Supplementary Materials

Materials and Methods

Animals and surgery

Male C57BL/6 mice (20–25 g, Grade II) were group-raised with 12-h on/off cyclic lighting; behavior experiments were conducted between 09:00 and 17:00. All experimental procedures were approved and complied with the Zhejiang University Animal Experimentation Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Before surgery, mice were anesthetized with pentobarbital (50 mg/kg) and mounted in a stereotaxic apparatus (Stoelting). Bipolar electrodes made of stainless-steel Teflon-coated wire (791500, A.M. Systems, USA; 0.125 mm in diameter, 0.5 mm between exposed tips) were implanted into the right CA3 subfield of the hippocampus (AP, –2.9 mm; ML, –3.0 mm; DV, –3.0 mm) and the subiculum (AP, –3.3 mm; ML, –2.0 mm; DV, –1.9 mm) [1]. Reference and ground screws were placed in the skull over the cerebellum. Mice were allowed to recover for 7 days before further experiments.

Hippocampal rapid kindling

The hippocampal rapid kindling was performed as in our previous studies [1-3]. First, afterdischarge (AD) threshold of each mouse was determined (monophasic square-wave pulses, 20 Hz, 1 ms/pulse, 40 pulses) with a constant-current stimulator (Nihon Kohden, Japan) and EEGs were recorded with a Neuroscan system

(Compumedics, Melbourne, Australia). The stimulus intensity was started at 40 μA and then increased in 20-μA steps every 1 min. The minimal intensity that produced at least 5-s AD was designated the AD threshold and mice with a value >200 μA were excluded. Subsequently, kindling was performed as follows: 6 suprathreshold stimulations per day, 1 ms square-wave pulses at 400 μA and 20 Hz inter-train frequency for 2 s every 30 min. Mice were then divided into two groups: the LTG group received an i.p. injection of a sub-therapeutic dose of LTG (5 mg/kg) thrice a day along with kindling, the first injection being given 30 min before the first kindling stimulation, the second and third injections immediately after second and fourth kindling stimulations. The vehicle group received the same volume vehicle instead of LTG (Fig. 1). Seizure severity was classified according to the Racine scale [4]: (1) facial movement; (2) head nodding; (3) unilateral forelimb clonus; (4) bilateral forelimb clonus and rearing; and (5) rearing and falling. Mice exhibiting three consecutive stage 5 seizures were regarded as fully kindled. The effect of LTG (15 mg/kg, i.p.) was further tested three times on fully-kindled mice at an interval of three days to avoid drug accumulation.

LFS delivery

LFS was carried out as in our previous reports [5-7]. Kindled mice in the LTG group were further divided into LFS and sham groups based on their AD thresholds. In the LFS group, mice were given a kindling stimulation at 400 μA, after which, LFS (monophasic square-wave pulses, 1 Hz, 0.1 ms/pulse, 300 μA, lasting 15 min) was

delivered to the subiculum through the implanted electrodes. Mice in the sham group were given false LFS. Kindling stimulation and LFS were given twice a day for 5 days with an interval of 3 h. Seizure stage, latency to generalized seizures (GSs), GS duration (GSD), and AD duration were recorded. After 10 LFS sessions, the effect of LTG (15 mg/kg, i.p.) was measured in mice from the LFS group.

EEG analysis

EEG recording and spectral analysis were as in our previous reports [8-10]. Seizure EEG in CA3 was recorded with a Neuroscan system (Compumedics, Melbourne, Australia) with a band-pass filter spanning 200 Hz and a sampling rate of 1000 Hz. Entire seizure EEGs were selected manually and analyzed offline using Scan 4.5. Each seizure EEG was digitally band-pass filtered from 0.3 to 100 Hz, then divided into consecutive 4-s epochs (4096 points) on which a fast Fourier transform (FFT) was run with a Hanning window to avoid edge effects. The FFT output provided the total power for each 4-s epoch, then these frequency bins were averaged in 6 frequency bands: delta, 0.5–4 Hz; theta, 4–8 Hz; alpha, 8–12 Hz; beta, 12–30 Hz; gamma, 30–100 Hz.

The sum of the distances between consecutive data points (coastline index) of each seizure EEG was calculated as in previous reports [11, 12]. An algorithm was used to calculate the sum of the absolute values of the distances from one data point to the next. The calculation was performed in MatLab 9.0 (MathWorks, USA).

Statistics

All data are presented as the mean ± SEM. Statistical comparisons were made with SPSS (ver. 19.0) or GraphPad prism (version 6.0). Student's *t* test was used for two-group comparisons. The χ^2 test was used for effective rate comparisons. The detailed statistics are indicated in the figure legends. $P < 0.05$ was considered a significant difference.

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