MORPHOLOGICAL ALTERATIONS IN A MURINE MODEL OF FOCAL CORTICAL DYSPLASIA

Prochazkova N. (1), Chvojka J. (1, 2, 3), Buran, P. (4), Heřmanovská, B. (2, 4, 5),
Novak O. (1), Balastik M. (4), Otahal J. (2), Jiruska P. (1, 2)

1 – Department of Physiology, Second Faculty of Medicine, Charles University, Prague, Czech Republic;

2 – Department of Developmental Epileptology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic;

3 – Department of Circuit Theory, Faculty of Electrical Engineering, Czech Technical University, Prague, Czech Republic;

4 – Department of Molecular Neurobiology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic;

5 – Department of Paediatric Neurology, Second Faculty of Medicine, Charles University, Prague, Czech Republic

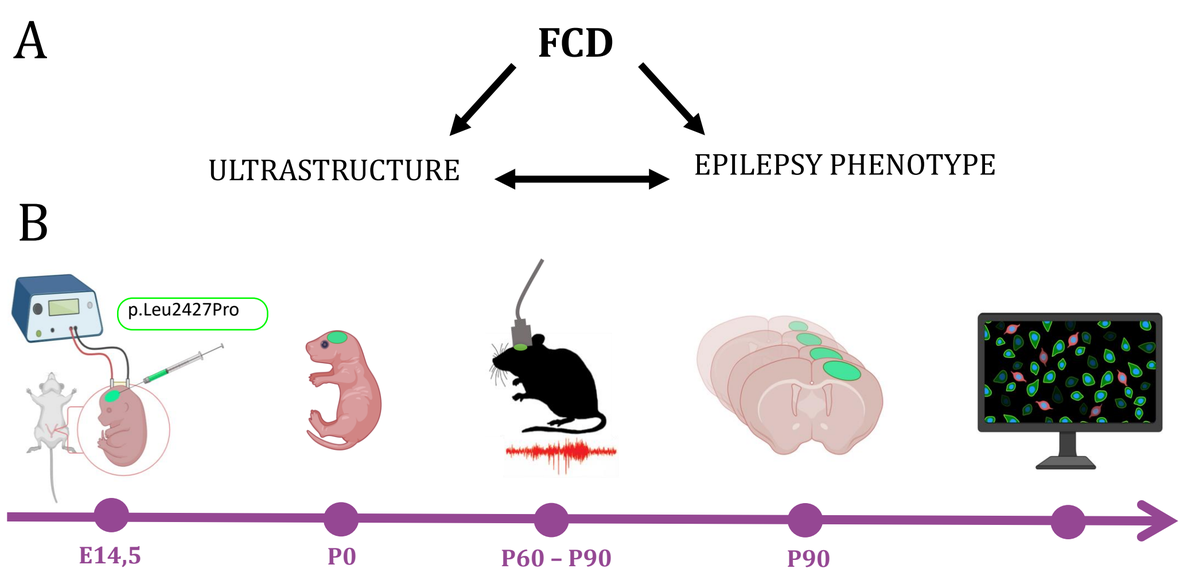
INTRODUCTION

It is estimated that malformations of cortical development cause up to 40% of pharmacoresisant focal epilepsy and focal cortical dysplasia (FCD) is the most common origin. FCD is a highly epileptogenic lesion and approximately 50% of pediatric epilepsy surgery cases are due to FCD, which is characterized by alterations of cytoarchitecture, presence of atypical neuronal types, the dysmorphic neurons. Structural and functional mechanisms, which account for the high epileptogenicity and pharmacoresistency of FCD, are not yet quite elucidated. The goal of this study is to assess detailed morphological analysis, including description of specific neuron type connectivity inside and outside the FCD lesion, and correlate these features to seizure activity of FCD lesion.

METODIKA

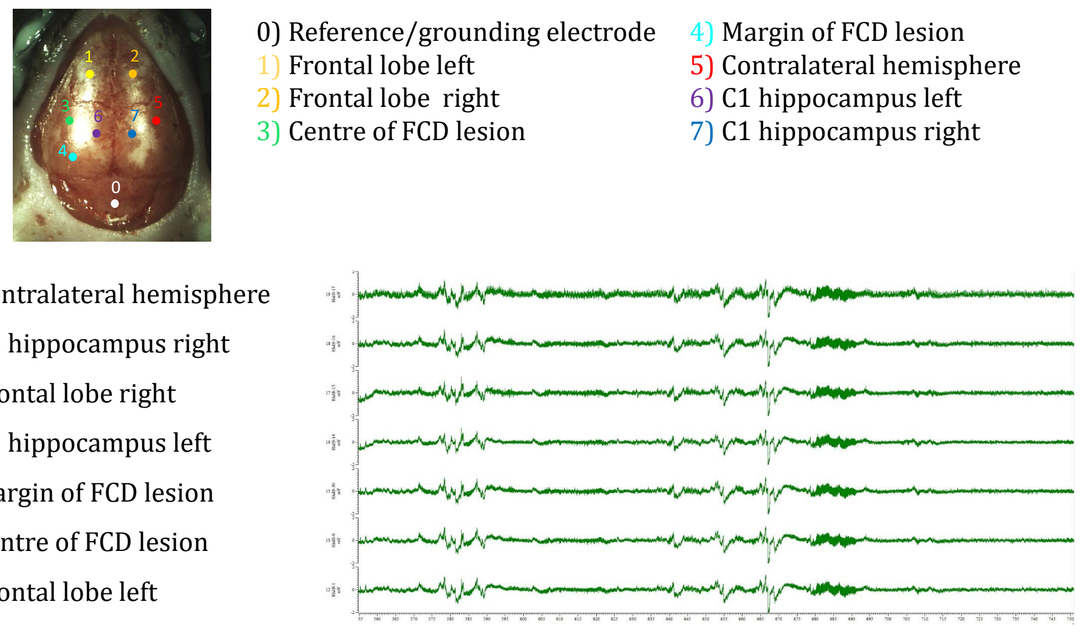
FCD IIb-type lesion is induced by the process of *in utero* electroporation. Mouse embryos at E14 are injected with plasmid carrying human mTOR mutation p.Leu2427Pro-mAmetrine and GFP, and electroporated by delivering five 35V 50ms pulses with 950ms interpulse periods. After birth, successful electroporation is verified with an epifluorescence lamp. Adult mice in the age of 6-8 weeks are implanted with epidural electrodes and chronically video-EEG monitored for 4 weeks. Brains of these mice are collected and slices are prepared to assess the morphology of the lesion using two-photon fluorescent microscopy.

WORKFLOW



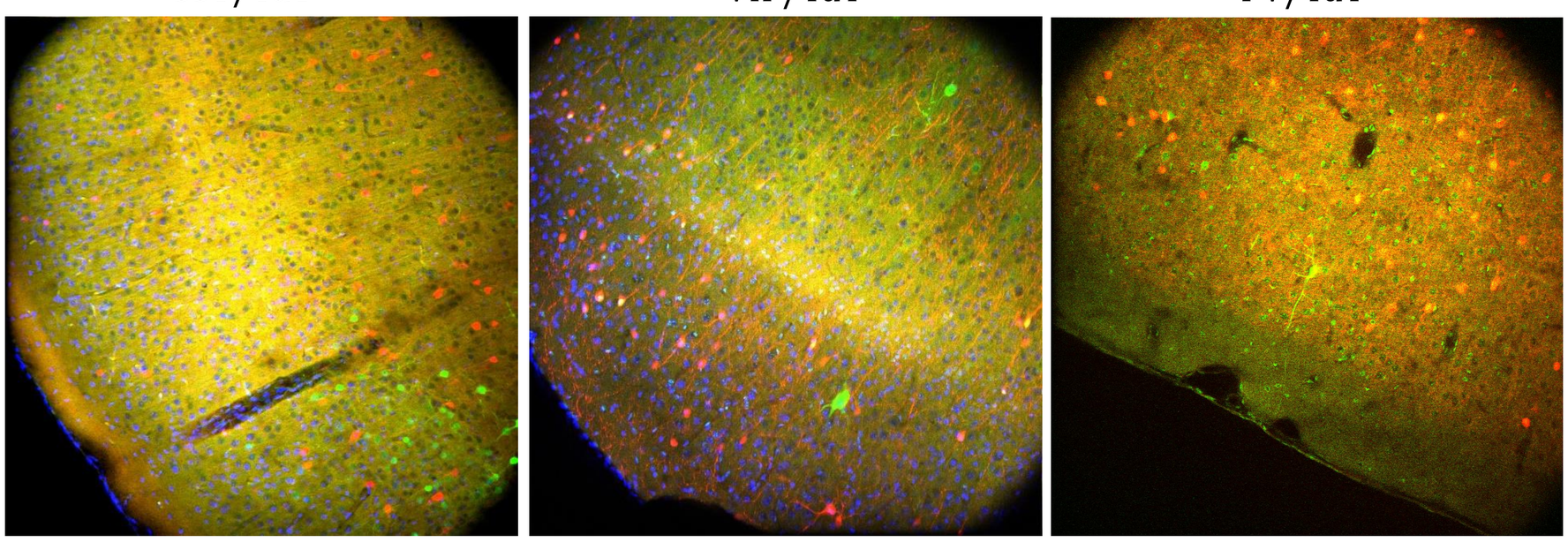
A. Size and extent of FCD lesion does not corresspond to the severity of epileptic phenotype. The goal of the study is to desctribe ultrastructure of the lesion in the context of epilepsy phenotype.
B. Experiment setup.

EPILEPTIFORMNÍ AKTIVITA



A. Chronically monitored EEG channels.
B. EEG monitoring data.

ULTRASTRUCTURE OF FCD LESION



The images of mouse brain slices show neurons electroporated with mTOR mutation and mAmetrine construct (green), cell nuclei (blue) and interneuron subpopulations (red).

The images of FCD lesion acquired with two-photon microscope will enable detailed morphological analysis. This analysis will bring unprecedented data of neuronal connectivity inside and outside FCD lesion, including new insight into the morphology of dysmorphic neurons, projection neurons and interneurons. Analysis will be assessed for dendritic arborization, axonal ramifications, and projections of individual cells. Moreover, the analysis will bring quantification of synaptic terminals on the membrane of principal cells and cell count of individual interneuron types. These new information are to be of great importance for future research of inhibion tone of FCD and future research of a novel therapy approach.

FUNDING

Supported by grants from the Ministry of Health of the Czech Republic (AZV CR17-28427A), Primus/19/med/003 and GAUK 1434720.

Email: ondrej.novak@lfmotol.cuni.cz,
natalie.prochazkova@lfmotol.cuni.cz

ZÁVĚR

Quantitative morphological analysis of FCD lesion in a murine model will bring new information about principal and dysmorphic neuron and also interneuron cytoarchitecture. These data will be correlated to EEG activity and severity of epileptic phenotype. This complex knowledge will enable specific future research of pathology and therapy of FCD.