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Cyto-, axo- and dendro-architectonic changes of neurons in the limbic system in the mouse pilocarpine model of temporal lobe epilepsy

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Summary While different hypotheses have been proposed to explain the mechanism of onset of temporal lobe epilepsy (TLE), most of them are based on structural, electrophysiological, cellular or molecular changes in one particular area. Extensive neuronal loss, axon reorganization, dendrite and dendritic spine growth make it impossible to apply one hypothesis to explain epileptogenesis for patients or animal models with different pathophysiological changes in the brain. It is therefore hypothesized that cyto-, axo- and dendro-architectonic changes at multiple brain regions may be involved in epileptogenesis in TLE. In the review, structural changes of the limbic system, in particular, hippocampus, entorhinal cortex, subiculum and amygdale, in the mouse pilocarpine model of TLE will be summarized. Their functional significance will be discussed. The final conclusion and future research directions will then be made.

Abbreviations: BDNF, brain-derived neurotrophic factor; CB, calbindin; BLA, basolateral amygdala; ChAT, choline acetyltransferase; CR, calretinin; CTB, cholera toxin B subunit; Dsub, dorsal subiculum; EC, entorhinal cortex; EEG, electroencephalography; fMRI, functional magnetic resonance imaging; GABA, gamma aminobutyric acid; GluR1, glutamate receptor 1; HBDs, hilar basal dendrites; HICAP cells, hilar interneurons with commissural—associational pathway-associated axon terminals; HIPP cells, hilar interneurons with perforant pathway-associated axon terminals; HS, hippocampal sclerosis; IML, inner molecular layer; IPI, initial precipitating injury; KA, kainic acid; LEnt, lateral entorhinal cortex; LSD, lateral septum; MFS, mossy fiber sprouting; MS-nDBB complex, medial septum and the nucleus of diagonal band of Broca; MTLE, mesial temporal lobe epilepsies; MTS, mesial temporal sclerosis; NPY, neuropeptide Y; PET, Positron emission tomography; PHA-L, phaseolus vulgaris leucoagglutinin; PISE, pilocarpine-induced status epilepticus; PRh, perirhinal cortex; PSA-NCAM, polysialylated neural cell adhesion molecule; PV, parvalbumin; SE, status epilepticus; SPECT, Single photon emission computed tomography; SRS, spontaneously recurrent seizures; TLE, temporal lobe epilepsy; vSub, ventral subiculum.

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Introduction

Cyto-architectonic changes of the limbic system, in particular, hippocampus (with pyramidal cell loss in CA1 and CA3 areas) in epilepsy has been reported as early as in 1880 (Sommer, 1880). While it was initially assumed that pyramidal cell loss was the cause of the epilepsy, subsequent studies indicated that cell loss in the hippocampus was due to epilepsy-related hypoxia (Foerster, 1926). Early studies have also suggested that birth or early infancy head trauma, febrile convulsions (Penfield and Jasper, 1954) and status epilepticus (Norman, 1964) are involved in hippocampal neuronal loss and epileptogenesis. However, until now, the mechanism of epileptogenesis after extensive neuronal loss in CA1 and CA3 areas is still not clear. Axo-architectonic changes in limbic system, such as mossy fiber sprouting in the inner molecular layer (IML) of the dentate gyrus have been observed first in the kainic acid animal model (Nadler et al., 1980) and later in patients with temporal lobe epilepsy (TLE) (Sutula et al., 1989), and were proposed to be involved in epileptogenesis. However, subsequent studies indicated that mossy fiber sprouting (MFS) is neither the cause, nor the consequence, of spontaneously recurrent seizures (SRS) in the pilocarpine model (Longo and Mello, 1997). A wealth of studies also indicated that granule cells became progressively less excitable, rather than hyperexcitable, as MFS progresses, and does not initiate spontaneous behavioral seizures. It therefore raised doubts about dentate granule cells as a source of spontaneous seizures in rats subjected to prolonged status epilepticus (SE), and suggested that dentate gyrus neuronal loss and MFS were not primary epileptogenic mechanisms in the animal model (Tuff et al., 1983; Tang and Lee, 2009). We now know that in patients with TLE, neuronal loss occurs not only in the hippocampus, but also in the subiculum (Dam, 1980), entorhinal cortex (Du et al., 1993; Tang et al., 2009), amygdale (Pitkänen et al., 1998) and thalamus (Margerison and Corsellis, 1966). Axo-architectonic changes are not restricted to mossy fibers, but also occur in axons of pyramidal neurons in CA1 and CA3 areas of hippocampus (Lehmann et al., 2001; Ma et al., 2006). It is therefore hypothesized that cyto-, axo- and dendro-architectonic changes at multiple brain regions may be involved in epileptogenesis in TLE. In this paper, I will first summarise our recent research findings in the mouse pilocarpine model of temporal lobe epilepsy before discussing their functional significances with collated by combining with results from many other epilepsy research groups. Finally I will make conclusions and propose further research directions for better understating on the mechanism of epileptogenesis for intractable epilepsy at the neural circuit level.

The main focuses of this paper will be on hippocampus, entorhinal cortex, subiculum and amygdale because they are the apparent sources of many of these spontaneous seizures.

Cyto-architectonic changes of the hippocampus, entorhinal cortex, subiculum and amygdale in the mouse model of temporal lobe epilepsy and their functional significances

The patterns of neuronal loss varied greatly in different animal strains, and also within the same animal strain used in different animal models of epilepsy. In the hippocampus of the mouse pilocarpine model at 2 months after pilocarpine-induced status epilepticus (PISE), we observed two types of neuronal loss: i.e., Type 1, partial neuronal loss in CA3 area in the entire hippocampus, and Type 2, almost complete neuronal loss in CA3 area in the temporal part of the dorsal hippocampus, while partial neuronal loss was observed in CA3 area of the septal part of the dorsal hippocampus and in the ventral hippocampus. In both types of neuronal loss, neuronal loss in the hilus of the dentate gyrus was drastic, and in Type 2, granule cells showed obvious dispersion (Zhang et al., 2009). The number of interneurons marked by calcium-binding proteins such as calbindin (CB), calretinin (CR) and parvalbumin (PV) in CA1-3 areas was also decreased (Tang et al., 2006). Mossy cells in the hilus of the dentate gyrus which were labeled by glutamate receptor 1 (GluR1) and CR had almost disappeared (Tang et al., 2005, 2006).

In the lateral entorhinal cortex (LEnt), significantly loss of the total neurons occurred in layers II—VI with 51% of reduction in layers II—III, and 53% in layers IV—VI in experimental mice at 2 months after PISE (Ma et al., 2008). In layers II—III and layers IV—VI, 49% and 53% of CB immunopositive neurons disappeared, 45% and 53% of CR-immunopositive neurons and 41% and 47% of PV-immunopositive neurons were lost. Furthermore, drastic loss of CR-immunopositive axonal plexuses was observed in layers V—VI (Ma et al., 2008).

In the dorsal subiculum(Dsub), $14.7\% \pm 3.6\%$ of neurons was lost at 2 months after PISE (He et al., 2009). The population of PV and CB immunopositive interneurons was decreased $34.4\% \pm 5.1\%$ and $46.5\% \pm 6.3\%$ respectively, whereas reduction of CR immunopositive neurons was $19.4\% \pm 4.0\%$. The percentage change of PV or CB immunopositive neurons was significantly more than that of NeuN positive neurons (He et al., 2009).

The total neuronal loss in the lateral, basal, and accessory basal nuclei of the amygdale was 34.4%, 28.7% and 43.1% respectively at 2 months after PISE. Of the three subtypes of CB, CR and PV-immunopositive interneurons in the lateral, basal or accessory basal nucleus, 33.5%, 13.5%, or 26.4% of CB, 48.1%, 36.9% or 36.4% of CR, 29.9%, 35.1% or 15.5% of PV-immunopositive neurons disappeared in each nucleus respectively (unpublished data).

Hippocampus

The histopathological classification system for hippocampal cell loss in patients suffering from mesial temporal lobe epilepsies (MTLE) has been proposed in previous study (Blümcke et al., 2007). Five distinct patterns were recognized: (1) hippocampi with no significant difference in neuronal cell densities compared to age-matched autopsy controls (no mesial temporal sclerosis or MTS) (~19%); (2) a classical pattern with severe cell loss in CA1 and moderate neuronal loss in all other subfields excluding CA2 (19%); (3) extensive neuronal cell loss in all hippocampal subfields (53%); (4) severe neuronal loss restricted to sector CA1 (10%, 6%); or (5) severe neuronal loss restricted to the hilar region (7%, 4%). Correlation with clinical data pointed to an early age of initial precipitating injury (IPI