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Research Paper

Altered intrinsic functional connectivity in the latent period of epileptogenesis in a temporal lobe epilepsy model

Hyoin Lee, Seungmoon Jung, Peter Lee, Yong Jeong *

Department of Bio and Brain Engineering, KI for Health Science and Technology, KAIST, Daejeon, Republic of Korea

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ABSTRACT

The latent period, a seizure-free phase, is the duration between brain injury and the onset of spontaneous recurrent seizures (SRSs) during epileptogenesis. The latent period is thought to involve several progressive pathophysiological events that lead to the evolution of the chronic epilepsy phase. Hence, it is vital to investigate the changes in the latent period during epileptogenesis in order to better understand temporal lobe epilepsy (TLE), and to achieve early diagnosis and appropriate management of the condition. Accordingly, recent studies with patients with TLE using resting-state functional magnetic resonance imaging (rs-fMRI) have reported that alterations of resting-state functional connectivity (rsFC) during the chronic period are associated with some clinical manifestations, including learning and memory impairments, emotional instability, and social behavior deficits, in addition to repetitive seizure episodes. In contrast, the changes in the intrinsic rsFC during epileptogenesis, particularly during the latent period, remain unclear. In this study, we investigated the alterations in intrinsic rsFC during the latent and chronic periods in a pilocarpine-induced TLE mouse model using intrinsic optical signal imaging (IOSI). This technique can monitor the changes in the local hemoglobin concentration according to neuronal activity and can help investigate large-scale brain intrinsic networks. After seeding on the anatomical regions of interest (ROIs) and calculating the correlation coefficients between each ROI, we established and compared functional correlation matrices and functional connectivity maps during the latent and chronic periods of epilepsy. We found a decrease in the interhemispheric rsFC at the frontal and temporal regions during both the latent and chronic periods. Furthermore, a significant decrease in the interhemispheric rsFC was observed in the somatosensory area during the chronic period. Changes in network configurations during epileptogenesis were examined by graph theoretical network analysis. Interestingly, increase in the power of low frequency oscillations was observed during the latent period. These results suggest that, even if there are no apparent ictal seizure events during the latent period, there are ongoing changes in the rsFC in the epileptic brain. Furthermore, these results suggest that the pathophysiology of epilepsy may be related to widespread altered intrinsic functional connectivity. These findings can help enhance our understanding of epileptogenesis, and accordingly, changes in intrinsic functional connectivity can serve as an early diagnosis.

1. Introduction

Temporal lobe epilepsy (TLE)—the most common drug-resistant type of epilepsy—is caused by abnormal electrical activity in the temporal lobe, which presents as epileptic foci, and is characterized by complex partial seizures and secondary generalization (French et al., 1993). Epilepsy and other brain disorders can be assumed as network disorders that involve not only epileptic foci, but also remotely connected brain regions (Holmes et al., 2013). Recently, researchers have investigated the changes in networks, particularly large-scale networks, by using resting-state functional magnetic resonance imaging (rs-fMRI) to build connectivity maps in the background of various neurological or psychiatric diseases (Rosazza and Minati, 2011). In cases of TLE, significant differences in the resting-state functional connectivity (rsFC) of the default mode network, dorsal attention, and executive network were noted, in comparison to healthy controls (Cataldi et al., 2013; Luo et al., 2012). This widespread involvement may explain the symptoms and signs of patients with TLE, such as loss of consciousness, impairments in learning and memory, emotions; and motor, sensory, or psychiatric symptoms (Kandratavicius et al., 2013; Maccotta et al., 2013), other than seizure episodes. Hence, it is important to monitor the changes in large-scale networks to understand the pathophysiology of TLE.

Some recent studies on rsFC in the mouse brain have monitored large-scale network features using intrinsic optical signal (IOS) imaging (White et al., 2011). This technique helps capture functional connectivity

* Corresponding author at: Laboratory for Cognitive Neuroscience and NeuroImaging, Department of Bio and Brain Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea.
E-mail address: yong@kaist.ac.kr (Y. Jeong).

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with better spatial and temporal resolutions in rodents (Bergonzi et al., 2014). In fact, alterations in the rsFC were observed in mouse models of diseases, such as Alzheimer's disease, stroke, and cortical spreading depression (Bauer et al., 2014; Bero et al., 2012; Li et al., 2012). To understand the progression of TLE, it is essential to observe the changes during the latent period, at which point epileptogenesis develops. At present, we believe that an initial injury, such as status epilepticus (SE), initiates the epileptogenesis during the latent period, after which spontaneous recurrent seizures (SRSs) occur as the disease progresses to the chronic period (French et al., 1993). Further understanding of the progression of epileptogenesis during the latent period would be vital to ensure the early diagnosis and management of this condition. Researchers have used animal models to study the epileptogenic mechanism during the latent period with in vitro and in vivo systems; mossy fiber sprouting, rewiring of synaptic circuits, interneuron loss, and glial cell activation have been found in these models (Covolan and Mello, 2000; Lévesque et al., 2016). Nonetheless, information on the latent phase via in vivo monitoring is still necessary, and a recent in vivo study reported that social behavior deficits and abnormal electroencephalographic (EEG) activity during the latent period were similar to those observed during the chronic period in a TLE mouse model (Seo et al., 2013). This previous study showed that behavioral pathogenesis continues to progress even in cases of no apparent seizure activity (Seo et al., 2013).

In the present study, we hypothesized that there are disruptions in the functional connectivity during the latent period. Hence, we sought to assess the altered intrinsic functional connectivity during the time course of epileptogenesis in a pilocarpine-induced TLE mouse model via IOS imaging.

2. Materials and methods

2.1. Epilepsy model

A pilocarpine-induced TLE model was used in this experiment. Pilocarpine (330 mg/kg; Sigma, St. Louis, MO) was injected intraperitoneally in C57BL/6 mice at the age of 5–7 weeks (Seo et al., 2013). To minimize the peripheral effects of pilocarpine, methyl-scopolamine (1 mg/kg; Sigma) was injected intraperitoneally 30 min prior to the pilocarpine injection. Following the onset of SE (the time point at which prolonged tonic-clonic seizures appeared), diazepam (5 mg/kg) was administered intraperitoneally, approximately after 40–60 min, to terminate the seizure. To reduce mortality and to help restore the normal state, 5% glucose solution was injected intraperitoneally into the mice for 2–3 days, until they could eat food pellets as usual. We divided the animals into three groups: normal control (no pilocarpine injection), latent (no ictal seizure activity at 5–7 days after SE), and chronic (after developing tonic-clonic seizure) groups (Fig. 1) (Seo et al., 2013).

Mice were housed under a 12 h light/dark cycle and had ad libitum access to food and water. All procedures and animal care were approved by the Institutional Animal Care and Use Committee of the Korea Advanced Institute of Science and Technology (KAIST).

2.2. Intrinsic optical signal imaging

For IOS imaging, mice were anesthetized via mask inhalation of 1% isoflurane. All surgeries were performed under head fixation with a stereotaxic frame (Kopf Instruments; Tujunga, CA). Body temperature was maintained at 37 °C, with a feedback controlled heating pad. The scalp was carefully removed with a midline incision to expose the skull. The intact skull was kept moist using mineral oil during the surgery. The area of the exposed skull was approximately 1 cm^2. A customized 3D printed chamber was attached to the skull with dental cement. The chamber was filled with mineral-oil and a round cover glass was secured to its surface.

We used the imaging methods described in previous reports (White et al., 2011). The white LED ring light source was placed approximately 10 cm above the mouse's head. Thereafter, the objective lens was focused at 500 μm under the pial surface to avoid emphasizing the surface vessel signal (Vanzetta, 2006). The field of view was approximately 11.5 × 8.25 mm, and included the entire cortex. The pixel size was approximately 13 × 13 μm. Images were acquired by a CCD camera (Andor Technology, Northern Ireland) at a frame rate of approximately 15 Hz. Using a beam splitter (DualView™, Visitron Systems GmbH, Germany), the reflected light from the skull surface was passed through a 568 ± 10 nm and 610 ± 10 nm filter (Kim and Jeong, 2013). Binning at 3 × 3 on the camera was performed to reduce the output image size from 654 × 900 pixels to 218 × 300 pixels, and reduce the noise and computation overload. Five-minute-long resting-state data sets were collected over 50–60 min.

2.3. Imaging data analysis

The spectroscopic analysis of images was performed as previously described (Bauer et al., 2014; Bero et al., 2012). In brief, the image light intensity was interpreted using the modified Beer-Lambert law, and converted to reflect the changes in hemoglobin concentration. Relative concentration changes in total hemoglobin (HbT) and deoxy-hemoglobin (HbR) were recorded (Fig. 2). The time traces of changes in ΔHbR at each pixel were used for further analysis. Images were smoothed with a Gaussian kernel of 3 × 3 pixels with a 1.5-pixel standard deviation.

For group comparisons, image sequences and the brain mask of each mouse were affine-transformed to a common atlas space determined by three anatomical landmarks, such as the positions of the junction between the coronal suture and sagittal suture, bregma, and lambda (Paxinos and Franklin, 2012). The intersection of every brain mask was calculated and all subsequent comparisons were performed on shared brain areas across all mice.

2.4. Functional connectivity measures

Functional connectivity analysis was performed, as previously described (Bergonzi et al., 2014). Before measuring functional connectivity, data were filtered through a functional connectivity band (0.009–0.08 Hz) by adopting human functional connectivity algorithms with ΔHbR data for analysis, and were resampled from 15 to 1 Hz. A common brain atlas was established for group analysis based on previous studies (Bauer et al., 2014). To remove the global source of variance, global signal regression was performed by averaging the time traces of all brain pixels.

To compare functional connectivity between the control and latent and chronic groups, seed-based connectivity analysis was performed. The averaged time traces of ROI of 0.46 × 0.46 mm (approximately 16 pixels) were extracted. A total of 14 ROIs were placed in the left and right hemisphere using a histological atlas (Paxinos and Franklin, 2012), which included the frontal, somatosensory, motor, cingulate, retrosplenial, temporal, and visual cortex regions. Functional connectivity analysis was performed by correlating the averaged time traces of the ROIs. To investigate the inter- and intra-hemispheric functional connectivity, a 14 × 14 functional connectivity matrix was generated using all ROIs and brain correlation maps, by correlating between the time traces of ROIs and all the other brain pixels via Pearson's correlation. Furthermore, asymmetricity was measured using Pearson's correlation by correlating extracted time traces between left and right in each brain region. This asymmetry r was calculated as follows:

$$r = \frac{\sum (L-\bar{L})(R-\bar{R})}{\sqrt{\sum (L-\bar{L})^2}\sqrt{\sum (R-\bar{R})^2}}$$

where L is defined as averaged time traces in the left brain region and R is defined as averaged time traces in the right brain region. Therefore, the correlation coefficient r ranges from −1 to 1. A value of −1 implies anti-correlation and a value of 1 implies linear positive correlation