Translocation of High Mobility Group Box 1 From the Nucleus to the Cytoplasm in Depressed Patients With Epilepsy

Xiao-Li Li1*, Shu Wang2,3*, Chong-Yang Tang3, Hao-Wei Ma3, Zi-Zhang Cheng3, Meng Zhao3, Wei-Jin Sun3, Xiong-Fei Wang3, Meng-Yang Wang4, Tian-Fu Li4,5,6, Xue-Ling Qi7, Jian Zhou3, Guo-Ming Luan3,5,6, and Yu-Guang Guan3,5,6

Abstract
Depression is a common psychiatric comorbidity in patients with epilepsy, especially those with temporal lobe epilepsy (TLE). The aim of this study was to assess changes in high mobility group box protein 1 (HMGB1) expression in epileptic patients with and without comorbid depression. Sixty patients with drug-resistant TLE who underwent anterior temporal lobectomy were enrolled. Anterior hippocampal samples were collected after surgery and analyzed by immunofluorescence (n = 7/group). We also evaluated the expression of HMGB1 in TLE patients with hippocampal sclerosis and measured the level of plasma HMGB1 by enzyme-linked immunosorbent assay. The results showed that 28.3% of the patients (17/60) had comorbid depression. HMGB1 was ubiquitously expressed in all subregions of the anterior hippocampus. The ratio of HMGB1-immunoreactive neurons and astrocytes was significantly increased in both TLE patients with hippocampal sclerosis and TLE patients with comorbid depression compared to patients with TLE only. The ratio of cytoplasmic to nuclear HMGB1-positive neurons in the hippocampus was higher in depressed patients with TLE than in nondepressed patients, which suggested that more HMGB1 translocated from the nucleus to the cytoplasm in the depressed group. There was no significant difference in the plasma level of HMGB1 among patients with TLE alone, TLE with hippocampal sclerosis, and TLE with comorbid depression. The results of the study revealed that the translocation of HMGB1 from the nucleus to the cytoplasm in hippocampal neurons may play a previously unrecognized role in the initiation and amplification of epilepsy and comorbid depression. The direct targeting of neural HMGB1 is a promising approach for anti-inflammatory therapy.

Keywords
high mobility group box 1, hippocampus, temporal lobe epilepsy, depression, comorbidity

Received April 20, 2022; Revised October 15, 2022; Accepted for publication October 17, 2022

1Department of Neurology, Affiliated ZhongDa Hospital, Southeast University, Nanjing, China
2Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University, Beijing, China
3Department of Neurosurgery, Sanbo Brain Hospital, Capital Medical University, Beijing, China
4Department of Neurology, Sanbo Brain Hospital, Capital Medical University, Beijing, China
5Beijing Key Laboratory of Epilepsy, Beijing, China
6Center of Epilepsy, Beijing Institute of Brain Disorders, Collaborative Innovation Center for Brain Disorders, Capital Medical University, Beijing, China
7Department of Pathology, Sanbo Brain Hospital, Capital Medical University, Beijing, China

*Xiao-Li Li and Shu Wang contributed equally to this work.

Corresponding Authors:
Yu-Guang Guan, Department of Neurosurgery, Sanbo Brain Hospital, Capital Medical University, Beijing, China; Beijing Key Laboratory of Epilepsy, Beijing, China; Center of Epilepsy, Beijing Institute of Brain Disorders, Collaborative Innovation Center for Brain Disorders, Capital Medical University, Beijing, China. Email: guanyg2020@ccmu.edu.cn

Guo-Ming Luan, Department of Neurosurgery, Sanbo Brain Hospital, Capital Medical University, Beijing, China; Beijing Key Laboratory of Epilepsy, Beijing, China; Center of Epilepsy, Beijing Institute of Brain Disorders, Collaborative Innovation Center for Brain Disorders, Capital Medical University, Beijing, China. Email: luangm@ccmu.edu.cn

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Introduction

Epilepsy is a serious chronic neurological disorder characterized by unpredictable and recurrent seizures (Wang et al., 2021b). Moreover, various associated comorbidities can increase the burden of epilepsy. Psychiatric comorbidities are particularly common and worrisome in patients with epilepsy (PWE) (Avalos et al., 2020; Jackson, 2005). Depression tends to be more prominent in individuals with refractory temporal lobe epilepsy (TLE) than in patients with genetic generalized epilepsies (Chang et al., 2020; Gill et al., 2017; Kwon & Park, 2014). Depression comorbid with TLE has been suggested to be associated with a decreased quality of life and a reduced quality of epilepsy outcomes (D’Alessio et al., 2019; Josephson et al., 2017). Thus, exploration of the mechanisms underlying depression comorbid with TLE is important for understanding the mechanisms of epilepsy.

The hippocampus is an important part of the temporal lobe, and volume reductions and dysfunction of the hippocampus are associated with psychological disorders including depression (Xu et al., 2019). However, it may not act as a unitary structure; instead, the dorsal (septal pole) and ventral (temporal pole) portions may take on different roles. There are substantial reports indicating that the ventral hippocampus (anterior in primates) is part of and modulates emotional and affective processes (Fanselow & Dong, 2010; Xu et al., 2019).

Human high mobility group box 1 (HMGB1) protein is a highly conserved DNA-binding protein consisting of 215 amino acids that, according to recent reports, acts as a mediator of proinflammatory processes (Nishibori et al., 2020; Paudel et al., 2019). Under normal physiological conditions, it is localized in the nucleus in most cells (Gou et al., 2020; Wang et al., 2022) and participate in DNA replication, repair, recombination, transcription, and genomic stability (Kang et al., 2014). Under damaging or pathological conditions, HMGB1 can translocate from the nucleus to the cytoplasm or be released into the extracellular environment and exert effects in many diseases, such as traumatic brain injury, ischemia, epilepsy, cognitive impairment, and other neuroinflammatory conditions that affect the central nervous system (Gou et al., 2020; Kang et al., 2014; Nishibori et al., 2020; Paudel et al., 2018a; Walker et al., 2016; Wang et al., 2021a).

There is also increasing evidence that HMGB1 is involved in epileptogenesis. Data from different experimental epilepsy models and epilepsy patients have shown that HMGB1 in the hippocampus plays an important role in the generation and recurrence of seizures (Kuehl et al., 1984; Li et al., 2021a; Maroso et al., 2010; Walker et al., 2014, 2016; Yang et al., 2017). In addition to the upregulation of HMGB1 expression, increased cytoplasmic localization of HMGB1 in neurons and astrocytes has been observed in children with refractory epilepsy caused by focal cortical dysplasia (Zhang et al., 2018; Zurolo et al., 2011). However, there are contradictory reports regarding HMGB1 levels in the brains of mice with acute and chronic epilepsy. Fu et al. reported that brain HMGB1 levels are decreased and plasma HMGB1 levels are increased in mice after pilocarpine treatment due to the translocation of HMGB1 from the cell nucleus to surrounding areas, including the bloodstream (Fu et al., 2017). In a pilocarpine-induced chronic epilepsy model, serum but not brain levels of total HMGB1 were significantly elevated (Walker et al., 2016).

Despite recent research advances, the exact roles of and changes in HMGB1 in PWE, in addition to comorbidities of epilepsy, remain elusive. In the present study, we investigated the change in the localization of HMGB1 in the hippocampi of drug-resistant TLE patients with and without comorbid depression after anterior temporal lobectomy (ATL).

Material and Methods

Patients and Ethics

Sixty patients with drug-resistant TLE were enrolled in this study. All patients underwent ATL, which included amygdalohippocampectomy, for the surgical treatment of drug-resistant TLE at the Epilepsy Center of our hospital between October 2019 and June 2021. Twenty-one samples were obtained from these patients after surgery. No randomization was performed in this study.

All the study procedures were conducted according to the guidelines outlined by the Code of Ethics of the World Medical Association (Declaration of Helsinki). The study protocols were approved by the Ethical Review Board of the hospital. Written informed consent was obtained from all participants or their guardians. Clinical characteristics, including sex, age, symptom duration, seizure types and frequency, and usage of antiepileptic drugs, were collected.

Inclusion and Exclusion Criteria. As described in our previous report (Li et al., 2021b), the patients enrolled in this study met the following inclusion criteria: (1) Patients who were diagnosed with TLE by at least two experienced epilepsy specialists based on the International League Against Epilepsy (ILAE) 2017 classification (Fisher et al., 2017). All patients were diagnosed with TLE based on ictal semiology, computed tomography (CT), magnetic resonance imaging (MRI), and video-electroencephalography (VEEG). (2) Patients with drug-resistant epilepsy, which was defined as “failure of adequate trials of two tolerated and appropriately chosen and
used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom” according to the ILAE 2010 definition (Kwan et al., 2010). (3) Patients who were considered good surgical candidates by the multidisciplinary team at the Epilepsy Center, which includes neurosurgery, neurology, anesthesiology, radiology, and neurophysiology specialists.

Patients with TLE were excluded if they met any of the following exclusion criteria: (1) coexisting serious psychiatric conditions or other severe general diseases (e.g., diabetes, chronic heart diseases, and asthma) that would increase the risk associated with surgery; (2) progressive neurological disease; (3) stroke or brain trauma in the prior 2 months; and (4) an intelligence quotient (IQ) less than 70.

**Neuroimaging Tests and VEEG.** All patients received assessments including CT, MRI, 64-channel interictal and ictal scalp VEEG, and neuropsychological tests before surgery. VEEG was performed to determine candidacy for epilepsy surgery. According to the 10–20 system, Nicolet electroencephalography (EEG) machines were used to confirm the seizure onset zone. Hippocampal sclerosis (HS) was defined according to the ILAE 2013 commission on diagnostic methods for HS (Blümcke et al., 2013). If MRI was negative or VEEG showed poorly localized ictal onset or multilocal onset, stereotactic depth electrodes were implanted for intracranial EEG recordings to further confirm the onset zone.

**Neuropsychological Assessment.** All patients included in this study underwent the full complement of routine neuropsychological assessments when they were admitted to our hospital. Two neuropsychology specialists who were blinded to the patient information performed neuropsychological assessments using several rating scales, including the Hamilton Rating Scale for Depression (HAMD), the Wechsler Adult Intelligence Scale-IV (WAIS-IV; Chinese version), and the Wechsler Memory Scale-IV (WMS-IV; Chinese version). According to Chinese norms, patients were diagnosed with depression when their HAMD scores were greater than 9 (sensitivity = 96.7%, specificity = 93.8%, positive predictive value = 74.4%, and negative predictive value = 99.3%) (Lin et al., 2018).

**Tissue Preparation**

Twenty-one hippocampal samples were collected from patients who underwent epilepsy surgery for drug-resistant TLE with and without comorbid depression. No statistical method was used to determine the sample size. The sample size was arbitrarily set to 21 (three groups with seven hippocampi each). Samples were selected and matched by gender and age. Based on the evaluation of HAMD scores and neuroimaging and pathological reports, the samples were first divided into a TLE without hippocampal sclerosis group (TLE-nHS group, n = 14) and a TLE with hippocampal sclerosis group (TLE-HS group, n = 7) to determine the relationship between hippocampal sclerosis and HMGB1 expression. Second, samples in the TLE-nHS group were further divided into a TLE without depression group (TLE group, n = 7) and a TLE with comorbid depression group (TLE-D group) to determine the relationship between depression and HMGB1 expression. This design was used to exclude the potential influence of hippocampal sclerosis on depression.

The patients received ATL, which included the removal of the anterior temporal lobe, amygdala, and hippocampus, for the treatment of drug-resistant TLE. After removal, the hippocampus was divided along the longitudinal axis into two subregions, the anterior and the posterior hippocampus. Hippocampal sclerosis was identified by MRI and neuropathological analysis. Anterior hippocampal tissues were quickly collected and immediately fixed in formalin for 24 h. After fixation, the tissue blocks were embedded in paraffin wax in preparation for coronal sectioning. The embedded tissues were sectioned at a thickness of 4 μm, mounted on Superfrost Plus glass slides, and deparaffinized for subsequent immunohistochemistry.

Serum samples from 21 patients were collected to test the HMGB1 levels in the three groups. After 20 min of centrifugation at 1000g at 4 °C, the supernatant was collected and stored at −80 °C until further use for enzyme-linked immunosorbent assay (ELISA) testing.

**Immunofluorescence Staining**

Immunofluorescence staining was performed according to a previous study by our laboratory (Li et al., 2021b). One in every ten sections from the paraffin-embedded tissue was selected for the following steps. Five sections from every patient were collected. The sections (4 μm thick) were dehydrated in a series of alcohol solutions of increasing concentration, treated with 0.03% H2O2 to inactivate endogenous peroxidase, and subjected to high pressure for antigen retrieval. The sections were blocked with goat serum at room temperature for 30 min prior to incubation with a rabbit anti-human neuron-specific nuclear protein (NeuN) antibody (1:200, Abcam, AB_2716282), mouse anti-human HMGB1 antibody (1:500, Abcam, AB_1566303), rabbit anti-human HMGB1 antibody (1:500, Abcam, AB_1566303), rabbit anti-human GFAP antibody (1:100, Abcam, AB_1209224), or mouse anti-human Iba-1 (1:300, Millipore, AB_10917271) for 1 h at 37 °C. After being washed, the sections were incubated with the appropriate peroxidase-linked secondary antibody conjugated to FITC or Cy3 (biotinylated anti-mouse or anti-rabbit IgG) for 1 h at room temperature. The slides were washed again with PBS prior to being counterstained with DAPI for 2 min and covered with glass coverslips.

**ELISA**

Samples were stored in 4 °C refrigerators one night prior to the ELISA. The HMGB1 concentrations in the plasma were measured using an ELISA (ELISA, HMGB1: E-EL-H1554c,
Elabscience) according to the manufacturer’s instructions. Each sample was tested in duplicate. The detection range was 31.25–2000 pg/mL. Briefly, 100 μL standard or plasma samples were added to the appropriate wells and incubated for 90 min at 37 °C. Each well was then filled with 100 μL of biotinylated detection antibody working solution after the liquid was decanted. Next, HRP conjugate working solution was added and incubated for 30 min at 37 °C. After that, a 15-min incubation at 37 °C was performed with substrate reagent, ensuring that the plate was protected from light. A total of 60 patients who underwent ATL, comprising 32 male patients and 28 female patients with a mean age of 30.08 ± 13.88 years and a mean seizure history of 12.02 ± 9.10 years, were recruited in this study. Among the 60 patients with TLE, 17 (28.3%) had a HAMD score greater than nine and were considered to have depression. In addition, 28 patients (46.7%) had hippocampal sclerosis. The characteristics and baseline scores of all included patients are shown in Table 1. Patients without HS but with depression (TLE-nHS/TLE) had higher HAMD scores than patients without HS and depression (TLE-nHS/TLE-D) (11.55 ± 1.69 vs. 5.38 ± 2.13, p < .001; unpaired Student’s t-test). Other characteristics did not show significant differences among patients with and without HS or patients with and without depression (all p > .05; unpaired Student’s t-test, the Mann-Whitney U-test, Pearson’s χ²-test, or Fisher’s exact test, as appropriate). Samples were obtained from sex- and age-matched patients and divided into three groups: the TLE-HS group (n = 7), the TLE-nHS/TLE group (n = 7), and the TLE-nHS/TLE-D group (n = 7). Neuroimaging tests followed by pathological verification showed that there was hippocampal sclerosis in the TLE-HS group (Figure 1). According to the ILAE classification, five samples in the TLE-HS group showed moderate segmental pyramidal cell loss, and two displayed severe neuronal loss. There were no significant differences in terms of sex; age at surgery; seizure history; or HAMD, WAIS, or WMS scores between patients in the TLE-HS group and those in the TLE-nHS group (all p > .05; unpaired Student’s t-test, Mann-Whitney U-test, Pearson’s χ²-test, or Fisher’s exact test, as appropriate). Samples from the TLE-nHS group were then divided into the TLE-nHS/TLE group (n = 7) and the TLE-nHS/TLE-D group (n = 7). The HAMD scores of patients in the TLE-nHS/TLE-D group ranged from 10 to 15, with a mean score of 12.00 ± 1.40, which was significantly higher than the mean score of patients in the TLE-nHS/TLE group (3.57 ± 1.40, p < .001; unpaired Student’s t-test). There were no significant differences in the other characteristics or neuropsychological assessment scores. The characteristics and baseline scores of patients from whom the samples were obtained (n = 21) are shown in Table 2. None of the TLE patients included in the present study had received treatment for depression before hospitalization.

### Statistical Analysis

Cell counting was completed using ImageJ software. The data were analyzed with GraphPad Prism version 8 and are presented as the mean ± standard error of the mean (SEM). Variables were tested for normality and homogeneity of variance. The unpaired Student’s t-test, the Mann–Whitney U-test, Pearson’s χ²-test, or Fisher’s exact test was performed as appropriate to analyze differences between the two groups. The ELISA results were compared among all three groups using one-way ANOVA followed by the Tukey multiple comparison test. Comparisons that had a two-tailed p < .05 were considered statistically significant. Descriptive statistics for patients’ demographic and clinical variables were obtained. The numbers of positive cells per field in five arbitrarily selected high-power fields (400×) were counted and averaged by two independent investigators blinded to the patients’ classifications. Only cells with visible nuclei in the images were analyzed.

### Results

#### Sample Characteristics and Neuropsychological Assessment

A total of 60 patients who underwent ATL, comprising 32 male patients and 28 female patients with a mean age of 30.08 ± 13.88 years and a mean seizure history of 12.02 ± 9.10 years, were recruited in this study. Among the 60 patients with TLE, 17 (28.3%) had a HAMD score greater than nine and were considered to have depression. In addition, 28 patients (46.7%) had hippocampal sclerosis. The characteristics and baseline scores of all included patients are shown in Table 1. Patients without HS but with depression (TLE-nHS/TLE) had higher HAMD scores than patients without HS and depression (TLE-nHS/TLE-D) (11.55 ± 1.69 vs. 5.38 ± 2.13, p < .001; unpaired Student’s t-test). Other characteristics did not show significant differences among patients with and without HS or patients with and without depression (all p > .05; unpaired Student’s t-test, the Mann-Whitney U-test, Pearson’s χ²-test, or Fisher’s exact test, as appropriate). Samples were obtained from sex- and age-matched patients and divided into three groups: the TLE-HS group (n = 7), the TLE-nHS/TLE group (n = 7), and the TLE-nHS/TLE-D group (n = 7). Neuroimaging tests followed by pathological verification showed that there was hippocampal sclerosis in the TLE-HS group (Figure 1). According to the ILAE classification, five samples in the TLE-HS group showed moderate segmental pyramidal cell loss, and two displayed severe neuronal loss. There were no significant differences in terms of sex; age at surgery; seizure history; or HAMD, WAIS, or WMS scores between patients in the TLE-HS group and those in the TLE-nHS group (all p > .05; unpaired Student’s t-test, Mann-Whitney U-test, Pearson’s χ²-test, or Fisher’s exact test, as appropriate). Samples from the TLE-nHS group were then divided into the TLE-nHS/TLE group (n = 7) and the TLE-nHS/TLE-D group (n = 7). The HAMD scores of patients in the TLE-nHS/TLE-D group ranged from 10 to 15, with a mean score of 12.00 ± 1.91, which was significantly higher than the mean score of patients in the TLE-nHS/TLE group (3.57 ± 1.40, p < .001; unpaired Student’s t-test). There were no significant differences in the other characteristics or neuropsychological assessment scores. The characteristics and baseline scores of patients from whom the samples were obtained (n = 21) are shown in Table 2. None of the TLE patients included in the present study had received treatment for depression before hospitalization.

### Table 1. Characteristics and Baseline Assessments of all Included Patients (All Patients, TLE-HS Patients, TLE-nHS Patients, and TLE-nHS/TLE-D Patients).

| Subject                                           | All (n = 60) | TLE-HS (n = 28) | TLE-nHS (n = 32) | TLE-nHS/TLE (n = 21) | TLE-nHS/TLE-D (n = 11) |
|---------------------------------------------------|--------------|----------------|------------------|----------------------|------------------------|
| Male/female, n                                    | 32/28        | 15/13          | 14/18            | 8/13                 | 6/5                    |
| Age at surgery, y, mean ± SD                      | 30.08 ± 13.88| 29.93 ± 9.89   | 32.03 ± 13.62    | 33.62 ± 15.06        | 29.09 ± 8.98           |
| Seizure history, y, mean ± SD                     | 12.02 ± 9.10 | 13.87 ± 9.68   | 11.52 ± 7.90     | 10.55 ± 8.65         | 12.36 ± 6.17           |
| With depression/without depression, n             | 17/43        | 6/22           | 11/21            | 0/21                 | 1/0                    |
| With HS/without HS, n                             | 28/32        | 28/0           | 0/32             | 0/21                 | 0/11                   |
| Neuropsychological assessments                    |              |                |                  |                      |                        |
| HAMD, mean ± SD                                   | 7.40 ± 3.36  | 7.29 ± 3.16    | 7.50 ± 3.57      | 5.38 ± 2.13          | 11.55 ± 1.69*          |
| WAIS, mean ± SD                                   | 88.10 ± 6.46 | 87.50 ± 6.45   | 88.63 ± 6.52     | 89.62 ± 6.58         | 86.73 ± 6.26           |
| WMS, mean ± SD                                    | 90.08 ± 8.58 | 89.21 ± 8.83   | 91.84 ± 9.12     | 92.29 ± 8.85         | 90.18 ± 8.04           |

**Abbreviations:** HS = hippocampal sclerosis; HAMD = Hamilton Rating Scale for Depression; WAIS = Wechsler Adult Intelligence Scale; WMS = Wechsler Memory Scale; SD = standard deviation.

* p < .01, TLE-nHS/TLE group versus TLE-nHS/TLE-D group.
Expression of HMGB1 in Neurons in the Anterior hippocampi of Patients with TLE

HMGB1 was ubiquitously expressed in neurons in all subregions of the anterior hippocampi of patients with TLE (Figures 2 to 4). An increased ratio of HMGB1-positive neurons was observed in patients with hippocampal sclerosis (TLE-HS group) \((p = .0004, \text{unpaired Student's } t\text{-test}; n = 7/\text{group; Figure 2})\). The percentage of HMGB1-positive neurons in the CA regions was significantly increased in patients with TLE and comorbid depression (TLE-nHS/TLE-D group) \((p = .0071, \text{unpaired Student's } t\text{-test}; n = 7/\text{group; Figure 3})\). However, there were no significant differences in the ratio of HMGB1-positive neurons in the dentate gyrus (DG) between the TLE-nHS and TLE-D groups \((p = .8537, \text{unpaired Student's } t\text{-test}; n = 7/\text{group; Figure 4})\).

HMGB1 was mainly localized in the nuclei of pyramidal neurons (Figure 3) and granule cells of the DG (Figure 4). We observed scattered cells with cytoplasmic
staining and neurons with both nuclear and cytoplasmic staining in the hippocampi of patients with TLE (Figures 2 to 4). The ratio of cytoplasmic HMGB1-positive neurons was significantly increased in the CA regions \(p = 0.0002\), unpaired Student’s \(t\)-test; \(n = 7\)/group; Figure 3) but not in the DG \(p = 0.454\), unpaired Student’s \(t\)-test; \(n = 7\)/group; Figure 4) in TLE patients with comorbid depression.

### Table 2. Characteristics and Baseline Assessments of Patients With Obtained Samples (\(n = 21\)).

| PT no. | Sex (M/F) | Age at surgery (y) | History (y) | Depression (Y/N) | HS (Y/N) | Neuropsychological assessment (mean ± SD) |
|--------|-----------|--------------------|-------------|------------------|----------|-----------------------------------------|
|        |           |                    |             |                  |          | HAMD | WAIS | WMS |
| 1      | M         | 31                 | 14          | Y                | Y        | 11  | 92  | 88  |
| 2      | F         | 54                 | 9           | Y                | Y        | 13  | 81  | 83  |
| 3      | M         | 36                 | 11          | Y                | Y        | 14  | 85  | 82  |
| 4      | F         | 35                 | 18          | N                | Y        | 6   | 94  | 95  |
| 5      | M         | 37                 | 16          | N                | Y        | 7   | 85  | 88  |
| 6      | M         | 55                 | 10          | N                | Y        | 6   | 91  | 94  |
| 7      | F         | 36                 | 23          | N                | Y        | 4   | 81  | 82  |
| TLE-HS | 4/3       | 40.57 ± 9.71       | 14.43 ± 5.00| 3/4              | 7/0      | 8.71 ± 3.90 | 87.00 ± 5.32 | 87.43 ± 5.47 |
| 8      | F         | 36                 | 18          | N                | N        | 4   | 88  | 92  |
| 9      | F         | 29                 | 7           | N                | N        | 2   | 95  | 97  |
| 10     | M         | 55                 | 8           | N                | N        | 6   | 92  | 94  |
| 11     | M         | 33                 | 31          | N                | N        | 3   | 82  | 84  |
| 12     | F         | 41                 | 20          | N                | N        | 2   | 88  | 85  |
| 13     | M         | 57                 | 11          | N                | N        | 4   | 91  | 93  |
| 14     | M         | 39                 | 15          | N                | N        | 4   | 94  | 96  |
| TLE-nHS/TLE | 4/3 | 41.43 ± 10.71 | 15.71 ± 8.32 | 0/7              | 0/7      | 3.57 ± 1.40 | 90.00 ± 4.43 | 91.57 ± 5.13 |
| 15     | M         | 49                 | 47          | Y                | N        | 14  | 93  | 89  |
| 16     | M         | 35                 | 7           | Y                | N        | 11  | 89  | 94  |
| 17     | F         | 37                 | 13          | Y                | N        | 12  | 92  | 91  |
| 18     | M         | 58                 | 15          | Y                | N        | 15  | 90  | 87  |
| 19     | M         | 40                 | 8           | Y                | N        | 10  | 88  | 89  |
| 20     | F         | 31                 | 10          | Y                | N        | 10  | 83  | 90  |
| 21     | F         | 38                 | 9           | Y                | N        | 12  | 82  | 85  |
| TLE-nHS/TLE-D | 4/3 | 41.14 ± 9.26 | 15.57 ± 14.14 | 7/0              | 0/7      | 12.00 ± 1.91 | 88.14 ± 4.22 | 89.29 ± 2.87 |
| All    | 12/9      | 10/11              | 7/14        | 7/0              |          | 12.00 ± 1.91 | 88.14 ± 4.22 | 89.29 ± 2.87 |

Abbreviations: PT = patient; HS = hippocampal sclerosis; HAMD = Hamilton Rating Scale for Depression; WAIS = Wechsler Adult Intelligence Scale; WMS = Wechsler Memory Scale; SD = standard deviation.

\(*p < .01, \text{TLE-nHS/TLE group versus TLE-nHS/TLE-D group.}\)

**Figure 2.** (A–H) High mobility group box protein 1 (HMGB1)-immunoreactive neurons in the CA3 region in TLE patients with and without hippocampal sclerosis. The ratio of HMGB1-immunoreactive neurons was significantly increased in the TLE-HS group \(n = 7\) patients/group, \(t = 3.736, p = 0.0004\), unpaired Student’s \(t\)-test). The arrows indicate normal pyramidal neurons in the CA3 region in the TLE-nHS group (D). TLE-nHS = TLE without hippocampal sclerosis; TLE-HS = TLE with hippocampal sclerosis.
Expression of HMGB1 in Glial Cells in the Anterior Hippocampi of Patients With TLE

In astrocytes, HMGB1 was expressed in the nuclei in both the TLE and TLE-D groups. Patients with TLE and depression had an increased ratio of HMGB1-positive astrocytes ($p = .0001$, unpaired Student’s $t$-test; $n=7$/group; Figure 5). However, there was no translocation of HMGB1 from the nucleus to the cytoplasm of astrocytes (Figure 5).

**Figure 3.** Distribution of HMGB1 in the neurons of CA regions of the anterior hippocampus of patients with TLE with and without comorbid depression. (A–H) The CA1 region. (B and D) HMGB1-immunoreactive pyramidal neurons from TLE patients without comorbid depression (thin arrows). (F and H) HMGB1-immunoreactive pyramidal neurons from TLE patients with comorbid depression (thick arrows). (I–P) The CA4 region in TLE patients with and without comorbid depression. The number of HMGB1-positive neurons (Q) and cytoplasmic HMGB1-positive neurons (R) were significantly increased in the TLE-D group ($t=2.776$ and $3.953$, $p=.0071$ and .0002, respectively; unpaired Student’s $t$-test; $n=7$ patients/group). TLE = temporal lobe epilepsy; TLE-D = TLE with comorbid depression; HMGB1 = high mobility group box protein 1.

**Figure 4.** Distribution of HMGB1 in the DG neurons of the anterior hippocampus of TLE patients with and without comorbid depression. (I) There was no significant difference in the number of HMGB1-positive neurons ($t=0.1852$, $p = .8537$, unpaired Student’s $t$-test) or (J) cytoplasmic HMGB1-positive neurons ($t=0.753$, $p = .454$, unpaired Student’s $t$-test) between the TLE and TLE-D groups ($n=7$ patients/group). DG = dentate gyrus; TLE = temporal lobe epilepsy; TLE-D = TLE with comorbid depression; HMGB1 = high mobility group box protein 1.
We also investigated the expression of HMGB1 in microglia. All the nuclei of microglia expressed HMGB1, and there was no significant difference in the number of HMGB1-positive microglia between the TLE patients and TLE-D patients ($t = 0.5293, p = .5984$, unpaired Student’s $t$-test; $n = 7$ patients/group). No cytoplasmic staining of HMGB1 was observed in the microglia of the hippocampus. TLE = temporal lobe epilepsy; TLE-D = TLE with comorbid depression; HMGB1 = high mobility group box protein 1.

Plasma Levels of HMGB1 in Patients With TLE
There was no significant difference in the plasma levels of HMGB1 among the TLE, TLE-HS, and TLE-D groups using one-way ANOVA ($p = .7106$, one-way ANOVA; $n = 7$/group).

Discussion
Depression is the most common psychiatric comorbidity of epilepsy and increases the burden of the disease. However, the molecular mechanisms underlying these pathophysiological processes are not fully understood. We assessed the expression of HMGB1 in the anterior hippocampal neurons of patients with TLE. We found that approximately 28.3% of patients with TLE had depression, exhibiting HAMD scores greater than nine. This percentage is similar to the percentages reported in previous studies, which range from 12% to 37%, and is two to three times higher than the rate in people without epilepsy (Avalos et al., 2020; Fiest et al., 2013; Josephson & Jetté, 2017). None of the patients with TLE in the present study had received treatment for depression. The high incidence of depression in PWE, especially in patients with drug-resistant TLE, indicates that depression and epilepsy might share a common pathogenic mechanism involving limbic structures (De Oliveira et al., 2010; Stretton et al., 2015).

HMGB1 has been found to act as a DNA chaperone, a chromosome “guardian,” and an initiator and amplifier of
neuroinflammation as well as a prototypic damage-associated molecular pattern (Kang et al., 2014; Paudel et al., 2019). The biological function of HMGB1 depends on its modifications, cellular localization, redox state, and binding partners. In the present study, we investigated the distribution of HMGB1 immunofluorescence in anterior hippocampal specimens and the concentration of this protein in plasma from patients with drug-resistant TLE with and without comorbid depression. We found that HMGB1-positive neurons and astrocytes were increased in PWE who had hippocampal sclerosis or comorbid depression. Moreover, translocation of HMGB1 from the nucleus to the cytoplasm was observed in neurons but not in glial cells in the hippocampus. We also investigated the plasma concentration of HMGB1 in PWE with hippocampal sclerosis with/without comorbid depression. The results showed that there was no significant difference among the three groups. Together, the results suggested that changes in HMGB1 expression and localization in neurons might play more important roles in epilepsy with comorbid depression.

HMGB1 is known to play important roles in events associated with DNA activity in the nucleus (Paudel et al., 2018a; Rana et al., 2021; Ye et al., 2019). The abundance of HMGB1-positive neurons was increased in PWE with hippocampal sclerosis. Unfortunately, we did not include normal control hippocampal specimens in the experiment, but previous studies have reported that HMGB1 participates in the chain of events leading to the precipitation and recurrence of seizure activity (Li et al., 2021a; Maroso et al., 2010; Walker et al., 2014; Zhang et al., 2018). Thus, the increase in nuclear HMGB1 expression in the hippocampus suggested that neuronal damage or stress was increased in patients with TLE and hippocampal sclerosis. A similar trend in the number of HMGB1-positive neurons in the CA regions and astrocytes was seen in depressed PWE compared to PWE without comorbid depression. In this analysis, none of the patients in either group had hippocampal sclerosis based on the results of neuroimaging and pathological analysis, allowing us to rule out any possible influence of hippocampal sclerosis. Our results, together with those of previous reports, indicate that HMGB1 participates in the pathological progression of drug-resistant TLE with comorbid depression or hippocampal sclerosis.

Furthermore, more neurons with cytoplasmic HMGB1 staining or with both nuclear and cytoplasmic HMGB1 staining were observed in depressed patients with TLE. HMGB1 is a multifunctional protein, and its functions depend on its localization in the cell (Ye et al., 2019). In addition to playing roles in the nucleus in the nonacetylated thiol form under normal conditions, HMGB1 in this form can translocate to the cytoplasm, where it can activate autophagy by interacting with beclin-1 (Kang et al., 2010). It has been reported that the translocation of HMGB1 occurs very quickly, is sensitive to hypoxia, and is a very early event among the responses to ischemia (Nishibori et al., 2020). Furthermore, HMGB1 can be released into the extracellular space and converted to disulfide HMGB1, which acts as a damage-associated molecular pattern that alerts nearby cells and the immune system to immediate danger, triggering inflammation (Venereau et al., 2016).

Cell activation, injury, or death could result in HMGB1 translocation from the nucleus to the cytoplasm or extracellular space due to the separation of HMGB1 from damaged DNA (Frank et al., 2015). Thus, the results of the present study demonstrated an increase in irreversible stress in hippocampal neurons in PWE with comorbid depression. When released or secreted into the cytoplasm or even the extracellular space, HMGB1 acts as a facilitator of neuroinflammation and participates in cell survival or death pathways (Kang et al., 2014; Ye et al., 2019; Zurolo et al., 2011). The expression of proteins related to the HMGB1 signaling pathway in lesioned tissue is significantly upregulated, and these changes are accompanied by increased cytoplasmic localization of HMGB1 in neurons and astrocytes in both epilepsy (Auvin et al., 2017; Maroso et al., 2010; Zhang et al., 2018) and depression (Frank et al., 2015; Rana et al., 2021).

It has been demonstrated that HMGB1 may be a target for both epilepsy and comorbid depression, which may share a bidirectional relationship with this protein (Paudel et al., 2018b, 2019). Several proteins have been indicated to take part in several pathways, including HMGB1 receptors such as receptor for advanced glycation end products (RAGE), Toll-like receptors 2 and 4, and chemokine receptor type 4 (Carty & Bowie, 2011; Rana et al., 2021). However, the exact neuronal subtypes and HMGB1 translocation signals involved in the diseases remain unclear. The goal to characterize the translocation of HMGB1-specific pathways in neurons via cutting-edge technology and analytics, for example, single-cell RNA sequencing, will provide more evidence on the mechanism of inflammation in epilepsy and comorbid conditions.

**Limitations**

The present study has some clear limitations, such as the small sample size of drug-resistant patients with TLE and the lack of control groups. Furthermore, the levels of cytoplasmic and nuclear HMGB1 were not measured in each group. In addition, it has been reported that HMGB1 increases the permeability of the blood–brain barrier by altering the morphological characteristics of vascular endothelial cells and pericytes, which is believed to be associated with epilepsy (Nishibori et al., 2020). However, we did not perform immunohistochemistry for vascular endothelial cells in the present study. Furthermore, the exact function and downstream pathways of translocated HMGB1 in TLE with comorbid depression were not explored.

**Conclusion**

In the present study, we investigated the distribution of HMGB1 in neurons and glial cells in the anterior
hippocampus of patients with TLE and comorbid depression. The abundance of HMGB1-positive neurons was found to be increased in patients with hippocampal sclerosis. Moreover, the numbers of HMGB1-positive neurons and astrocytes were increased in PWE who had comorbid depression. Increased cytoplasmic HMGB1 was observed in the CA regions in TLE patients with comorbid depression. Translocation of HMGB1 can occur very early after stress, such as ischemia (Nishibori et al., 2020). However, the cause and timing of HMGB1 translocation in pyramidal neurons in the anterior hippocampus in TLE patients with or without comorbid depression remain elusive, reinforcing the need for a deeper understanding of this protein.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Beijing Natural Science Foundation (grant no. 7222098).

Ethical Statements
All the study procedures were conducted according to the guidelines outlined by the Code of Ethics of the World Medical Association (Declaration of Helsinki). The study protocols were approved by the Ethical Review Board of Sanbo Brain Hospital, Capital Medical University (Beijing, China).

Research Ethics and Patient Consent
All the study procedures were conducted according to the guidelines outlined by the Code of Ethics of the World Medical Association (Declaration of Helsinki). The study protocols were approved by the Ethical Review Board of Sanbo Brain Hospital, Capital Medical University (Beijing, China). Written informed consent was obtained from all participants or their guardians.

Significance Statement
HMGB1 might play important role in the development of hippocampal sclerosis and depression as a comorbidity of TLE.

ORCID iD
Yu-Guang Guan https://orcid.org/0000-0001-9945-2872

References
Auvin, S., Walker, L., Gallentine, W., Jozwiak, S., Tombini, M., & Sills, G. J. (2017). Prospective clinical trials to investigate clinical and molecular biomarkers. *Epilepsia*, 58, 20–26. https://doi.org/10.1111/epi.13782
Avalos, J. C., Silva, B. A., Tevés Echazu, M. F., Rosso, B., Besocke, A. G., & del Carmen Garcia, M. (2020). Quality of life in patients with epilepsy or psychogenic nonepileptic seizures and the contribution of psychiatric comorbidities. *Epilepsy & Behavior: E&B*, 112, 107447. https://doi.org/10.1016/j.ybepb.2020.107447
Blümcke, I., Thom, M., Aronica, E., Armstrong, D. D., Bartolomei, F., Bernasconi, A., Bernasconi, N., Bien, C. G., Cendes, F., Coras, R., Cross, J. H., Jacques, T. S., Kahane, P., Mathern, G. W., Miyata, H., Moshé, S. L., Oz, B., Özkara, Ç, Perucca, E., Sisodiya, S., Wiebe, S., & Sprefacio, R. (2013). International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: A task force report from the ILAE commission on diagnostic methods. *Epilepsia*, 54, 1315–1329. https://doi.org/10.1111/epi.12220
Carty, M., & Bowie, A. G. (2011). Evaluating the role of toll-like receptors in diseases of the central nervous system. *Biochemical Pharmacology*, 81, 825–837. https://doi.org/10.1016/j.bcp.2011.01.003
Chang, B. S., Krishnan, V., Dulla, C. G., Jette, N., Marsh, E. D., Dacks, P. A., Whittemore, V., & Poduri, A. (2020). Epilepsy benchmarks area I: Understanding the causes of the epilepsies and epilepsy-related neurologic, psychiatric, and somatic conditions. *Epilepsy Currents / American Epilepsy Society*, 20, 55–135. https://doi.org/10.1077/l335759719895280
D’Alessio, L., Konopka, H., Solís, P., Scévola, L., Lima, M. F., Nuñez, C., Seoane, E., Oddo, S., & Kochen, S. (2019). Depression and temporal lobe epilepsy: Expression pattern of calbindin immunoreactivity in hippocampal dentate gyrus of patients who underwent epilepsy surgery with and without comorbid depression. *Behavioural Neurology*, 2019, 1–12. https://doi.org/10.1155/2019/7396793
De Oliveira, G. N. M., Kummer, A., Salgado, J. V., Portela, E. J., Sousa-Pereira, S. R., David, A. S., & Teixeira, A. L. (2010). Psychiatric disorders in temporal lobe epilepsy: An overview from a tertiary service in Brazil. *Seizure*, 19, 479–484. https://doi.org/10.1016/j.seizure.2010.07.004
Fanselow, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron*, 65, 7–19. https://doi.org/10.1016/j.neuron.2009.11.031
Fiest, K. M., Dykeman, J., Patten, S. B., Wiebe, S., Kaplan, G. G., Maxwell, C. J., Bulloch, A. G. M., & Jette, N. (2013). Depression in epilepsy: A systematic review and meta-analysis. *Neurology*, 80, 590–599. https://doi.org/10.1212/WNL.0b013e31827b1ae0
Fisher, R. S., Cross, J. H., French, J. A., Higurashi, N., Hirsch, E., Jansen, F. E., Lagae, L., Moshé, S. L., Peltola, J., Roulet Perez, F., Bernasconi, A., Bernasconi, N., Bien, C. G., Cendes, F., Coras, R., Cross, J. H., Jacques, T. S., Kahane, P., Mathern, G. W., Miyata, H., Moshé, S. L., Oz, B., Özkara, Ç, Perucca, E., Sisodiya, S., Wiebe, S., & Sprefacio, R. (2013). International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: A task force report from the ILAE commission on diagnostic methods. *Epilepsia*, 54, 1315–1329. https://doi.org/10.1111/epi.12220
Frank, M. G., Weber, M. D., Watkins, L. R., & Maier, S. F. (2015). Stress sounds the alarmin: The role of the danger-associated molecular pattern HMGB1 in stress-induced neuroinflammatory priming. *Brain Behavior and Immunity*, 48, 1–7. https://doi.org/10.1016/j.bbi.2015.03.010
Fu, L., Liu, K., Wake, H., Teshigawara, K., Yoshino, T., Takahashi, H., Mori, S., & Nishibori, M. (2017). Therapeutic effects of anti-HMGB1 monoclonal antibody on pilocarpine-induced status epilepticus in mice. *Scientific Reports*, 7, 1–13. https://doi.org/10.1038/s41598-016-0028-x
Gill, S. J., Lukmanji, S., Fiest, K. M., Patten, S. B., Wiebe, S., & Jetté, N. (2017). Depression screening tools in persons with
epilepsy: A systematic review of validated tools. *Epilepsia*, 58, 695–705. https://doi.org/10.1111/epi.13651

Gou, X., Ying, J., Yue, Y., Qiu, X., Hu, P., Qu, Y., Li, J., & Mu, D. (2020). The roles of high mobility group box 1 in cerebral ischemic injury. *Frontiers in Cellular Neuroscience*, 14, 602080. https://doi.org/10.3389/fncel.2020.602080

Jackson, M. J. (2005). Depression and anxiety in epilepsy. *Journal of Neurology Neurosurgery & Psychiatry*, 76, i45–i47. https://doi.org/10.1136/jnnp.2004.060467

Josephson, C. B., & Jetté, N. (2017). Psychiatric comorbidities in epilepsy. *International Review of Psychiatry*, 29, 409–424. https://doi.org/10.1080/09540261.2017.1302412

Josephson, C. B., Lowerison, M., Vallerand, I., Sajobi, T. T., Patton, S., Jette, N., & Wiebe, S. (2017). Association of depression and treated depression with epilepsy and seizure outcomes. *JAMA Neurology*, 74, 533–539. https://doi.org/10.1001/jamaneurol.2016.5042

Kang, R., Chen, R., Zhang, Q., Hou, W., Wu, S., Cao, L., Huang, J., Yu, Y., Fan, X., Yan, Z., Sun, X., Wang, H., Wang, Q., Tsung, A., Billiar, T. R., Zeh, H. J., Lotze, M. T., & Tang, D. (2014). HMGB1 in health and disease. *Molecular Aspects of Medicine*, 40, 1–116. https://doi.org/10.1016/j.mam.2014.05.001

Kang, R., Livesey, K. M., Zeh, H. J., Lotze, M. T., & Tang, D. (2010). HMGB1: A novel Beclin 1-binding protein active in autophagy. *Autophagy*, 6, 1209–1211. https://doi.org/10.4161/auto.6.8.13651

Kuehl, L., Salmond, B., & Tran, L. (1984). Concentrations of high-mobility-group proteins in the nucleus and cytoplasm of several rat tissues. *Journal of Cell Biology*, 99, 648–654. https://doi.org/10.1083/jcb.99.2.648

Kwan, P., Arzimanoglou, A., Berg, A. T., Brodie, M. J., Hauser, W. A., Mathern, G., Moshé, S. L., Perucca, E., Thompson, P. J., & Wiebe, S. (2010). *Toll-like receptor 4 (TLR4) and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures*. *Nature Medicine*, 16, 413–419. https://doi.org/10.1038/nm.2127

Liu, X. L., Tang, C. Y., Wang, S., Zhao, M., Wang, X. F., Li, T. F., Qi, X. L., Luan, G. M., & Guan, Y. G. (2021b). Regulation of HMGB1 and autophagy. *Frontiers in Neurology*, 12, 1–19. https://doi.org/10.3389/fnlmn.2021.695

Nishibori, M., Wang, D., Ousaka, D., & Wake, H. (2020). High mobility group box-1 and blood–brain barrier disruption. *Cells*, 9, 2650. https://doi.org/10.3390/cells9122650

Paudel, Y. N., Seemple, B. D., Jones, N. C., Othman, I., & Shaikh, M. F. (2019). High mobility group box 1 (HMGB1) as a novel frontier in epileptogenesis: From pathogenesis to therapeutic approaches. *Journal of Neurochemistry*, 151, 542–557. https://doi.org/10.1111/jncc.14663

Paudel, Y. N., Shaikh, M. F., Chakraborti, A., Kumari, Y., Aledo-Serrano, Á, Aleksovska, K., Alvim, M. K. M. H., & Othman, I. (2018a). HMGB1: A common biomarker and potential target for TBI, neuroinflammation, epilepsy, and cognitive dysfunction. *Frontiers in Neuroscience*, 12, 1–19. https://doi.org/10.3389/fnins.2018.00628

Paudel, Y. N., Shaikh, M. F., Shah, S., Kumari, Y., & Othman, I. (2018b). Role of inflammation in epilepsy and neurobehavioral comorbidities: Implication for therapy. *European Journal of Pharmacology*, 837, 145–155. https://doi.org/10.1016/j.ejphar.2018.08.020

Rana, T., Behl, T., Mehta, V., Uddin, M. S., & Bungau, S. (2021). Molecular insights into the therapeutic promise of targeting HMGB1 in depression. *Pharmaceutical Reports*, 73, 31–42. https://doi.org/10.1007/s43440-020-00163-6

Stretton, J., Pope, R. A., Winston, G. P., Sidhu, M. K., Symms, M., Duncan, J. S., Koeppe, M., Thompson, P. J., & Foong, J. (2015). Temporal lobe epilepsy and affective disorders: The role of the subgenual anterior cingulate cortex. *Journal of Neurology Neurosurgery & Psychiatry*, 86, 144–151. https://doi.org/10.1136/jnnp-2013-306966

Venerareu, E., De Leo, F., Mezzapelle, R., Caregica, G., Musco, G., & Bianchi, M. E. (2016). HMGB1 as biomarker and drug target. *Pharmacological Research*, 111, 534–544. https://doi.org/10.1016/j.phrs.2016.06.031

Walker, L., Tse, K., Ricci, E., Thippeswamy, T., Sills, J. G., White, S. H., Antoine, D. J., Marson, A., & Pirmohamed, M. (2014). High mobility group box 1 in the inflammatory pathogenesis of epilepsy: Profiling circulating levels after experimental and clinical seizures. *Lancet (London, England)*, 383, S105. https://doi.org/10.1016/S1474-4422(13)72346-8

Walker, L. E., Janigro, D., Heinemann, U., Riikonen, R., Bernard, C., & Patel, M. (2016). WONOEP Appraisal: Molecular and cellular biomarkers for epilepsy. *Epilepsia*, 57, 1354–1362. https://doi.org/10.1111/epi.13460

Wang, S., Guan, Y., & Li, T. (2021a). The potential therapeutic role of the HMGB1–TLR pathway in epilepsy. *Current Drug Targets*, 22, 171–182. https://doi.org/10.2174/1214899517666666669

Wang, S., Guan, Y. G., Zhu, Y. H., & Wang, M. Z. (2022). Role of high mobility group box protein 1 in depression: A mechanistic and therapeutic perspective. *World Journal of Psychiatry*, 12, 779–786. https://doi.org/10.5498/wjp.v12.i6.779

Wang, S., Zhao, M., Li, T., Zhang, C., Zhou, J., Wang, M., Wang, X., Ma, K., Luan, G., & Guan, Y. (2021b). Long-term efficacy and cognitive effects of bilateral hippocampal deep brain stimulation in patients with drug-resistant temporal lobe epilepsy. *Neurological Sciences*, 42, 225–233. https://doi.org/10.1007/s10072-020-04554-8

Xu, P., Chen, A., Li, Y., Xing, X., & Lu, H. (2019). Medial prefrontal cortex in neurological diseases. *Physiological Genomics*, 51, 432–442. https://doi.org/10.1152/physiogenomics.00006.2019
Yang, W., Li, J., Shang, Y., Zhao, L., Wang, M., Shi, J., & Li, S. (2017). HMGB1-TLR4 axis plays a regulatory role in the pathogenesis of mesial temporal lobe epilepsy in immature rat model and children via the p38MAPK signaling pathway. *Neurochemical Research, 42*, 1179–1190. https://doi.org/10.1007/s11064-016-2153-0

Ye, Y., Zeng, Z., Jin, T., Zhang, H., Xiong, X., & Gu, L. (2019). The role of high mobility group box 1 in ischemic stroke. *Frontiers in Cellular Neuroscience, 13*, 127. https://doi.org/10.3389/fncel.2019.00127

Zhang, Z., Liu, Q., Liu, M., Wang, H., Dong, Y., Ji, T., Liu, X., Jiang, Y., Cai, L., & Wu, Y. (2018). Upregulation of HMGB1-TLR4 inflammatory pathway in focal cortical dysplasia type II. *Journal of Neuroinflammation, 15*, 1–11. https://doi.org/10.1186/s12974-017-1027-y

Zurolo, E., Iyer, A., Maroso, M., Carbonell, C., Anink, J. J., Ravizza, T., Fluitter, K., Spliet, W. G. M., Van Rijen, P. C., Vezzani, A., & Aronica, E. (2011). Activation of toll-like receptor, RAGE and HMGB1 signalling in malformations of cortical development. *Brain, 134*, 1015–1032. https://doi.org/10.1093/brain/awr032