A novel kindling model of temporal lobe epilepsy in rhesus monkeys induced by Coriaria lactone

Zhen Hong a,1, Tian-Hua Yang a,1, Ming-Hai Tang b,c, Heng Zhang d, Hong-Xia Li e, Lei Chen a, Qin Chen a, Dong Zhou a,c,

Abbreviations: TLE, Temporal lobe epilepsy; CL, Coriaria lactone; HE, Hematoxylin–eosin.

1 Corresponding author at: Department of Neurology, West China Hospital, Sichuan University, No. 37 Guoxue Road, Chengdu, Sichuan 610041, People’s Republic of China.
2 State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, People’s Republic of China.
3 Department of Life Sciences, Sichuan University, Chengdu, Sichuan 610041, People’s Republic of China.
4 Department of Neurosurgery, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, People’s Republic of China.
5 National Chengdu Center for Safety Evaluation of Traditional Chinese Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, People’s Republic of China.

A novel kindling model of temporal lobe epilepsy in rhesus monkeys subjected to large numbers of kindling stimuli displayed mitochondrial damage and astrocyte morphological alterations in the hippocampus. Thus, this model might be used in future investigations of the mechanisms involved in the epileptogenesis of TLE and in the development of new antiepileptic drugs.

Due to the heterogeneity of the species or the unexpected lesions that remain disparities with epilepsies observed in humans, particularly due to the progressive development of spontaneous recurrent seizures from temporal lobe foci but is also pathologically characterized by specific morphological alterations in the hippocampus. However, the detailed pathogenic mechanisms remain poorly understood, which limits the development of new AEDs. In addition, difficulties in obtaining human samples, particularly those in the initiation or early stages of epilepsy, hinder studies designed to further understand epileptogenesis and pharmacoresistance. Thus, appropriate animal models are required for studies investigating the underlying mechanisms of epileptogenesis and for the development of more effective therapeutic strategies.
are induced in the brain tissue. Models of TLE are most often induced by either chemical substances, e.g., kainic acid and pilocarpine, or electrical methods [5–7], and most of these methods are performed on rodents. Neurons in the hippocampus and in the nearby entorhinal cortex of rodents and primates are similar in many respects; however, there are also differences in their neuronal anatomy and function that are potentially important for TLE [8–10]. This raises questions regarding the suitability of rodents for modeling certain aspects of human TLE. To resolve this issue, nonhuman primate models of TLE must be developed. However, few studies have attempted to develop nonhuman models of TLE. On the other hand, primate models of TLE in rhesus macaques have been produced using stereotaxic injections of an alumina gel into the temporal lobe or electrical stimulation of the amygdala or other brain regions [11–15]. However, the generation of direct mechanical lesions in the brain tissue, which are not evident in the human disorder, could not be avoided in these models [5]. In addition, difficulties in manipulation during the model establishment process and the corresponding low reproducibility significantly decreased the value of these models. Recently, a new nonhuman primate model of TLE was induced using pilocarpine injection (i.p.) in the marmoset; however, it demonstrated a comparatively lower seizure-induction rate (50%) and a higher animal mortality rate (25%) [16]. Thus, the purpose of this study was to establish a new primate animal model of TLE, an animal model of epileptogenesis.

**Coriaria lactone (CL)** consists of the active components of the plant Loranthus that grows on *Coriaria sinica Maxim*, a medicinal herb. The main components include tutin (C₂₈H₁₉O₁₆) and coriamyrtin (C₁₉H₁₂O₁₀), which both belong to a family of naturally occurring picrotoxane compounds (picrotoxinin, picrotin, tutin, and coriamyrtin) [17]. Tutin is a hydroxycoriamyrtin [18] and functions mainly as a GABA antagonist [19]. Different extraction methods result in the contents of tutin and coriamyrtin to likewise differ. In the preparation used in this study, the content of tutin was 50%. When used as a folk remedy for schizophrenia treatment in China, epileptiform seizures, which exhibited features of TLE, were observed in humans [19–21]. This phenomenon suggested that CL could be a convulsive agent in primate animals and that a CL-induced animal model should be more relevant to human disease given that it has been shown to definitively induce epileptiform seizures in humans.

In addition, direct or indirect periodic administration of subconvulsive stimuli to specific brain structures resulted in the development and progressive intensification of elicited motor seizures—this phenomenon has been termed “kindling” [22]. Kindling is the most widely studied model of TLE and is particularly useful for studying epileptogenesis because animals can be studied at particular stages in the kindling process, up to and including the emergence of spontaneous motor seizures (overkindling) [23]. Based on our previous studies of CL in rodents [24–28], we established a novel kindling TLE model in rhesus monkeys using repeated intramuscular injections of CL. This model was easily and efficiently produced (with a lower mortality rate and a higher percentage of animals with spontaneous seizures), and the animals demonstrated the behavioral, electrographic, and anatomical characteristics of human TLE.

### 2. Materials and methods

#### 2.1. Animals

Adult male rhesus monkeys (n = 12; weight: 4–5 kg, obtained from the Experimental Animal Center, Sichuan University, P.R. China) aged 3–4 years were used in this study. The animals were housed in individual cages in a controlled environment (constant temperature, 22–25 °C; humidity, 55 ± 5%; 12-h light/dark cycle with light on at 7 a.m.). The scalp electroencephalogram (EEG) and neurological examinations were performed on all of the animals prior to the experiment to exclude abnormal animals. The animals had free access to standard laboratory food and water and were allowed to adapt to laboratory conditions for at least one week before the beginning of the experiments. In the handling and care of the animals, all possible steps were taken to avoid the animals suffering at any stage of the experiment, and efforts were made to use a minimum number of animals. All of the procedures were approved by the Institutional Animal Care and Use Committee of Sichuan University and Project of Sichuan Animal Experiment Committee, License 045, China. This study was performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

#### 2.2. Drug treatment

*Coriaria* lactone was provided by the Pharmaceutical Company of Sichuan University (CL injection ampoules, 1 ml = 5 mg; lot no. 980715) and was administered through intramuscular injection as previously described [25]. Our preliminary studies confirmed that a CL dose >3.5 mg/kg induced acute stage 5 (Racine scale) seizures in rhesus monkeys. A dose of 1.5 mg/kg was the optimal subthreshold dose for kindling [25]. Thus, the kindling process began with an already-verified subthreshold dose of CL.

Intramuscular injection of subthreshold doses of CL (0.75, 1.50, and 3.00 mg/kg) was used to kindle three groups of monkeys. A total of 12 animals were randomly divided into a CL 0.75-mg/kg group (n = 3), a CL 1.50-mg/kg group (n = 3), a CL 3.00-mg/kg group (n = 3), and a normal saline (NS) control group (n = 3). Different doses of CL and NS injections were administered in the same volume once every 72 h to each animal group until five or more consecutively kindled seizures were elicited in the seizure group (stage 5 seizure according to the Racine seizure severity classification scale) [29]. The injection interval (72 h), which was determined in our preliminary test, was associated with low mortality and higher kindling rates (as shown in Fig. 1).

#### 2.3. Behavioral and electroencephalographic monitoring

Behavioral and electroencephalographic monitoring was performed as previously described [25]. The severity of the behavioral seizures was graded according to Racine [29]: stage 1 — immobility, eye closure, twitching of vibrissae, sniffing, facial clonus; stage 2 — head nodding associated with more severe facial clonus; stage 3 — clonus of one forelimb; stage 4 — rearing, often accompanied by bilateral forelimb clonus; and stage 5 — all of the previous stages plus loss of balance and falling, accompanied by generalized clonic seizures [16,29]. Behavioral changes of each rhesus monkey were continuously monitored by Panasonic WV-CL350 video cameras (Osaka, Japan) and quantified by a technician who was blinded to the treatment conditions of the CL/NS.

After a maximum of 60 injections, all of the experimental animals were divided into the kindled group, nonkindled group, and NS control group. Three monkeys from the kindled group were randomly selected for EEG recording. After sedation with ketamine, the monkeys were transferred from their home cage to a primate chair to allow easy access to the electrical contacts. Electroencephalograms were recorded after the monkeys recovered from sedation. The scalp EEG recording was performed by a certified EEG technician on an EB/Mizzar amplifier (EBNeuro S.p.A., Florence, Italy) with the software of Galileo NT. Scalp electrodes were placed in relation to standard landmarks, and the same measurements were used for each testing to ensure proper alignment. Nine scalp electrodes, including Fp1, Fp2, F3, F4, T3, T4, P3, P4, and Cz, were recorded, with Cz as the referential electrode. Standard filters with a range of 0.5–70 Hz were used as necessary. Sensitivity ranged from 2 to 25 µV/mm. For the EEG, the time in the primate chair was 1–2 h.

#### 2.4. Ultrastructural observation

Three animals from the kindled group were randomly selected for pathological study and sacrificed. The monkeys were anesthetized with i.p. 3% pentobarbitalum natricum. When the animals were in a
state of deep anesthesia, their brains were removed from the skull, and the hippocampal tissues were rapidly dissected and immediately submerged in 4% glutaraldehyde (0.1-M sodium cacodylate buffer, pH 7.2). The hippocampi were processed for electron microscopy as previously reported [30]. Each specimen was trimmed and embedded in Spurr’s medium. Tissue blocks were postfixed with osmium, en bloc stained with uranyl acetate, and poststained with uranyl acetate and lead citrate. Tissue sections were cut to a thickness of 90 nm and viewed on 300-mesh-coated grids by using a JEOL JEM-2100F (Tokyo, Japan) transmission electron microscope. Ten sections from CA1–CA3 of the hippocampus were evaluated from each rhesus monkey, and 3 animals were included in each group.

A comparative evaluation of the electron micrographs was performed in a blinded manner by two different observers who assigned a single score to each of the areas based on the criteria of Kloner et al. [31]. A grading scale of 0–4 depending on the degree of mitochondrial morphologic damage was used with the following definitions: (0) normal mitochondria; (1) early swelling as manifested by separation of cristae and clearing of matrix density; (2) more marked swelling than in grade 1; (3) massive swelling with architectural disruption; and (4) findings in grade 3 plus rupture of inner and outer mitochondrial membranes. The average obtained from the two observers was expressed for each grade as a percentage of the total number of mitochondria counted per sample.

2.5. Hematoxylin–eosin (HE) staining and immunohistochemistry

When the animals were in a state of deep anesthesia, their brains were removed from the skull. The cortical temporal lobe, thalamus, striatum tissues, and hippocampus were rapidly dissected and immediately fixed with 4% paraformaldehyde in 0.1 M at 4 °C for 24 h. The fixed brain tissues were embedded in paraffin, and 5-μm sections were made using a microtome.

Hematoxylin–eosin staining was performed as previously described [32]. Immunoreactivity of glial fibrillary acidic protein (GFAP, a marker of activated astrocytes) was determined using the avidin–biotin–peroxidase method. Briefly, the paraffin-embedded brain sections were deparaffinized with xylene and rehydrated by ethanol at graded concentrations of 100–70% (v/v), followed by washing with water. High-temperature antigen retrieval was performed in 1-mM citrate buffer for 15 min. To block endogenous peroxidase activity, sections were incubated for 30 min in 1% H2O2. After being blocked with 5% (v/v) normal goat serum in PBS for 1 h at 37 °C, sections were incubated with a mouse anti-GFAP monoclonal antibody (Dako, Glostrup, Denmark, 1:500 dilution) at 4 °C for 24 h. Biotinylated antibodies were detected with the avidin–biotin–horseradish peroxidase complex detection kit (Elite ABC Kit, Vector Labs, USA). 3,3’-diaminobenzidine tetrahydrochloride (DAB kit, Vector Labs, USA) was used as the colorimetric substrate. Sections were mounted on gelatin-coated slides, dehydrated with alcohol gradients, cleared with xylene, and coverslipped using DPX. The intensity of GFAP-immunoreactive cells and fibers was subsequently determined using a computerized image analysis system (Olympus SP-1000, Japan) as described previously [33]. Every seventh section spanning the tissue clot was assessed for the intensity of GFAP-immunoreactive astrocytes, and six sections in total were analyzed per animal. All sections were blindly coded and examined.

2.6. Statistical analysis

The statistical analyses were performed using SPSS for Windows (version 13.0). The distribution of animals and the number of mitochondria in each group were analyzed using the Fisher exact probability and Pearson χ2 tests, respectively. Changes in the seizure number, intensity, and duration in kindled monkeys among the groups were compared using the Mann–Whitney U test, including the post hoc analysis of the significance between different animal groups. The intensity of GFAP protein expression in different groups was expressed as a mean ± SEM. A Student’s t-test or one-way analysis of variance (ANOVA) (multigroup) was used to compare the intergroup differences. Repeated measures were performed at least three times in all

Fig. 1. Experimental scheme. CL, Coriaria lactone; valproate; carbamazepine; levetiracetam; zonisamide; NS, normal saline; HE, hematoxylin–eosin.
of the experiments. A two-sided \( p \)-value of <0.05 was considered to be significant.

3. Results

3.1. Establishment of the kindling model

3.1.1. The rate of kindling

A total of 12 monkeys were enrolled in this study, and 3 of the monkeys received normal saline injections; the other monkeys received CL injections. No obvious changes in the clinical phenomena were observed in the 3 monkeys of the NS control group before and during the NS injection. Moreover, the EEG remained normal in animals that received NS injections.

Our preliminary studies confirmed that a CL dose >3.5 mg/kg induced acute stage 5 (Racine scale) seizures in rhesus monkeys. In addition, 1.5 mg/kg was the optimal subthreshold dose concentration for kindling in rats [25]. Therefore, in this study, the kindling process began with a verified subthreshold dose of CL.

Nine monkeys received CL injections (3 animals per 0.75-, 1.50-, and 3.00-mg/kg i.p. injection group). After a maximum of 60 CL injections with a median latency period of 145 days (48 injections, 41–60 injections), 7 monkeys had been kindled with a seizure severity of stage 5. The total kindled rate was 78%. In the 0.75-mg/kg group, the kindled rate was only 33%. However, in both the 1.50- and 3.00-mg/kg groups, the kindle rates were 100%. In all the three CL-treated groups, none of the animals died because of CL toxicity during the nonseizure period, and only one animal from the 3.00-mg/kg group died because of status epilepticus. As shown in Table 1, the minimally effective dose of CL that can produce kindling without substantial lethality is 1.5 mg/kg.

Table 1

<table>
<thead>
<tr>
<th>Dose</th>
<th>Kindled number (%)</th>
<th>Nonkindled number (%)</th>
<th>Death (%)</th>
<th>Median injection times to be kindled (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75 mg/kg</td>
<td>1 (33%)(^a)</td>
<td>2 (66%)</td>
<td>0 (0%)</td>
<td>60</td>
</tr>
<tr>
<td>1.50 mg/kg</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>48 (45–56)</td>
</tr>
<tr>
<td>3.00 mg/kg</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
<td>1 (33%)</td>
<td>43 (41–50)</td>
</tr>
<tr>
<td>Total</td>
<td>7 (78%)</td>
<td>2 (22%)</td>
<td>1 (11%)</td>
<td>48 (41–60)</td>
</tr>
</tbody>
</table>

\(^a\) The intergroup comparison of kindled rates: Fisher exact probability test, \( p < 0.05 \).

Fig. 2. Representative electroencephalogram (EEG) recordings of Coriaria lactone (CL)-kindled monkeys. (A) Normal baseline recording of EEG prior to CL injection. (B) Approximately 7 min after CL injection. The EEG shows a regular appearance of epileptiform monospikes to bursts of polyspikes. The animal shows mastication, blinking accompanied with sound, and “wet dog shakes”. (C) Approximately 10 min after CL injection. The monkey begins to stretch and manifest tonic seizures with generalized high-amplitude seizure patterns on EEG recordings. (D) Eleven minutes after CL injection. The monkey falls down and has a generalized tonic–clonic seizure. The EEG shows high-amplitude seizure patterns mixed with muscular artifact.
3.1.2. Behavioral and electrophysiological manifestations

All of the monkeys showed normal baseline behavior and normal baseline EEG recordings (Fig. 2A). For each CL-kindled animal, clinical signs of seizure activity were observed. In addition, all of the animals exhibited a well-defined pattern of TLE behavior after CL treatment. After approximately 2 min, the animals showed immobility, prostration, and subsequent nausea and vomiting with increased saliva secretion. In the following 5–10 min, the vomiting decreased, and the animals showed mastication and facial clonus accompanied with or without clonus of one forelimb. These phenomena were classified as seizures of stages 1 to 3. During this period, the EEG recordings showed bursts of focal sharp spikes and polyspikes (Fig. 2B and Supplemental figure), initially over the mesial-inferior temporal electrodes and then rapidly over all of the electrodes. The focal sharp waves were similar in morphology to the epileptiform abnormalities observed in human TLE. The fully kindled rhesus monkeys then progressed to episodes of rearing that were often accompanied by bilateral forelimb clonus around 10–15 min after injection of CL. A loss of balance, falling, and generalized tonic–clonic seizures were then observed with continuous high-amplitude discharges of epileptic seizure patterns on the EEG recordings (Figs. 2C, D and Supplemental figure). The generalized tonic phase normally lasted 5–15 s, followed by a gradual slowing clonus. In most cases, such an episode might occur several times with varied durations and intervals in one animal after a single CL injection. After nearly 60–90 min of a motionless groveling condition, the animals gradually went into the normal state with a recovery in the EEG. There was only one animal from the 3.00-mg/kg group which progressed to status epilepticus and died because of it, as mentioned in Section 3.1.1.

3.2. Spontaneous seizures

To assess whether CL-induced seizures in rhesus macaques were followed by spontaneous seizures, we performed continuous video recordings over a period of several weeks. From our preliminary experiments as well as previous reports from the literature [25], seizures induced by one CL injection primarily occurred within the first 2 h after injection. Thus, spontaneous seizures in the present study were defined and quantified when they occurred, excluding those within 2 h after the injection. After a maximum of 112 CL injections with a median latency period of 291 days (average: 97 injections, 89–112 injections), spontaneous seizures were observed in all the 6 kindled animals (see Fig. 1). During

Fig. 3. Representative images of HE staining in the hippocampal hilus. (A) Paraformaldehyde-fixed, paraffin-embedded sections were stained and viewed using light microscopy. The images in the left column (a, c) show a normal structure in the CL-treated epileptic monkey group. The images in the right column show a similar structure in the control animal group (b, d). The images in c and d are an enlargement of the rectangles in a and b, respectively. No significant changes in structure were observed between the two groups. The scale bar in a and b represents 1000 μm. The scale bar in c and d represents 100 μm. (B) Quantitative determination of cell numbers in both of the groups. A total of six sections were analyzed per animal. All of the values are expressed as the mean ± SEM. No significant difference between the two groups (p > 0.05).
the 28 days of video-camera monitoring after the first spontaneous seizure observed, a total of 89 spontaneous seizures from 6 kindled monkeys were recorded that ranged from stages 1 to 3. The average occurrence rate was 0.53 seizures/day/monkey, and most of the seizures (85%) occurred during daytime. The mean duration of the spontaneous seizures recorded in this CL model was 30 s.

3.3. Changes in the hippocampus induced by CL

3.3.1. HE staining
Temporal lobe epilepsy was not only characterized clinically by the progressive development of spontaneous recurrent seizures from temporal lobe foci but was also pathologically characterized by specific morphological alterations in the hippocampus [2]. Hematoxylin–eosin staining was performed to investigate the structural changes after the kindling model was established. There was no significant change between the hippocampal samples from the rhesus monkey model of TLE and those from the control animals (Fig. 3).

3.3.2. Ultrastructural observation
As an independent confirmation of the changes in the hippocampus of each animal, we used electron microscopy to examine the extent of hippocampal damage. In the CL-treated group, the mitochondrial ultrastructures of both neurons and glial cells were severely damaged. This common feature of mitochondrial damage was invariably associated with significant swelling of all mitochondrial spaces and disintegration of the mitochondrial cristae. In the more severe cases, mitochondrial swelling was accompanied by a disruption in membrane integrity. Conversely, we found no evidence of mitochondrial damage in the saline-treated group (Fig. 4A). Statistically significant differences were also found between the saline and CL-treated groups when grading the mitochondrial damage from 0 to 4 (Pearson $\chi^2$, $p < 0.01$) (Fig. 4B).

Fig. 4. Ultrastructural damage in the hippocampus of CL-based kindling TLE rhesus model. (A) Representative electron photomicrographs of mitochondrial ultrastructure in the CA1 region of the monkey hippocampus (magnification 12,000×). Note the intact mitochondrial ultrastructure of the control hippocampus, significant swelling of all mitochondrial spaces, and disintegration of the mitochondrial cristae of the hippocampus in the CL-induced TLE animal. (B) Semiquantitative determination of mitochondrial damage in both of the groups. Ten sections from CA1–CA3 of the hippocampus were evaluated from each rhesus monkey, and 3 animals were included in each group. The average obtained from the two observers was expressed for each grade as a percentage of the total number of mitochondria quantified per sample. Grading: (0) normal mitochondria; (1) early swelling as manifested by separation of cristae and clearing of matrix density; (2) more marked swelling compared to grade 1; (3) massive swelling with architectural disruption; and (4) findings in grade 3 plus rupture of inner and outer mitochondrial membranes. *$p < 0.01$ vs. the vehicle-treated control group.

Fig. 5. Immunohistochemical analysis validates the differential expression of GFAP. (A) Representative images of GFAP protein expression in the stratum oriens of the hippocampus, external granular layer of the cortical temporal lobe (TL), middle section of the thalamus, and striatum of the control and CL-based kindling TLE rhesus model. Paraffin-embedded sections were stained using mouse monoclonal antibody GFAP. All of the tissues were counterstained with hematoxylin and viewed using light microscopy. The images in the left column showed GFAP expression in the normal control group. The images in the right column showed GFAP expression in the CL-based kindling TLE rhesus model group. Note the significant glial responses triggered by CL treatment. The scale bar represents 100 μm. (B) Histograms demonstrating the effect of CL on GFAP protein expression. These results were expressed as the intensity of immunoreactive GFAP. Three animals in each group. Note that CL i.m. injection significantly enhanced the intensity of immunoreactive GFAP in the hippocampus, cortical temporal lobe (TL), and thalamus but not in the striatum, although an increasing trend was observed in the striatum. All of the values are expressed as the mean ± SEM. *$p < 0.01$ vs. the vehicle-treated control group.
3.3.3. CL-induced activation of astrocytes

Glial fibrillary acidic protein, a major component of neurofilament, is a marker of astrocyte activity. Our results showed that GFAP-positive astrocytes exhibited stellate morphology, slim processes, and small cellular bodies in the control group (Fig. 5A). Intramuscular injection of CL significantly enhanced the intensity of reactive astrocytes in the
hippocampus, cortical temporal lobe, and thalamus but not in the striatum, although a trend toward an increase was observed. In the CL-treated TLE groups, reactive astrocytes underwent morphological transformations, including intense GFAP immunolabeling, enlarged cell soma, and many thickened cell processes (Fig. 5A). In addition to the formations, including intense GFAP immunolabeling, enlarged cell tumor, although a trend toward an increase was observed. In the CL-hippocampus, cortical temporal lobe, and thalamus but not in the striatum – a pattern that was similar to the neuropathological changes characteristic of the human disorder.[37,38]. Lastly, a relatively higher seizure-induction rate (100%) and a lower animal mortality rate (thus far, 0%–11.1%) support its use as an animal TLE model. In this study, a dose of 1.50-mg/kg CL is recommended to kindle a rhesus monkey TLE model because of its low animal mortality (0%) and high seizure-induction (100%) rates. Thus, this animal model does not exhibit the limitations of previous animal models (e.g., disparities of species, the unexpected lesion of brain tissue, or the higher mortality and low seizure-induction rates). This kindling-based TLE model largely mimics human TLE. This model might be used in future investigations of the mechanisms involved in TLE and in the development of new AEDs.

Another advantage of the present kindling model is that it is particularly useful for the study of epileptogenesis because the animals can be studied at particular stages in the kindling process up to and including the emergence of spontaneous motor seizures (i.e., overkindling) [23]. Spontaneous seizures develop after many kindling stimulation treatments. Kindled animals in a stage without spontaneous seizures induced are not truly epileptic. Bragin and colleagues hypothesized that kindling accounts for the latent period in patients [39]. Kindling has been induced by electrical or chemical stimulation of the pyriform cortex, amygdala, entorhinal cortex, ventral hippocampus, olfactory bulb, septum, caudate, and anterior neocortex in species such as frogs, mice, gerbils, rats, rabbits, cats, dogs, rhesus monkeys, and baboons [5]. However, this kindling epilepsy model in nonhuman primates by peripheral chemical administration (repeated intramuscular injections) does not require stereotaxic implantation of an electrode or the delivery of a chemical to particular brain loci and, thus, is more easily performed.

In a previous report, an average of 14 stimulations was required for amygdala kindling in rats, 25 in cats, and 196 in rhesus macaques [5]. In the present study, the numbers of stimulations required for both the kindling and spontaneous seizures were fewer than those described in these reports. This disparity is primarily due to the heterogeneity of several parameters, including the species used, the different brain regions that were targeted by the kindling, the different kindling protocols used, etc.

Compared with previous well-established kindling models, for example, the amygdala electrical kindling rat model of TLE [40–43], which are less expensive and have a rich history of experimental data, the advancement of the present model probably includes the following several points: Advancement of species: As mentioned in the Introduction section, the neuronal anatomy and function of the hippocampus and the nearby entorhinal cortex between rodents and primates are different in many aspects, which is potentially important for TLE [8–10]. This raises questions regarding the suitability of rodents for modeling certain aspects of human TLE. 2) Advancement of constructive method: The amygdala electrical kindling rat model needs to stereotaxically implant an electrode in the amygdala, which is not easily performed and will lead to the unexpected lesion of brain tissue. 3) Coriaria lactone has been shown to definitively induce epileptiform seizures in humans, which exhibited features of TLE [19–21]. However, in our study, only surface EEGs were recorded, which is not the optimal approach for localizing suspected seizure onset to deep structures of the mesial temporal lobes. In addition, another significant limitation of the study is the small sample size which is common in primate research. Further research by using larger size of samples is necessary to further verify the reproducibility and efficacy of the model.

In the present study, there was no significant structural change in the CL-based kindling TLE model, suggesting that neuronal death was unlikely required for the early stage of the epileptogenic process. Importantly, the idea that epileptogenesis is independent of neuronal death has been suggested in several genetic epilepsy models in the mature brain (e.g., GAERS, WAG/Rij) [44]. In addition, sparse cell death was also found after kindling in the rat [45,46]. Previous studies have reported a selective degeneration of pyramidal neurons in the CA1 and CA3 regions of the hippocampus in epilepsy, which resulted from a very late stage of epilepsy [47]. This proposal was consistent with our study and other converging studies on epileptogenesis, which propose enduring functional changes manifested by altered programs of gene expression (protein) as the foundation of the epileptogenic process.

Our ultrastructural observations revealed a dysfunction in mitochondria in the CL-based kindling TLE monkey model. In addition, in the CL-treated TLE groups, GFAP, a marker of astrocytic activity, was significantly enhanced, and reactive astrocytes underwent morphological transformations in the hippocampus. These pathologic results were consistent with previous results on human patients with TLE. However, it is still unclear whether the reported mitochondrial dysfunction and glial alterations serve as an initiator or are merely consequences of the pathology and subsequently contribute to the vicious circle during epileptogenic progression. However, our model provides insight into the exploration of this condition in the brain tissue during the early stage of epileptogenesis (before spontaneous seizures are induced).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yebeh.2013.07.028.

Acknowledgments

This work was supported by grants obtained from the National Natural Science Fund of China (no. 30900471), China Postdoctoral Foundation (no. 20090451411) and Sichuan Science and Technology Support Program (no. 2013SZ0003). We are very grateful to Dr. Li Li for technical assistance. We also deeply appreciate the animals that have given their lives for our studies. We are grateful to Prof. Ley Sander and Jing Zhang for their helpful comments.
References


[18] Department of Psychiatry SU. In: Department of Psychiatry, Sichuan Medical University, editor. Clinical observation of Coriaria lactone injection on schizophrenia patients. Chengdu: Sichuan Medical University Publishing House; 1977.


