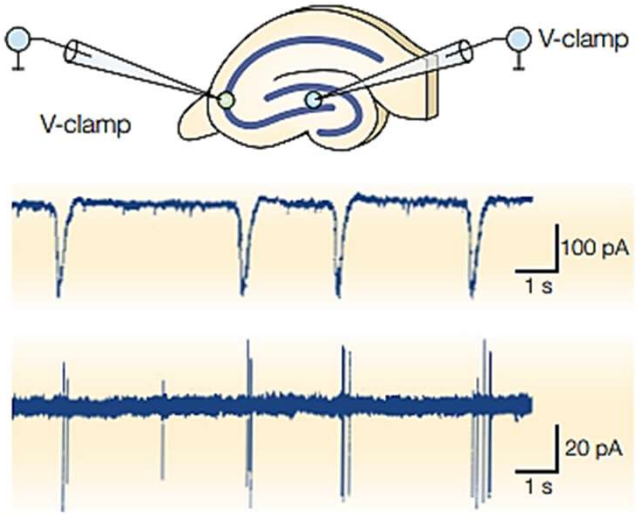
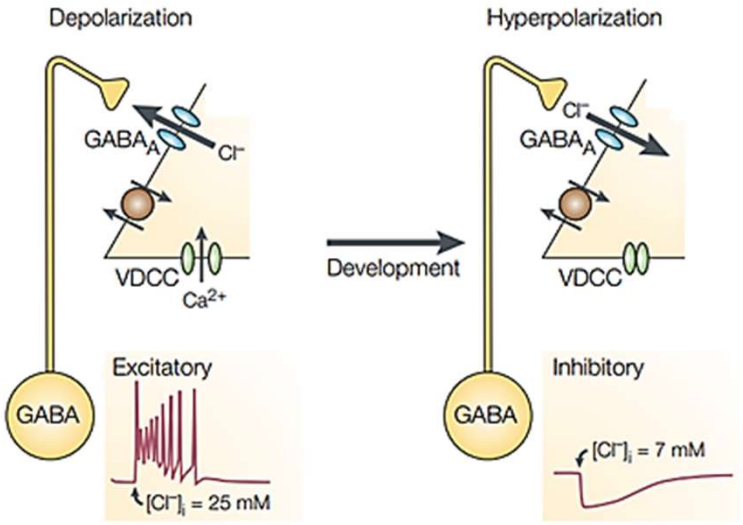
Master in Neuroscience

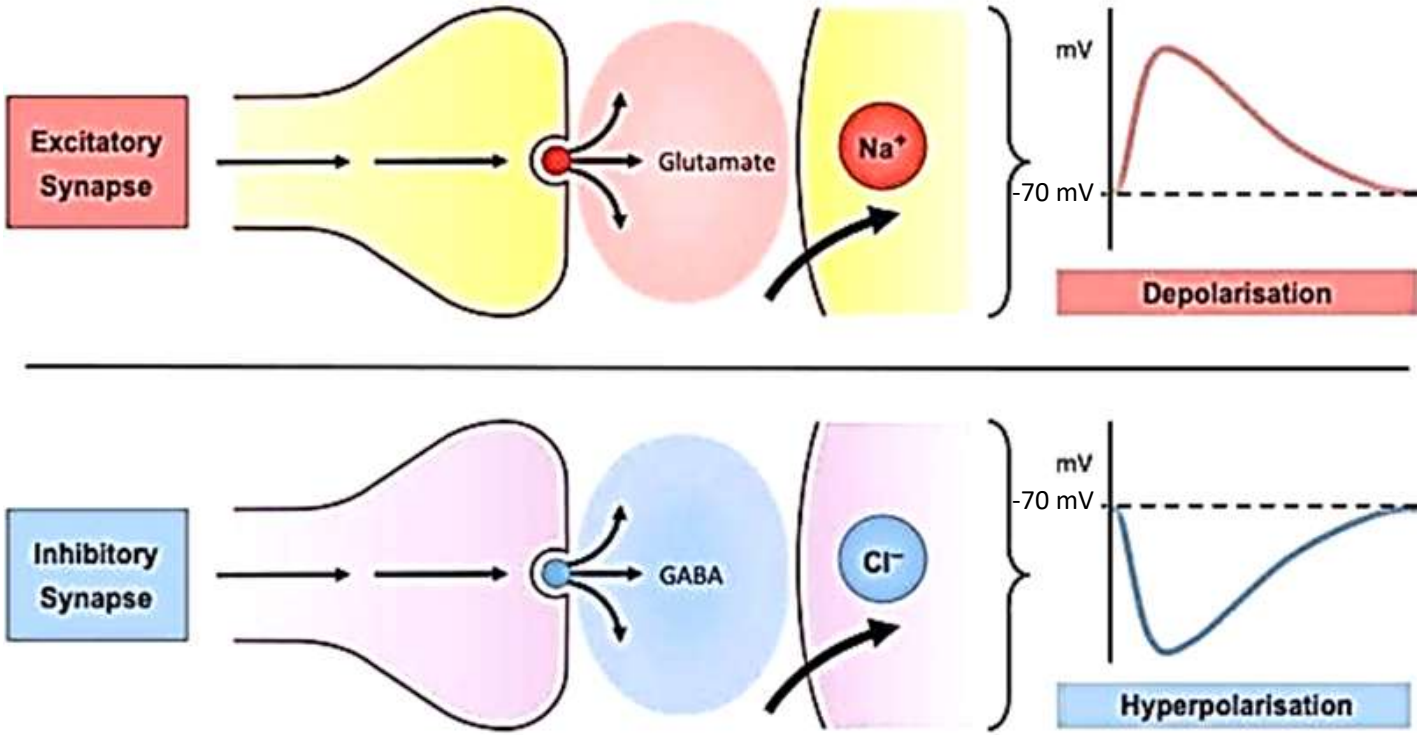
# *MOLECULAR NEUROPHYSIOLOGY -lesson 2-*




Prof. G. Cellot



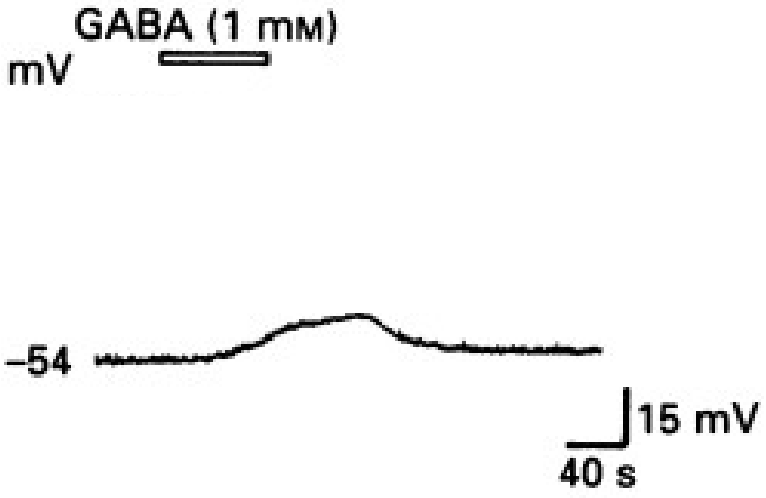
# How do synapses form and consolidate during early development?




*Excitatory and inhibitory synapses in the adult brain*

# During early postnatal development GABA exerts a depolarizing action

Intracellular recordings- CA3 pyramidal cells




Ben Ari et al., J Physiol, 1989

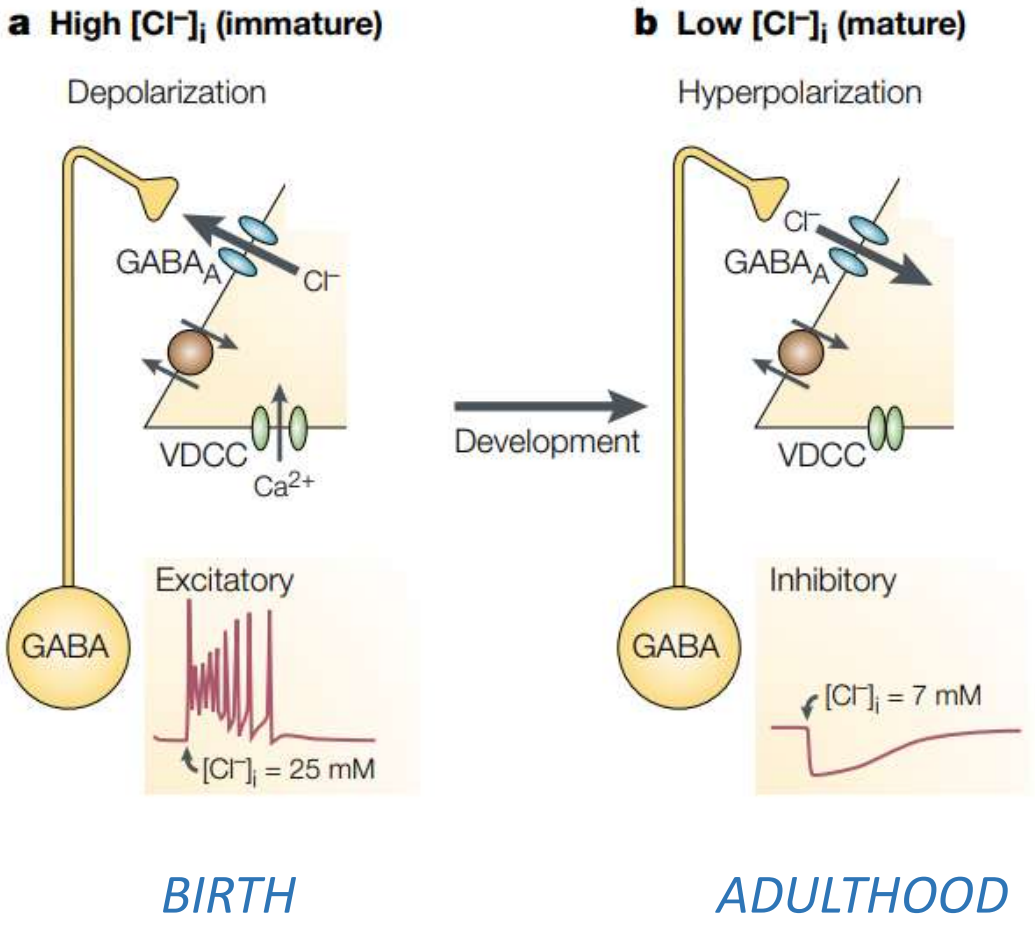
Acute hippocampal slices
(birth-two postnatal weeks)

- ✓ Found in a wide range of brain structures
- ✓ Also glycine has a depolarizing action in early development
- ✓ Conserved among species

**GABA depolarization might have a fundamental role in the maturation of neuronal circuits**

# During development, GABA depolarizing action relies on its high intracellular concentration

At the resting membrane potential….




*BIRTH* *ADULTHOOD*

Modified from Ben-Ari, Nature, 2002

The direction and magnitude of ion diffusion is determined by its **electro-chemical equilibrium**:

1. the concentration gradient of the ion
2. The value of membrane potential

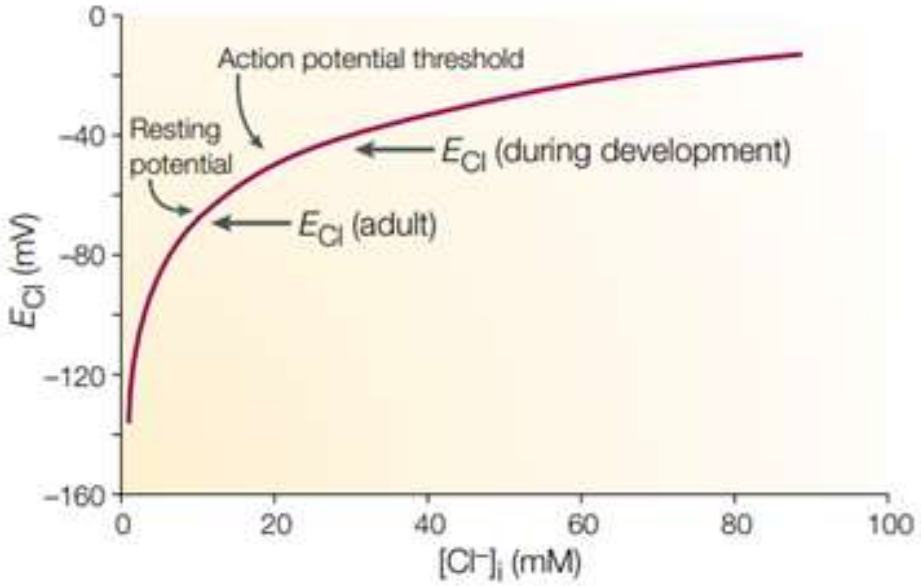
| | Immature brain | Mature brain |
|---|---|---|
| Extracellular Chloride concentration ($[Cl^-]o$) | 140 mM | 140 mM |
| Intracellular Chloride concentration ($[Cl^-]i$) | 25 - 40 mM | 7 mM |

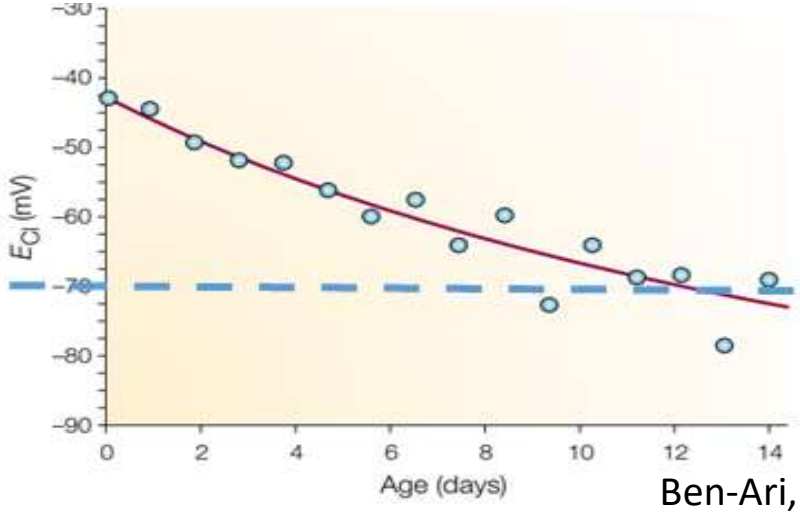
# GABA depolarizing action relies on its high intracellular concentration during development

The **Nernst equation** defines the electrochemical equilibrium between a concentration gradient and an electrical potential, for one particular ion.

$$E_{Cl} = \frac{RT}{(-1)F} \ln \frac{[Cl^-]_o}{[Cl^-]_i}$$

$$\frac{RT}{F} \approx 0.027 \frac{Joule}{Coulomb} = 0.027 \frac{Coulomb \cdot Volt}{Coulomb} = 27\,mV$$



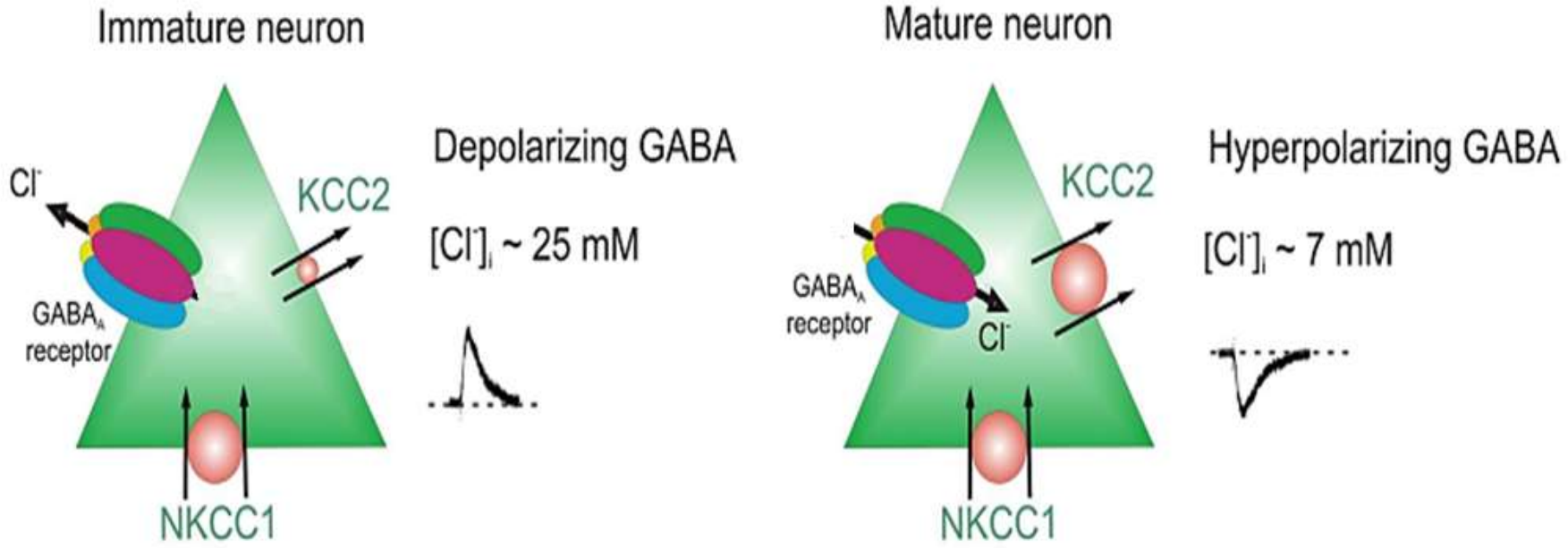




Resting membrane potential

Ben-Ari, Nature, 2002

# Intracellular chloride concentration is controlled by two developmentally regulated cation chloride co-transporters

- $Na^+–K^+–2Cl^–$ co-transporter type 1 (NKCC1): intrudes $Cl^–$ and raises $[Cl^–]i$
- $K^+–Cl^–$ co-transporters type 2 (KCC2): extrudes $Cl^–$ and lowers $[Cl^–]i$



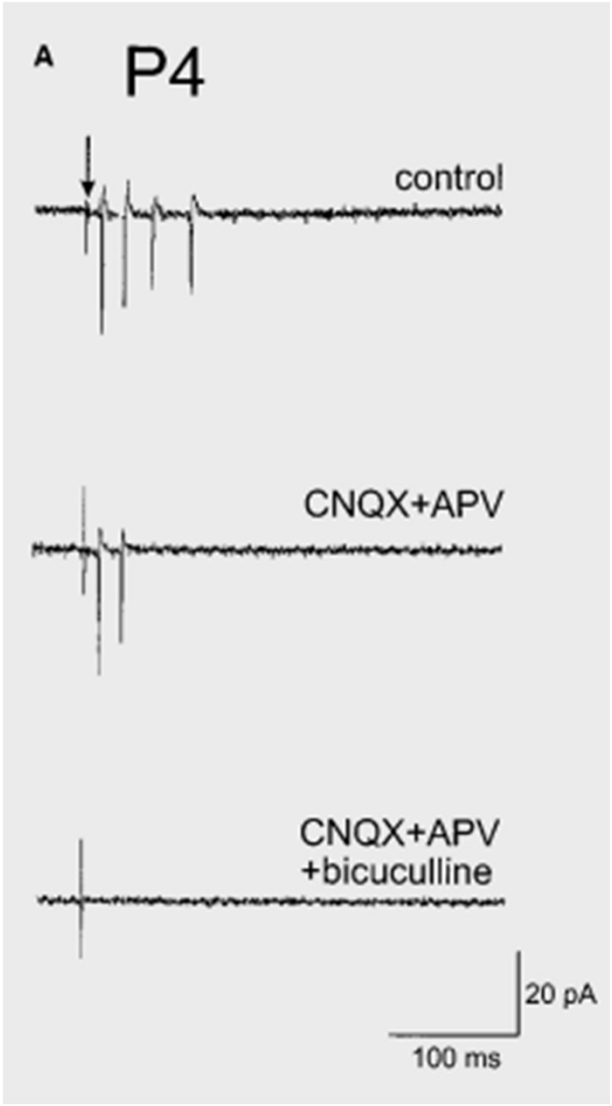

**KCC2 expression increases during development, leading to decrease in $[Cl^–]i$**

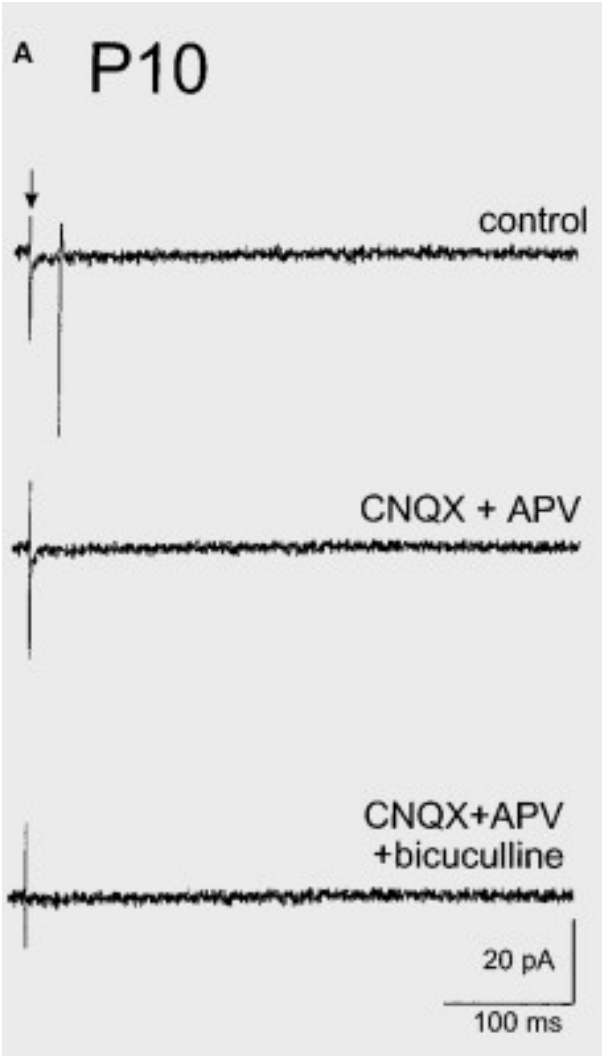
Cellot and Cherubini, Frontiers in Pediatrics, 2014

Electrical stimulation

Acute hippocampal slices, CA3 pyramidal cells
One stimulating electrode, one recording electrode

At early developmental stage, GABA depolarizing action results in excitatory neuronal activity



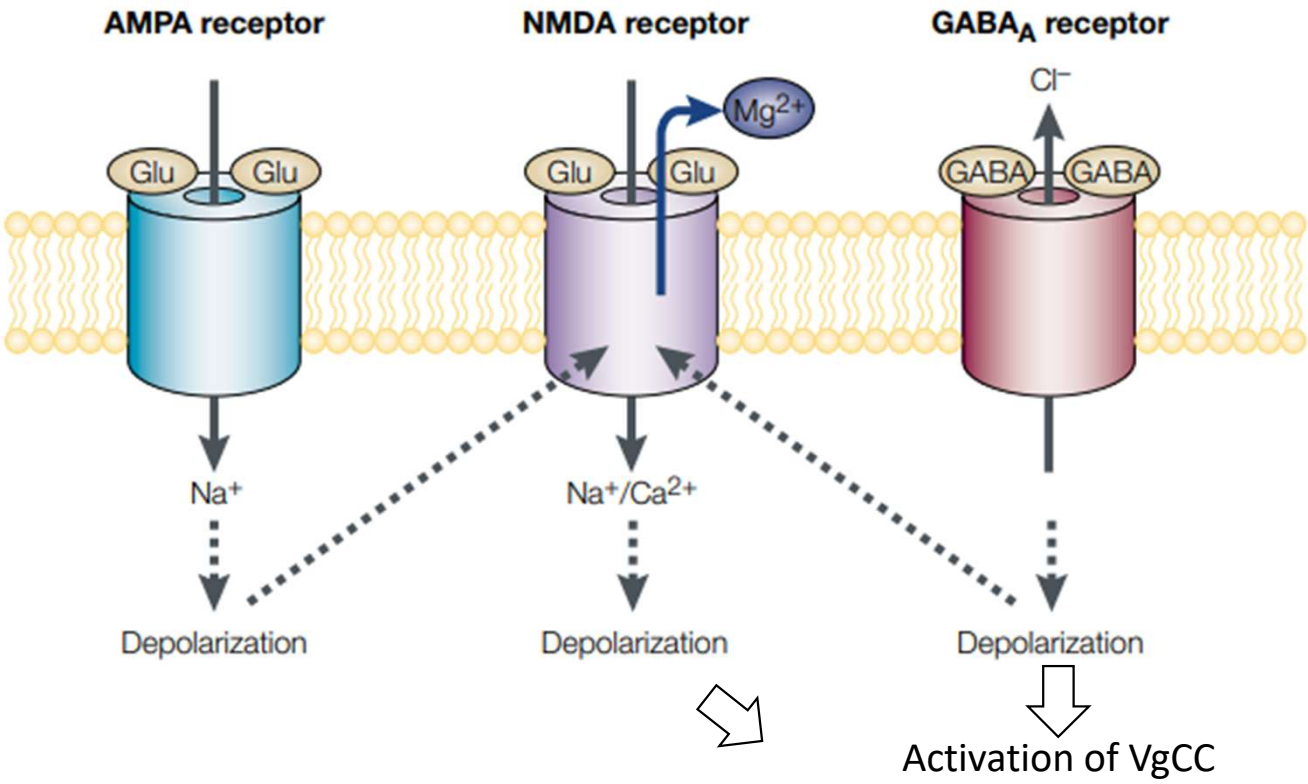

Khalilov et al., Dev Neurosci, 1999

# Synergistic actions of GABA, NMDA and AMPA receptors in developing neurons

At early developmental stage, GABAergic excitatory action is sufficient to activate voltage gated calcium channels (VgCC) and, in synergy with AMPA receptors, to remove $Mg^{2+}$ inhibition from NMDA receptors

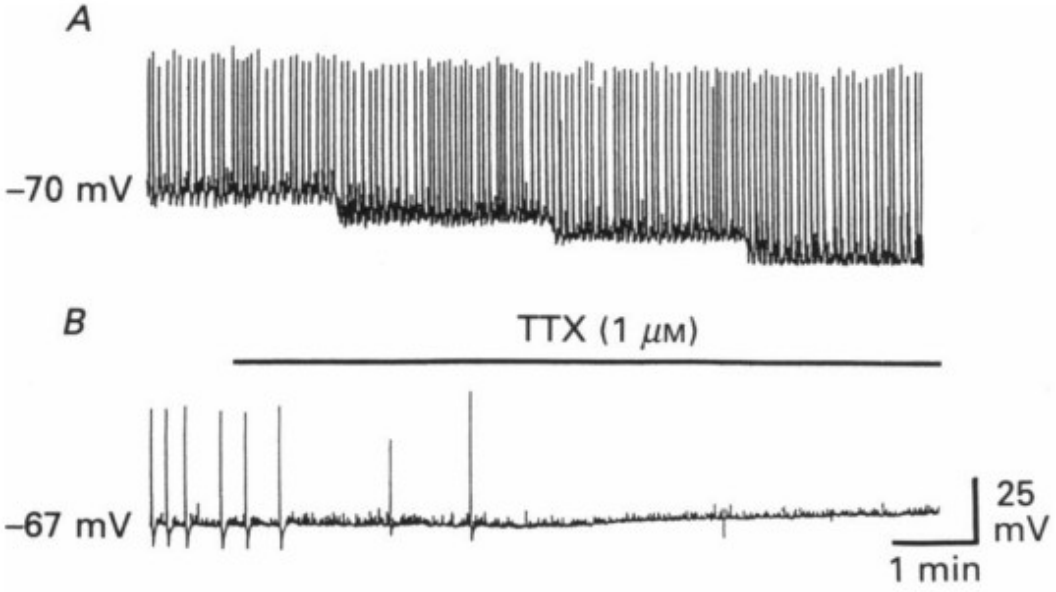
Recurrent and synchronized network activity, responsible for prolonged calcium transients

***GIANT DEPOLARIZING POTENTIALS (GDPs)***

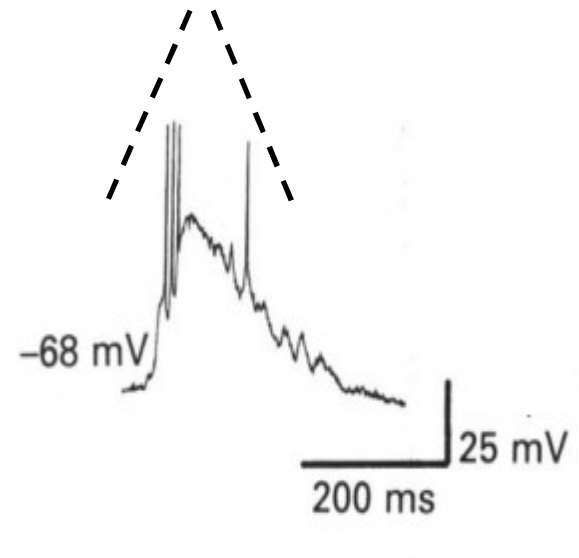


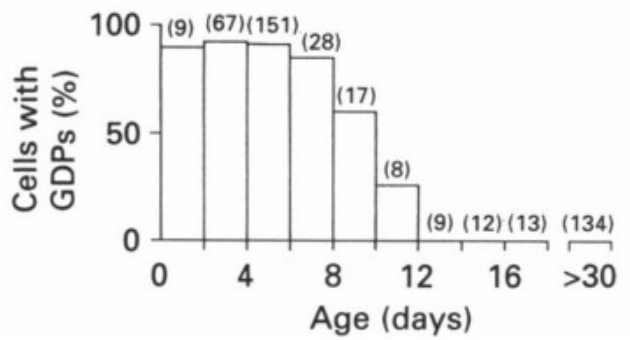

Ben-Ari, Nature, 2002

# GIANT DEPOLARIZING POTENTIALS (GDPs): properties



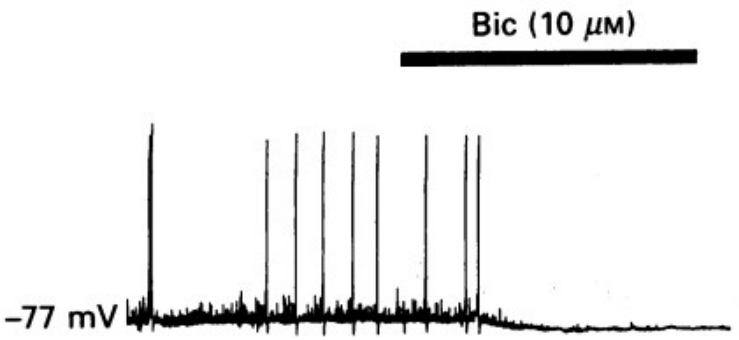

- Independent on the membrane potential
- Synaptic activity driven
- Age dependent (as variation in $[Cl^-]i$ )
- Inhibited by $GABA_A$ r antagonists, but not from glutamatergic blockers.



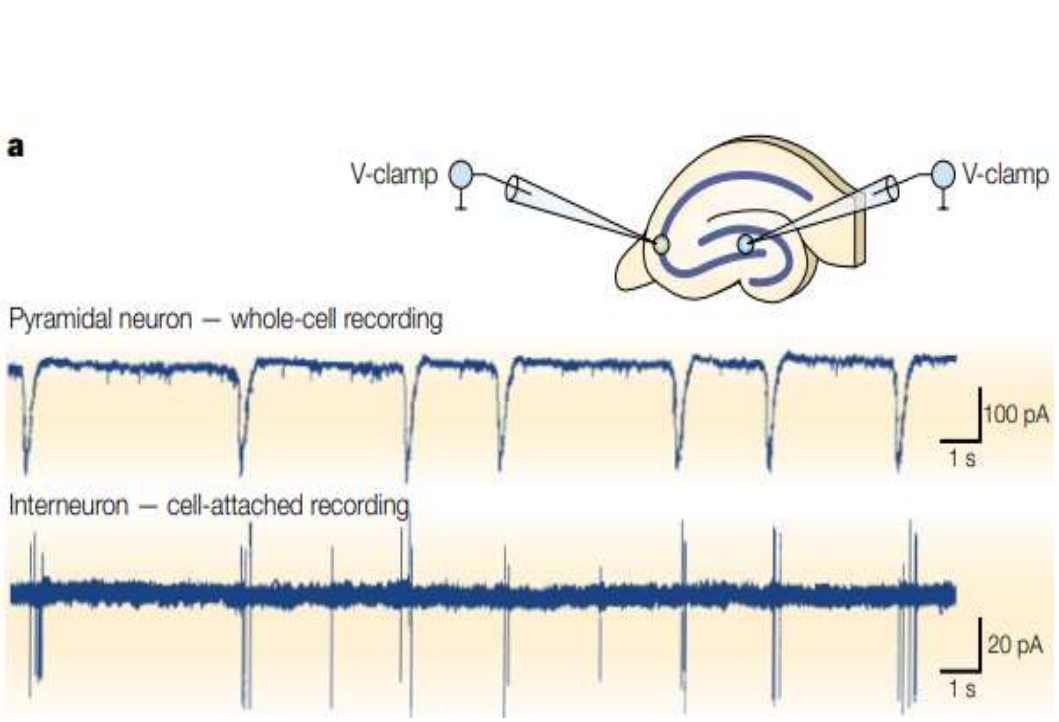

Ben Ari et al., J Physiol, 1989

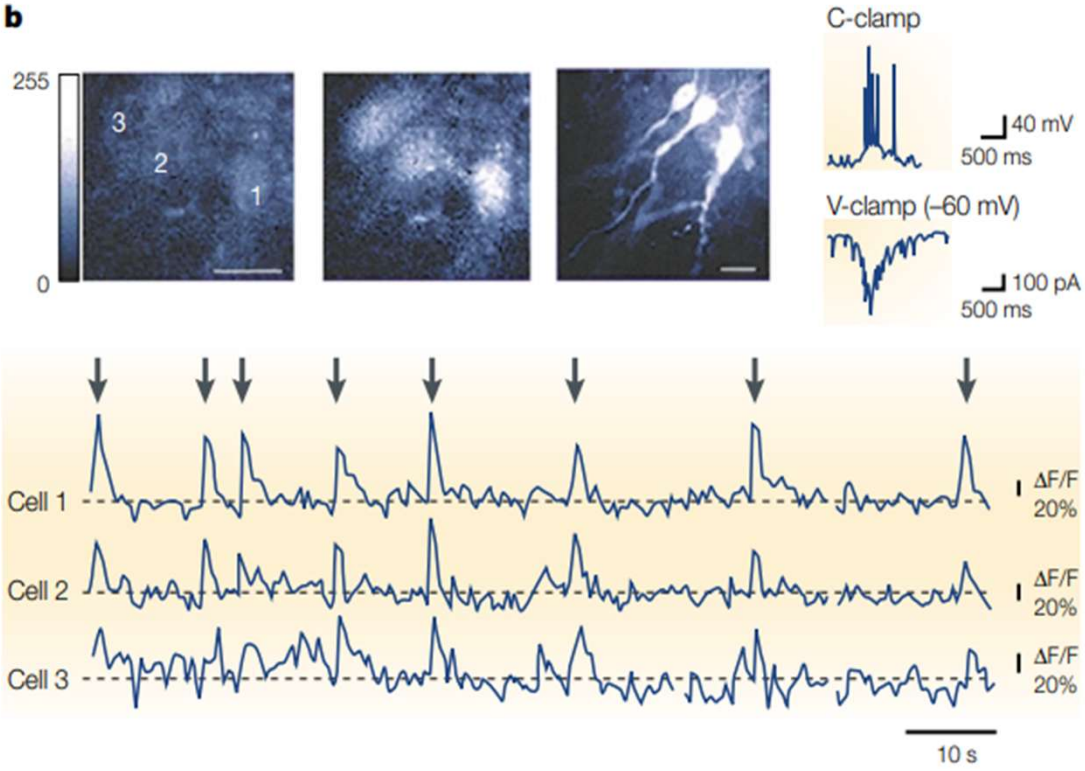
*Early stage of development*



# GIANT DEPOLARIZING POTENTIALS (GDPs) and their contribution to the generation of intracellular calcium transients

Calcium sensitive dye Fluo3-AM

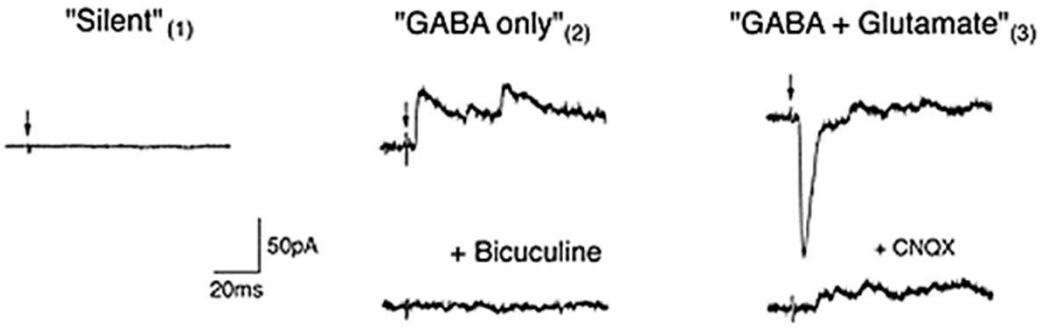


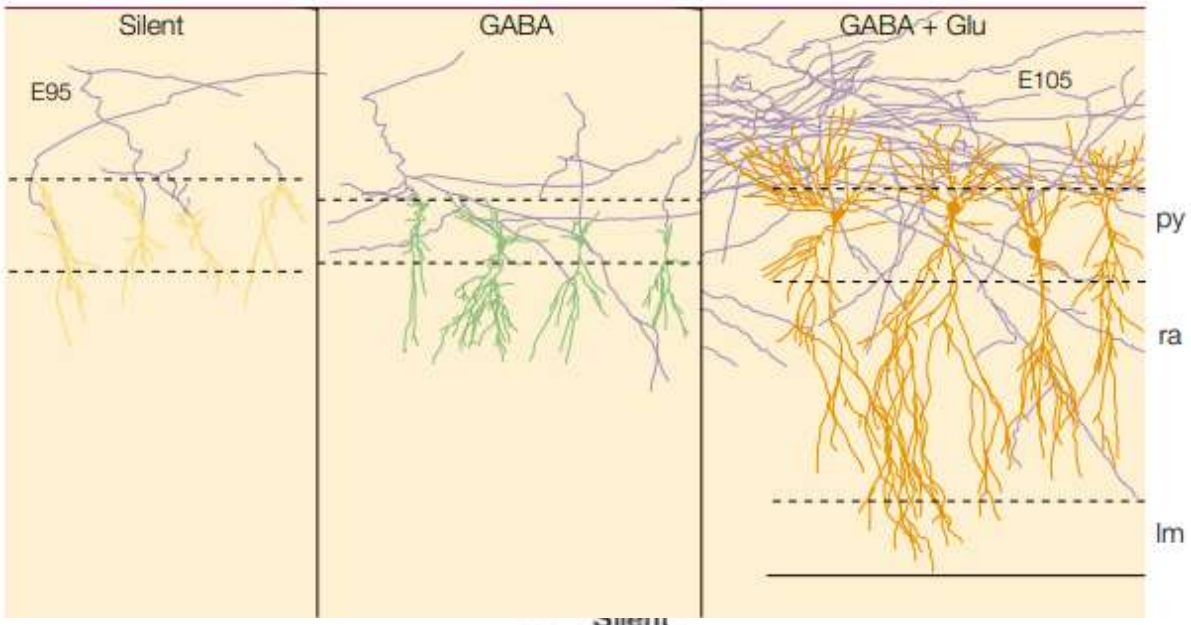



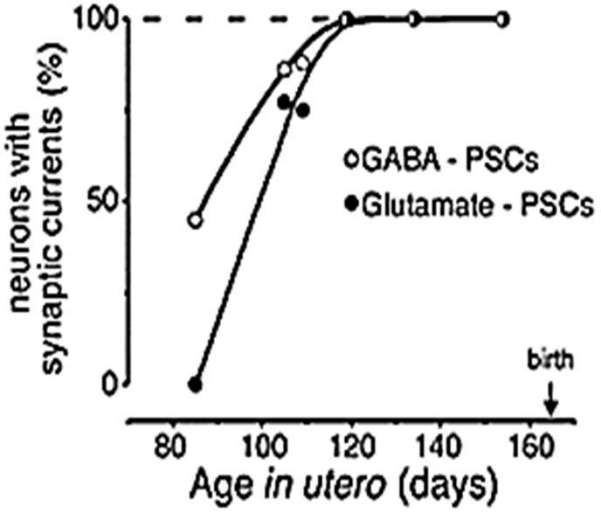

Leinekuge et al , Neuron, 1997

# Sequential formation of GABA and glutamate synapses in the developing hippocampus

CA1 pyramidal neurons



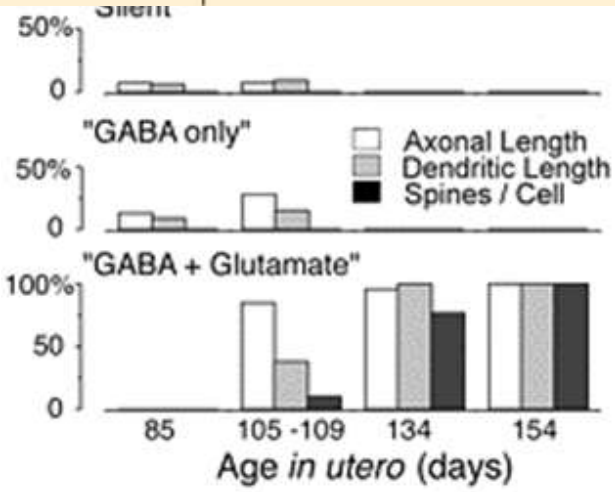




Khazipov et al., J Neurosci, 2001

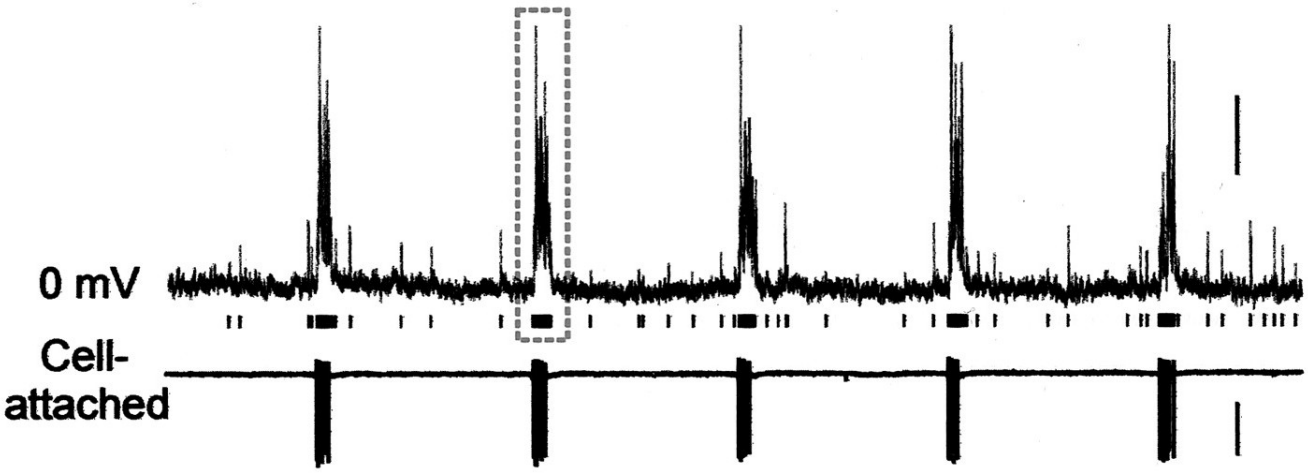
Hippocampal slices of macaque monkey fetuses delivered by cesarean section during the second half of gestation

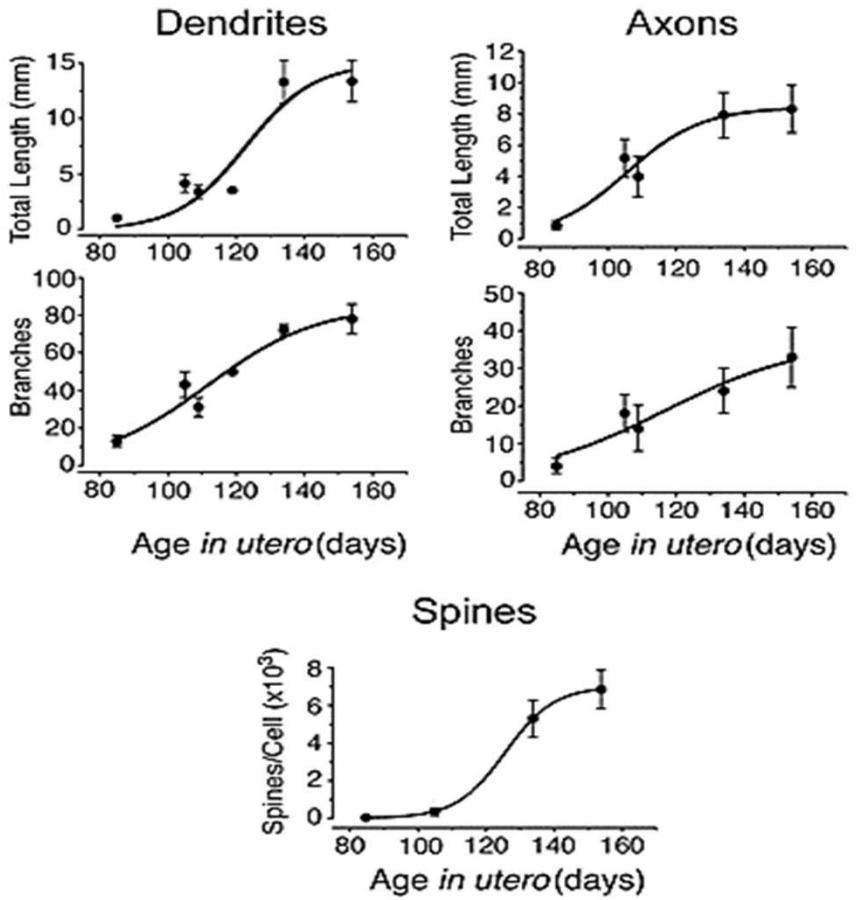
*Gestation duration= 165 days*



# Synchronized GABA driven network activity support neuronal maturation

After E105, appearance of GDPs like activity



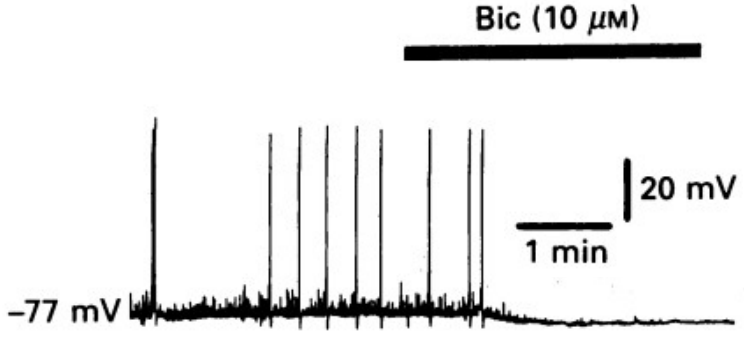

…fundamental for the maturation of excitatory synapses through a process of synaptic plasticity

Khazipov et al., J Neurosci, 2001

# Blockade of $GABA_A$ receptors suppresses GDPs and induces an epileptiform activity.

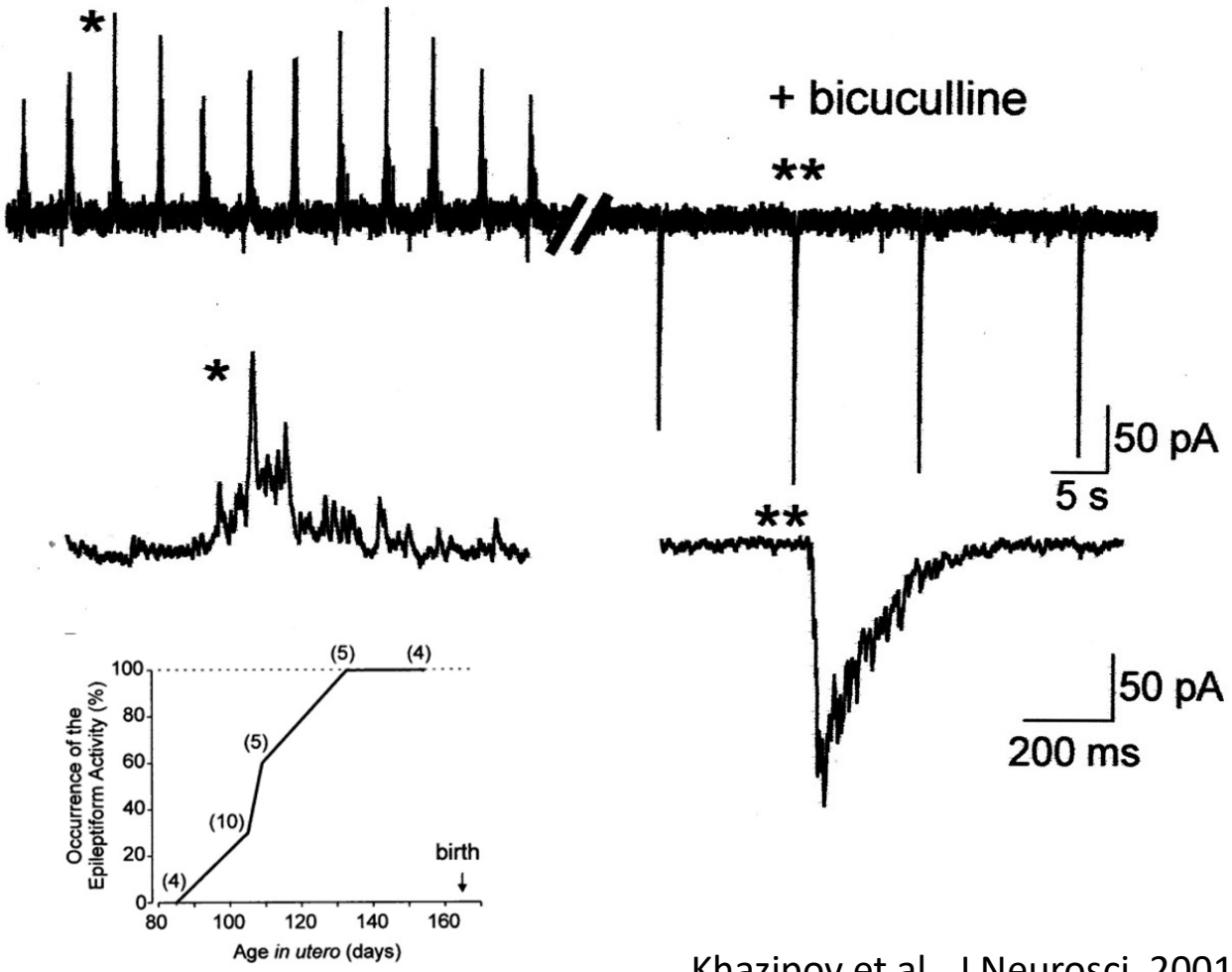
Consolidation of glutamatergic synapses (outgrowth of axonal collaterals of pyramidal cells) modifies the neuronal activity emerging from the hippocampal network

*Early stage of development*




Ben Ari et al., J Physiol, 1989

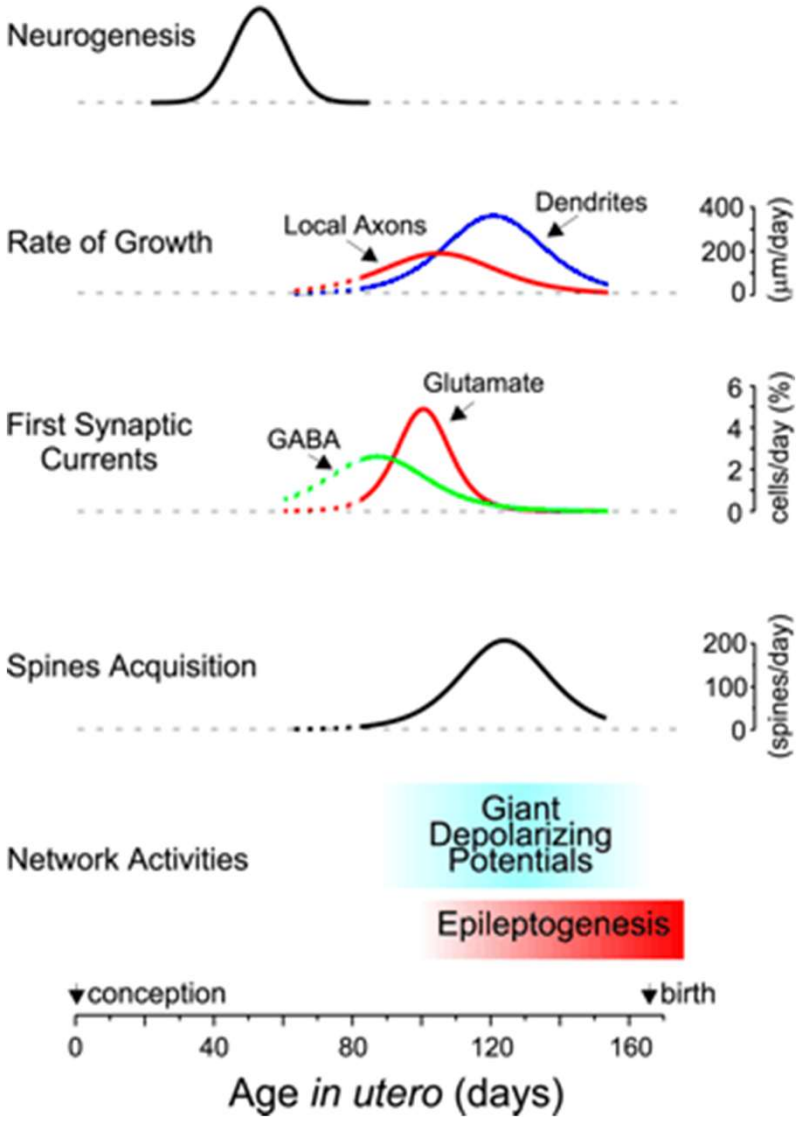
A month before birth…




Khazipov et al., J Neurosci, 2001

# Summarizing the role of GABA during development

1. GABA is excitatory as a result of a high intracellular concentration of chloride ([Cl–]i ).
2. GABA-releasing and glutamatergic synapses are formed sequentially.
3. Appearance of a primitive network-driven pattern of electrical activity— the giant depolarizing potentials (GDPs),relying on excitatory actions of GABA.
4. GABA action switches from excitatory to inhibitory, thanks to the operativity of a chloride-extruding system




Khazipov et al., J Neurosci, 2001