

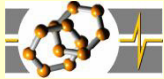
Synchronization of GABAergic Inputs to CA3 Pyramidal Cells Precedes Seizure-Like Event Onset

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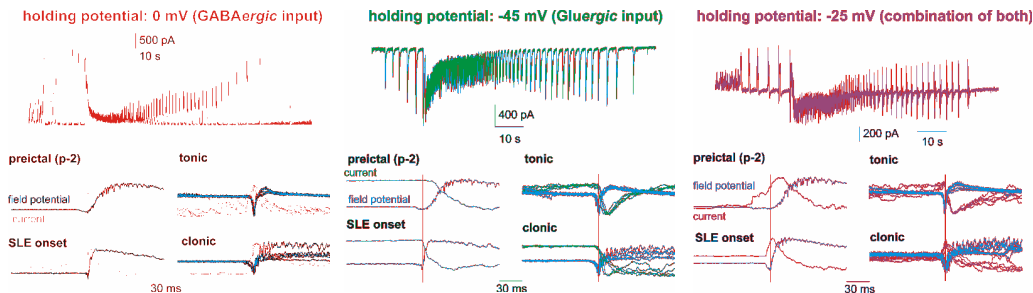


INTRODUCTION

Although the susceptibility to seizures of the juvenile brain is well documented, information on the short-term dynamics of Gluergic and/or GABAergic synaptic drive contributing to ictogenesis is much more scarce. Interestingly, experimental data supported either excitatory (Dzhala et al. 2003; Khazipov et al. 2004) or inhibitory/shunting (Rivera et al. 1999; Tyzio et al. 2007) actions of GABAergic transmission at this age. Juvenile rat (P10-13) hippocampal slices bathed in low-[Mg²⁺] artificial cerebrospinal fluid (ACSF) express preictal discharge trains and confinement of field potential (FP) high-frequency oscillations (HFOs, 400-800 Hz) to the start of discharges (Lasztóczy et al. 2004). Accomplished by a detailed analysis of the temporal relationships between pyramidal cell firing, FP activity and synaptic input sequences during SLEs, the aim of the present study was to dissect GABAergic and Gluergic contributions to SLE dynamics.

2. Voltage clamp recordings:

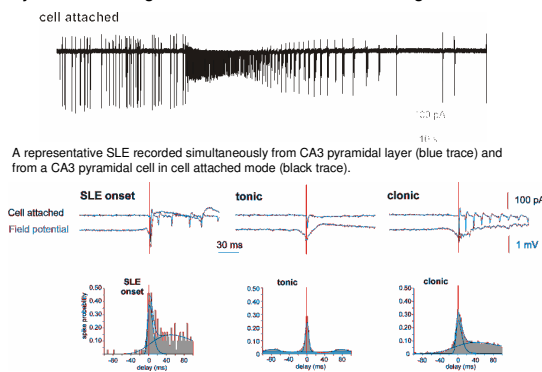
GABAergic currents peaked at the start of FP discharges, while Glutamatergic currents lagged behind



Representative SLEs recorded from a voltage clamped CA3 pyramidal cell. The cell was voltage clamped at 0 mV (red traces), -45 mV (green traces) or -25 mV (purple traces) to record pure, GABAergic (outward), pure glutamatergic (inward) or combined currents, respectively. SLEs were recorded simultaneously from CA3 pyramidal layer (field potential; blue traces).

4. Cell attached recordings:

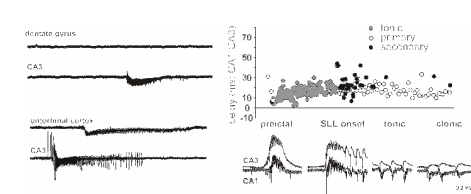
Pyramidal cell firing coincide with the start of FP discharges



FP (blue traces) and cell attached (black traces) recordings of individual discharges time-aligned to the start of FP discharges (vertical red lines).

Spike probability histograms deduced from cell attached recordings for different discharge categories (as indicated). Red lines indicate the time of discharge starts in the FP trace. Black traces are fits and their sums. Spike probability histograms peaked at 0 ms calculated for the SLE onset, tonic and clonic discharges.

7. SLEs are generated within the network of the CA3 region

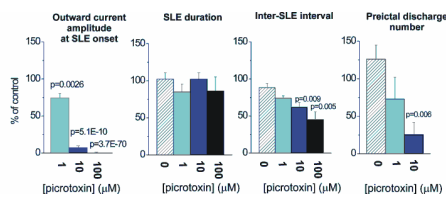


Top: Dual FP recording from the dentate gyrus (upper trace) and CA3 (lower trace). Bottom: Dual FP recording from the entorhinal cortex (upper trace) and CA3 (lower trace).

METHODS

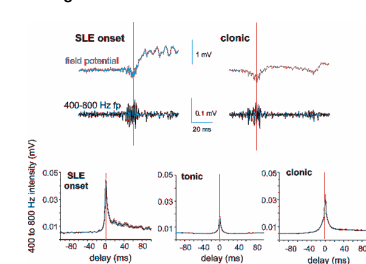
To evoke spontaneous recurrent SLEs, juvenile (P10-13) rat hippocampal slices were bathed in low-[Mg²⁺] ACSF. For voltage clamp measurements, pipettes (4-6 M Ω) were filled with a solution containing (in mM) 130 CsMeSO₄, 10 NaCl, 0.05 CaCl₂, 2 ATP (magnesium salt), 1 EGTA and 10 HEPES 5 mM QX 314. Cells were voltage-clamped around 0 mV, -45 mV or -30 mV to record GABAergic (outward), Gluergic (inward) or combined (outward-inward) synaptic currents, respectively. For current clamp recordings, pipettes (3-5 M Ω) contained (in mM) 135 KCl, 10 NaCl, 0.05 CaCl₂, 2 ATP, 1 EGTA and 10 HEPES. We placed the extracellular electrode at <100 μ m distance from the recorded cell.

5. The effect of GABA is mainly inhibitory on SLE genesis in juvenile rat hippocampal slices



Effect of different concentrations (1 μ M, 10 μ M and 100 μ M) of a GABA_A receptor inhibitor: picrotoxin on the amplitude of outward currents associated with the SLE onset, on duration of SLEs, on inter-SLE intervals and on the number of preictal discharges observed before the SLEs. All values (mean \pm SE) are expressed as the percent of control values, measured before drug application.

8. HFOs at the SLE onset are confined to the start of discharge



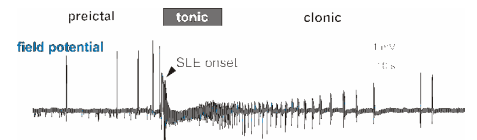
Top: Field potential recording (blue trace) from the CA3 pyramidal layer and field potential band-pass filtered between 400 and 800 Hz. The vertical red lines mark the discharge start.

Bottom: Average intensities of activity in 400-800 Hz band during different discharge categories. The grey shaded areas represent the \pm SE ranges. The vertical red lines mark the point of alignment (discharge start).

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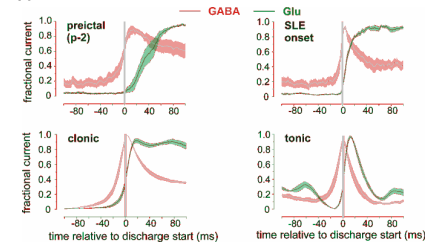
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1. Perfusion of juvenile (P10-13) rat hippocampal slices with low-[Mg²⁺] ACSF resulted in spontaneous SLEs



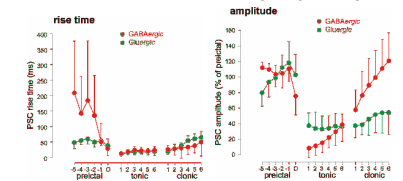
Representative FP recording of an SLE from the CA3 pyramidal layer of a hippocampal slice from a P12 rat. The bar above marks different SLE periods

3. Kinetic comparison: GABAergic input precedes glutamatergic input on CA3 pyramidal neurons



Mean fractional GABAergic (red line) and glutamatergic (green line) current transients associated with discharges of different SLE periods. Red and green shaded areas represent the \pm SE range. The zero bin (from -1.5 to +1.5 ms) is indicated by the grey vertical bar. For the ease of kinetic comparison both outward and inward currents are presented in upward direction.

6. Synchronization of GABAergic inputs: the rise time of outward currents decreases during the preictal period



Mean rise-time and amplitude of GABAergic (red) and glutamatergic (green) current transients associated with discharges of different SLE periods. Amplitudes were normalized to the mean of preictal period. 0= SLE onset.

CONCLUSIONS

- SLEs are generated within the network of the CA3 region and the effect of GABA is mainly inhibitory on SLE genesis in juvenile hippocampal slices evoked by low [Mg²⁺] ACSF.
- Temporal organization of synaptic currents arriving onto a single CA3 pyramidal cell at the SLE onset is characterized by the primary arrival of GABAergic input followed by Gluergic excitation.
- Pyramidal cell firing and the 400-800 Hz field potential HFO at the SLE onset coincides with a strong GABAergic synaptic input to CA3 pyramidal cells.
- Dynamic changes of GABAergic input set the threshold for excitation and regulate precise timing of CA3 pyramidal cell firing, and are thus key players in the extreme susceptibility to seizures of the juvenile hippocampus.

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