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EPILEPTOGENESIS AFTER CORTICAL PHOTOTHROMBOTIC BRAIN LESION IN RATS

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Abstract—We investigated epileptogenesis after cortical photothrombotic stroke induced with Rose Bengal dye in adult Sprague–Dawley rats. To detect spontaneous seizures, video-electroencephalograms were recorded at 2, 4, 6, 8, and 10 months for 7–14 days (24 h/day). At the end, spatial and emotional learning and memory were assessed using the Morris water-maze and fear-conditioning test, respectively, and the brains were processed for histologic analysis. Seizures were detected in 18% of rats that received photothrombosis. The average seizure frequency was 0.39 seizures per recording day and mean seizure duration was 117 s. Over 60% of seizures occurred during the dark hours. Rats with photothrombotic lesions were impaired in the water-maze ($P<0.05$) but not in the fear-conditioning test as compared with controls. Histology revealed that lesion depth varied from cortical layers I to VI in photothrombotic rats with epilepsy. Epileptic rats had light mossy fiber sprouting in the inner molecular layer of the dentate gyrus both ipsilateral and contralateral to the lesion. This study extends the current understanding of epileptogenesis and functional impairment after cortical lesions induced by photothrombosis. Our observations support the hypothesis that photothrombotic stroke in rats is a useful animal model for investigating the mechanisms of post-stroke epileptogenesis.

Key words: animal models, brain ischemia, electroencephalography, epilepsy, seizures, stroke.

Brain injury precedes epileptogenesis in approximately half the cases of focal epilepsy in adult humans (Hauser, 1992). In 30% of cases, the preceding insult is cerebrovascular disease (Hauser, 1992). The initial injury is followed by an epileptogenesis phase and eventually by recurrent spontaneous seizures (i.e. epilepsy) in 2% to 14% of ischemic stroke survivors (Kotila and Waltimo, 1992; Burn et al., 1997; Bladin et al., 2000; Dhanuka et al., 2001; Lossius et al., 2002; Lamy et al., 2003). Although the importance of stroke as an etiologic factor for epilepsy is widely acknowledged, relatively few data are available regarding the mechanisms of post-stroke epileptogenesis. This is partly due to the fact that the development of animal models of post-stroke epilepsy is still in its infancy despite the large number of clinically relevant stroke models available (Ginsberg and Busto, 1998).

Recently, it was suggested that combined occlusion of the middle cerebral and common carotid arteries does not induce epilepsy in rats during a 6-month follow-up (Kelly et al., 2006). Similar findings were obtained with the intraluminal filament model of middle cerebral artery occlusion followed by 10 months of video-electroencephalogram (EEG) recordings (Karhunen et al., 2003). Further, the endothelin-1 model of middle cerebral artery occlusion induced late seizures in one of eight rats during 12 months of video-EEG recordings (Karhunen et al., 2006). In contrast to middle cerebral artery occlusion models, a model of small cortical photothrombotic lesions with Rose Bengal dye induces epilepsy in up to half of the animals (Kelly et al., 2001; Kharlamov et al., 2003). These studies show that post-stroke epilepsy can be induced in rats using cortical photothrombosis. There are only few data available, however, regarding the association of epilepsy with brain pathology and behavioral impairments in these animals.

The present study aimed to investigate whether the lesion characteristics or hippocampal pathology differs between epileptic and non-epileptic rats after photothrombotic stroke. Photothrombotic stroke was induced in 40 adult rats in two separate experiments, and the development of epilepsy was followed with video-EEG monitoring for up to 10 months. In addition to epileptogenesis, we assessed sensorimotor performance as well as spatial and emotional learning and memory. Cortical and hippocampal pathology was examined histologically.

EXPERIMENTAL PROCEDURES

As summarized in the study design shown in Fig. 1, data were collected from two separate experiments (experiments 1 and 2). Experiment 1 was a preliminary study aiming at investigating whether photothrombotic stroke triggers epileptogenesis at all. Experiment 2 extended experiment 1, and included also a control group that underwent the same study protocol as the rats with cortical photothrombotic stroke. In addition to video-EEG monitoring, animals included in experiment 2 underwent behavioral testing. At the end of both experiments, rats were perfused for histological analysis.

Animals

A total of 51 male Sprague–Dawley rats (Harlan Netherlands B.V., Horst, the Netherlands) weighing 310–380 g (age 9–12 weeks) at the time of surgery were used for the experiments. Rats were

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Abbreviations: CS, conditioned stimulus; EEG, electroencephalogram; TLE, temporal lobe epilepsy.

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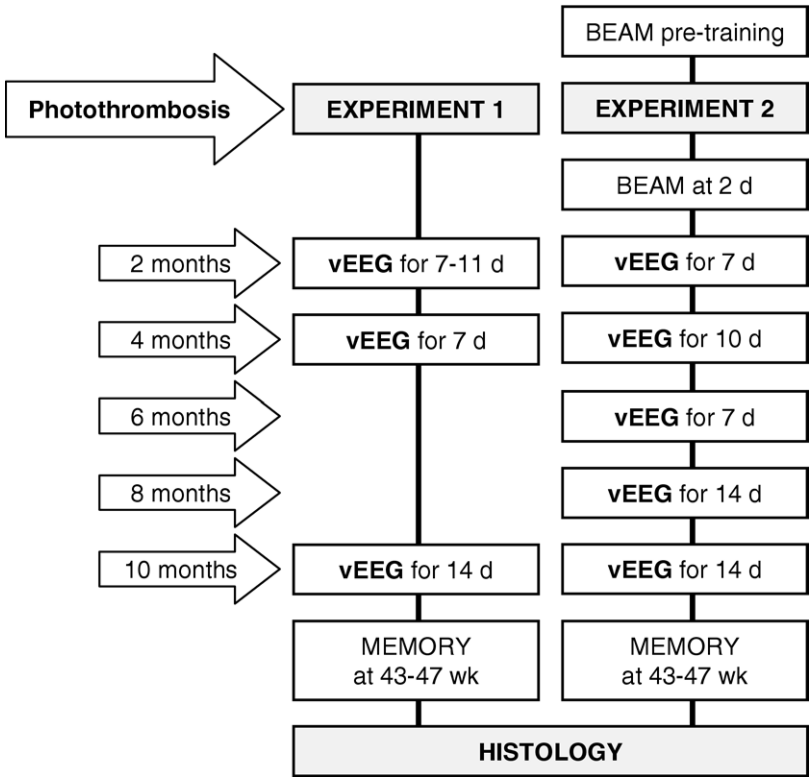

Fig. 1. Study design of two separate experiments showing the timing of video-EEG monitoring, behavioral tests, and histology after cortical photothrombosis. Control rats in experiment 2 underwent similar follow-up protocol as the rats with photothrombotic lesion. Abbreviations: beam, tapered beam walking test; FC, fear-conditioning test; WM, water-maze test; vEEG, video-electroencephalography.

single housed in a controlled environment with free access to pellet food and bottled water (lights on 07:00–19:00 h, temperature 22±1 °C, humidity 50%–60%). All experimental procedures were conducted in accordance with the European Community Council directives 86/609/EEC and were approved by the Committee for the Welfare of Laboratory Animals of the University of Kuopio and the Provincial Government of Kuopio, Finland. All efforts were made to minimize the number of animals used and their suffering.

Surgical procedures

Induction of photothrombotic stroke. Rats were anesthetized with i.p. injection of a mixture (6 ml/kg) of sodium pentobarbital (58 mg/kg), chloral hydrate (60 mg/kg), magnesium sulfate (127.2 mg/kg), propylene glycol (42.8%), and absolute ethanol (11.6%) and placed in a stereotaxic frame (Kopf, Tujunga, CA, USA; incisor bar −3.3 mm according to Paxinos and Watson, 1997).

Cortical brain infarction was induced as described by Watson et al. (1985) and modified by Zhao et al. (2005). Briefly, an incision was made along the midline of the head to expose the skull. Fresh Rose Bengal dye (20 mg/kg; 20 mg/ml; Sigma-Aldrich, Munich, Germany) was injected into the femoral (experiment 1) or saphenous vein (experiment 2) at a rate of 150 μl/min. The center of the light beam (4 mm in diameter; Olympus, Glostrup, Denmark) was focused on 1.8 mm posterior and 2.2 mm lateral to bregma corresponding to the posterior somatosensory and motor areas. During the 10-min photoactivation, the skull surface was cooled with air.

Electrode implantation. Electrodes were implanted immediately after photothrombosis. A custom-made bipolar electrode with nylon-insulated wires (0.127 mm in diameter) was soldered to a gold pin (Franco Corradi, Milano, Italy) and inserted into the ipsilateral ventral hippocampus (6.0 mm posterior, 4.6 mm lateral from bregma, and 7.0 mm ventral from the skull surface). A stainless steel screw electrode (Plastics One Inc., Roanoke, VA, USA) was implanted into the skull over the contralateral cortex homotopic to the lesion (1.8 mm posterior and 2.2 mm lateral from bregma). Two screws located above the cerebellum served as indifferent and ground electrodes (10.3 mm posterior and±2 mm lateral to bregma). The pins of the electrodes were attached to a plastic pedestal (Plastics One Inc.), and dental acrylic was used to secure the headset in place (Selectaplus CN, Dentsply DeTrey GmbH, Dreieich, Germany). A local anesthetic cream (2% lidocaine hydrochloride, Orion, Turku, Finland) was spread over the edges of the incision.

The study included two control groups. In one control group (n=6), surgical operations similar to the induction of photothrombosis were performed (including injection of Rose Bengal dye and electrode implantation), but the light was not turned on and thus, photoactivation leading to ischemia did not occur. The other control group (n=5) had electrodes implanted into the hippocampus and the skull.

Video-EEG recording and analysis

Timing and duration of video-EEG monitoring after photothrombosis are shown in Fig. 1. The preliminary experiment 1 suggested that seizure frequency is relatively low in rats that develop epilepsy after cortical photothrombosis. Therefore, video-EEG monitoring was more frequent in experiment 2, and also, the duration of monitoring session was longer. For the video-EEG monitoring, the rat was placed in a plastic cage where it could move about