seizures [31]. This model now allows us to answer the following questions: Is neurogenesis acutely suppressed by a status epilepticus sufficient to provoke hippocampal sclerosis, and is neurogenic potential compromised in rats that later develop hippocampal sclerosis?

Materials and methods

Experiments were performed in accordance with protocols approved by the Legacy Institutional Animal Care and Use Committee and RCSI Research Ethics Committee (under license from the Department of Health, Ireland; B100/3248). Rats were housed in a facility on a 12:12 light/dark cycle and provided access to food and water *ad libitum*. Seizure induction

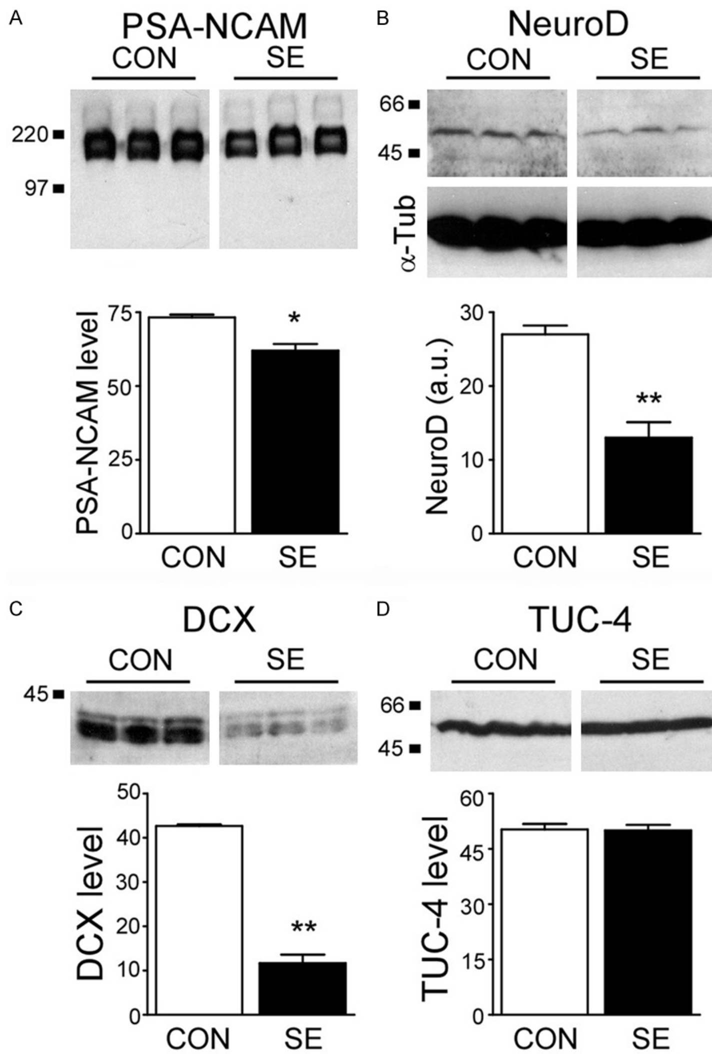

Figure 2. *Reduced neurogenesis marker proteins 72 h after status epilepticus in P10 rat pups.* Graphs show representative immunoblots for neurogenesis marker proteins (A) PSA-NCAM, (B) NeuroD, (C) DCX and (D) TUC-4, in vehicle-injected controls (CON) and rat pups that underwent status epilepticus induced by intra-amygdala KA (SE). Tubulin is included as a guide to loading. Unpaired *t* test with Welch's correction. $^{*}P < 0.05$; $^{**}P < 0.01$ (n = 3 per group). TUC-4 (non-significant; P = 0.88).

Table 1. Summary of immunoblot data on neurogenesis gene responses at 3 and 45 days after *status epilepticus* in P10 rats

Gene	*Acute (P13)*	*Chronic (P55)*
PSA-NCAM	↓	n.d.
NeuroD	↓	n.a.
Doublecortin	↓	n.d.
TUC-4	n.d.	n.d.

Abbreviations: *n.d.* not different. *n.a.* not assessed.

was by intra-amygdala injection of KA, modified from previous studies by this group [31]. All experiments were performed on male or female Sprague-Dawley rats (Harlan) on P10 or P11. On the day of the experiment, pups were removed from the dam, weighed, and anesthetized using isoflurane (5% induction, 1 - 2% maintenance in 100% O_2). Pups were placed in a stereotaxic frame equipped with a neonatal rat adaptor (David Kopf Instruments, Tujunga, CA), and body temperature was maintained at 37 ± 0.5°C by means of a homeostatic blanket (Harvard Apparatus, Boston, MA) and heat lamp. A complete craniectomy was drilled for placement of the injection cannula. Coordinates for the burr hole from Bregma were as follows: AP ± 1.44 mm; L ± 2.85 mm. Pups were then removed from the frame and placed in an open recording arena. Intraamygdala injections were performed with a 31-gauge internal cannula inserted into the guide and lowered below cortical surface to the injection point in the amygdala. Pups received either 0.2 µl of vehicle (phosphate buffer, pH 7.4) or KA (2 µg; Sigma-Aldrich). Following injection, lidocaine was applied and the wound sutured before the animal was placed in an incubator (35 ± 0.5°C) for behavioural analysis of seizures before being returned to the dam.

Video analysis of epileptic seizures

The sub-group of animals receiving thymidine analogues CldU + IdU were monitored for 48 h at P55-56 to determine the presence of spontaneous seizures (see also **Figure 4A**). Video was recorded using a Philips SPC 900NC PC camera and saved onto a PC in .avi format for offline analysis. A safe light was used to allow recording during darkness hours. The videos were then viewed by an experienced technician and assessed for spontaneous behavioural seizures. Behavioural seizures were determined to be present if the animal displayed behaviour corresponding to Racine Grade 3 or higher (Grade 3. Bilateral forelimb clonus; Grade 4.

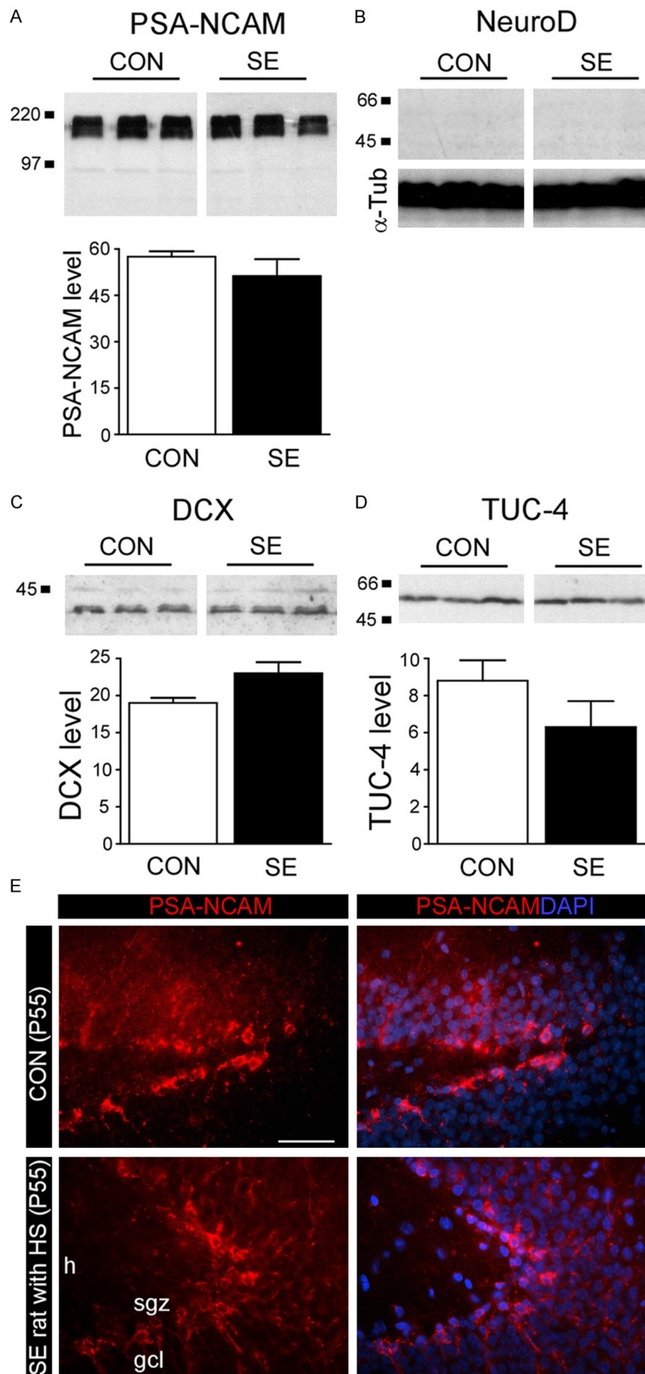

Figure 3. *Restitution of neurogenesis marker proteins at P55 in rats that underwent status epilepticus at P10.* Graphs and representative immunoblots for a selection of neurogenesis marker proteins (A) PSA-NCAM, (B) NeuroD, (C) DCX and (D) TUC-4 in the hippocampus of P55 rats that received either vehicle (CON) or intra-amygdala KA to induced status epilepticus (SE) at P10. Data were analysed using unpaired t test. Tubulin is included as a guide to loading. Unpaired *t* test with Welch's correction (*n* = 4 per group). PSA-NCAM (P = 0.34); NeuroD (not tested/no signal); DCX (P = 0.07); TUC-4 (P = 0.21). (E) Representative immunostaining for PSA-NCAM showing similar level of staining in a CON and a rat with hippocampal sclerosis at P55. *h*, hilus; *sgz*, subgranular zone; *gcl*, granule cell layer. Bar, 70 μm.

Forelimb clonus and rearing; Grade 5. Forelimb clonus with rearing and loss of posture). Following sacrifice of these animals, coronal sections at the level of the dorsal hippocampus were examined under a brightfield microscope to determine the presence of hippocampal sclerosis.

Injection of thymidine analogues

Three different thymidine analogs (5-bromo-2-deoxyuridine (BrdU), chlorodeoxyuridine (CldU), iododeoxyuridine (IdU)) were used to investigate cell proliferation and survival. For BrdU analysis, P12 rats were injected with BrdU (Sigma, Ireland) dissolved in 0.9% saline (50 mg/kg), 4 injections at 2 h intervals. Animals were deeply anaesthetised (sodium pentobarbital, 200 mg/kg) and transcardially perfused with 4% paraformaldehyde in PBS, pH 7.4. The brains were removed from the skull and post-fixed in 4% paraformaldehyde in PBS, pH 7.4 at 4°C overnight. Brains were then immersed in 20% sucrose in PBS overnight at 4°C.

To determine cell proliferation at different time-points following SE in the same animal we used a dual labelling protocol, with modifications [33, 34]. Briefly, rats received 42.75 mg/kg CldU (dissolved in 0.9% saline and 0.007N NaOH) 24 h post KA at P10 (3 injections at 4 h intervals) and followed up with 57.65 mg/kg IdU (dissolved in 0.9% saline, 0.2 N NaOH and normalised to pH 7 with 10 N HCl) at P55 (3 injections at 4 h intervals), before the animals were then sacrificed 7 days later (**Figure 4A**). Animals were perfused and fixed with PFA as described above with the BrdU animals. Brains from both the BrdU and CldU + IdU groups were then sectioned on a cryostat (25 μm) and stored in glycerol at -20°C for immunohistochemical staining.

Immunofluorescence

Sections at the level of dorsal hippocampus from control and KA rats at P55 were fixed, blocked and incubated with primary antibodies against PSA-NCAM (Monoclonal; Chemicon) followed by second-

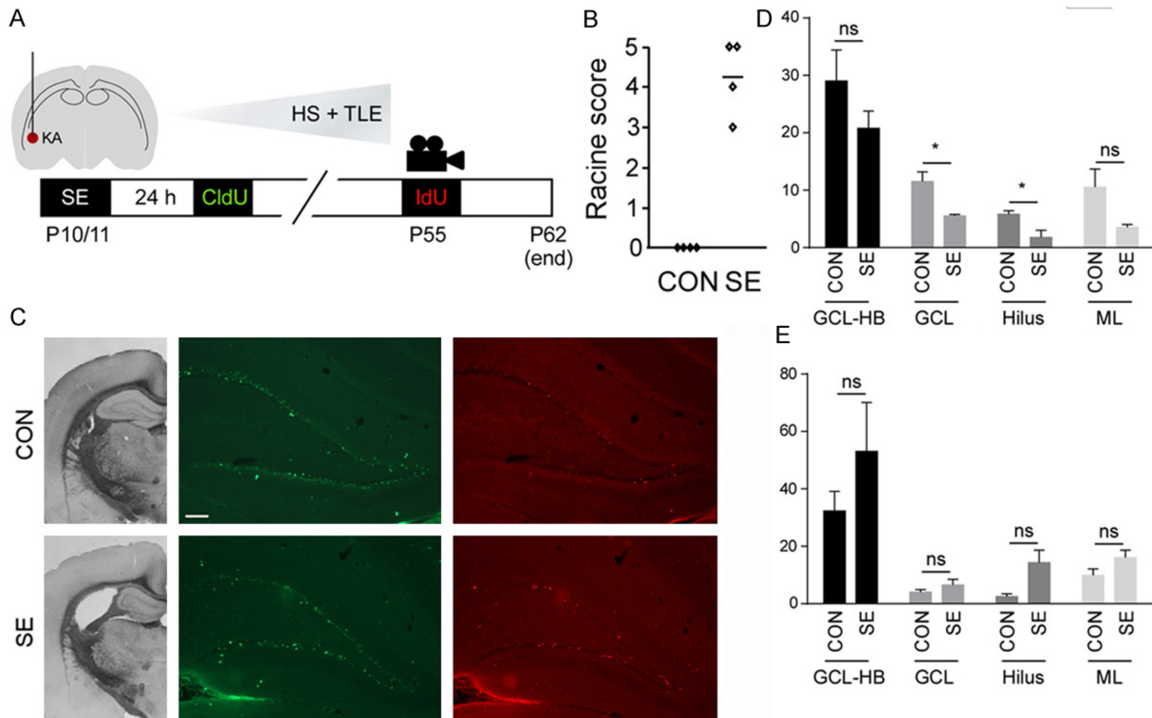

Figure 4. *Restitution of neurogenesis in rats with hippocampal sclerosis subjected to status epilepticus at P10*. A. Cartoon shows pulse-chase thymidine analogue experiment paradigm. Rats underwent status epilepticus at P10 induced by intra-amygdala KA and were given the thymidine analogue CldU at 24 h to label dividing neurons. At P55 (when hippocampal sclerosis had developed) rats were monitored for the occurrence of spontaneous seizures and then injected with IdU and were killed 7 days later. B. Behavioral scoring of spontaneous seizures in rats at P55 which were not observed in controls. C. Representative CldU and IdU staining from a control and status epilepticus rat. Note slightly reduced CldU staining in status epilepticus rats whereas IdU staining is somewhat higher in status epilepticus rats. Bar, 100 µm. D. Graph shows reduced neurogenesis rates in the animals labelled 24 h after status epilepticus. E. In contrast, IdU labelling at P55 indicates normal or slightly elevated neurogenesis rates at adulthood despite presence of hippocampal sclerosis. Student's unpaired t test with Welch's correction. $^{*}P < 0.05$ ($n = 4$ per group). GCL-HB; granule cell layer - hilar border; ML, molecular layer.

ary antibodies coupled to AlexaFluor 568 (BioSciences) and mounted with 4',6-diamidino-2-phenylindole (DAPI; Vector laboratories). Images were captured using a imaged using a Nikon 2000 s epifluorescence microscope with a Hamamatsu Orca 285 camera (Micron-optica, Enniscorthy, Ireland) [31].

For BrdU, IdU and CldU immunohistochemistry slices were incubated in 2 N HCl at 37°C for 30 min to denature the DNA and blocked with 1% bovine serum albumin (BSA) in 0.1% Triton X-100 in PBS. This was followed by incubation with rat anti-BrdU monoclonal antibody (1:500; Axyll Labs) to detect CldU and mouse anti-BrdU monoclonal antibody (1:500; BD Biosciences) to detect IdU overnight at 4°C. Previous work has shown that this staining protocol has high specificity with minimal cross reaction between the antibodies and the respective thymidine analogues [33]. Other sections were used to co-stain with antibodies against the neuronal marker NeuN (1:400; Millipore). Sections were washed and incubated with goat polyclonal antibodies coupled to AlexaFluor 488 or AlexaFlour 568 (BioSciences Ltd). Sections were mounted in aqueous mounting medium (Flurosave, Calbiochem). Staining was examined and images captured as above. Counts for individual subfields were the mean of the total from adjacent sections from *n* animals assessed by a blinded observer.

Western blot

Western blotting was undertaken as described previously [31]. Protein was extracted from the whole hippocampi in lysis buffer containing a protease inhibitor mixture (Sigma-Aldrich) and 50 µg samples separated by 12 to 15% SDS-PA-

GE. Proteins were transferred to polyvinylidene difluoride membranes and incubated with specific anti-bodies against the following: α-tubulin, NeuroD and DCX (Santa Cruz Biotechnology, Santa Cruz, CA), PSA-NCAM and TUC-4 (Chemicon International). Protein levels were detected using chemiluminescence, imaged and semiquantitative densitometry undertaken on timematched gels using AlphaEaseFC 4.0 software.

Statistics

Data are presented as mean ± standard error of the mean (SEM). Data were compared using unpaired t test (Welch corrected for non-equal SDs). Significance was accepted at $P < 0.05$.

Results

Status epilepticus triggered by intra-amygdala kainic acid in P10

Figure 1A shows the experimental paradigm. P10/11 rat pups received intra-amygdala KA injection to induce status epilepticus. Rats later display unilateral hippocampal sclerosis (e.g. at P55). Representative EEG traces from P10 rats are shown in **Figure 1B** confirming the development of high amplitude high frequency spiking after KA injection. Electrographic seizure activity evolved over the first hour after KA injection, becoming continuous and eventually abating after 4-24 h [31, 32]. For the thymidine labelling studies, pups were not equipped with EEG but were observed for 40 min after KA injection and animals retained in which there was confirmed seizure-like behaviour including loss of posture, free running, clonus and vocalisations. We previously reported that rat pups analysed 72 h later display permanent injury within the CA3, CA1 and hilus of the ipsilateral hippocampus, as assessed using either Fluoro-jade B or the TUNEL assay for double-stranded DNA fragmentation [31, 32].

In the post-natal brain, neurogenesis rates peak in the first week but remain elevated thereafter. To confirm we could label new neurons using thymidine analogues, we injected rat pups with BrdU at P12 and then analyzed staining at P13 (**Figure 1C**). As expected, extensive BrdU labelling of cells was observed in the hilus of sections from P13 rats (**Figure 1C**).

Next, we performed nissl staining of tissue sections from rats killed 45 days after status epilepticus. As reported [31], rats that underwent status epilepticus at P10 later displayed hallmark hippocampal sclerosis that was restricted to the side ipsilateral to original KA injection (**Figure 1D**). Rats that received intra-amygdala vehicle injection at P10 displayed a normal-appearing hemisphere (**Figure 1D**).

Reduction in the expression of neurogenesis markers following status epilepticus in P10 rats

To understand the effects of status epilepticus on neurogenesis in this model we first examined the expression of a number of key markers of neurogenesis in the hippocampus. We selected markers with roles in key steps in proliferation and migration [7], for which data were also available in resected hippocampus from patients with temporal lobe epilepsy [20]. These were: (i) PSA-NCAM (polysialated neural cell adhesion molecule (CD56), a large plasma membrane glycoprotein normally present in areas of neurogenic potential such as the subgranular zone of the dentate gyrus. (ii) NeuroD (Neurogenic differentiation factor 1), a 40 kD neurogenic basic helix-loop-helix protein required for differentiation of hippocampal granule neurons. (iii) doublecortin, a ~43 kD microtubule-associated protein found in immature migrating and differentiating neurons. (iv) TUC-4 (turned on after division 4) which encodes a ~60 kD protein that is expressed early in differentiation and a marker of immature, potentially new neurons.

Western blot analysis of hippocampal samples obtained 72 h after status epilepticus (i.e. P13) revealed decreased expression of PSA-NCAM (**Figure 2A**), NeuroD (**Figure 2B**) and DCX (**Figure 2C**) compared with controls. No difference in TUC-4 expression was observed between the groups (**Figure 2D** and see **Table 1**).

Normal expression of neurogenesis markers at P55 in rats that underwent status epilepticus at P10

We next investigated expression of the same markers in control and status epilepticus rats killed at P55 (**Figure 3**). Protein levels of PSA-NCAM (**Figure 3A**), doublecortin (**Figure 3C**) and TUC-4 (**Figure 3D**) were not significantly different between controls and rats that underwent status epilepticus at P10. We could not detect a protein band for NeuroD in either group (**Figure 3B**). Supporting these findings, sections

from control and status epilepticus rats at P55 showed similar PSA-NCAM staining (**Figure 3E**).

Pulse-chase experiments suggest acute suppression of neurogenesis following status epilepticus at P10

To support these protein data we used a thymidine analogue technique to separately label proliferating cell populations at two time-points (**Figure 4A**). CldU was injected 24 h after status epilepticus or vehicle at P10, and then IdU was injected at P55 into both groups (n = 4 for both groups). All animals were assessed for the occurrence of spontaneous seizures at P55 (**Figure 4B**). No seizure-like behaviour was observed in the control group. Furthermore hippocampal sclerosis and hydrocephalus *ex-vacuo* was present in all the KA-treated animals upon sacrifice at P62 (**Figure 4C** and data not shown). There was no significant difference in body weight between the groups (CON: 215 ± 4 g; SE: 208 ± 24 g).

Counts of CldU positive cells in sections of the ipsilateral dentate gyrus of rats at P62 that underwent status epilepticus at P10 were significantly lower than controls (**Figure 4C**, **4D**). This effect was most obvious in the granule cell layer and hilus but a similar trend was found in the molecular layer and granule cell layer-hilar border (**Figure 4D**).

Normal neurogenesis at P55 in animals with hippocampal sclerosis following status epilepticus at P10

Last, we examined IdU staining, which marked proliferating cells at P55 following status epilepticus at P10. In contrast to findings with CldU, numbers of IdU-positive cells in rats with hippocampal sclerosis that underwent status epileptius at P10 were not significantly different from controls. There was, however, a consistent trend, particularly in the hilus (P = 0.06), for increased IdU labelling.

Discussion

The effect of prolonged seizures on neurogenesis in the developing brain is of significant interest since it may affect learning and memory, repair capacity and the pathogenesis of epilepsy. Here we show that status epilepticus in P10 rat pups induced by intra-amygdala KA results in a transient suppression of neurogenesis. Surprisingly, we found that levels of neurogenesis proteins and labelling of proliferating cells were similar to controls rats at P55, despite the presence of hippocampal sclerosis and epileptic seizures. Together these data suggest that prolonged seizures in the developing brain temporarily interfere with neurogenesis but the hippocampus can retain full neurogenic potential despite significant damage and ongoing seizures.

The impact of seizures on the developing brain is of major clinical importance as there continues to be concern that prolonged seizures in children can result in mesial temporal sclerosis, a common epilepsy syndrome that is particularly refractory to medication [2]. Fortunately, recent human imaging data show that prolonged febrile seizures rarely cause hippocampal sclerosis [4]. Nevertheless, early-life seizures may promote epileptogenesis through other mechanisms, including by disturbing neurogenesis. Research to date has shown that while neurogenesis is increased in the adult brain after seizures, early-life seizures often suppress neurogenesis. What has been difficult to determine until now, however, is whether neurogenic function can recover in an injured hippocampus. This is because few animal models at the age where neurogenesis is reduced by early-life prolonged seizures, later display hippocampal sclerosis [22]. The model used in the present study is therefore important because it features prolonged early life seizures that leads to unilateral hippocampal sclerosis and epilepsy. We selected 10 day old rat pups because they are thought to be correspond to the end of the neonatal period and infancy in humans, when neonatal and febrile seizures occur [21, 22].

A first major finding in the present study was that protein markers of neurogenesis were reduced in rats 72 h after status epilepticus in the model. This was seen for three of the four genes investigated and is suggestive of transient suppression of the production and/or migration of new brain cells. Since this period of time (second week of life) is critical for cell proliferation and migration this could have profound effects on hippocampal development that would alter hippocampal excitability, learning and memory [7]. Surprisingly, TUC-4 levels were unaffected in the rats. The function of TUC-4 is rather less well known than other markers such as doublecortin and PSA-NCAM but it has been shown to be involved in axon guidance and

extension [35]. It is possible that TUC-4 protein is more stable than other tested markers or that the processes it is involved in are not affected during the period of study. Regardless, our data suggest there is a reduction in neurogenesis shortly following prolonged seizures in P10 rats which is consistent with labelling and marker work in other models [26, 28, 29]. It is difficult to directly compare our protein data to measures of neurogenesis in other studies. However, our results seem to suggest similar if somewhat more severe suppression [28, 29]. Beyond a certain duration or severity, seizures may produce a similar inhibitory effect on neurogenesis in the developing brain.

We found lower numbers of CldU-labelled cells in rats that underwent status epilepticus at P10 compared to controls. A simple interpretation of this data is that seizures at P10 supressed neurogenesis. This would be consistent with the protein marker data and fits with studies in other models in which seizures in rodents below two weeks of age reduce neurogenesis [26-29]. This also fits with some human data on reduced neurogenesis markers in children with epilepsy [30]. In contrast, neurogenesis was reported to be increased following prolonged seizures in rats of two weeks or older, regardless of damage [23]. This suggests the neurogenic function of the hippocampus has a specific period of vulnerability to seizures which, if also true in the developing human brain, could have implications for the treatment of seizures and long-term prognosis. Because of the experimental design of our study, we cannot exclude that labelling rates in seizure rats were similar to control at P11/12, but that the survival of these cells in the post-status epilepticus brain was poorer. Certainly, significant numbers of new born cells do not survive when born into a post-status epilepticus damaged hippocampus [36]. This idea could be tested in future studies, for example by performing analyses at a shorter survival time.

The second major finding in the present study was that marker proteins and labelling of proliferating cells at P55 in rats previously subject to status epilepticus at P10 was similar to control animals. This was unexpected, particularly because the hippocampus of these rats had experienced very severe injury at P10 and displayed unilateral hippocampal sclerosis. These rats were also epileptic. This suggests the neurogenic potential of the hippocampus was either not permanently damaged by the seizures at P10 or that the neurogenic niches underwent some degree of repair. This fits with the remarkable robustness of the neurogenic niche, which continues to maintain or even increase cell production despite severe hippocampal pathology [37]. Our data contrast somewhat with the majority of data from adult seizure models in which neurogenesis is invariably increased [38]. They also differ from findings at adulthood following early-life seizures where again neurogenesis levels were elevated [26]. This difference is unlikely to be due to rates of epileptic seizures since animals in our studies all had recent seizures. We may conclude, therefore, that although neurogenic function is largely preserved in this model, the extensive hippocampal injury may blunt epileptic seizure-related increases.

There are a number of limitations to the present study. Our model uses KA to trigger prolonged seizures and this lacks physiological relevance when compared to models in which milder seizures are induced through methods such as hyperthermia [2]. Second, the group sizes in the thymidine studies were small. We saw a consistent trend toward increased numbers of IdU-positive cells in animals with hippocampal sclerosis and it is possible that with larger group sizes we would have statistical evidence for increased neurogenesis in these animals. The array of molecular analyses we performed was quite limited and restricted to measurement of protein levels. We did not measure gene transcription and a number of other genes relevant to neurogenesis in epilepsy are known, including Numb [20]. We did not perform functional analyses of new born neurons and studies consistently show such cells, when born into a post-status epilepticus environment, display an immature phenotype with firing properties that may influence hippocampal excitability [13, 14]. Although we did not undertake a widespread analysis of the placement of proliferating cells in this model, it is interesting to note the increased level of CldU positive cells present in the hilus of the KA-treated animals. Ectopic granular cells have been implicated in epileptogenesis following neonatal seizures [12]. It would be of interest in future studies to trace aberrant cell migration in this model and determine how this is affected by the developing hippocampal sclerosis.

In conclusion, these studies reveal a bi-phasic neurogenesis response following status epilep-

ticus in P10 rat pups in the intra-amygdala KA model. Initially, neurogenesis may be suppressed. It appears to recover, however, and neurogenic potential appears restored in the sclerotic hippocampus of rats with later life epilepsy. Together these data indicate a remarkable resilience of neurogenesis after early-life status epilepticus that leads to temporal lobe epilepsy with hippocampal sclerosis.

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Address correspondence to: Dr. David C Henshall, Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland. Tel: +353 1 402 8629; Fax: +353 1 402 2447; E-mail: dhenshall@rcsi.ie

References

[1] Chin RF, Neville BG, Peckham C, Bedford H, Wade A and Scott RC. Incidence, cause, and short-term outcome of convulsive status epilepticus in childhood: prospective population-based study. Lancet 2006; 368: 222-229.
[2] Patterson KP, Baram TZ and Shinnar S. Origins of temporal lobe epilepsy: febrile seizures and febrile status epilepticus. Neurotherapeutics 2014; 11: 242-250.
[3] Yoong M, Martinos MM, Chin RF, Clark CA and Scott RC. Hippocampal volume loss following childhood convulsive status epilepticus is not limited to prolonged febrile seizures. Epilepsia 2013; 54: 2108-2115.
[4] Lewis DV, Shinnar S, Hesdorffer DC, Bagiella E, Bello JA, Chan S, Xu Y, MacFall J, Gomes WA, Moshe SL, Mathern GW, Pellock JM, Nordli DR Jr, Frank LM, Provenzale J, Shinnar RC, Epstein LG, Masur D, Litherland C, Sun S and Team FS. Hippocampal sclerosis after febrile status epilepticus: the FEBSTAT study. Ann Neurol 2014; 75: 178-185.
[5] Lado FA, Laureta EC and Moshe SL. Seizure-induced hippocampal damage in the mature and immature brain. Epileptic Disord 2002; 4: 83-97.
[6] Wasterlain CG, Niquet J, Thompson KW, Baldwin R, Liu H, Sankar R, Mazarati AM, Naylor D, Katsumori H, Suchomelova L and Shirasaka Y. Seizure-induced neuronal death in the immature brain. Prog Brain Res 2002; 135: 335-353.
[7] Lledo PM, Alonso M and Grubb MS. Adult neurogenesis and functional plasticity in neuronal circuits. Nat Rev Neurosci 2006; 7: 179-193.
[8] Parent JM and Kron MM. Neurogenesis and Epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's Basic Mechanisms of the Epilepsies. 4th. Bethesda (MD); 2012.
[9] Scharfman HE and McCloskey DP. Postnatal neurogenesis as a therapeutic target in temporal lobe epilepsy. Epilepsy Res 2009; 85: 150-161.
[10] Houser CR. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. Brain Res 1990; 535: 195-204.
[11] Lurton D, El Bahh B, Sundstrom L and Rougier A. Granule cell dispersion is correlated with early epileptic events in human temporal lobe epilepsy. J Neurol Sci 1998; 154: 133-136.
[12] Koyama R, Tao K, Sasaki T, Ichikawa J, Miyamoto D, Muramatsu R, Matsuki N and Ikegaya Y. GABAergic excitation after febrile seizures induces ectopic granule cells and adult epilepsy. Nat Med 2012; 18: 1271-1278.
[13] Scharfman H, Goodman J and McCloskey D. Ectopic granule cells of the rat dentate gyrus. Dev Neurosci 2007; 29: 14-27.
[14] Kokaia M. Seizure-induced neurogenesis in the adult brain. Eur J Neurosci 2011; 33: 1133-1138.
[15] Mikkonen M, Soininen H, Kalvianen R, Tapiola T, Ylinen A, Vapalahti M, Paljarvi L and Pitkanen A. Remodeling of neuronal circuitries in human temporal lobe epilepsy: increased expression of highly polysialylated neural cell adhesion molecule in the hippocampus and the entorhinal cortex. Ann Neurol 1998; 44: 923-934.
[16] Crespel A, Rigau V, Coubes P, Rousset MC, de Bock F, Okano H, Baldy-Moulinier M, Bockaert J and Lerner-Natoli M. Increased number of neural progenitors in human temporal lobe epilepsy. Neurobiol Dis 2005; 19: 436-450.
[17] Liu YW, Curtis MA, Gibbons HM, Mee EW, Bergin PS, Teoh HH, Connor B, Dragunow M and Faull RL. Doublecortin expression in the normal and epileptic adult human brain. Eur J Neurosci 2008; 28: 2254-2265.
[18] Hattiangady B, Rao MS and Shetty AK. Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. Neurobiol Dis 2004; 17: 473-490.
[19] Fahrner A, Kann G, Flubacher A, Heinrich C, Freiman TM, Zentner J, Frotscher M and Haas CA. Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients. Exp Neurol 2007; 203: 320-332.
[20] Engel T, Schindler CK, Sanz-Rodriguez A, Conroy RM, Meller R, Simon RP and Henshall DC.

Expression of neurogenesis genes in human temporal lobe epilepsy with hippocampal sclerosis. Int J Physiol Pathophysiol Pharmacol 2011; 3: 38-47.

[21] Baram TZ, Gerth A and Schultz L. Febrile seizures: an appropriate-aged model suitable for long-term studies. Brain Res Dev Brain Res 1997; 98: 265-270.

[22] Haut SR, Veliskova J and Moshe SL. Susceptibility of immature and adult brains to seizure effects. Lancet Neurol 2004; 3: 608-617.

[23] Sankar R, Shin D, Liu H, Katsumori H and Wasterlain CG. Granule cell neurogenesis after status epilepticus in the immature rat brain. Epilepsia 2000; 41 Suppl 6: S53-56.

[24] Porter BE, Maronski M and Brooks-Kayal AR. Fate of newborn dentate granule cells after early life status epilepticus. Epilepsia 2004; 45: 13-19.

[25] Gray WP, May K and Sundstrom LE. Seizure induced dentate neurogenesis does not diminish with age in rats. Neurosci Lett 2002; 330: 235-238.

[26] Dong H, Csernansky CA, Goico B and Csernansky JG. Hippocampal neurogenesis follows kainic acid-induced apoptosis in neonatal rats. J Neurosci 2003; 23: 1742-1749.

[27] Xiu-Yu S, Ruo-Peng S, Ji-Wen W. Consequences of pilocarpine-induced recurrent seizures in neonatal rats. Brain Dev 2007; 29: 157-163.

[28] McCabe BK, Silveira DC, Cilio MR, Cha BH, Liu X, Sogawa Y and Holmes GL. Reduced neurogenesis after neonatal seizures. J Neurosci 2001; 21: 2094-2103.

[29] Lauren HB, Ruohonen S, Kukko-Lukjanov TK, Virta JE, Gronman M, Lopez-Picon FR, Jarvela JT and Holopainen IE. Status epilepticus alters neurogenesis and decreases the number of GABAergic neurons in the septal dentate gyrus of 9-day-old rats at the early phase of epileptogenesis. Brain Res 2013; 1516: 33-44.

[30] Mathern GW, Leiphart JL, De Vera A, Adelson PD, Seki T, Neder L and Leite JP. Seizures decrease postnatal neurogenesis and granule cell development in the human fascia dentata. Epilepsia 2002; 43 Suppl 5: 68-73.

[31] Dunleavy M, Shinoda S, Schindler C, Ewart C, Dolan R, Gobbo OL, Kerskens CM and Henshall DC. Experimental neonatal status epilepticus and the development of temporal lobe epilepsy with unilateral hippocampal sclerosis. Am J Pathol 2010; 176: 330-342.

[32] Mesuret G, Engel T, Hessel EV, Sanz-Rodriguez A, Jimenez-Pacheco A, Miras-Portugal MT, Diaz-Hernandez M and Henshall DC. P2X7 receptor inhibition interrupts the progression of seizures in immature rats and reduces hippocampal damage. CNS Neurosci Ther 2014; 20: 556-564.

[33] Vega CJ and Peterson DA. Stem cell proliferative history in tissue revealed by temporal halogenated thymidine analog discrimination. Nat Methods 2005; 2: 167-169.

[34] Tuttle AH, Rankin MM, Teta M, Sartori DJ, Stein GM, Kim GJ, Virgilio C, Granger A, Zhou D, Long SH, Schiffman AB and Kushner JA. Immunofluorescent detection of two thymidine analogues (CldU and IdU) in primary tissue. J Vis Exp 2010; 7.

[35] Quinn CC, Gray GE and Hockfield S. A family of proteins implicated in axon guidance and outgrowth. J Neurobiol 1999; 41: 158-164.

[36] Ekdahl CT, Zhu C, Bonde S, Bahr BA, Blomgren K and Lindvall O. Death mechanisms in status epilepticus-generated neurons and effects of additional seizures on their survival. Neurobiol Dis 2003; 14: 513-523.

[37] Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC and Greenberg DA. Increased hippocampal neurogenesis in Alzheimer's disease. Proc Natl Acad Sci U S A 2004; 101: 343-347.

[38] Parent JM, Elliott RC, Pleasure SJ, Barbaro NM and Lowenstein DH. Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. Ann Neurol 2006; 59: 81-91.