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Characteristics of Epileptiform Spike-wave Discharges and Chronic Histopathology in Controlled Cortical Impact Model of Sprague–Dawley Rats

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Abstract

Post-traumatic epilepsy (PTE) is a serious complication that can occur following traumatic brain injury (TBI). Sustained secondary changes after TBI promote the process of PTE. Here, we aim to evaluate changes in behavior, electrocorticogram, and histomorphology in rats following chronic TBI models. We observed intensive 7–8 Hz spike-wave-discharges (SWDs) at frontal recording sites and quantified them in SD rats with different degrees of TBI and compared them with age-matched sham rats to evaluate the association between SWDs and injury severity. Notably, although SWDs were even presented in the sham group, the number and duration of events were much lower than those in the TBI groups. SWDs have numerous similarities to absence seizures, such as abrupt onset, termination, and lack of postictal suppression, which may be the non-convulsive characteristics of PTE. Retigabine, a novel antiepileptic drug, is ineffective in reducing SWDs. In addition, we examined chronic histopathological changes in TBI rats. Rats subjected to moderate and severe TBI exhibited significantly impaired neurological function, which was accompanied by marked cortical injury, hippocampus deformation, reactive gliosis, and mossy fiber sprouting. Long-term progressive structural changes in the brain are one of the characteristics of epileptogenesis after TBI. Our study provided the potential value of epileptiform SWDs in reflecting the nonconvulsive characteristic of PTE and highlighted the vital role of chronic pathological changes, such as reactive gliosis, in promoting the epileptogenesis following TBI.

Keywords Traumatic brain injury · Nonconvulsive seizure · Spike-wave discharges · Chronic histopathology · Reactive gliosis

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Abbreviations

TBI	Traumatic brain injury
PTE	Post-traumatic epilepsy
CCI	Controlled cortical impact
DG	Dentate gyrus
ASs	Absence seizures
SWDs	Spike-wave-discharges
ECoG	Electrocorticogram
GAERS	Genetic Absence Epilepsy Rats from Strasbourg
WAG/Rij	Wistar Albino Glaxo Rats from Rijswijk
AP	Anteroposterior
ML	Mediolateral
mNSS	Modified Neurological Severity Score
PFA	Paraformaldehyde
Iba1	Ionized calcium binding adaptor molecule 1
GFAP	Glial fibrillary acidic protein
MFS	Mossy fiber sprouting
ZnT3	Zinc transporter 3

FPI	Fluid percussion injury
GABA	Gamma-aminobutyric acid
eGABA$_A$R	Extrasynaptic GABA$_A$ receptors

Introduction

Traumatic brain injury (TBI) is a primary cause of acquired epilepsy [1], which is characterized by spontaneous repetitive seizures that are usually focal and nonconvulsive but can be generalized as convulsive seizures. Post-traumatic epilepsy (PTE) is often refractory to anti-seizure medications or surgery. Therefore, it is crucial to use appropriate preclinical models to explore the mechanisms underlying the development of PTE. One of the most common animal models for TBI is the controlled cortical impact (CCI) model, which mimics penetrating brain trauma and is significantly more likely to produce PTE than other models [2–4]. To date, spontaneous convulsive (infrequent and delayed) and nonconvulsive seizures have been reported in CCI rats [5, 6], and the increase in the risk of seizures depends on injury severity [5–7]. The CCI model is well characterized histopathologically and has been used successfully in rats to demonstrate the development of PTE. Brain structural changes, such as neuronal injury and reactive gliosis, have been observed in the ipsilateral cortex, hippocampus (CA1–CA3 and dentate gyrus [DG] regions), and thalamus [6].

Absence seizures (ASs) are a form of nonconvulsive seizure characterized by impaired consciousness, sudden arrest of ongoing behavior, and occurrence of bilaterally synchronous spike-wave-discharges (SWDs) in the electrocorticogram (ECoG) [8, 9]. The SWDs of ASs are typically episodes of sudden onset, termination, and lack of postictal suppression that last from seconds to minutes and mainly occur during light sleep and passive wakefulness [10]. These events are often accompanied by behavioral arrest (interruption of ongoing movement) with or without rhythmic facial twitching. Inherent 7–12 Hz SWDs are commonly recorded in both inbred (Genetic Absence Epilepsy Rats from Strasbourg [GAERS] and Wistar Albino Glaxo Rats from Rijswijk [WAG/Rij]) and outbred (Sprague–Dawley and Long-Evans) rat strains. Moreover, SWDs are recorded in both sham and TBI rats and are identical to those recorded in photothrombotic brain infarction models of post-stroke epilepsy [11, 12]. However, the association between SWDs and TBI still remains unknown. TBI can lead to acute cell death, BBB damage, and excitatory toxicity shortly after injury. Nevertheless, chronic neuropathology, such as neuronal injury, hippocampal cell loss, mossy fiber sprouting (MFS), and synaptic reorganization, may be primarily involved in the process of post-traumatic epileptogenesis. Therefore, in this study, we visually examined continuous video-ECoG recordings for possible seizure attacks and

quantified the frequency and duration of SWDs to describe the electrographic signatures of SWDs and examine the relation between SWDs and TBI. In addition, we tested the effect of a novel drug, retigabine, on reducing SWDs. At last, we also discussed the corresponding neuropathological changes associated with chronic TBI to establish relationship between structure and function.

Materials and Methods

Animals

A total of 24 adult male Sprague–Dawley rats weighing 250 ± 10 g (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) were housed in an animal facility in standard laboratory conditions (temperature 22 °C $\pm$ 1 °C; humidity 50–60%; 12-h light–dark cycle) with food and water ad libitum. All procedures were performed in accordance with the guidelines of the Capital Medical University Animal Care and Use Committee. Rats were randomly divided into the following groups: sham-operated ($n = 6$), CCI-mild ($n = 7$), CCI-moderate ($n = 7$), and CCI-severe ($n = 4$).

Controlled Cortical Impact Injury

We used the CCI model of TBI. Rats were anesthetized with 3–5% isoflurane, fixed in a stereotaxic frame, and maintained with 1.5% isoflurane via a nose cone throughout the surgery. A 6-mm diameter craniotomy was centered at anteroposterior (AP) − 3.0 mm and mediolateral (ML) + 3.0 mm, with the dura mater kept intact. Cortical contusion was produced using CCI equipment (Leica 9969S) with a 5 mm-diameter tip. The impact velocity was 4.0 m/s, and the contact duration was 100 ms. The impact depth was 1.0, 2.0, or 3.0 mm (mild, moderate, or severe injury, respectively). The rats were placed in a heating pad for recovery post-TBI. Sham-injured rats received identical surgical preparation but were not subjected to CCI injury.

Behavioral Test

The modified Neurological Severity Score (mNSS) test [13] was performed in all rats before TBI and at 1, 3, 7, 10, and 14 days following TBI by two investigators who were blinded to experimental group. The mNSS comprehensively evaluates neurological functions, such as motor, sensory, reflex, and balance functions. Scores range from 0 to 18 (minimum 0, maximum 18) and higher scores indicate more serious injury.

Chronic Video-ECoG Recording

Ten months after injury, rats were implanted with intracranial screw electrodes (1 × 4 mm, Plexon). Two recording electrodes were placed over the ipsilateral frontal (AP 3.0 mm, ML 2.5 mm) and contralateral frontal (AP 3.0 mm, ML − 2.5 mm) cortices. A reference screw and a ground screw were implanted in the bilateral occipital bones, respectively. Screws were wrapped with silver wire, which was welded to a multi-pin connector and secured using dental acrylic. After a 1-week recovery period, video-ECoGs were recorded for 7 days using the Omniplex Server System (Plexon, USA) at a sampling rate of 2000 Hz. On the final day of video-ECoG monitoring, rats received a single intraperitoneal [i.p.] injection of retigabine (10 mg/kg; R823985, Macklin, Shanghai, China), followed by video-ECoG monitoring for 4 h to observe changes in SWDs and behavior.

Quantification of SWD Events

At the end of video-ECoG monitoring, 3-day (6 h/day) long records were extracted for each rat and manually reviewed for subsequent SWD-event and related behavior analyses. An SWD event was defined as abnormal rhythmic discharge with high-amplitude spikes that are distinct from baseline activity and have a minimum duration of 10 s. The asynchronicity and synchronicity of SWDs were assessed by comparing SWD initiation between the two electrodes. If there was no detectable SWD delay between the two recording electrodes, onsets were considered synchronous, whereas an SWD delay exceeding 100 ms was considered an asynchronous onset. The behavior of rats during SWDs was also observed using time-locked video.

Histological Assessment

TBI-moderate rats were deeply anesthetized with 10% chloral hydrate (0.04 ml/kg, i.p.) and transcardially perfused with 0.9% NaCl and 4% paraformaldehyde (PFA). The brains were removed and placed in 4% PFA at 4 ℃ to be post-fixed overnight. Brains were cryosectioned (CM1950, Leica, Bensheim, Germany) into serial coronal slices (25 μm). The sections were placed onto gelatin-covered microscope slides and stained with Cresyl Violet for morphological analysis. The damage volume was calculated by the formula: $[(VC − VL)/VC] × 100\%$ (VC: volume of the contralateral hemisphere; VL: volume of the ipsilateral hemisphere). Numbers of neurons in the hippocampal CA1 region were counted with ImageJ and analyzed with Prism (GraphPad Software, San Diego, CA, USA).

Floating slices were washed three times with PBSTT (phosphate-buffered saline [PBS] with 0.3% Triton X-100 and 0.1% Tween). Slices were blocked with 1% goat serum to avoid binding of non-specific antibodies and incubated at 4 ℃ overnight in a solution containing the primary antibody for ionized calcium binding adaptor molecule 1 (Iba1; CST, 17,198, 1:300), glial fibrillary acidic protein (GFAP; Invitrogen, 13–0300, 1:300) and zinc transporter 3 (ZnT3; SySy, 197,002, 1:300). After thoroughly washing in PBSTT, the slices were incubated with secondary antibodies (Alexa Fluor 488 donkey anti-rat or goat anti-rabbit immunoglobulin G) for 1.5 h and counterstained with 4',6-diamidino-2-phenylindole. Images were captured using a confocal microscope (LSM710, Zeiss).

Statistical Analysis

Statistical analysis was performed using SPSS version 22 and GraphPad Prism 8. Data were analyzed using ANOVA, and t test. Two-tailed Fisher's exact tests were used to evaluate the incidence of SWDs in each group. Results are expressed as Means ± SEM, and $p < 0.05$ was considered statistically significant.

Results

CCI Injury and Neurological Dysfunction

The design of the study is illustrated in Fig. 1A. The rats were subjected to different degrees (sham, mild, moderate, and severe) of unilateral cortical contusion using the CCI model, and body weight and neurological function were evaluated before injury and 1, 3, 7, 10, and 14 days after injury. The body weight of the rats decreased on the first day following TBI and gradually increased during subsequent days, with no significant difference between groups (Fig. 1B). The mNSS test was used to evaluate the behavior of rats at different injury time points. Severe brain injury rats showed obvious neurological dysfunction, which did not recover at 14 days after TBI, which resulted in obvious sequelae. Rats subjected to moderate TBI exhibited significantly impaired neurological function on days 1 and 3 post-TBI and largely recovered after 7 days. There was no significant difference in neurological function between the mild injury and sham groups (Fig. 1C). To effectively measure parameters of the chronic CCI models, we should take the mortality into consideration. Due to a mortality rate of up to 25%, rats in the severe injury group were not appropriate for long-term observation. Therefore, we recruited rats in mild and moderate groups for the subsequent procedure.

Appearance and Characteristics of SWD Events

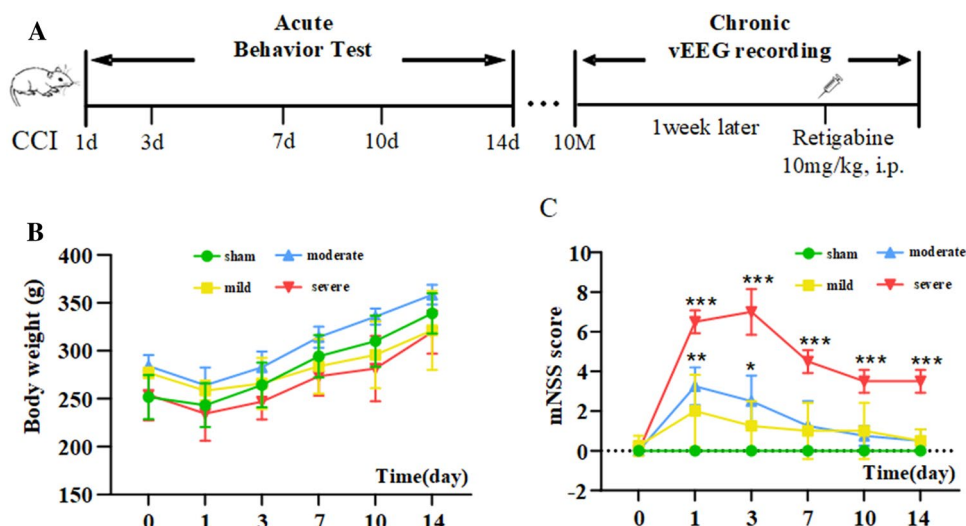At 10 months after TBI, rats received video-ECoG monitoring to assess spontaneous seizures. Two rats (one in

Fig. 1 Controlled cortical impact (CCI) injury and neurological dysfunction. **A** Study design. The rats were subjected to different degrees (sham, mild, moderate and severe) of CCI injury. Behavioral test was performed before CCI and 1, 3, 7, 10, and 14 days after CCI. Skull electrodes were implanted at 10 months post-traumatic brain injury (TBI). Video-electrocorticogram(v-ECoG) was monitored for 1 weeks, then followed by retigabine injection(10 mg/kg). **B** The body weight of the rats decreased on the first day after TBI and then gradually increased, with no significant differences among groups. **C** Behavioral test, indicated by modified Neurological Severity Score (mNSS), was assessed at the time points mentioned above. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. the sham group)

the sham group and one in the moderate TBI group) were excluded from analysis because of neocortical damage that occurred during electrode implantation surgery. In one case, the headstage detached from the rat 3 days after the electrodes were implanted. The other rats all showed spontaneous SWD bursts in the continuously recorded video-ECoG, which typically had large amplitudes, high-frequency activity, abrupt onset, and termination. Figure 2A and B shows a 200-s ECoG recording and corresponding power spectra of sham and moderately injured rats 10 months following TBI. SWDs were characterized by abrupt initiations and terminations and no suppression post-event and had spectral power in the theta (4–8 Hz) and beta bands (12–18 Hz). Waveforms were selected from the sham and TBI groups and expanded at different time-scales, as shown in grey box of Fig. 2. The spike frequency was approximately 7 Hz (6–8 Hz), and morphology did not appear significantly different, which is consistent with previous reports [14]. Another feature of these abnormal discharges was that they could be interrupted by warning stimuli, such as sudden changes in noise or light (Fig. 3A). The corresponding videos showed stereotyped behaviors of rats, such as exhibiting a freezing-like posture (interruption of ongoing movement), vibrissa extension, and slight vibrational tremor, which is similar to non-convulsive seizures (Fig. 3B), which have been described in many other studies [15, 16].

TBI Increased the Frequency and Duration of SWD Events

The SWD incidence rate was no significant difference between TBI rats (80%) and sham rats (75%) (Fisher's Exact Test, $p = 1.0$). Most SWD events began synchronously, and few initiated asynchronously (Fig. 4A and B). To compare SWD events among the sham, mild, and moderate injury groups, we measured the number and duration of SWD events in each group that were > 10 s. The comparison of the number of SWD events between the different groups showed that the TBI moderate injury group had significantly more SWD events than did the sham ($p = 0.0042$) and mild injury groups ($p = 0.0216$) (Fig. 4C). The total SWD duration per day of the TBI moderate injury rats differed significantly from that of the sham ($p = 0.0087$) and mildly injured rats ($p = 0.0212$) (Fig. 4D). In addition, the longest SWD event recorded in the sham group lasted 38.4 s, whereas that in the moderate injury group lasted 154.9 s.

Retigabine Increases the Frequency of SWD Events

Retigabine, a novel anti-seizure medication, has been reported to be effective in controlling convulsive seizures in animal models [17, 18]. However, the efficacy of retigabine in the treatment of nonconvulsive seizures remains unclear. Having observed SWDs with characteristics similar to ASs, we tested whether retigabine can reduce SWDs in these TBI rats. Therefore, we administered an anticonvulsant,

Fig. 2 Characteristics of spike-wave-discharges (SWD) events. **A–B** A sample 200-s ECoG recording and corresponding power spectra from sham and TBI moderately injured rats. Grey boxes showed the extended waveforms from the sham and TBI groups at different time scales
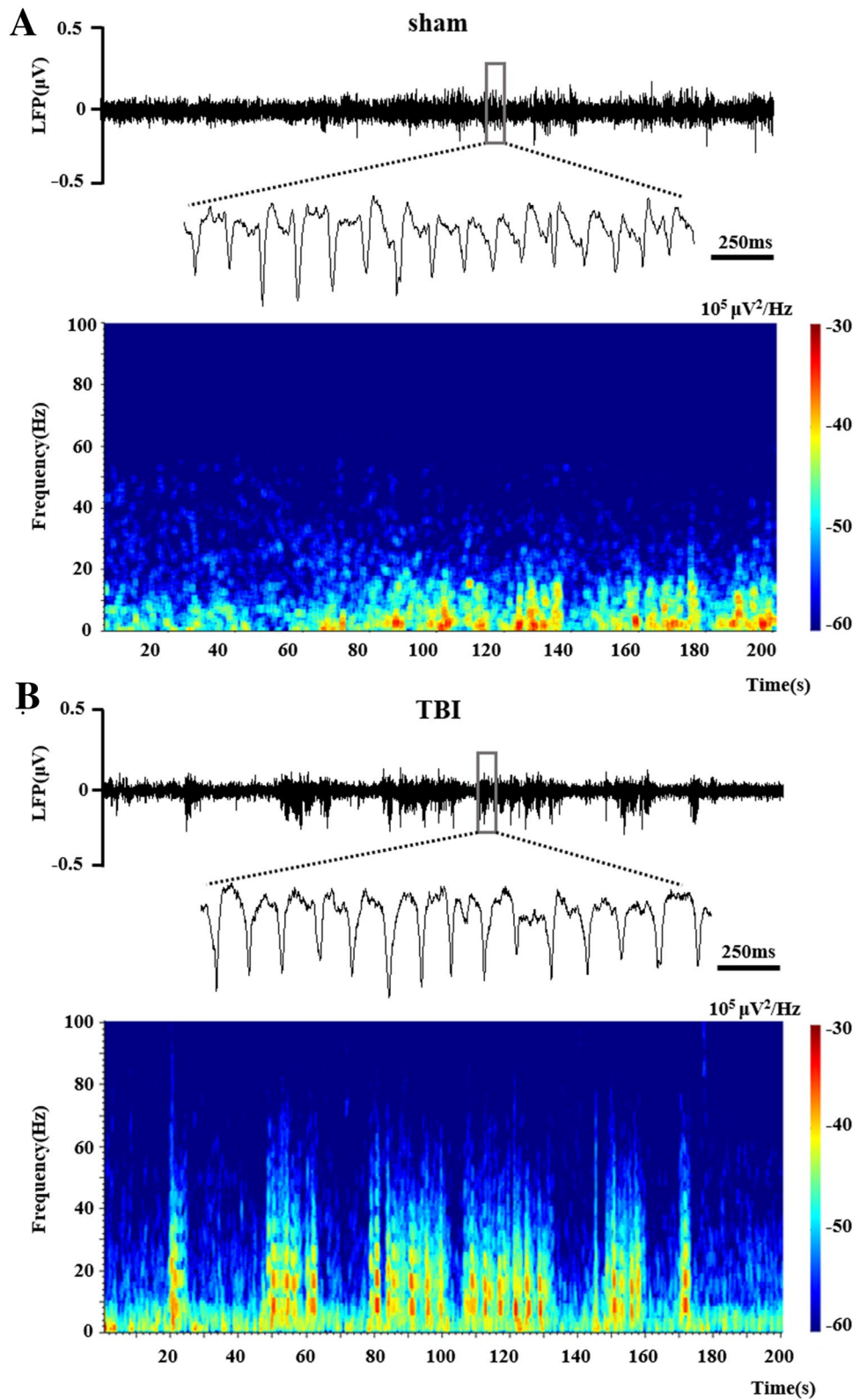
retigabine (10 mg/kg; i.p.) to rats at 40 ± 8 weeks post-TBI and monitored rats via video-ECoG. After 2 h of baseline recording, retigabine was injected, followed by 4 h of

observation. We found that the frequency of SWD events was significantly increased after retigabine intervention. Figure 5A and B shows the ECoG and the corresponding power

Fig. 3 Interruption of ongoing SWD events with noise. **A** SWDs interrupted by warning stimuli were recorded using ECoG and spectrogram. A noise occurred (red line) and interrupted the SWD episode. **B** Corresponding videos showed stereotyped behaviors in rats, such as exhibiting a freezing-like posture (interruption of ongoing movement), vibrissa extension, and slight vibrational tremor
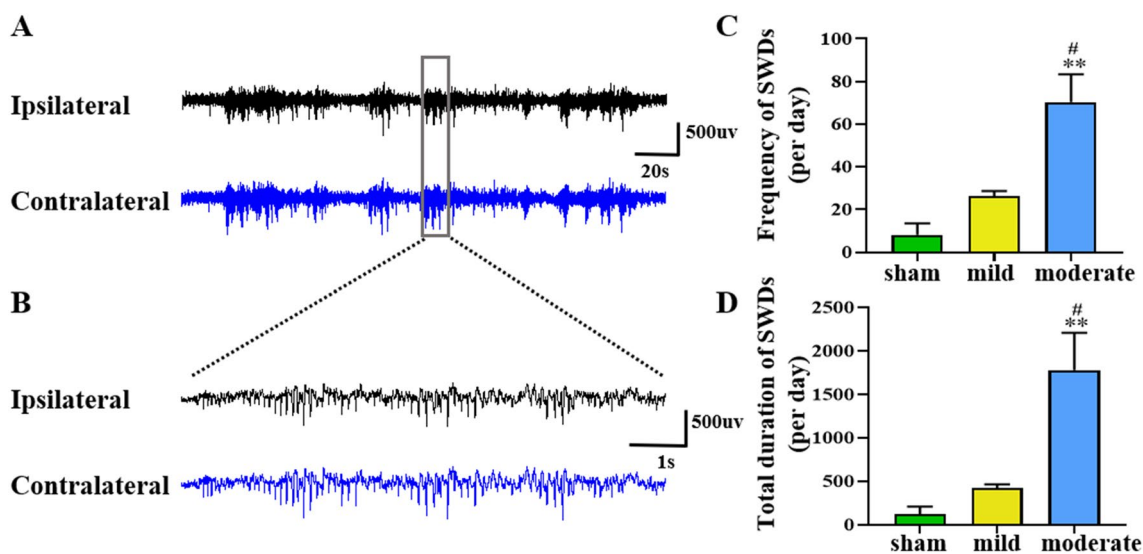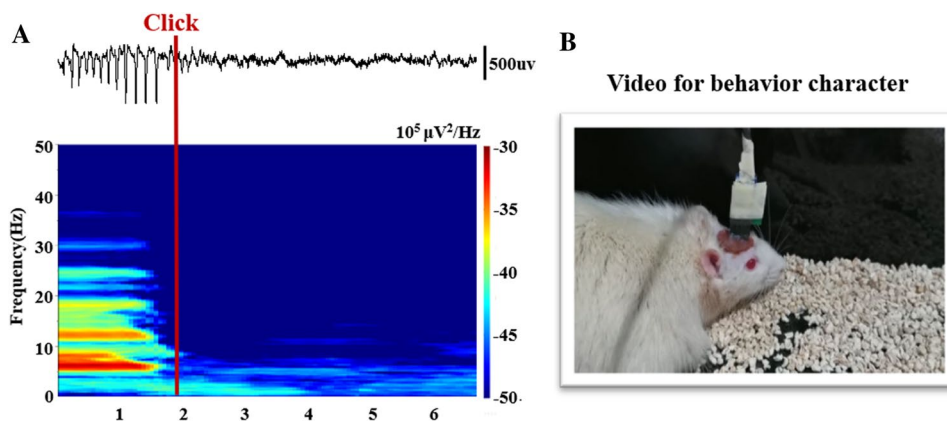

Fig. 4 TBI injury increased the frequency and duration of SWD events. **A** Spontaneous SWD bursts occurred in ipsilateral and contralateral hemispheres of TBI rat brains. **B** Expansion of approximately 9 s of samples showed that SWD events appeared to start synchronously. **C** Comparisons of the frequency of SWD events among different groups showed that moderate injury rats exhibited significantly more SWD events. **D** SWD duration was longer in moderate group than that in sham and mild groups. (**$p < 0.01$ vs. the sham group; #$p < 0.05$ vs. the mild group)

spectra before and after retigabine intervention. However, SWD morphology did not differ significantly between before and after the intervention. Overall, the data indicated that administering retigabine post-TBI aggravates spontaneous SWDs; however, further studies are necessary to clarify the possible mechanisms.

Chronic Neuropathology after TBI

Nissl staining performed on the brain slices of chronic moderate TBI and age-matched sham rats. The analysis of cortical loss and hippocampal damage, which is considered to be associated with epileptogenesis following TBI, indicated that compared with the sham group, there was a significantly greater volumetric loss of focal cortex and more pronounced hippocampal deformation in

the TBI group, particularly in the ipsilateral brain tissue (Fig. 6A and C). The results of quantitative analysis show that compared with the sham group, the TBI group showed significantly reduced cortical tissue (Fig. 6B). In the TBI group, the number of neurons in the ipsilateral CA1 was significantly lower than that in the contralateral CA1, which was calculated using the stereological strategy. No significant differences between the contralateral and ipsilateral sides were observed in the sham group (Fig. 6D). Immunofluorescence for Iba1 and GFAP in the damaged cortex was elevated in the TBI group compared with that in the sham group (Fig. 7A and B). Hippocampal astrocyte proliferation based on GFAP were evident in TBI rats, particularly in the CA3 region (Fig. 8A). MFS was detected by staining hippocampal sections with ZnT3, a member of

Fig. 5 Retigabine aggravated SWDs in TBI rats. **A** Spontaneous SWD bursts were recorded using ECoG, the frequency of SWD events significantly increased following retigabine intervention (10 mg/kg; i.p.). **B** The corresponding power spectra are shown before and after retigabine intervention
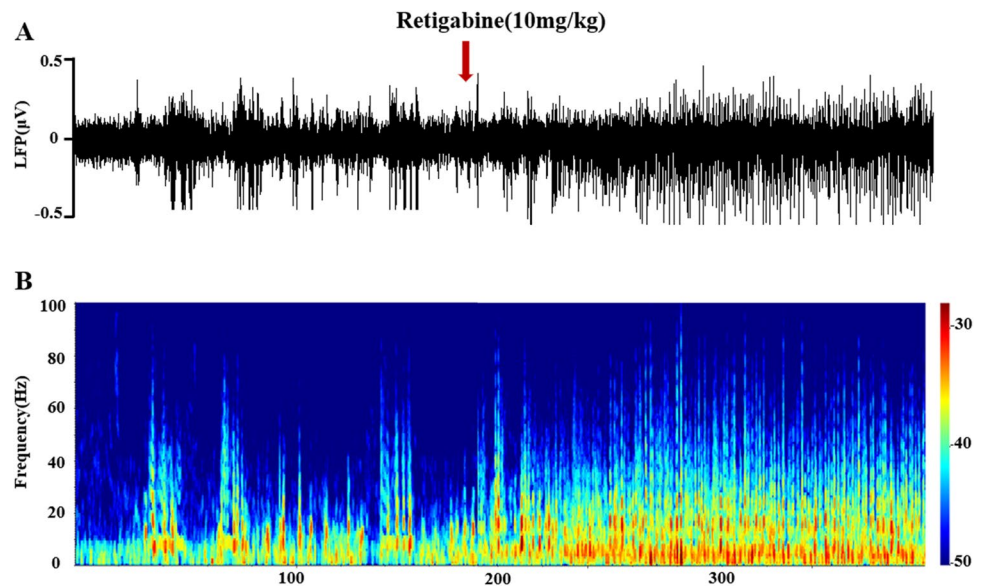

Fig. 6 Chronic structural abnormalities in TBI rats. **A** Tissue loss were investigated using Cresyl Violet staining at 10 months after TBI in the sham and TBI groups. **B** Compared with the sham group, the cortical tissue of TBI group decreased significantly. **C** Hippocampal neuronal loss was significant in the TBI group, particularly in the ipsilateral brain tissue. **D** The number of neurons in CA1 was quantified using the stereological strategy and was significantly lower in the ipsilateral than in the contralateral hemisphere in the TBI group. (*$p < 0.05$, **$p < 0.01$)
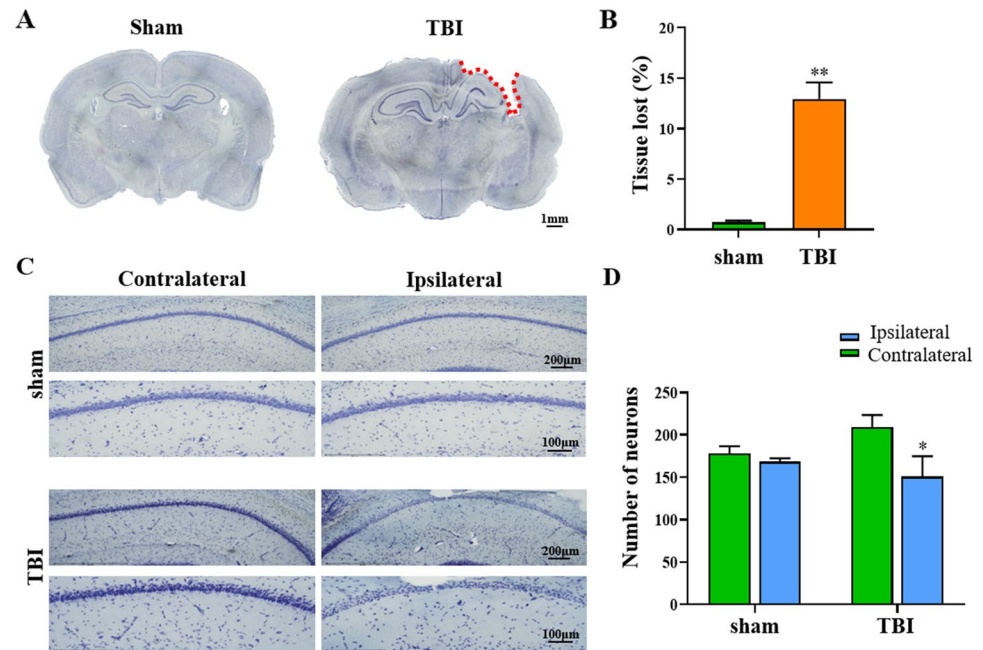
mammalian zinc transporters, which is expressed in reactive zinc-enriched brain regions such as entorhinal cortex, hippocampus and amygdala (as observed previously with Timm staining) [19, 20]. ZnT3 is enriched in mossy fibers that project from hippocampal dentate granule cells to hilar interneurons and CA3 pyramidal neurons [20]. MFS in the CA3 was detected in TBI rats (Fig. 8B), which may create a functional excitatory feedback loop and contribute to epileptogenesis.

Discussion

PTE is a chronic recurrent spontaneous brain dysfunction caused by cellular and molecular alterations of the brain and increased neuronal excitability following brain trauma. In this study, we evaluated changes in behavior, ECoG, and histomorphology in chronic TBI models. High amplitude and frequent SWDs of 7–8 Hz were observed at frontal recording sites, and they were correlated with the degree of injury. SWDs were characterized by abrupt onset and termination, and were often accompanied by behavioral arrest, vibrissa extension, and slight facial twitching. In

Fig. 7 Reactive gliosis in peripheral cortex after TBI. **A-B** Microglia and astrocytes all increased in damaged cortex, the fluorescence intensity of ionized calcium binding adaptor molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP) in the ipsilateral cortex of the TBI group were significantly higher than that of the sham group
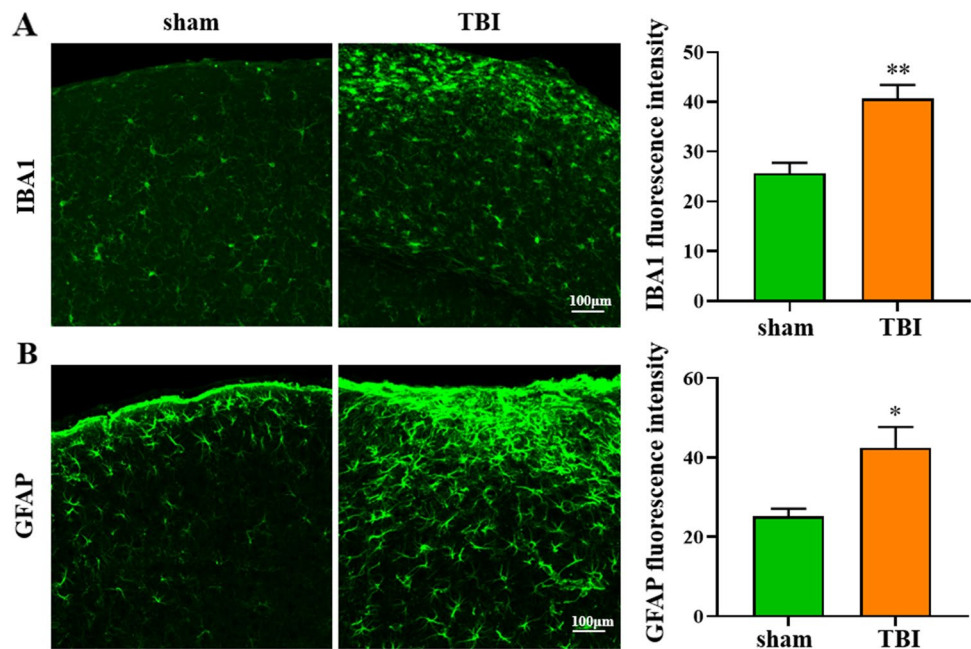
addition, Food and Drug Administration-approved anticonvulsant drug, retigabine, was ineffective in reducing these SWDs. Histopathological results showed significant cortical damage, hippocampal deformation, glial cell proliferation and MFS in chronic moderate TBI model.

SWD after TBI May Reflect ASs

In one study, two distinct types of spontaneous seizures were observed in adult rats months (rather than weeks) after TBI. These types of seizures included convulsive seizures, which are infrequent but last for several minutes, and nonconvulsive seizures, which are frequent but only last for tens of seconds. Nonconvulsive seizures were four times more frequent and two times longer in the TBI rats than in the uninjured rats [21]. D'Ambrosio and Miller [22] observed transient rhythmic electrical activity accompanied by behavioral arrest in rats following lateral fluid percussion injury (FPI), which they considered similar to SWDs and ASs. Our study findings are consistent with SWDs previously observed in Sprague–Dawley rats [12]. The observed SWD characteristics can be summarized as follows: (1) high-voltage rhythmic and frequent SWDs of 7–8 Hz were observed at frontal recording sites, which correlated with the degree of TBI; (2) SWDs were equally prominent in age-matched sham rats, which had a shorter duration and lower frequency, and had similar morphological and behavioral features to the SWDs of the TBI group; (3) most SWD events in both the sham and TBI groups started synchronously; those that were not if not synchronous differed by only several hundreds milliseconds; (4) SWDs were often accompanied by stereotyped behaviors of rats, such as behavioral arrest (interruption of ongoing movement), vibrissa extension, and slight facial twitching, which are similar to the behaviors of ASs; (5) sensory stimuli, such as a click, can interrupt the development of SWDs.

ASs, a common form of genetic epilepsy, can result in impaired attention and emotional and social deficits [23]. ASs are characterized by a short duration (a few seconds) and are usually accompanied by behavioral interruptions and impairment in consciousness [24, 25]. One of the most striking electrographic features of ASs is synchronous 3 Hz SWDs across the cerebral cortex [26]. There has been controversy over whether SWDs at 6–8 Hz in rodents is a pathological or normal rhythm [27]. Some researchers suspect that these events are not a pathological characteristic (lack of postictal suppression) of seizures that are observed clinically in posttraumatic epilepsy or other forms of acquired epilepsy [28, 29]. In addition, these high-voltage rhythmic spikes, which are described as SWDs, are present in uninjured rats, including Sprague–Dawley rats used in previous FPI experiments. Two rat strains, GAERS and WAG/Rij, have been used as animal models for genetic absence epilepsy, which also exhibit spontaneous SWDs at 7–12 Hz, accompanied by behavioral arrest. However, a counterargument is that rodent SWDs resemble human ASs: (1) SWDs recorded in TBI rats occur numerous times per day and last for several seconds with rhythmic spikes in the range of 6–8 Hz, which are similar to ASs; (2) SWDs with an abrupt onset, termination, and lack of postictal suppression are properties that are identical to classical ASs; (3) SWDs can be blocked by anti-absence medications, such as ethosuximide, which strengthen their association with ASs [10]. Although focal onsets may

Fig. 8 Astrocyte proliferation and mossy fiber sprouting (MFS) in the ipsilateral hippocampus after TBI. **A** Compared with the sham group, the immunofluorescence of GFAP increased significantly more in the TBI group. **B** Region-matched hippocampal sections were stained for Zinc transporter 3 (ZnT3), a zinc transporter that is abundant in synaptic vesicles of mossy fibers, to examine MFS. Compared with sham rats, MFS was evident in TBI rats, especially in the CA3 region (white arrow)
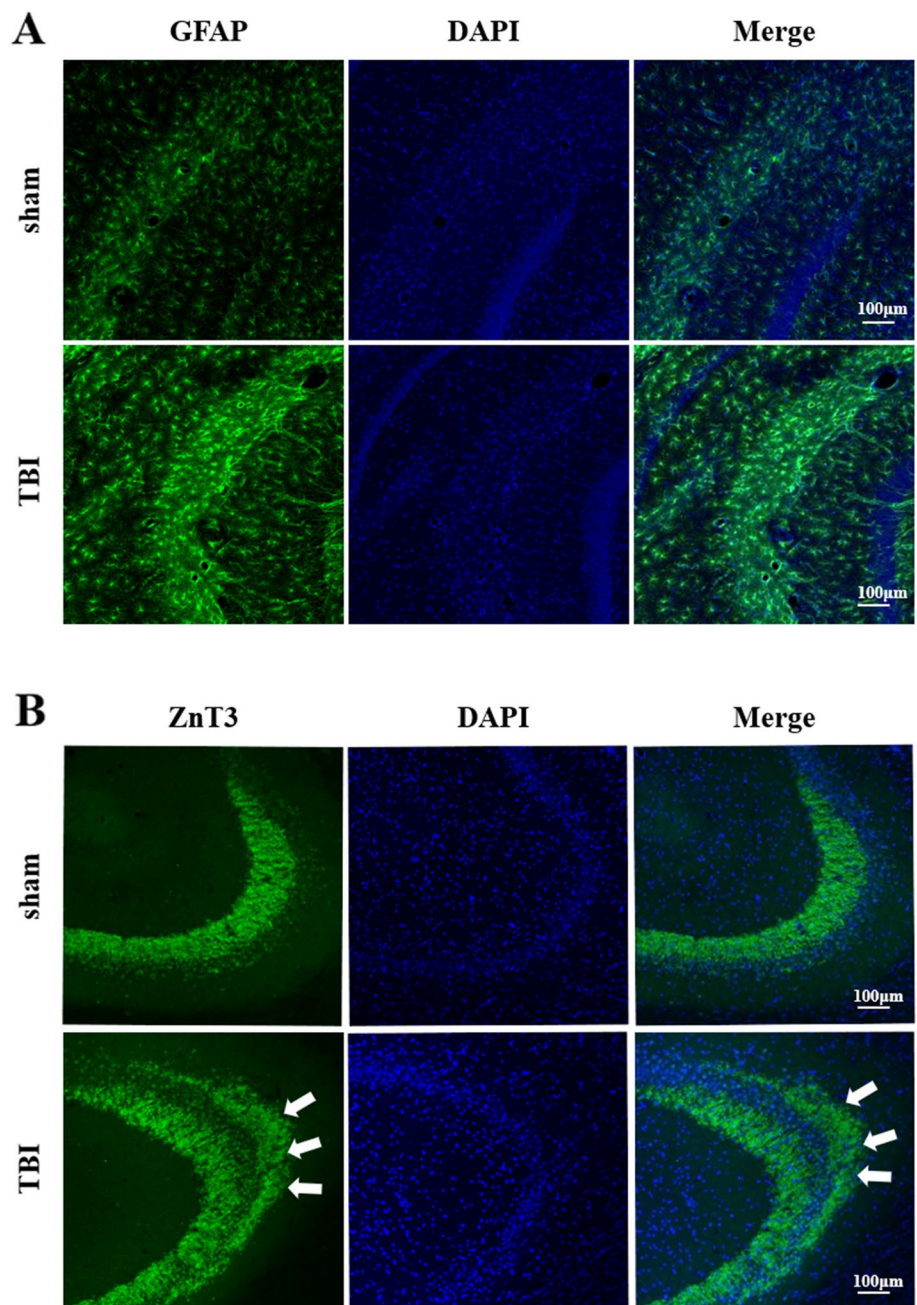
be a key characteristic to distinguish between subconvulsive events after injury and ASs, studies have shown that ASs in rodents start asynchronously; moreover, the 'cortical focus' theory suggests that cortical focus drives the widespread corticothalamic network during ASs [29, 30]. Furthermore, signal analytical techniques have demonstrated that a local cortical onset zone of SWD exists in both rodents and humans, albeit in different locations, and SWDs occur through repetitive cyclic activity of the cortico–thalamo–cortical circuits [31].

Retigabine Increases the Frequency and Duration of SWD Events

Although the mechanisms that give rise to the paroxysmal oscillations of ASs are not fully understood, evidence suggests that synchronous bidirectional cortico–thalamo–cortical circuits are crucial [32]. ASs constitute a specific type of epilepsy that is likely associated with excess gamma-aminobutyric acid (GABA)ergic transmission in the thalamus. Studies have shown that the inhibition and disinhibition

mediated by extrasynaptic GABA$_A$ receptors (eGABA$_A$R) in thalamocortical (TC) neurons play a bidirectional role in the generation of ASs. The anticonvulsant retigabine is a novel antiseizure drug that is used for partial-onset seizures in adults with epilepsy. Its anticonvulsive effects involve Kv7 channels, which are encoded by KCNQ genes, as well as eGABA$_A$R [33]. Retigabine is effective for nearly all types of seizure models except ASs, even though Kv7 channels in the thalamus are highly expressed [17]. Retigabine is a subtype-selective regulator of GABA$_A$R, with a preference for extrasynaptic δ-containing receptors [33]. Although enhanced eGABA$_A$R activity in TC neurons is a typical pathophysiological mechanism in several absence epilepsy models, drugs that activate δ-containing GABA$_A$R can aggravate ASs [34, 35]. This explains our results where retigabine exacerbated seizure expression (reflected by increases in both the duration and number of SWDs). Consistent with these findings, we also found that the frequency and duration of SWD events were significantly increased following retigabine intervention, although SWD morphology did not change. This indicates that using retigabine for patients with absence-like seizures after TBI is not recommended.

Histopathology of the Post-TBI Epileptic Rat Brain

The reason for the high frequency of SWDs in TBI rats is not well understood. However, it is hypothesized that it is related to the severity of brain injury and the degree of midline shift. Although damage following CCI is largely unilateral, moderate or severe injury may cause relative widespread damage of the ipsilateral brain, which includes the medulla, thalamus, and hippocampus. Before spontaneous seizures occur, progressive brain damage occurs over months to years. Magnetic resonance images from 3 h to 60 days after FPI shows that lesions involve the damaged cortex, hippocampus, and thalamus (with hemi-atrophy occurring over time) [36]. Our study found the chronic structural abnormalities in TBI rats, including the cortical tissue loss, hippocampal deformation, and reactive gliosis in the injured cerebral cortex after brain trauma. These findings suggest that persistent progressive structural change is one of the characteristics of epileptic seizures after brain injury.

At the cellular level, TBI leads to neuronal injury [37] and reactive gliosis [38], which are characteristics of the human PTE brain. Variable subpial and subcortical gliosis were observed in ten epileptic patients who underwent focal cortical resection surgery [39]. One study showed that astrocytes begin to increase at approximately 7–10 days after injury, and in the subsequent weeks, months, and even years, astrocytes increase in number and fibrillary appearance, which causes glial scarring in and around the damaged area and eventually promotes seizures [40]. Conversely, long-term chronic epileptic seizures

can also result in astrocyte proliferation involved in hippocampal sclerosis, which affects the normal physiological regulation of the brain and aggravates seizures. Moreover, in addition to gliogenesis and neurogenesis, MFS plays a vital role in promoting epileptogenesis. Mossy fibers are axons emitted by granule cells in the hippocampal DG. Typically, few synapses are established between granule cells in the DG; however, sprouted mossy fibers form an abnormal positive feedback circuit among granule cells, which significantly promotes the spread of seizure-like activity [19, 41]. Consistent with previous studies, we observed intense glial GFAP + immunoreactivity as well as significant MFS in the hippocampus during the chronic period following injury.

Controversial Role of SWDs in TBI Model of SD Rats and Limitations

The spontaneous occurrence of SWDs in normal non-pathological rats complicates the interpretation of nonconvulsive seizures after TBI. Although the current studies of SWDs cannot confirm that TBI causes de novo nonconvulsive seizures, as Sick's study suggests, TBI may deteriorate pre-existing epileptic or hyperexcitable states [42]. For example, it has not been established from previous studies whether TBI causes changes in brain excitability, or leads to conversion of normal brain to epileptic brain that increase SWDs in the brain already expressing these events. However, our data provided the indirect evidence that cortical injury significantly increased the incidence of SWDs, which was worsened by increased injury severity. In addition, the age-dependent expression of SWDs in rats also makes the occurrence of acquired epileptogenesis after TBI difficult to explain, since the time course of this acquired epilepsy is very similar to normal aging. Thus, caution is required when using this model to study acquired epilepsy after TBI.

Astrocytes play a crucial role in neuronal growth, restruction and homeostatic balance. It is well known that TBI causes reactive gliosis, which is primarily involved in boundary formation around areas of tissue damage or inflammation [43]. The upregulation of GFAP in reactive astrocytes is perhaps the best-known hallmark of reactive gliosis after brain trauma [44]. In this study, we described the chronic histopathological alterations in TBI rats, such as reactive gliosis in peripheral cortex. However, one specific limitation was that we were unable to explain the possible causal relationship between SWDs expression and these histopathological changes after TBI. Therefore, given the complicated interaction between astrocytes and neurons, dissecting the cellular and molecular mechanisms and exploring the role of reactive glial cells in the acquired nonconvulsive seizures represents key goals of future research.

Conclusion

The behavioral, electrographic, and structural abnormalities observed in this study may reflect the dynamic functional reorganization of the brain post-TBI that leads to epileptogenesis. High-voltage rhythmic and frequent SWDs of 7–8 Hz were recorded in both sham and TBI rats and were characterized by abrupt onset and termination, variable duration, and lack of postical suppression. These were accompanied by behavioral arrest and slight facial twitching, which are properties that are identical to classical ASs. In addition, the frequency and duration of SWDs increased with the increasing severity of TBI. These measures may serve as predictive markers of trauma severity, advanced post-traumatic pathology, or other disorders. Consistent with previous studies, brain structural changes, such as glial proliferation and MFS, were observed in the cortex and hippocampus during the chronic period following injury, which are characteristics of human PTE. The generation of SWDs depends on the impairment of the corticothalamic circuit, which may be related to excessive GABAergic transmission in the thalamus. The novel antiepileptic drug, retigabine, was not only ineffective for SWDs but also exacerbated SWDs expression (duration and number); thus, its administration requires clinical vigilance.

Author Contributions LS and RL conducted literature review and wrote the initial draft of the manuscript. HY and TYmade preliminary revision. JW and QW made critical revision. All authors contributed to manuscript revision and approved the submitted version.

Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no conflict of interest.

Ethical Approval All procedures were approved by the Animal Care and Use Committee of Capital Medical University (Beijing, China).

Consent to participate Not applicable.

Consent to publish Not applicable.

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