Supp_movie 1. ENO movie

Representative movie of the spontaneous activity in a rat somatosensory cortical slice in control conditions one day after birth (P2). Slice was cut with a horizontal orientation. Cells were loaded with Fura2-AM and the slice was imaged using multibeam two-photon excitation with a 20X objective. Acquisition rate was 164msec/frame. Total recording duration was 492 seconds. Recurrent calcium waves (cENOs) repetitively invade the entire network.

Supp_movie 2. GDP movie

Representative movie of the spontaneous activity in a rat somatosensory cortical slice in control conditions at the end of the first postnatal week (P8). Slice was cut with a horizontal orientation and imaged area corresponded to deeper cortical layers. Cells were loaded with Fura2-AM and the slice was imaged using multibeam two-photon excitation with a 20X objective. Acquisition rate was 164 ms/frame. Total recording duration was 492 seconds. Fast calcium transients frequently synchronizing part of the network are clearly visible (cGDPs).

Supp_Fig 1. Tonic activation of NMDA-Rs by ambient glutamate mediates a depolarizing drive on cortical neurons at stages when cENOs are observed.

A.1.Whole cell current-clamp recording from a cortical neuron in a P2 somatosensory horizontal slice. Cell was recorded at resting membrane potential (-55 mV) and displayed cENO-associated membrane potential oscillations (indicated by *). 2. Recordings are displayed below on an expanded timescale. Application of the NMDA-R antagonist (D-APV, 40μM) for about 10 minutes reversibly hyperpolarizes the membrane potential of the recorded neuron and prevents the occurrence of cENOs. B. Bath application of the NMDA-R antagonist D-APV (40 μM) blocks a tonic current in a cortical neuron from a P2 horizontal held at +40 mV in voltage-clamp mode. C. Same as (A) but current-clamp recordings were from a cortical neuron in a P8

somatosensory horizontal slice. Application of the NMDA-R antagonist (D-APV, 40µM) for 10 minutes did not affect the membrane potential of the recorded neuron.

Supp_Fig 2. High potassium conditions change the spatio-temporal dynamics of synchronous neuronal activity.

A. Histograms indicating the fraction of imaged cells detected as being active for each movie frame as a function of time in a P1 somatosensory horizontal slice. The occurrence of cENOs (peaks of synchrony, left histogram) was significantly reduced when the NMDA-R antagonist (D-APV, 40µM) was added to the saline (middle histogram) and network synchronization was restored when potassium (8mM *in fine*) was added to the saline (right histogram). Note that the dynamics of these synchronizations was different from control ones. B. Same type of histograms as in (A) but in P8 somatosensory horizontal slices, showing that high potassium conditions altered the dynamics of synchronous activity events (cGDPs in control, left), after their blockade with the $GABA_A$-R antagonist gabazine (10 µM, middle histograms). Dynamics could be different in two ways: 1. similar amplitude but high frequency synchronous events were produced in 8mM extracellular potassium conditions (right) as compared with control (left) or 2. hypersynchronous, low frequency events were produced in elevated extracellular potassium conditions (right) as compared to control.

Supp_Fig 3. The enzymatic glutamate scavenger selectively affects extracellular glutamate without altering NMDA-R-mediated synaptic transmission.

A. Representative traces of voltage clamp recordings at +40mV from a cortical neuron in a P2 cortical slice showing that GPT (5U/ml together with pyruvate 2mM) does not significantly affect the synaptic current evoked by electrical stimulation (arrows). Stimulation was performed once every 30 seconds in the presence of gabazine (10µM) to isolate glutamatergic currents. B.1. Histogram showing the averaged baseline current as a function of time during the experiment

illustrated in (A). Note that a significant outward current could be observed in the presence of the glutamate scavenger. 2. Bar histograms showing the averaged amplitude of the evoked EPSC at +40 mV in control and in the presence of GPT. No significant difference was measured ($p>0.5$).