Region-specific vulnerability to endoplasmic reticulum stress-induced neuronal death in rat brain after status epilepticus

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We sought to clarify the involvement and the intra-cerebral distribution variability of C/EBP homologous protein (CHOP), a representative molecule related to endoplasmic reticulum (ER) stress-induced cell death signalling pathways, in neuronal death resulting from status epilepticus in rats. The expression patterns of CHOP and glucose-regulated protein (GRP) 78, a good marker of ER stress, were assessed by Western blotting, real-time PCR, Hoechst and immunohistochemistry in the hippocampus, cortex and striatum on a status epilepticus (SE) model. Double-fluorescent staining of CHOP and the terminal deoxynucleotidyl transferase-mediated DNA nick-end labelling (TUNEL) method were performed to clarify the involvement of CHOP in cell death. SE resulted in a time-dependent increase in the expression of GRP78 and CHOP. The expression of GRP78 protein was increased at 3, 6 and 12 h after SE and no brain region variability was found. The expression of CHOP protein was also increased, reached its peak at 24 h and remained high at 48 h. CHOP protein expression, however, showed brain region variability with highest expression noted in the hippocampus followed by the striatum, and lowest in the cortex. The up-regulation of CHOP occurring at the transcriptional level was demonstrated by real-time PCR. Double fluorescence showed that CHOP expression strongly correlated with neurons undergoing apoptosis. The results indicated that SE compromises the function of the ER and that the hippocampus is more vulnerable than the cortex and the striatum.

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1. Introduction

Status epilepticus (SE) is a devastating neurological condition with a high morbidity and mortality if not treated immediately, with the mortality ranging from 7.6% to 4.3% (Chin *et al.* 2004). We defined SE as 'an acute epileptic condition characterized by continuous seizures (partial or generalized, convulsive or non-convulsive) for at least 30 min of intermittent seizures' (Wasterlain and Chen 2006). SE can result in neuronal cell death (Yang *et al.* 2007; Gao and Geng 2013) and *sensitivity to a chronic epileptogenic influence* (Dedeurwaerdere *et al.* 2012). An episode of SE can be associated with the development of temporal lobe epilepsy (Ryley Parrish *et al.* 2013). And an association of early SE with a lower seizure threshold and spontaneous was reported by Williams *et al.* (Rattka *et al.* 2013).

In spite of numerous studies focusing on epileptogensis both in adult and immature brain (Lopes *et al.* 2013; Laurén *et al.* 2013), its cellular and molecular mechanisms have remained largely undiscovered. Neuronal loss and microglial activation are well-described features of epilepsy, and contribute to epileptogenesis (Rattka *et al.* 2013; Kim *et al.* 2011). However, the mechanisms that induce apoptosis in the development of SE-induced brain damage are not fully characterized. Recently, endoplasmic reticulum (ER) stress is increasingly recognized as a major trigger for apoptosis

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(Roussel *et al.* 2013). This ER-specific apoptosis pathway is different from the well-known death receptor ('extrinsic') and mitochondrial ('intrinsic') apoptotic pathways (Liu *et al.* 2013a, b).

The endoplasmic reticulum performs several functions, including protein folding and trafficking and regulating intracellular calcium levels (Xu *et al.* 2005). Seizure activity results in the activation of an endoplasmic reticulum stress response (Pelletier *et al.* 1999; Kitao *et al.* 2001; Yamamoto *et al.* 2006; Sokka *et al.* 2007). Endoplasmic reticulum stress is normally sensed by three upstream signalling proteins; the kinases PERK [protein kinase RNA (PKR)-like ER (endoplasmic reticulum) kinase] and IRE1 (inositol-requiring protein1) and the transcription factor ATF6 (activating transcription factor 6) (Yamamoto *et al.* 2006). Together, these initiate the unfolded protein response that functions to up-regulate endoplasmic reticulum chaperones, such as glucose-regulated protein 78, inhibit most protein translation and activate proteases involved in the degradation of misfolded proteins (Xu *et al.* 2005; Hetz 2012).

When cells are subjected to intense and prolonged ER stresses, apoptosis is induced to eliminate the damaged cells in order to protect the organism (Hwang *et al.* 2007). One of the most important apoptotic pathways activated by ER stress involves CHOP. CHOP, also called GADD 153 (growth arrest and DNA damage-inducible gene 153), is a proapoptotic transcription factor expressed ubiquitously at very low levels under physiological conditions, but strongly induced in response to ER stress (Oyadomari and Mori 2004; Liu *et al.* 2013a, b). As one of the components of the ER stress-mediated apoptosis pathway, CHOP mediated apoptosis seems to be involved in some human diseases like diabetes mellitus, spinal cord injury (Wang *et al.* 2013) and neurodegenerative diseases (Xiong *et al.* 2013). Furthermore, a selective neuronal apoptosis was induced in the different brain regions upon damage caused by transient brain ischemia (Ayuso *et al.* 2013) or hypoxia (O'Connor 2013).

However, the involvement of the ER stress in neuronal apoptosis following SE has remained poorly analysed. In the present study, we showed that SE can cause ER stress responses in the brain that lead to cell death. Our study also uncovers the determinant role of CHOP in the fate of the neuron and the distribution variability of CHOP in the brain following SE.

2. Materials and methods

2.1 *Animals and induction of status epilepticus*

This study was carried out in strict accordance with the Animal Management Rule of the People's Republic of China and the Care and Use of the Laboratory Animals Guide of Nanjing Medical University. The protocol was approved by the Committee on the Ethics of Animal Experiments of Nanjing Medical University (Permit Number: 2008-0004). All surgery was performed under chloral hydrate anesthesia, and all efforts were made to minimize suffering. Newborn Sprague-Dawley male rats were housed in cages with their mothers under standard laboratory conditions until they were weaned at 21 days (body weight, ~100 g). Animals aged 21 days were used in the experiments. Rats (n=80) were randomly divided into the following 2 groups: control and SE groups, with 40 rats each *group and 8 rats at each time point.* SE rats were given a single intraperitoneal (i.p.) dose (80mg/kg) of pentylenetetrazol (Sigma, USA). Control animals (n=40) were treated similarly with an equal volume of saline instead of pentylenetetrazol. Seizure manifestations included twitching of facial muscles, mastication, head nodding, clonic movements of forelimbs, tail erection and 'swimming' movements. To prevent the death due to status epilepticus, diazepam was injected i.p. an hour after the onset of status epilepticus. Rats of both groups were all sacrificed at either 3, 6, 12, 24 and 48 h post-seizure.

2.2 *Histological analysis*

Some animals were anesthetized with 10% chloral hydrate (0.35 mL/100 g, i.p.), perfused transcardially with saline, and followed by the administration of 4% paraformaldehyde in 0.01 M phosphate-buffer saline (PBS, pH 7.4) for 30 min. The rat brains were maintained in the same fixative for 20 h and then successively dehydrated overnight in 20% and 30% sucrose in PBS; the brains were finally frozen in liquid nitrogen (not inducing ice crystal in the brains and showing the texture of the brain tissue better) and stored at –80°C for further analysis. The brains were sectioned into 8-μm-thick coronal sections using a cryostat.

For single immunostaining, a goat polyclonal anti-GRP78 (1:100, Santa Cruz Biotech, USA) was used overnight at 4°C. After subsequent washing in PBS, the sections were incubated with a secondary rabbit anti-goat antibody, conjugated to a peroxidase labled dextran polymer (Goat Polymer Kit, Zhongshan Goldenbridge Biotechnology, China) at 37°C for 30 min. 3,3-diaminobenzidine tetrahydrochloride was then added to the sections. As a negative control, an immunohistochemical assay was performed without a primary antibody.

For the determination of apoptosis, TUNEL assay was performed using the *In Situ* Cell Death Detection Kit, Fluorescein (Roche Applied Science, Germany) according to the protocol provided by the manufacturer. For the negative staining controls, the TdT reaction mixture was omitted. To analyse the morphological changes in the nuclear chromatin of cells undergoing apoptosis, the sections were stained with the Hoechst 33528 fluorochrome (Applygen Technologies Inc., China) for 10 min.

For double immunofluorescence of neuronal nuclei (NeuN), a well-established neuronal marker, and CHOP, the brain sections were successively treated with 0.3% H_2O_2/methanol, 0.1% Triton X-100/PBS, and 5% BSA/PBS. Primary antibodies were applied to the sections, and the sections were incubated overnight at 4°C (rabbit polyclonal anti-CHOP antibody, 1:200, Santa Cruz, USA; mouse monoclonal anti-NeuN antibody, 1:1000, Chemicon International, USA). The sections were visualized by incubation with rhodamine-labelled anti-rabbit immunoglobulin (IgG) and fluorescein isothiocyanate (FITC)-labelled anti-mouse IgG (1:50, Zhongshan Goldenbridge) at room temperature for 45 min. For double immunostaining of CHOP and TUNEL, CHOP immunostaining was performed using the anti-CHOP antibody (1:500) and rhodamine fluorescence systems, followed by the previously described TUNEL method. All fluorescence images of the sections were obtained with a confocal laser scanning microscope (Olympus Fluoview FV1000, Japan).

2.3 *Western blot analysis*

Another part of rat (n=80) were deeply anesthetlzed with 10% chloral hydrate (0.35 mL/100 g, i.p.) and decapltated at 3, 6, 12, 24, or 48 h after seizure. Brains were removed, frozen in liquid nitrogen and stored in a deep freezer. These brain tissues were homogenized on ice in a lysis buffer [50 mM Tris-HCl, pH 7.9; 150 mM NaCl; 1 mM ethylenediaminetetraacetic acid (EDTA); 1% nonidet (NP)-40; 0.25% sodium deoxycholate; and 1 mM phenylmethylsulfonyl fluoride (PMSF)] with protease inhibitors (10 μg/mL pepstatin, 10 μg/mL leupeptin, and 5 μg/mL aprotinin). After centrifugation at 13,000*g* at 4°C for 20 min, the clear supernatant was used for western blot analysis. Protein concentration was measured by Bradford's method (Bradford 1976). Aliquots of each sample (80 μg of protein) were added to the sample buffer. The mixture was boiled for 5 min and loaded onto a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel. Following electrophoresis, the separated proteins were transferred onto a nitrocellulose membrane (Amersham Biosciences, UK) using a wet transfer method. After blocking [5% non-fat milk, 0.05% Tween 20 in Tris-buffered saline (TTBS)], the blots were incubated with a rabbit monoclonal anti-GADD153 (1:1000, Sigma, USA) or a rabbit polyclonal anti-GRP78 (Cell Signalling Technology, USA) at 4°C overnight. To verify equal protein loading, blots were probed for a rabbit polyclonal anti-actin (1:2000, Sigma, USA). After washing with TTBS, the membranes were incubated with a 1:10,000 dilution of horseradish peroxidase-conjugated anti-rabbit secondary antibody (Santa Cruz) in TTBS at room temperature for 1 h with agitation. Protein bands were detected with ECL Western blotting detection reagents (Zhongshan Goldenbridge) on an X-ray film (Koda, USA).

2.4 *Quantitative real-time reverse transcription-polymerase chain reaction*

Total RNA from the control and treated samples of hippocampal tissue was extracted by the TRIzol methods (Invitrogen, USA) at different time points according to the manufacturer's instructions. Total RNA (2 μg) was incubated with 1 μL oligo(dT) (0.5 mg/mL, Promega, USA) at 70°C for 5 min and then at 4°C for 5 min. A reverse transcription reaction mixture was added to the RNA-oligo(dT) sample to achieve a final volume of 25 μL, containing 10 mM dithiothreitol (DTT), 5 mM dNTP, and 200 U/μL Moloney murine leukemia virus reverse transcriptase (M-MLVase). Polymerase chain reaction (PCR) was performed in a thermal cycler as follows: 42°C for 60 min and 95°C for 5 min. Samples were then stored at −20°C until further use.

PCR amplifications were performed with a TaqMan probe on an ABI 7300 real-time PCR system (Applied Biosystems, USA), using 96-well microtiter plates. The amplifications were performed in a reaction mixture (total volume, 50 μL) containing 2 mM $MgCl_2$, 10×PCR buffer, 5 μL, 6-carboxy-X-rhodamine (ROX), 1 μL; 7.5 μM each of the forward and reverse gene-specific primers 2 μL; 5μM TaqMan probe, 2 μL, 2.5 mM dNTP mixture, 4 μL; cDNA 4 μL; Taq DNA polymerase, 2.5U and RNase-free water. PCR amplifications were always performed in triplicate wells using the following universal temperature cycles: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. A sample without cDNA was used as the negative control. Primers for all target sequences (table 1) were designed using the computer Primer Express 3.0 software program specially provided with the 7300 Sequence Detection System (Applied Biosystems).

2.5 *Statistical analysis*

All data were expressed as mean±SD. Statistical differences were evaluated by one-way analysis of variance (ANOVA) followed by a *post hoc* comparisons (LSD test) among the treatment groups. Comparisons between 2 groups involved the use of the Student's *t*-test. A value of $P<0.05$ was considered statistically significant. The analyses were performed using the SPSS 16.0 software (SPSS, USA).

The differences in the CHOP protein expressions in the hippocampus, striatum and cortex at the same time point were compared using One-way ANOVA along with *post hoc* test.

Table 1. Primers and probes sequences used in quantitative real-time RT-PCR

Target gene	Accession no.	Orientation	Sequence (5′–3′)	Product size (bp)
β-Actin	NM031144	Fw	CCCTGGCTCCTAGCACCAT	77
		Rev	AGAGCCACCAATCCACACAGA	
		TaqMan probe	AAGATCATTGCTCCTCCTGAGCGCAAGTA	
GRP78	NM013083	Fw	TGCCCACCAAGAAGTCTCAGA	121
		Rev	TCAAATGTACCCAGAAGGTGATTG	
		TaqMan probe	CTTCTCCACAGCTTCTGATAATCAGCCCAC	
CHOP	NM024134	Fw	GGCAGCGACAGAGCCAAA	111
		Rev	CAGCTGGACACTGTCTCAAAGG	
		TaqMan probe	AACAGCCGGAACCTGAGGAGAGAGAAAC	

Fw, forward primer; Rev, reverse primer; GRP78, glucose-regulated protein 78; CHOP, C/EBP homologous protein.

3. Results

3.1 *Assessment of apoptosis by TUNEL and Hoechst staining*

To investigate area specificity of SE-induced neurotoxicity, neuronal death in the brain was assessed by TUNEL staining. In the control group, a few TUNEL positive cells were observed in the hippocampus, striatum and cortex. SE resulted in a time-dependent TUNEL fluorescence changes. The number of apoptotic neurons in the hippocampus, especially in the CA1 subregion, was remarkably increased at 12, 24 and 48 h after the seizures, as compared with the control rats. In contrast, the fluorescence intensity in the striatum and the cortex was increased and reached the highest point at 24 h, which was still higher than the control group at 48 h and no change was observed at 12 h in the two regions.

As compared with the hippocampus, the fluorescence intensity at 24 h and 48 h was lower in the striatum, and lowest in the cortex (figure 1A). Hoechst 33528 staining showed chromatin condensation and breakdown of the nuclei in SE-induced apoptotic neurons (figure 1B).

3.2 *Induction and regional distribution of CHOP protein in rat brain after status epilepticus*

In order to compare the expression of CHOP protein, an ER-resident molecule, in various brain regions, tissue lysates were prepared from the cerebral cortex, striatum and hippocampus at either 3, 6, 12, 24 or 48 h post-treatment from the control and SE groups and Western blotting analysis was performed. Western blotting using a monoclonal CHOP antibody revealed that CHOP proteins in different regions were all increased and reached the maximum point at 24 h after SE and remained high at 48 h. Although there were similar changes in time-dependent manner in different brain regions, the up-regulation of CHOP protein was predominantly located in the hippocampus and lowest in cortex. Meanwhile, CHOP protein was found to be expressed at nearly undetectable levels in the different regions in the control group (figure 2A and D).

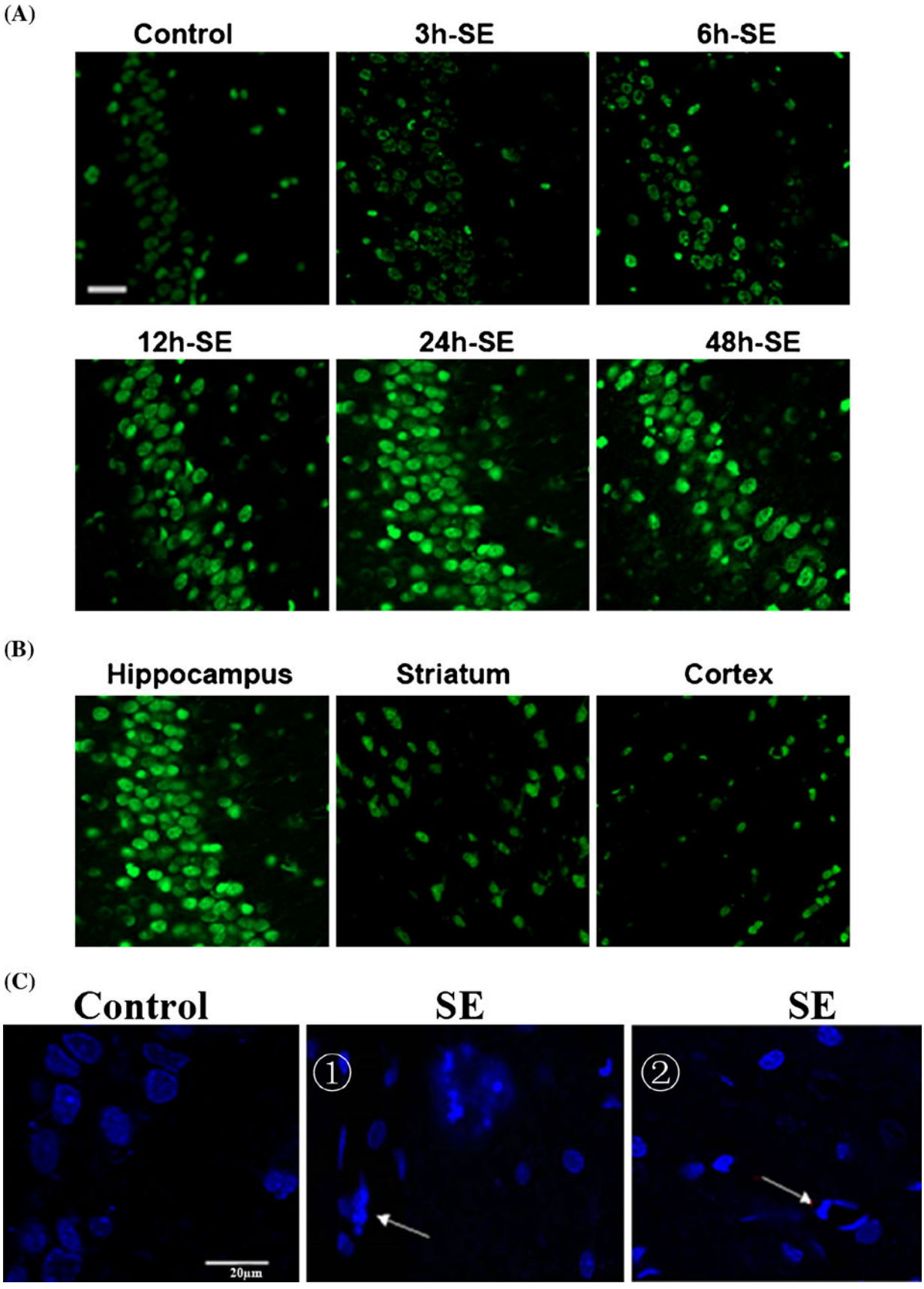
Next, the distribution of CHOP protein in the rat brain was examined using immuno- fluorescence analysis. The number of CHOP-positive cells was the highest in the hippocampus and mostly localized in the CA1 region. However, in the striatum and cerebral cortex, CHOP-positive cells were distributed ubiquitously with the fewest cells in the cortex (figure 2B).

CHOP is a transcription factor that migrates to the nucleus after activation. As shown in figure 2, CHOP fluorescence localizes specifically in the nucleus (figure 2C). Double-immunofluorescence of CHOP and NeuN suggested that most of CHOP-positive cells were neurons (figure 2C).

3.3 *Up-regulation of CHOP occurs at the transcriptional level*

One possible explanation for the increased levels of CHOP protein in neurons is that SE induces transcription of the CHOP gene. We therefore performed quantitative real-time PCR to determine CHOP mRNA level in the neurons of the brain after SE.

Figure 1. The intra-cerebral variability in apoptosis induced by SE. ▶ (**A**) TUNEL staining showed a remarkable increase in the number of apoptotic neurons in CA1 subregion in the hippocampus at 12 h, 24 h, and 48 h after SE. (bar = 30 μm). (**B**) The number of apoptotic cells at 24 h was higher in hippocampus than those in the striatum and cortex, and lowest in the cortex. (**C**) Hoechst 33528 staining (bar = 20 μm) of neurons from different regions in the hippocampus: Arrows showing nuclear breakdown (see: ①) and chromatin condensation (see: ②) in the apoptotic cells induced by SE.


(A)
Control
3h-SE
6h-SE
12h-SE
24h-SE
48h-SE
(B)
Hippocampus
Striatum
Cortex
(C)
Control
SE
SE
①
②
20μm

The ratio of CHOP mRNA to internal control (β-actin) mRNA, which indicate the relative amounts of the target mRNA, was calculated from the cycle of threshold values. Results, represented in figure 2, showed that SE can induce *CHOP* expression in different brain regions in a time-dependent manner (figure 2E). The expression of *CHOP* was increased and reached the maximum level at 24 h, at which CHOP mRNA reached values that were almost 3–5 times higher than those of controls. And the basal *CHOP* expression was detected at a low level in various brain regions in control groups (figure 2E). These results suggested that the higher CHOP mRNA expression resulted in higher CHOP protein levels.

3.4 *Increased CHOP production mediates SE-induced neuron death*

In order to elucidate the contribution of CHOP to SE-induced neuronal death, we performed double-fluorescence of CHOP and TUNEL in the hippocampus at 24 h after SE, the time point at which we observed both the highest number of CHOP-positive cells and numerous TUNEL-positive cells. We also found that most of CHOP-positive cells exhibited nuclear fragmentation detectable by the TUNEL method (figure 2C).

3.5 *Induction of GRP78 protein in various rat brain regions after status epileticus*

GRP78, an ER-resident Hsp70 family member, is induced by ER stress, and is in fact known to be a major marker of such stress. To examine the induction of GRP78 protein after status epilepticus, we performed Western blotting analysis and immunochemistry.

In various brain regions, total tissue lysates were prepared from the cerebral cortex, hippocampus and striatum from rats of both groups and Western blotting analysis was performed. The level of GRP78 protein was all elevated at 3 h SE, reaching the highest point at 6 h after SE. The level remained high for 6 more hours and returned afterwards to basal levels in the regions shown in figure 3A and C. Unlike CHOP, GRP78 protein is distributed ubiquitously in the brain, and no difference in GRP78 protein level was observed among the brain regions investigated (figure 3B). Using quantitative real-time PCR to measure GRP78 transcript in the three regions, we found changes very similar to those seen in Western blotting (figure 3C).

4. Discussion

SE is an acute neurological emergency in children. Recent data demonstrate that SE or recurrent febrile seizures occurring at an early age in rats cause long-term functional impairment without any clear histologically assessed neuronal loss (Nehlig and Pereira de Vasconcelos 1996), except in the hippocampus (Sankar *et al.* 1998; Lado *et al.* 2002; Han *et al.* 2005). The present study was designed to test the hypothesis that SE at an early age leads to neuronal degeneration in brain areas that have previously not been explored in detail.

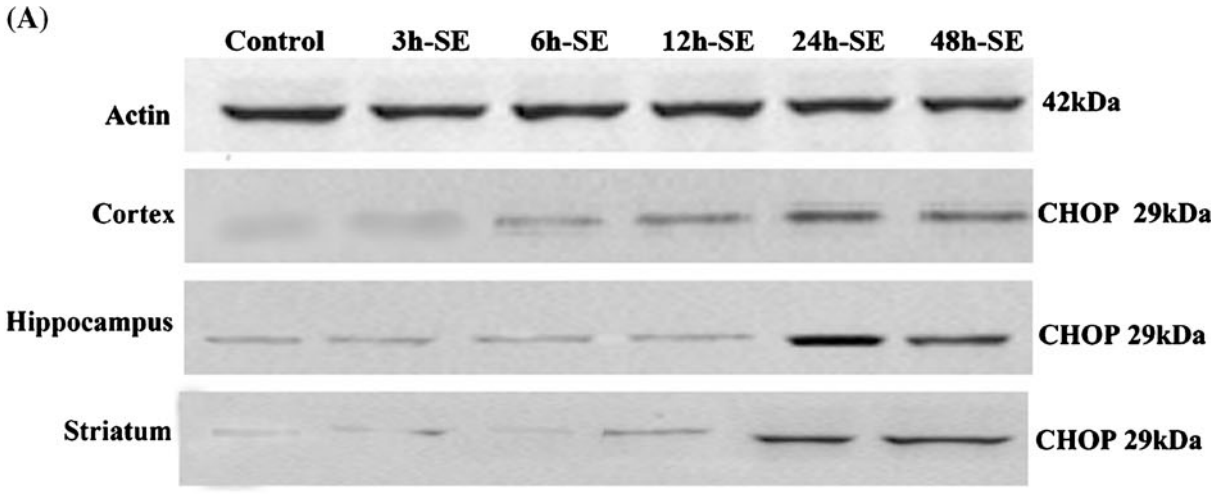
Animal studies have clearly established that status epilepticus induces neuronal injury and that the degree of damage varies depending on the age of animals, type and duration of seizure, the causality of seizure, and genetic factors (Holmes 2002). During seizure, the combination of decreased oxygen delivery and excitation secondary to excessive glutamate secretion result in increase of intracellular calcium.

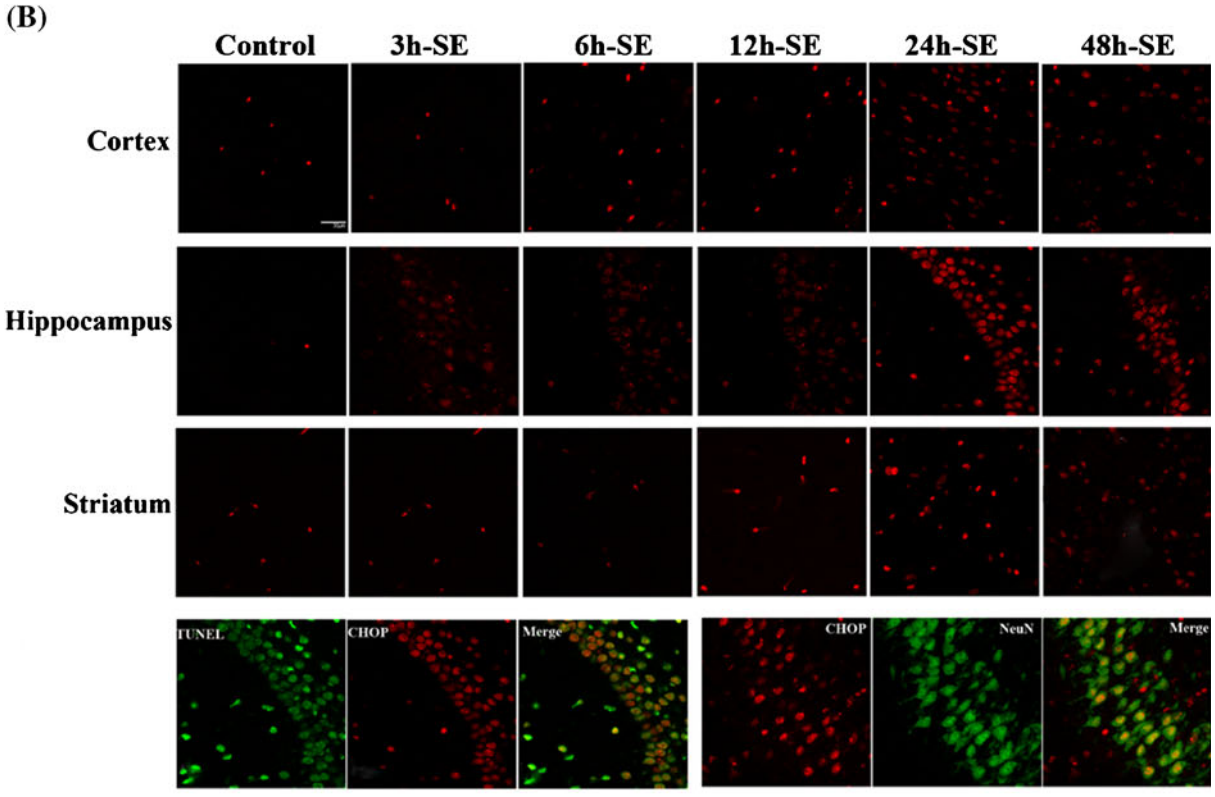
This generates reactive oxygen species by mediating the activation of nitric synthase, oxidative phosphorylation of mitochondria, and activation of lipase, protease, endonuclease and other enzymes which are harmful to cellular metabolism, resulting in neuronal cell injury and reorganization of the neuronal synaptic network (Zhao and Brinton 2007).

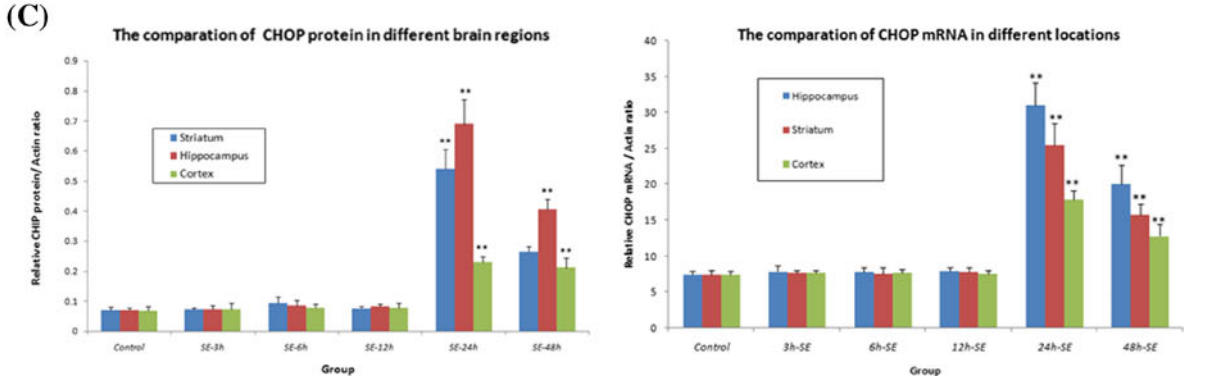
In the current study, we demonstrated that our SE model resulted in enhanced expression of Bip/GRP78, which establishes that ER stress was induced in our experimental paradigm. The results also showed that the express of GRP78 was increased at the early stage (peak at 6 h), and not changed at the late stage (24 h, 48 h). GRP78, as an anti-apoptotic protein (Ni *et al.* 2011), was induced highly to facilitate cell adaptation to stress for survival by attenuating protein translation when cells respond to mild ER stresses.

But prolonged endoplasmic reticulum stress, as well as DNA damage, can induce apoptosis. So we focused on one of the ER-derived apoptosis signals, CHOP/ GADD153. The level of CHOP/GADD153 protein was significantly increased at 12h, reached its peak at 24 h, and remained high

Figure 2. There was regional variability in the activation of CHOP by SE. (**A**) CHOP protein was analysed by Western blotting in the two groups. CHOP protein was all increased in all the brain regions investigated, and reached its peak value at 24 h and remained high at 48 h in the hippocampus, striatum and cortex. The level of up-regulated CHOP protein was most prominent in the hippocampus, and the lowest in the cortex. (**B**) Immunofluorescence showed CHOP expression in the striatum, hippocampus and cerebral cortex (bar=30μm). (**C**) Double-fluorescence with CHOP and TUNEL indicated that CHOP was involved in the cell death. Double-fluorescence with CHOP and NeuN showed that the most of CHOP-positive cells were neurons. (**D**) The expression of CHOP protein was increased at 24 h and 48 h after SE, and the rank order was the hippocampus>striatum>cortex. **, *vs.* control group, $P < 0.01$. (**E**) Quantitative real-time RT-PCR results showed that changes of CHOP mRNA in hippocampus were very similar to those of CHOP protein assayed by Western blot in (D). **, $P<0.01$, *vs.* control group.


(A)
Control
3h-SE
6h-SE
12h-SE
24h-SE
48h-SE
Actin
42kDa
Cortex
CHOP 29kDa
Hippocampus
CHOP 29kDa
Striatum
CHOP 29kDa


(B)
Control
3h-SE
6h-SE
12h-SE
24h-SE
48h-SE
Cortex
Hippocampus
Striatum
TUNEL
CHOP
Merge
CHOP
NeuN
Merge


(C)
The comparation of CHOP protein in different brain regions
Striatum
Hippocampus
Cortex
Group
The comparation of CHOP mRNA in different locations
Hippocampus
Striatum
Cortex
Group

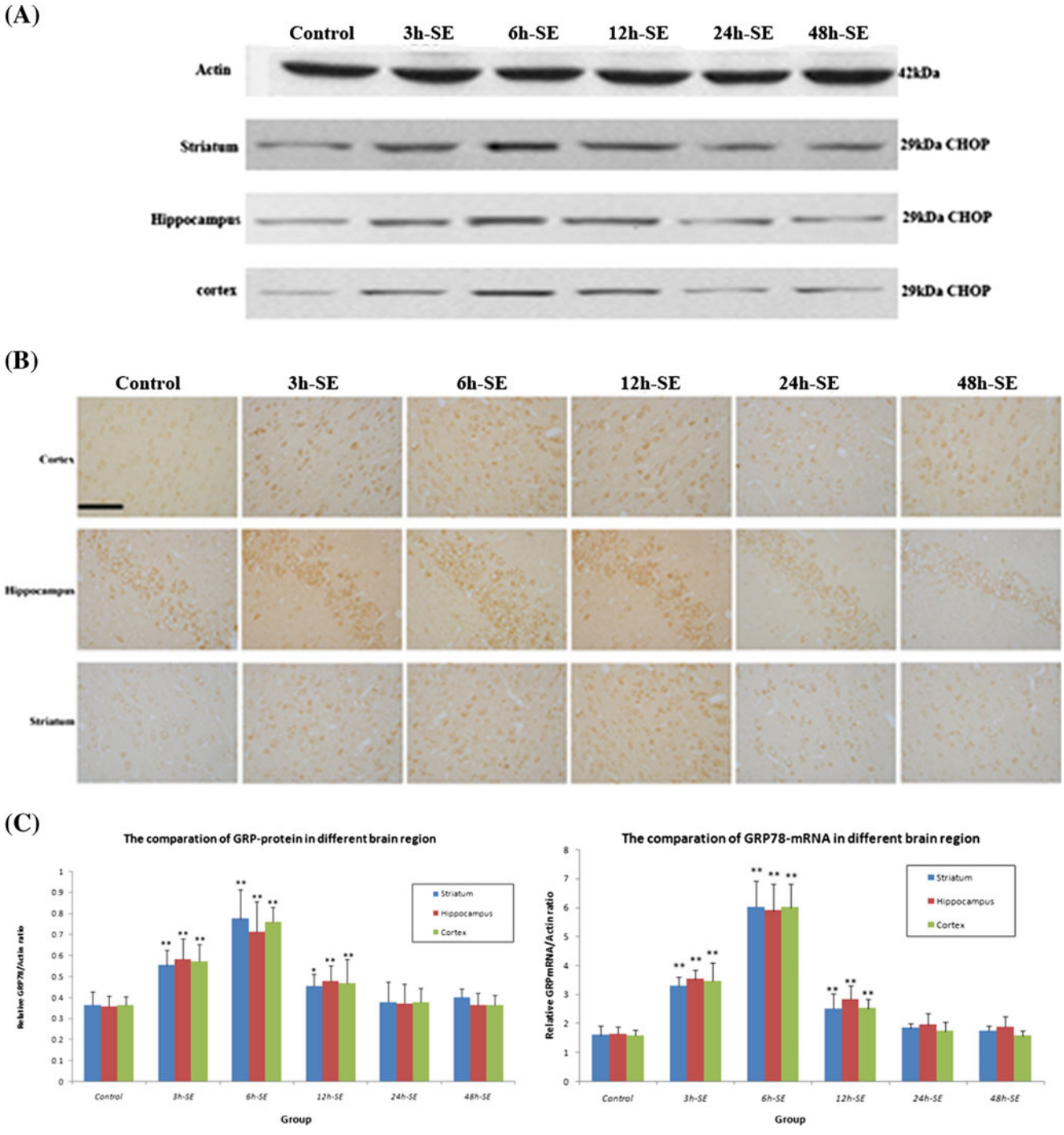


Figure 3. The expressions of GRP78 in different brain regions. (**A**) Western blotting showed that GRP78 protein was increased at 3 h, 6 h and 12 h, the highest point was at 6 h after SE. (**B**) Immunohistochemistry revealed that GRP78 protein is distributed ubiquitously in the brain, and no difference in GRP78 protein level was observed among the brain regions investigated (bar = 25 μm). (**C**) Quantitative real-time PCR results showed that the changes of GRP78 mRNA in the hippocampus are very similar to those of GRP78 protein assayed by Western blot. **, $P<0.01$, vs. control group.

up to 48 h after SE in different regions of rat brain. The increase in protein level was apparently the result of an increase in CHOP/GADD153 gene transcription, since the changes in CHOP/GADD153 mRNA level were very similar to those of CHOP/GADD153 protein assayed by Western blotting. Furthermore, the expression of CHOP/GADD153 was localized mainly in the hippocampal neurons. As we know, CHOP is a transcription factor that migrates to the nucleus after activation. CHOP is a basic leucine zipper (bZIP)-containing transcription factor that was identified as a member of the CCAAT/enhancer binding protein (C/EBP) family (Ron and Habener 1992). CHOP is also known as the growth-arrest and DNA-damage-inducible gene 153 (GADD153), although it is induced by ER stress more than by growth arrest or DNA damage (Eizirik *et al.* 2013). During prolonged ER stress, CHOP is one of the most highly up-regulated genes (Tabas and Ron 2011). Several studies have implicated CHOP in ER stress-induced apoptosis. CHOP-/- mouse embryonic fibroblasts (MEFs) are partially resistant to ER stress and have reduced ER stress-induced apoptosis (Ma *et al.* 2002). Conversely, over-expression of CHOP promotes apoptosis in response to ER stress caused by thapsigargin and tunicamycin (Quick and Faison 2012; Ono *et al.* 2012). In our study, the increased CHOP/GADD153 likely contributed to the neuronal death caused by SE, as evidenced by the large number of CHOP-positive cells exhibiting DNA fragmentation as detected by the TUNEL method. These results imply that CHOP/GADD153 is involved in the pathophysiology of SE-induced brain injury.

Another interesting finding from the present study is that the extent of SE-induced changes in CHOP protein and mRNA levels differed considerably in the cortex, striatum, and hippocampus. The higher CHOP mRNA and protein expression in the hippocampus suggested that the hippocampus was more vulnerable to SE-induced brain damage than the striatum and the cerebral cortex. No variation was however observed in the levels of GRP78 in the different areas of the brain investigated.

SE is a state of energy depletion during which the ER calcium homeostasis is disturbed, because ATP is needed to fuel the ER Ca^{2+}-ATPase which pumps back calcium ions from the cytoplasm into the ER. A depletion of ER calcium stores resulted in a rise in CHOP mRNA levels (Park *et al.* 2009).

In summary, CHOP protein and mRNA levels were found to be significantly increased in the brain after SE. Since induction of CHOP expression is a specific marker for the stressed ER, it is concluded that SE causes disturbances of ER functions. After SE, the relative rise in CHOP protein and mRNA levels was most pronounced in the hippocampus implying that the hippocampus is more vulnerable to the stress than the striatum and cerebral cortex.

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References

Ayuso MI, Martínez-Alonso E, Cid C, de Leciñana MA and Alcázar A 2013 The translational repressor eIF4E-binding protein 2 (4E-BP2) correlates with selective delayed neuronal death after ischemia. *J. Cereb. Blood Flow Metab*. **33** 1173–1181

Bradford MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. **72** 248–254

Chin RF, Neville BG and Scott RC 2004 A systematic review of the epidemiology of status epilepticus. *Eur. J. Neurol*. **11** 800–810

Dedeurwaerdere S, Callaghan PD, Pham T, Rahardjo GL, Amhaoul H, Berghofer P, Quinlivan M, Mattner F, *et al.* 2012 PET imaging of brain inflammation during early epileptogenesis in a rat model of temporal lobe epilepsy. *EJNMMI Res*. **2** 60

Eizirik DL, Miani M and Cardozo AK 2013 Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. *Diabetologia* **56** 234–241

Gao H and Geng Z 2013 Calpain I, activity and its relationship with hippocampal neuronal death in pilocarpine-induced status epilepticus rat mode. *Cell Biochem. Biophys*. **66** 371–377

Han Y, Qin J, Chang X, Yang Z, Tang X and Du J 2005 Hydrogen sulfide may improve the hippocampal damage induced by recurrent febrile seizures in rats. *Biochem. Biophys. Res. Commun.* **327** 431–436

Hetz C 2012 The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol*. **13** 89–102

Holmes GL 2002 Seizure-induced neuronal injury: animal data. *Neurology* **59** S3–6

Hwang D, Seo S, Kim Y, Kim C, Shim S, Jee S, Lee S, Jang M, *et al.* 2007 Selenium acts as an insulin-like molecule for the down-regulation of diabetic symptoms via endoplasmic reticulum stress and insulin signalling proteins in diabetes-induced non-obese diabetic mice. *J. Biosci.* **32** 723–35

Kim JE, Ryu HJ, Yeo SI and Kang TC 2011 P2X7 receptor differentially modulates astroglial apoptosis and clasmatodendrosis in the rat brain following status epilepticus. *Hippocampus* **21** 1318–1333

Kitao Y, Ozawa K, Miyazaki M, Tamatani M, Kobayashi T, Yanagi H, Okabe M, Yamashima T, *et al.* 2001 Expression of the endoplasmic reticulum molecular chaperone (ORP150) rescues hippocampal neurons from glutamate toxicity. *J. Clin. Invest*. **108** 1439–1450

Lado FA, Laureta EC and Moshe SL 2002 Seizure-induced hippocampal damage in the mature and immature brain. *Epileptic Disord*. **4** 83–97

Laurén HB, Ruohonen S, Kukko-Lukjanov TK, Virta JE, Grönman M, Lopez-Picon FR, Järvelä JT and Holopainen IE 2013 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*. **1516** 33–44

Liu CY, Yang JS, Huang SM, Chiang JH, Chen MH, Huang LJ, Ha HY, Fushlya S, *et al.* 2013a Smh-3 induces G(2)/M arrest and apoptosis through calciummediated endoplasmic reticulum stress and mitochondrial signaling in human hepatocellular carcinoma Hep3B cells. *Oncol. Rep*. **29** 751–762

Liu D, Zhang M and Yin H 2013b Signaling pathways involved in endoplasmic reticulum stress-induced neuronal apoptosis. *Int. J. Neurosci*. **123** 155–162

Lopes MW, Soares FM, de Mello N, Nunes JC, Cajado AG, de Brito D, de Cordova FM, da Cunha RM, *et al.* 2013 Time-dependent modulation of AMPA receptor phosphorylation and mRNA expression of NMDA receptors and glial glutamate transporters in the rat hippocampus and cerebral cortex in a pilocarpine model of epilepsy. *Exp. Brain Res.* **266** 153–163

Ma Y, Brewer JW, Diehl JA and Hendershot LM 2002 Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *J. Mol. Biol.* **318** 1351–1365

Nehlig A and Pereira de Vasconcelos A 1996 The model of pentylenetetrazol-induced status epilepticus in the immature rat: short- and long-term effects. *Epilepsy Res.* **26** 93–103

Ni M, Zhang Y and Lee AS 2011 Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. *Biochem. J.* **434** 181–188

O'Connor JJ 2013 Targeting tumour necrosis factor-α in hypoxia and synaptic signaling. *Ir. J. Med. Sci.* **182** 157–162.

Ono Y, Shimazawa M, Ishisaka M, Oyagi A, Tsuruma K and Hara H 2012 Imipramine protects mouse hippocampus against tunicamycin-induced cell death. *Eur. J. Pharmacol*. **696** 83–88

Oyadomari S and Mori M 2004 Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ.* **11** 381–389

Park KS, Poburko D, Wollheim CB and Demaurex N 2009 Amiloride derivatives induce apoptosis by depleting ER, Ca(2+) stores in vascular endothelial cells. *Br. J. Pharmacol.* **156** 1296–1304

Pelletier MR, Wadia JS, Mills LR and Carlen PL 1999 Seizure-induced cell death produced by repeated tetanic stimulation in vitro: possible role of endoplasmic reticulum calcium stores. *J. Neurophysiol*. **81** 3054–3064

Quick QA and Faison MO 2012 CHOP and caspase 3 induction underlie glioblastoma cell death in response to endoplasmic reticulum stress. *Exp. Ther. Med*. **3** 487–492

Rattka M, Brandt C and Loscher W 2013 The intrahippocampal kainate model of temporal lobe epilepsy revisited: epileptogenesis, behavioral and cognitive alterations, pharmacological response, and hippoccampal damage in epileptic rats. *Epilepsy Res.* **103** 135–152

Ryley Parrish R, Albertson AJ, Buckingham SC, Hablitz JJ, Mascia KL, Davis Haselden W and Lubin FD 2013 Status epilepticus triggers early and late alterations in brain- derived neurotrophic factor and NMDA glutamate receptor Grin2b DNA methylation levels in the hippocampus. *Neuroscience* **248** 602–619

Ron D and Habener JF 1992 CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors CEBP and LAP and functions as a dominant-negative inhibitor of gene transcription. *Genes Dev.* **6** 439–453

Roussel BD, Kruppa AJ, Miranda E, Crowther DC, Lomas DA and Marciniak SJ 2013 Endoplasmic reticulum dysfunction in neurological disease. *Lancet Neurol*. **12** 105–118

Sankar R, Shin DH, Liu H, Mazarati A, Pereira de Vasconcelos A and Wasterlain CG 1998 Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J. Neurosci*. **18** 8382–8393

Sokka AL, Putkonen N, Mudo G, Pryazhnikov E, Reijonen S, Khiroug L, Belluardo N, Lindholm D, *et al.* 2007 Endoplasmic reticulum stress inhibition protects against excitotoxic neuronal injury in the rat brain. *J. Neurosci*. **27** 901–908

Tabas I and Ron D 2011 Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat. Cell Biol*. **13** 184–190

Wang Z, Zhang C, Hong Z, Chen H, Chen W and Chen G 2013 C/EBP homologous protein (CHOP) mediates neuronal apoptosis in rats with spinal cord injury. *Exp. Ther. Med*. **5** 107–111

Wasterlain CG and Chen JW 2006 Definition and classification of status epilepticus; in *Status epilepticus: Mechanisms and management* 1st edition (eds) CG Wasterlain and DM Treiman (Cambridge, MA: The MIT Press) pp 11–16

Xiong R, Siegel D and Ross D 2013 The activation sequence of cellular protein handling systems after proteasomal inhibition in dopaminergic cells. *Chem. Biol. Interact*. **204** 116–124

Xu C, Bailly-Maitre B and Reed JC 2005 Endoplasmic reticulum stress: cell life and death decisions. *J. Clin. Invest*. **115** 2656–2664

Yamamoto A, Murphy N, Schindler CK, So NK, Stohr S, Taki W, Prehn JH and Henshall DC 2006 Endoplasmic reticulum stress and apoptosis signaling in human temporal lobe epilepsy. *J. Neuropathol. Exp. Neurol.* **65** 217–225

Yang J, Huang Y, Yu X, Sun H, Li Y and Deng Y 2007 Erythropoietin preconditioning suppresses neuronal death following status epilepticus in rats. *Acta. Neurobiol. Exp. (Wars)* **67** 141–148

Zhao L and Brinton RD 2007 Estrogen receptor alpha and beta differentially regulate intracellular Ca(2+) dynamics leading to ERK phosphorylation and estrogen neuroprotection in hippocampal neurons. *Brain Res.* **1172** 48–59

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