Characterization of a mice model of human epilepsy with Multi-Electrode Arrays

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We applied microelectrode array (MEA) recordings to study the generation and propagation of epileptform activity in various connected regions of cortico-hippocampal slices obtained from Synapsin I/II/III knockout (TKO) mice and the effects of the synaptic vesicle-targeted antiepileptic drug levetiracetam (LEV). Synapsins (SynI, SynII and SynIII) are synaptic vesicle phosphoproteins playing a role in synaptic transmission and plasticity. TKO mice display an epileptic phenotype and mutation of the *SYN1* gene is associated with epilepsy in man. We found that both interictal (IIC) and ictal (IC) discharges induced by 4AP were more pronounced and widespread in TKO mice, revealing a state of hyperexcitability of TKO networks. To get insight into the frequencies characterizing the IC seizures, we analyzed the average IC power spectral density (PSD) in the 10-50 Hz range in different cortical regions. TKO slices exhibited an increase of power for frequencies above 20Hz with respect to Wild-Type (TWT). To determine whether the hyperexcitability of TKO slices is also reflected by an increased spread of IC discharges and taking advantage of the spatial resolution of the MEA device, we measured the percentage of electrodes recording IC discharges over the total number of cortical electrodes. The spread of excitation was significantly higher in TKO slices than in TWT ones and treatment with LEV decreased the spread of IC discharges in the entorhinal of TKO slices. In order to better characterize the propagation of the IIC events in the hippocampus, we recently coupled MEA recordings with optical imaging using voltage-sensitive dyes by exploiting the possibility of simultaneous recordings with a high spatial and temporal resolution to reveal more detailed patterns of propagation.

1 Introduction

Genetically engineered mouse lines have provided a number of valuable epilepsy models with the potential to link epileptogenesis to changes in both selected genes and neuronal function (Noebels, 2003). The genetic deletion of the Syns in mice is of particular interest in this respect, because it represents the first model of epilepsy based on concomitant alterations of the release of excitatory and inhibitory neurotransmitters and because of the widespread distribution of these SV proteins in the synapses of the central nervous system. TKO provide a unique animal model to investigate cellular and network mechanisms underlying epileptogenesis, because mice are normal at birth but, from the second month of age, they start showing epileptic attacks whose severity increases with age. Moreover a missense mutation of the SYN1 gene was identified in a four generation family whose male members were affected by partial temporal lobe or frontal lobe epilepsy or by various combinations of epilepsy, learning difficulties, macrocephaly and aggressive behavior (Garcia et al., 2004)

2 Materials and Methods

Acute horizontal cortico-hippocampal slices (250 µm thick) were obtained from 3 weeks-old TWT and TKO mice. Slices were transferred over a planar MEA (500-30 TiN internal reference, Multi Channel System® - MCS, Reutlingen, Germany) coated with poly-ornithine (500µg/ml), and fixed by the use of a little platinum anchor. Experiments were performed at 34°. Spontaneous and electrically evoked activity was recorded on brain slices treated with 4-aminopyridine (4AP), a potassium channels blocker, that elicited typical epileptiform waveforms consisting of sporadic long-lasting IC seizures and frequent short-lasting IIC events [4]. IC discharges were principally present in the cortex, while IIC events were recorded from both hippocampus and cortex, sometimes propagating among these regions. Evoked epileptic activity was recorded for 3 sec after biphasic stimulation (amplitude: 1mA, duration: 40 µs for each phase) applied on the hilus. PSD analysis has been performed on the frequency range from 10 to 50Hz and the comparison among slices of different genotypes, treated or not with LEV was made with Kolmogorov-Smirnov test on the integral of the PSD distribution. Further experiments were performed on

hippocampal slices by coupling MEA recordings with voltage sensitive dye optical imaging. The voltage sensitive dye RH 795 (Invitrogen) was used to label cell membranes at a concentration of 0.1mg/ml. IIC events, evoked by stimulating in the hilus, were optically monitored with a CCD camera (Red Shirt imaging).

3 Results

Average power spectral density (PSD) histogram and average PSD integral in the 10-50 Hz frequency band were calculated for IC seizures recorded in 3-weeks-old TWT and TKO cortex (Fig. 1A,B). The PSD analysis revealed that IC events in slices from TKO mice were composed of a higher frequency components with respect to TWT (Fig. 1B). Cortical spread of IC events (Fig.1C), calculated as the mean percentage of electrodes recording IC discharges over the total number of the electrodes covering the specific brain area, was used as an estimation of the IC spreading in the entorhinal cortex of TWT, TWT+LEV, TKO and TKO+LEV mice.

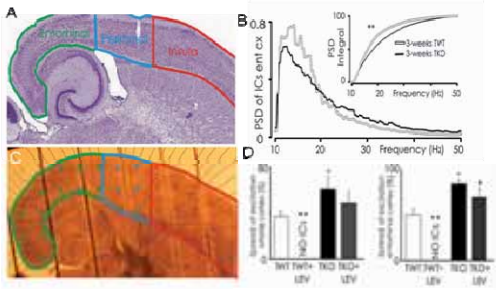

Fig. 1. Anatomical distinction of the regions composing the cortico-hippocampal slices tested in our experiments (A). Analysis of PSD profile (B) and spread of excitation (C, D) of IC seizures

The spread of excitation analysis showed a wider area involved with IC discharges for TKO with respect to TWT in the whole cortex, while LEV succeeded in confining the spread in a area similar to TWT in entorhinal cortex. (Fig. 1D).

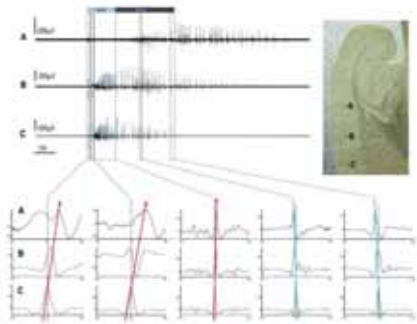

Fig. 2. Propagation of 4-AP induced IC discharges recorded in acute cortico-hippocampal brain slices obtained from 3-weeks-old TKO mice

Fine spatio-temporal monitoring of the propagation of IC events is allowed by MEA recordings. Multi-site recording and cross-correlation algorithms let us measure not only the direction but also the delays of each phase of the IC discharges.

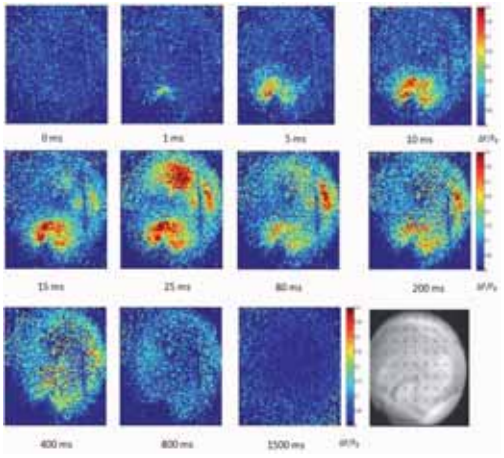

Fig 3. Propagation of evoked epileptic activity in the hippocampus after stimulation in the region of hilus which involves multiple areas. Stimulation on slices treated with 4-AP induces a long lasting depolarization which lasts up to 1 sec.

Moreover, a more precise estimation of the propagation trajectories of IIC events as well as propagation velocities can be evaluated with the voltage sensitive dye optical imaging (Fig.3).

4 Conclusions

MEA recording combined with voltage sensitive dye optical imaging is a very efficient approach to define neurophysiological mechanisms underlying *in vitro* epilepsy in slice preparation. This technique permit to precisely analyse parameters, such as the spread of excitation and cross-correlation among different electrodes, and to address confining and propagation issues on IC discharges.

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