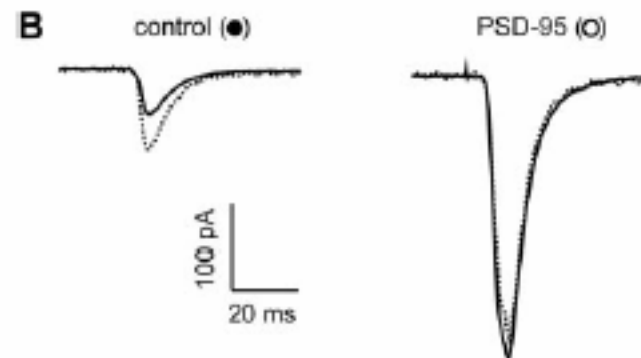
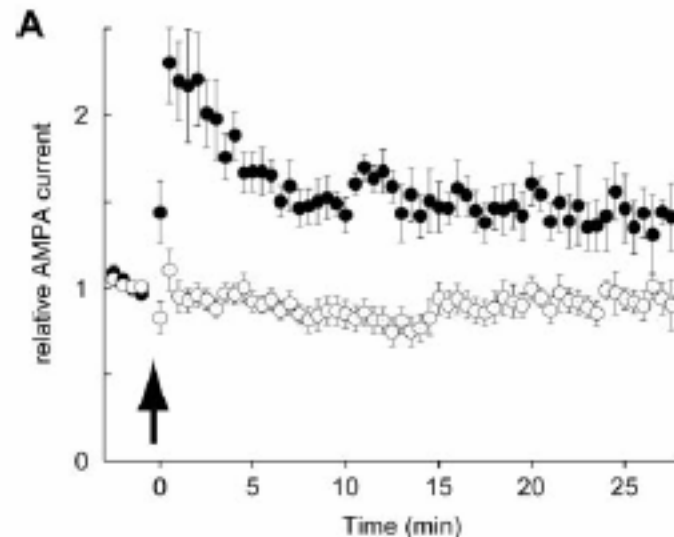


Figure 3. Expression of PSD-95 occludes LTP. *A*, Graph comparing LTP recorded simultaneously from control neurons (closed circles) and neurons expressing PSD-95 (open circles). The number of cells for both graphs is initially 10 and decreases to five by the end. Arrow indicates the time of pairing. *B*, Sample records from a pair of neurons showing EPSCs recorded before pairing (solid lines) and after pairing (dotted lines).

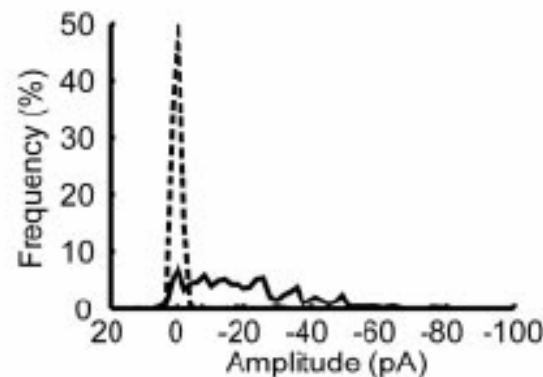
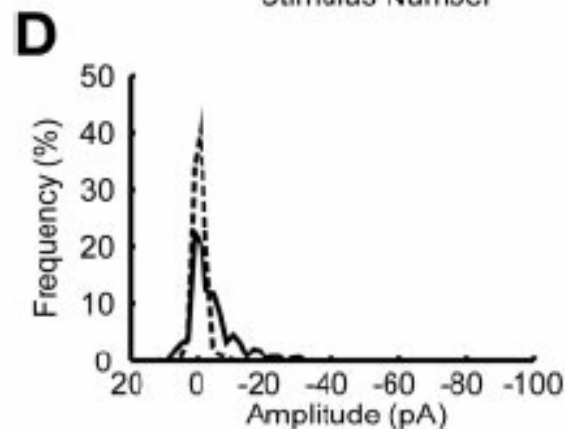
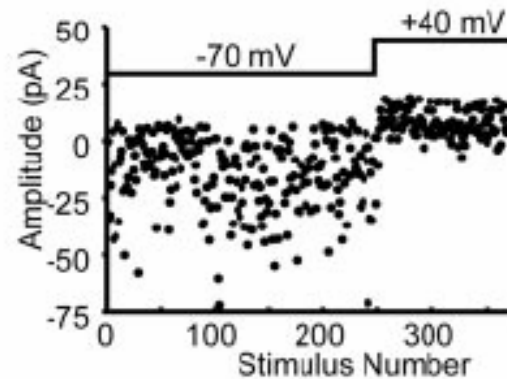
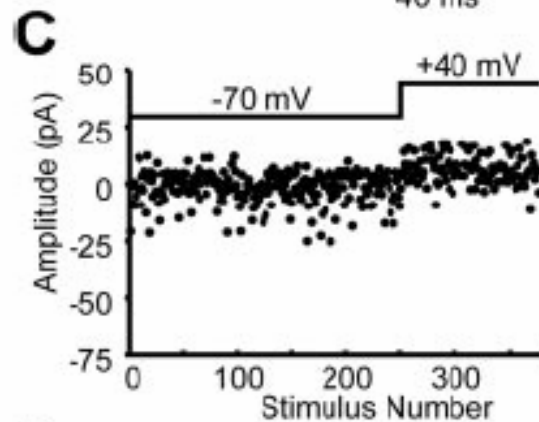
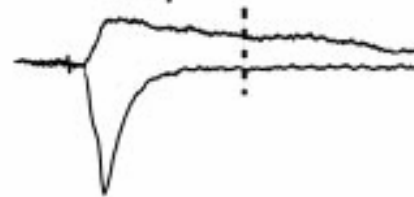
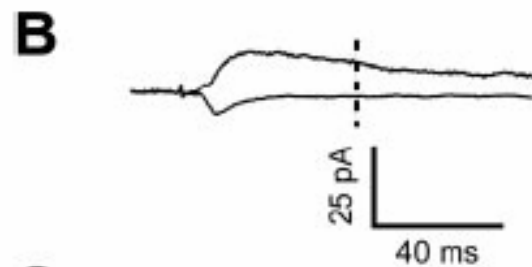
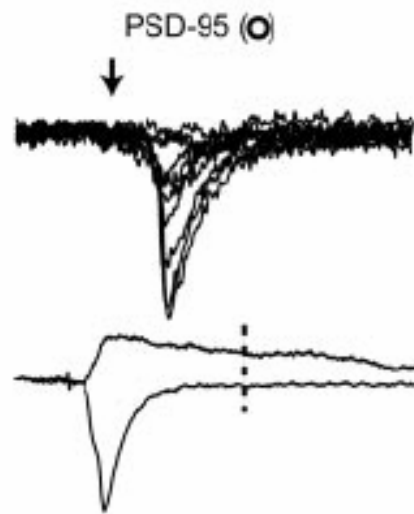
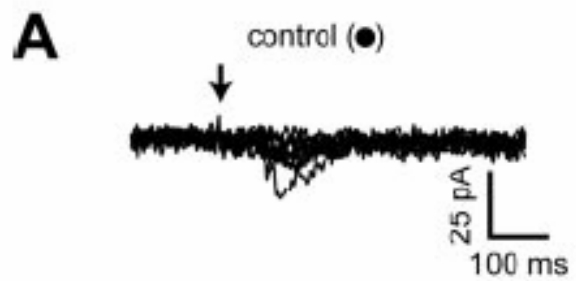


When cells are already maximally potentiated LTP is occluded.

Stein, V et al., (2003) J Neurosci, 23:5503-6606.

Also found in Rat Barrel Cortex

Ehrlich & Malinow (2004) J. Neurosci. 24:916-927



Over-expression of PSD-95 also decreases the number of synapses that are silent in response to minimal stimulation.

AND LTD IS ENHANCED !

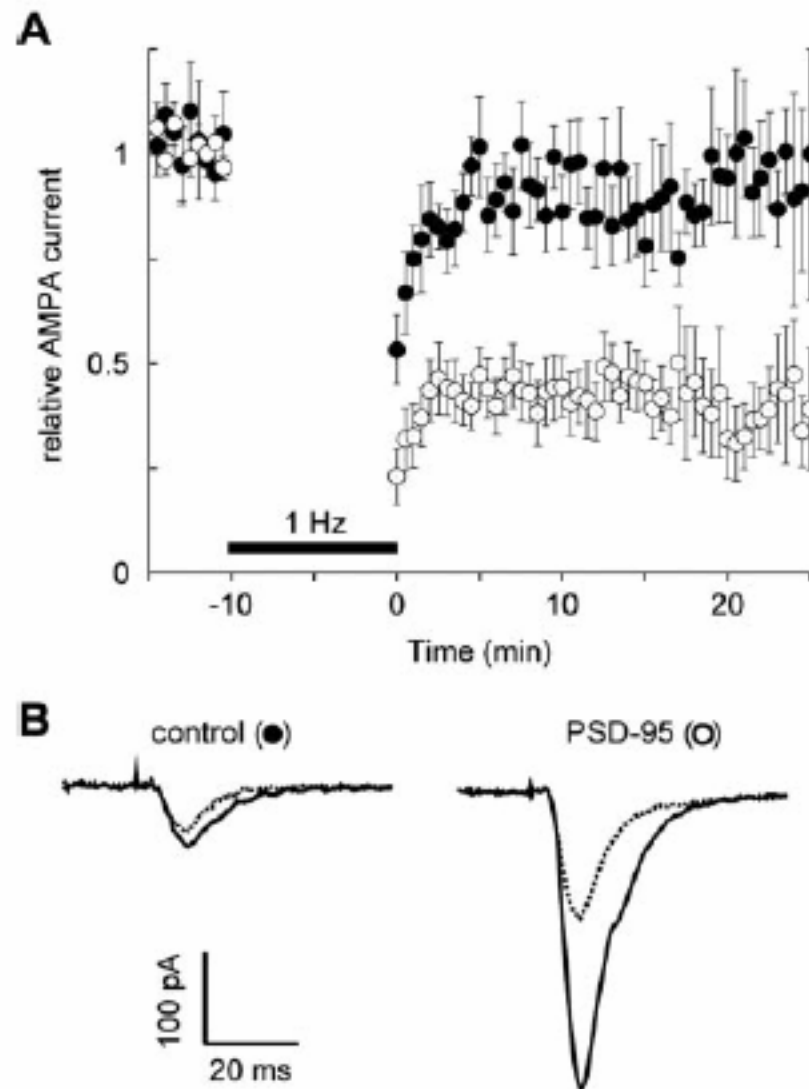
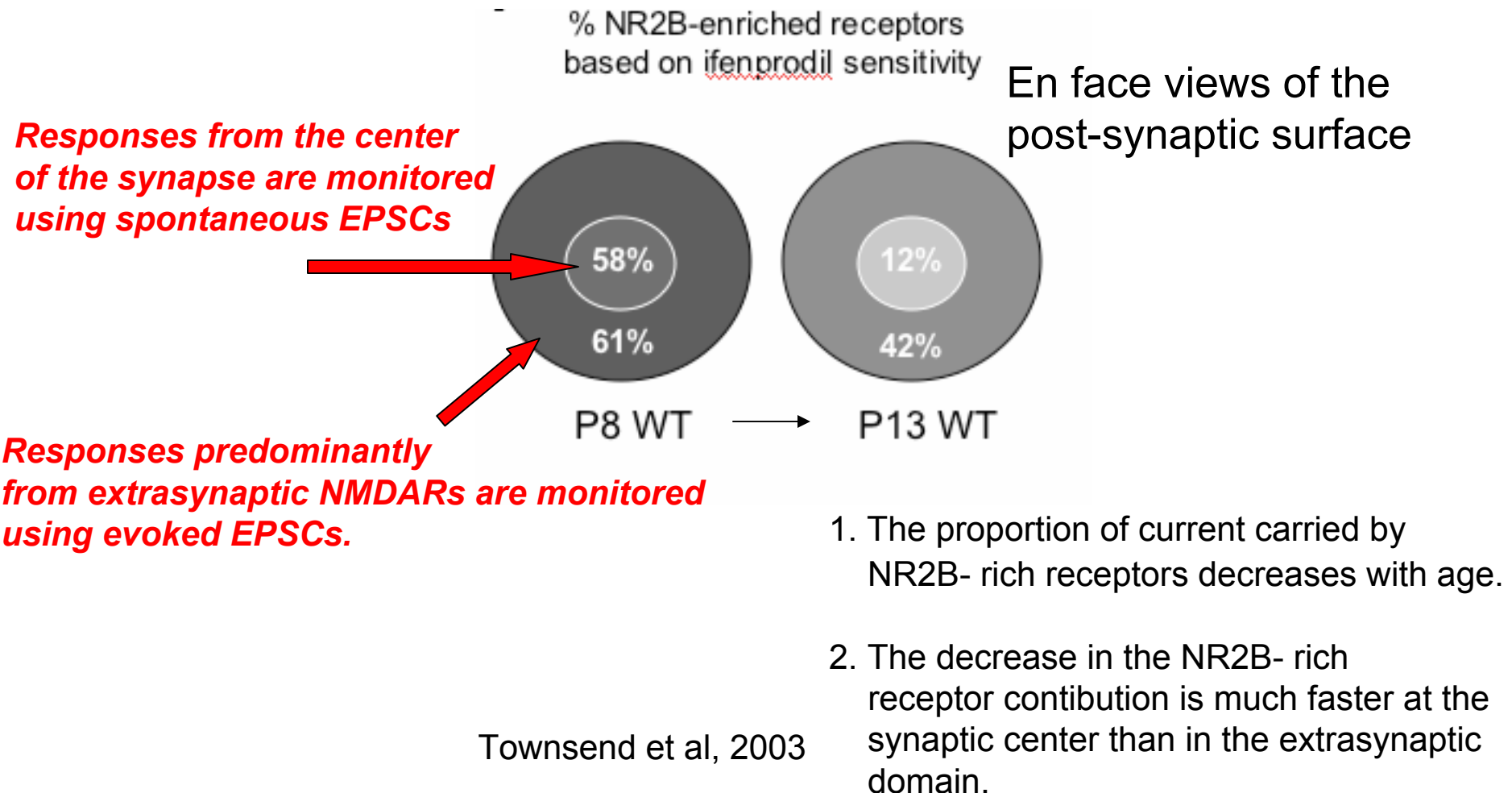
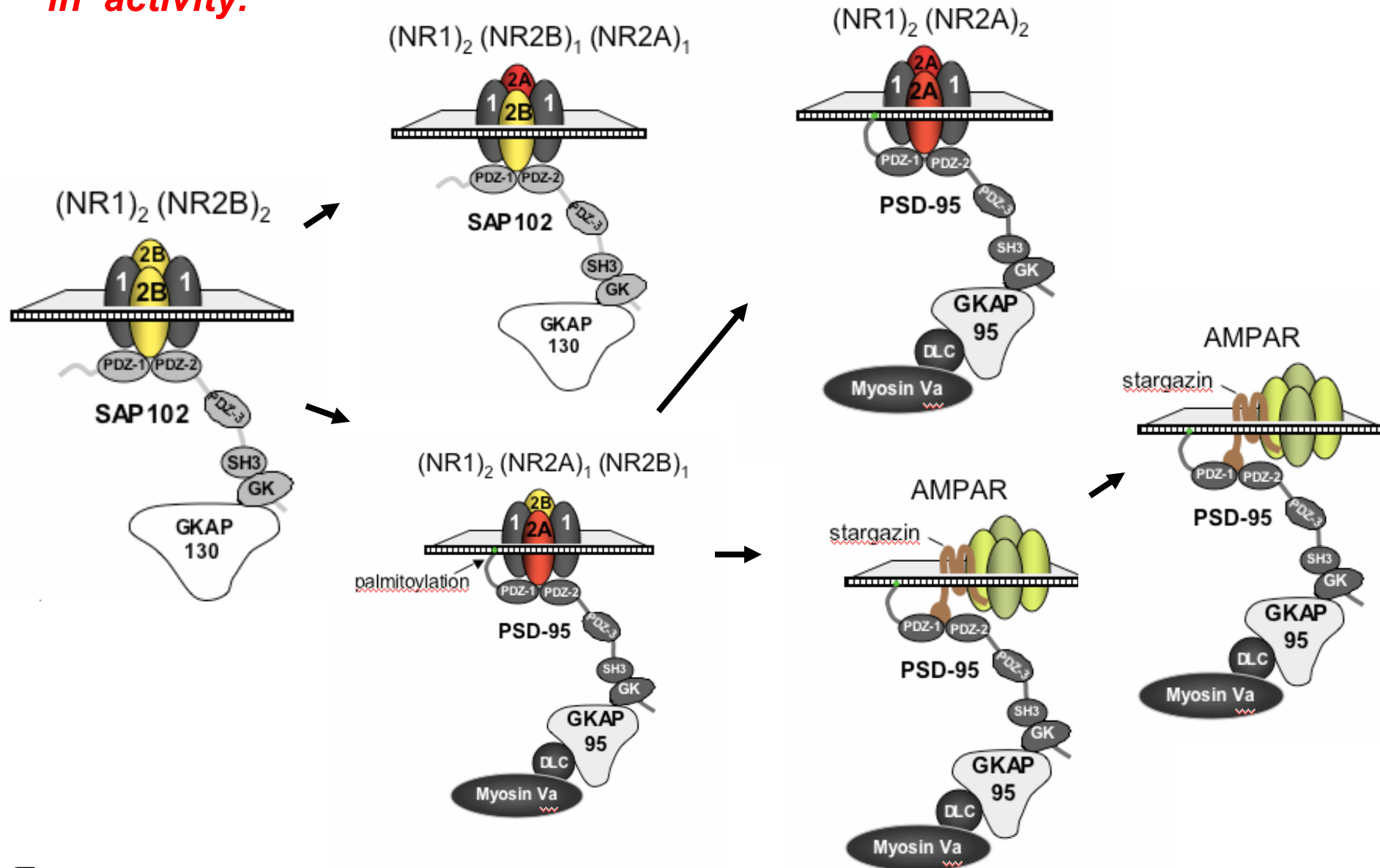


Figure 4. Expression of PSD-95 enhances LTD. *A*, Graph comparing LTD recorded simultaneously from control neurons (closed circles) and from neurons expressing PSD-95 (open circles). The number of cells for the control graph is initially 11 and decreases to six by the end of the experiments. For the graph of the cells expressing PSD-95, the number of cells is initially 11 and decreases to five by the end of the experiment. *B*, Sample records from a pair of neurons showing EPSCs recorded before pairing (solid lines) and after pairing (dotted lines).

Ifenprodil blocks current through NR2B-rich NMDARs. Therefore, it can be used to determine how much of the NMDAR current is carried by NR2B-rich NMDARs.

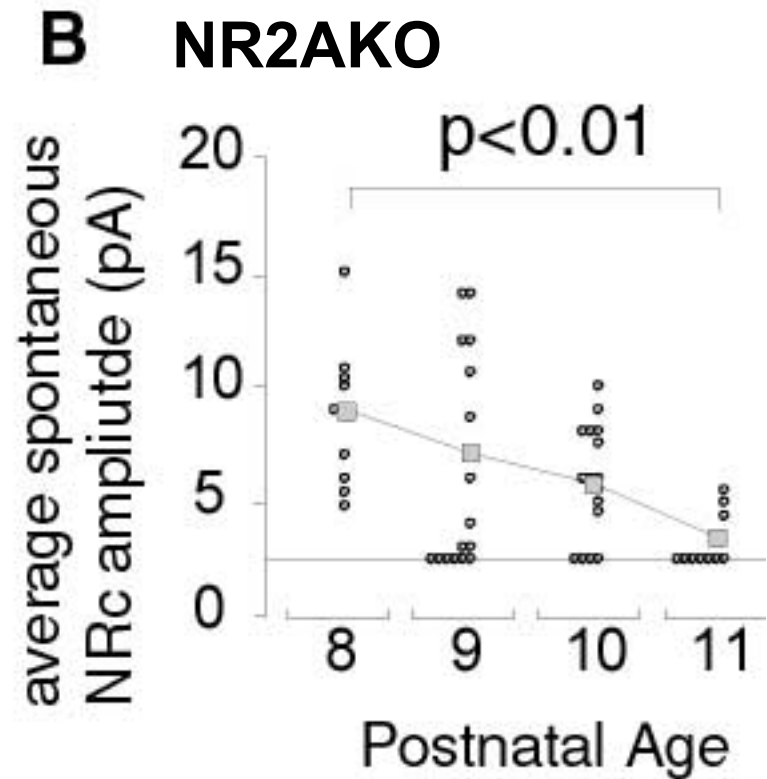
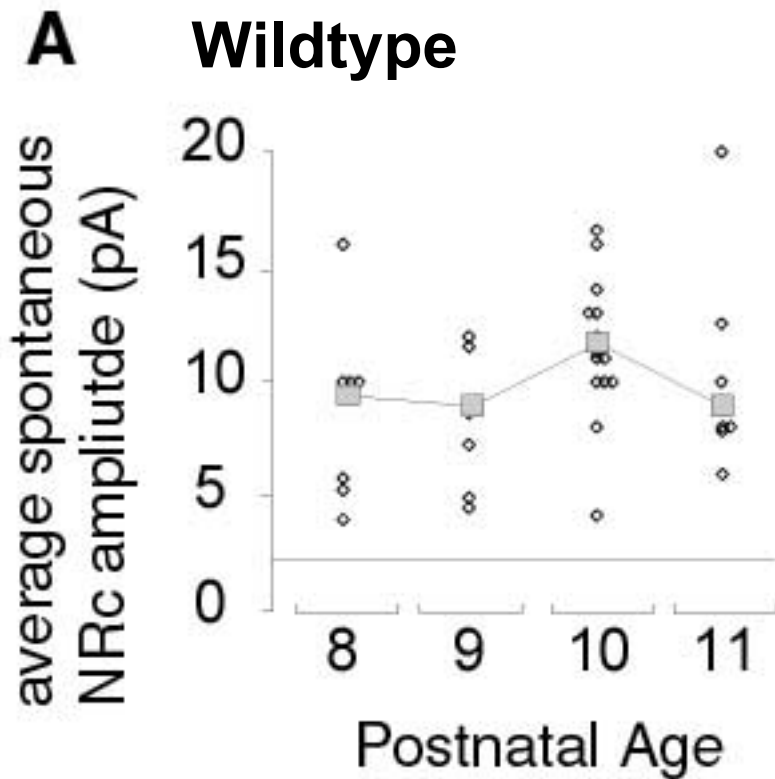


Hypothesis: The entire ionotropic glutamate receptor scaffolding, trafficking, and signaling complex changes with developmental increases in activity.

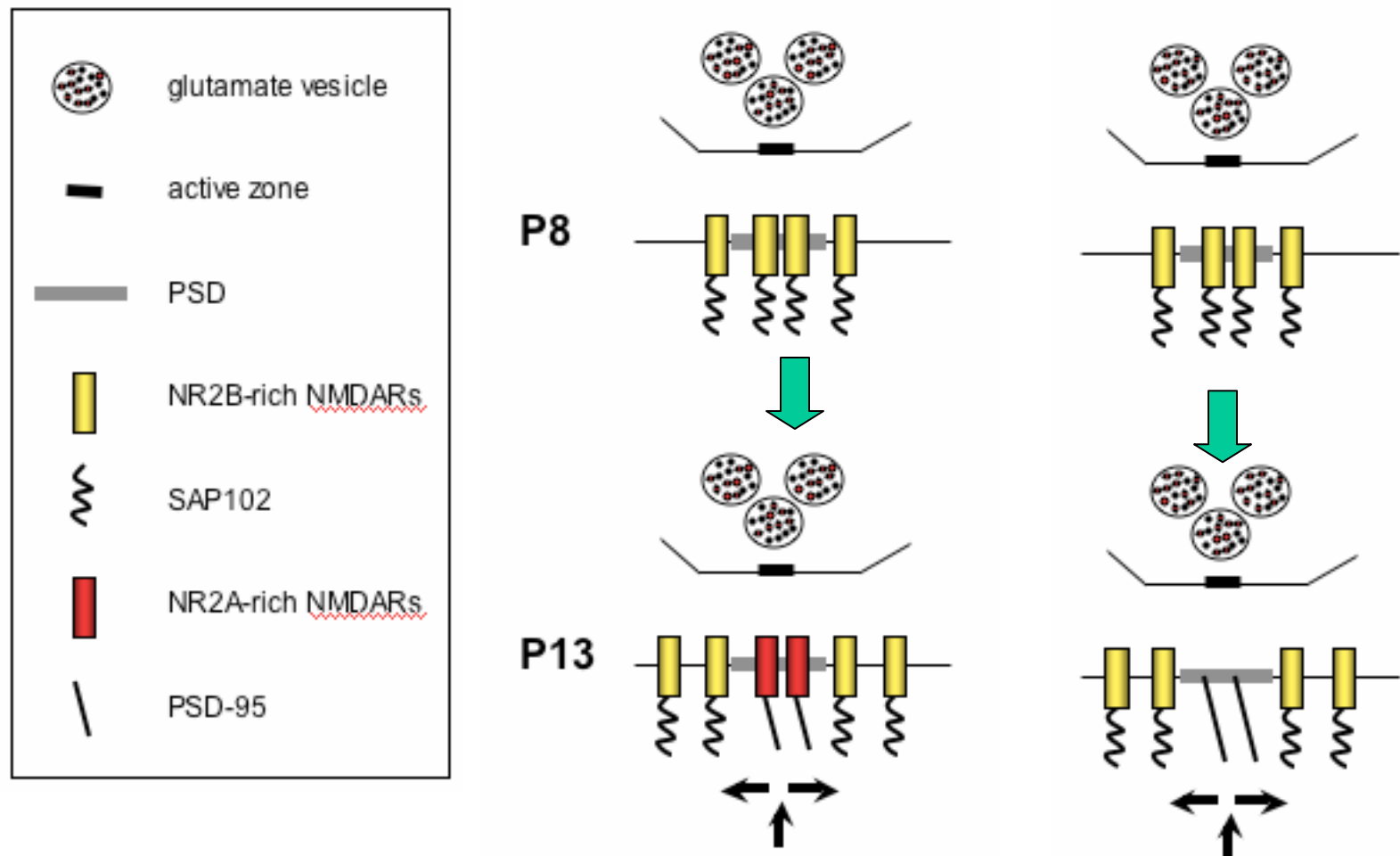


From Van Zundart, B. et al., (2004) TINS (in press)

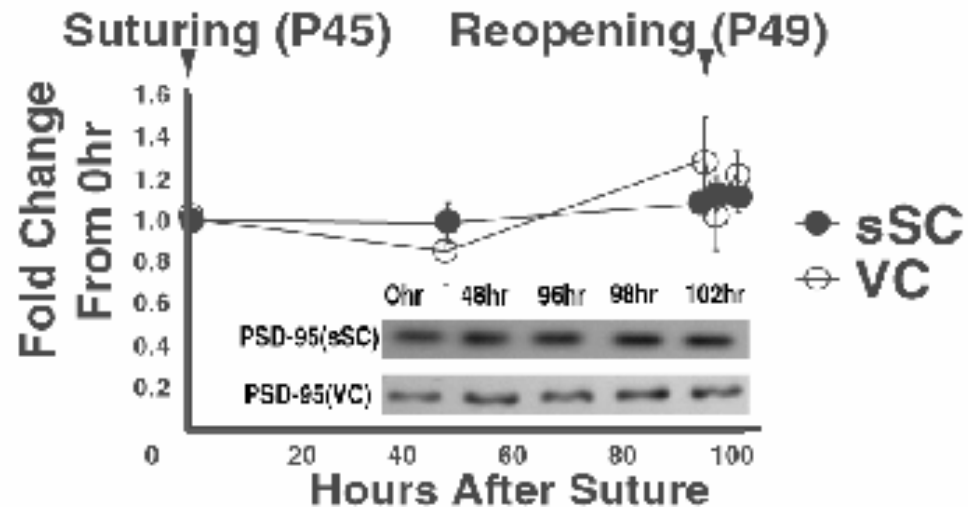
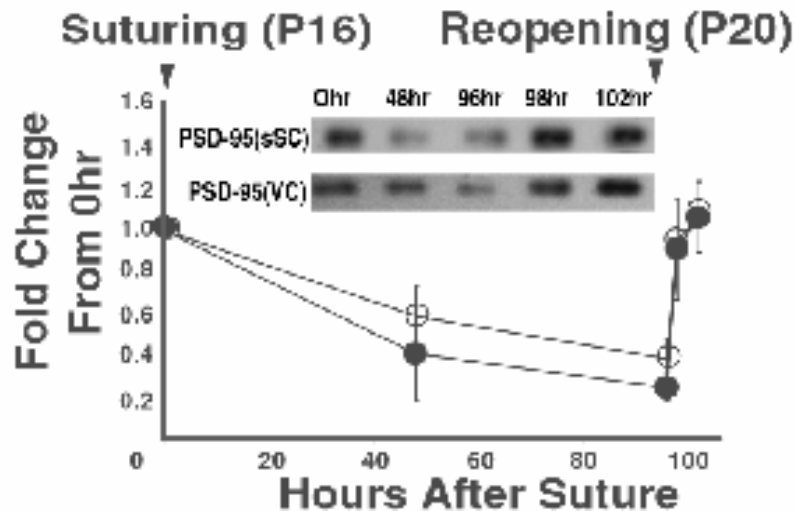
Miniature NMDAR currents in the NR2AKO mouse disappear in the interval between the onset of light-driven visual responses and eye-opening (P8 and P13)



Different mechanisms of trafficking SAP102 and PSD-95 produce the switch from NR2B to NR2A NMDAR subunits with activity and, at the same time, are changing the molecules through which the NMDAR signals.



Synaptic plasticity on a molecular level: PSD-95 is rapidly removed from visual dendrites with pattern vision deprivation in juvenile animals, but it remains stable over the same interval in young adults



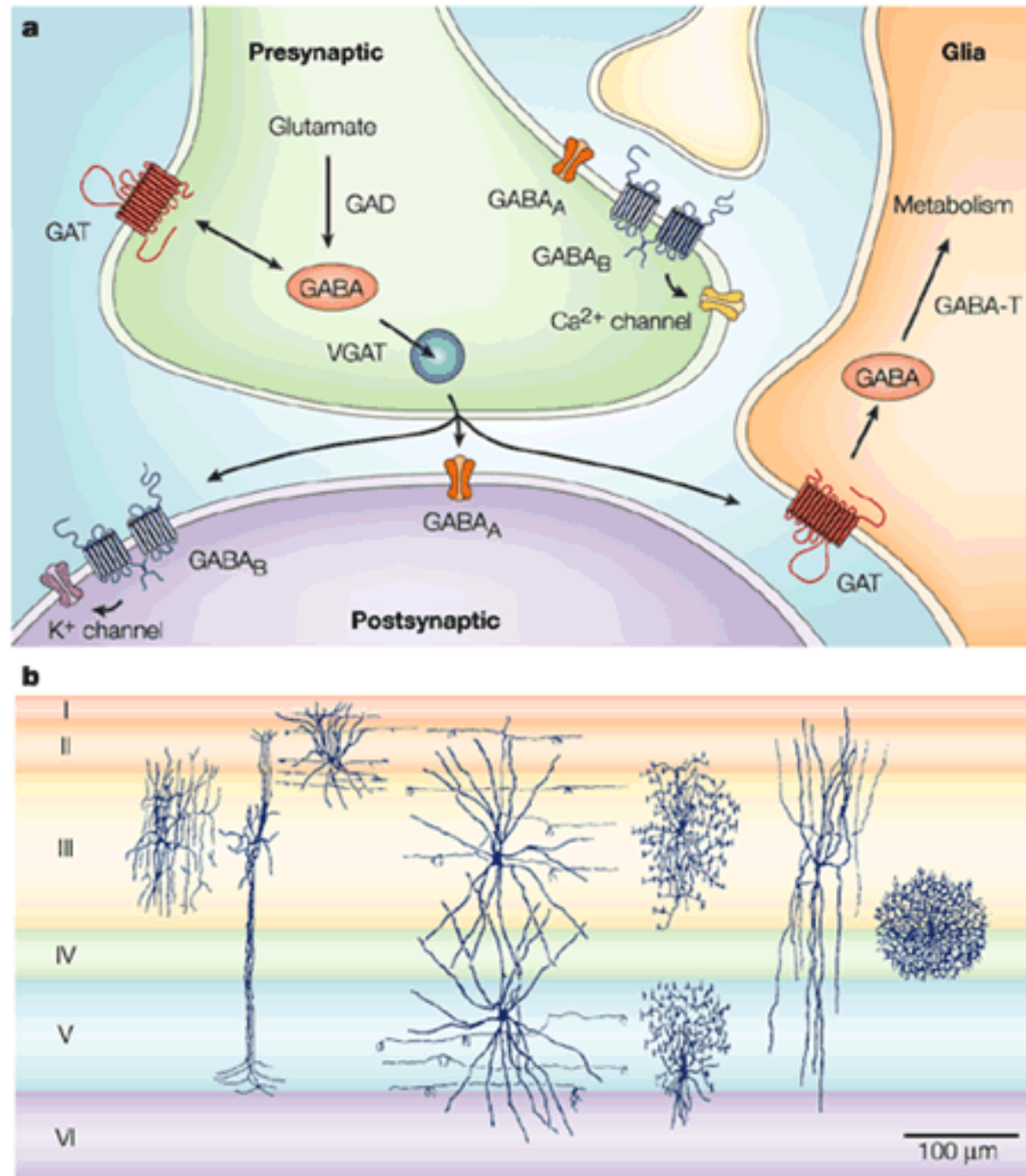
Yoshii et al., 2003

LECTURE 8:

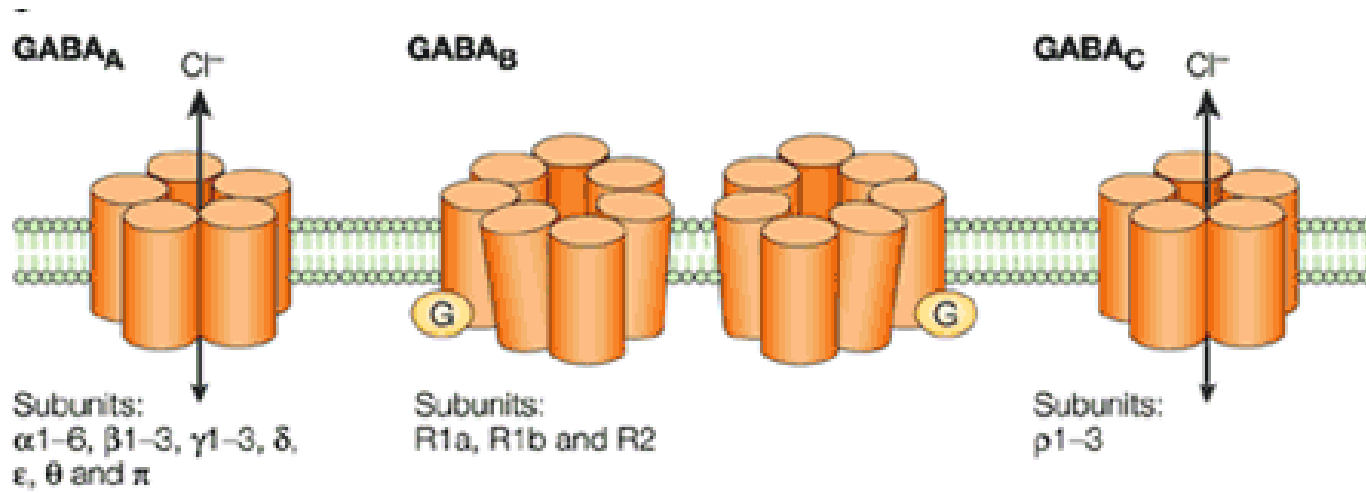
GABA mediated Cl⁻ Currents in Synaptogenesis and Plasticity

GABA and glutamate synapses show many of the same functions

In most regions of the CNS there are many different types of GABA expressing neurons.



GABA_A and GABA_C receptors are “depolarizing” when the membrane potential of the post-synaptic neuron is more negative than the Cl⁻ equilibrium potential of the cell.

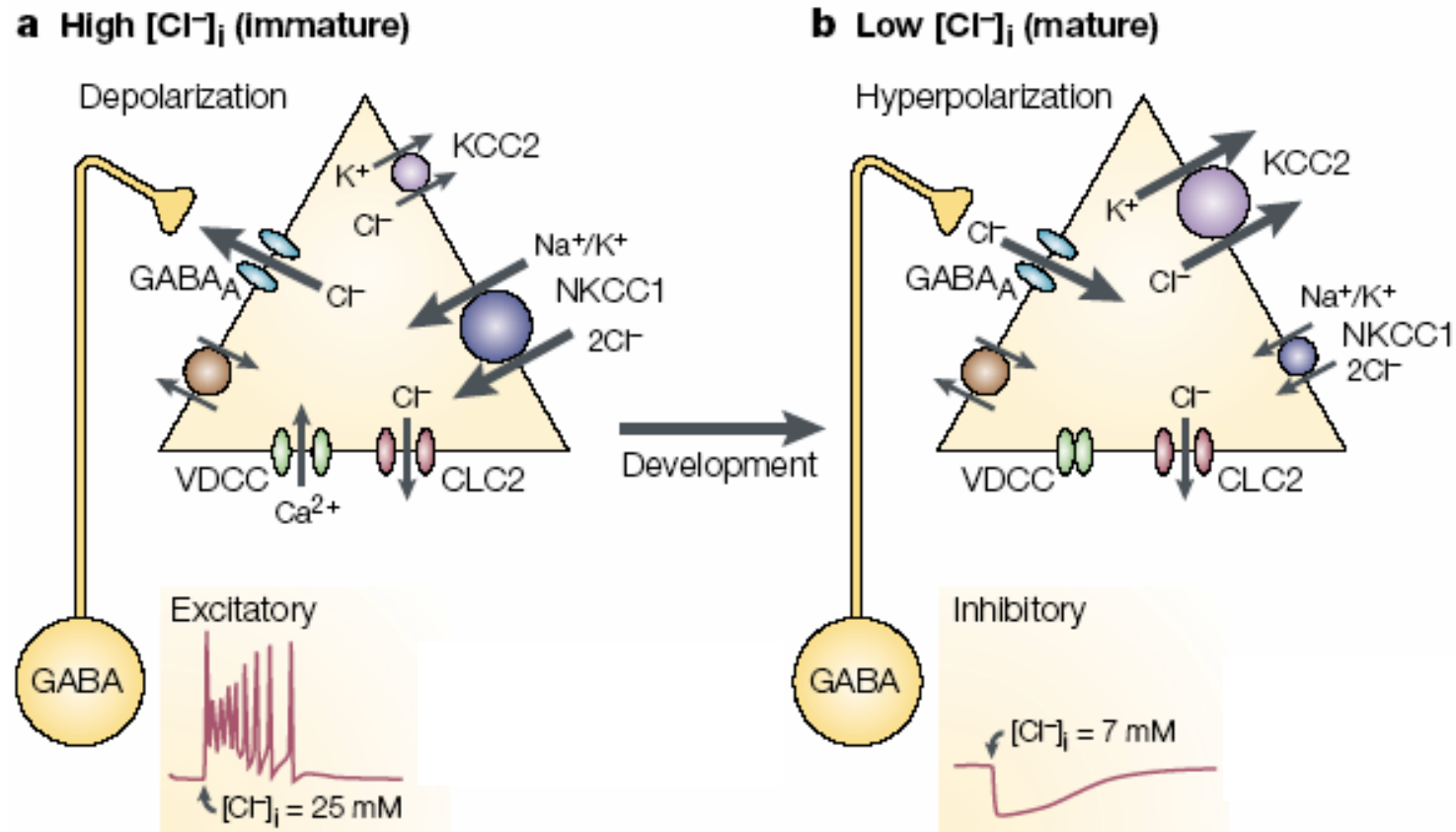


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Shifts in the relative expression of two Cl^- transporters are responsible for developmental changes in the chloride equilibrium potential

Transporters are not pumps. They couple the energy generated by ions moving down their electrochemical gradient to transport other ions up their eletrochemical gradient.

Early high expression of the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter (NKCC1) increases $[\text{Cl}^-]_i$ and shifts E_{rev} for Cl^- to a potential less negative than the resting membrane potential. Consequently, when the GABAA or GABAC chloride channels open Cl^- moves along its electrochemical gradient and depolarizes the cell.

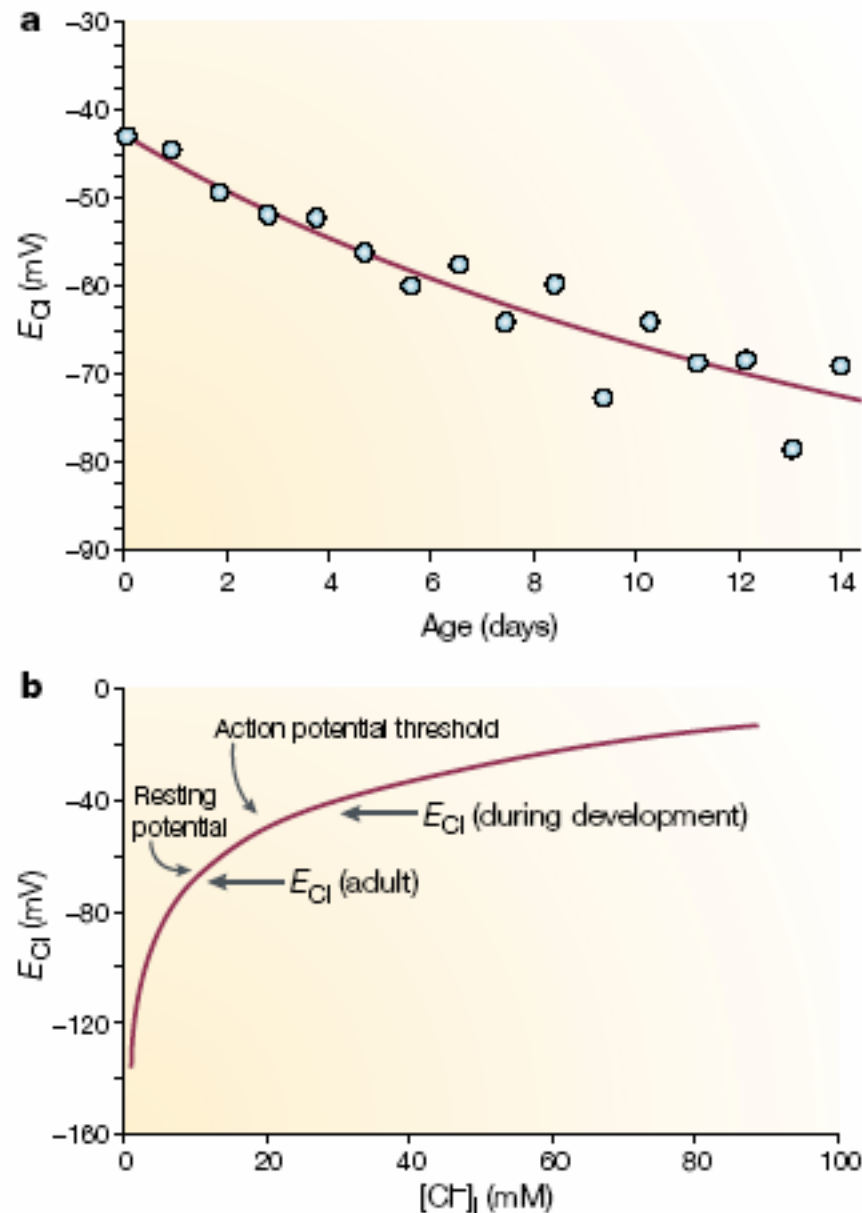


Later expression high of the $\text{K}^+\text{-Cl}^-$ cotransporter (KCC2) decreases $[\text{Cl}^-]_i$ and shifts E_{rev} for Cl^- to a potential more negative than the resting membrane potential. Consequently when Cl^- channels open the membrane hyperpolarizes.

Anions are distributed differentially across the cell membrane. The main anions of the intracellular fluid are organic molecules, such as negatively charged amino acids, proteins and nucleic acids, whereas chloride is the principal anion in the extracellular fluid. Under physiological conditions, the concentration gradient for chloride — that is, the difference between the external and internal concentrations — is 140 mM – 7 mM, so there will be an influx of chloride when chloride-permeable channels, such as GABA (γ -aminobutyric acid) type A receptors, open. However, the direction and magnitude of ion diffusion will be determined by both the concentration gradient and the membrane potential (V_m), which forces ions to move in a particular direction according to their charge. The electrochemical equilibrium potential (E_m ; also known as the reversal potential) for a given ion is the membrane potential at which the concentration-gradient force that tends to move a particular ion in one direction is exactly balanced by the electrical force that tends to move the same ion in the reverse direction. For cations, these values are 0 mV for sodium and 100 mV for potassium — far from a typical resting potential (V_{rest}) of –65 mV. By contrast, E_{Cl} in the adult is only a few mV more hyperpolarized than V_{rest} (that is, –75 mV), so the net driving force is small. In the rodent hippocampus, we have shown that E_{Cl} decreases with age during the postnatal period (see part a of the figure).

Developing neurons have a higher intracellular concentration of chloride ($[Cl^-]_i$) than adult neurons. To estimate $[Cl^-]_i$, recordings are made using the perforated-patch-clamp technique, in which a solution is used to make perforations in the membrane that are not permeable to chloride and so do not change the genuine $[Cl^-]_i$. In spite of their limitations for small neurons, perforated-patch recordings indicate that $[Cl^-]_i$ is in the order of 20–25 mM in young hippocampal neurons (part b; adapted, with permission, from REF 45 © 2001 Macmillan Magazines Ltd). An important feature of chloride gradients is that even small changes in $[Cl^-]_i$ can have profound consequences. Indeed, the curve that relates the reversal potential to the transmembrane chloride concentration (the Nernst equation) is steep at physiological concentrations of chloride⁴⁵. So, small changes in $[Cl^-]_i$ are sufficient to cause the GABA reversal potential to be either below or above the resting membrane

The internal concentration of Cl^- is measured using a cell-attached patch electrode configuration with gramicidin in the pipette solution. Gramicidin makes holes in the neuron plasma membrane that will allow current to flow from the cytoplasm to the patch pipette thus creating a whole-cell recording configuration. However, the holes made by gramicidin are too small to allow chloride ions to flow. Therefore, the intracellular Cl^- concentration is not altered. GABA_B receptors are then blocked if necessary and GABA is applied to the cell when the cell membrane is voltage clamped at different membrane potentials. The point at which the Cl^- current reverses is E_{Cl^-} . You then know the external Cl^- concentration plus E_{Cl^-} and from the Nernst Equation you can calculate $[\text{Cl}^-]_\text{i}$.



Cells recorded in developing hippocampus

GABA_A mediated currents are among the first post-synaptic currents recorded in developing hippocampal neurons

Rat PO

pyramids

interneurons

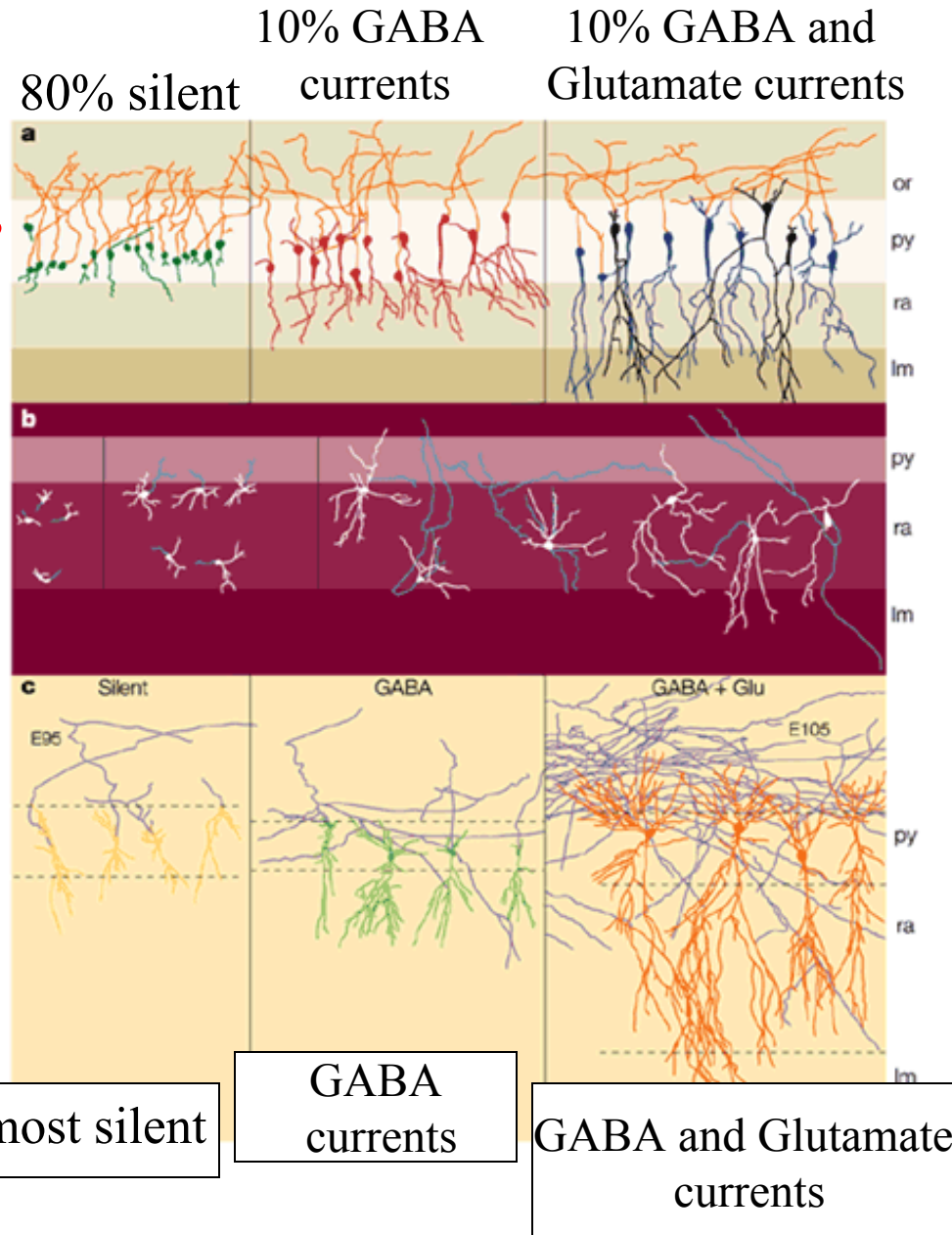
Only 3% silent,
most GABA+Glutamate
currents

Primate E 95

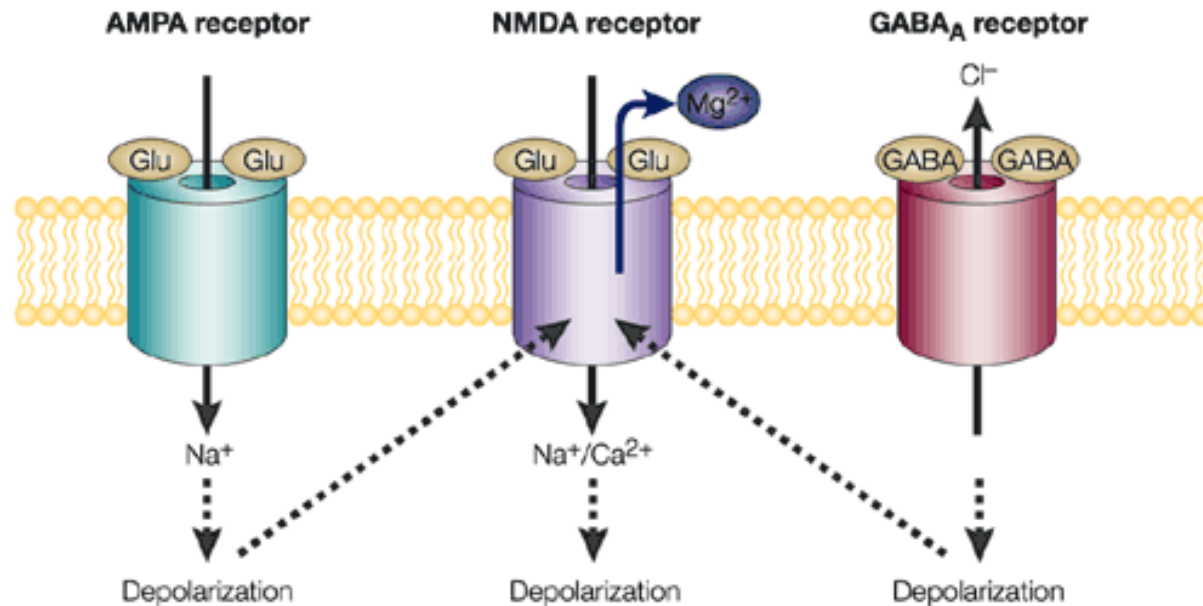
(mid-embryonic)

pyramids

Synaptic current maturation correlates with morphological maturation.



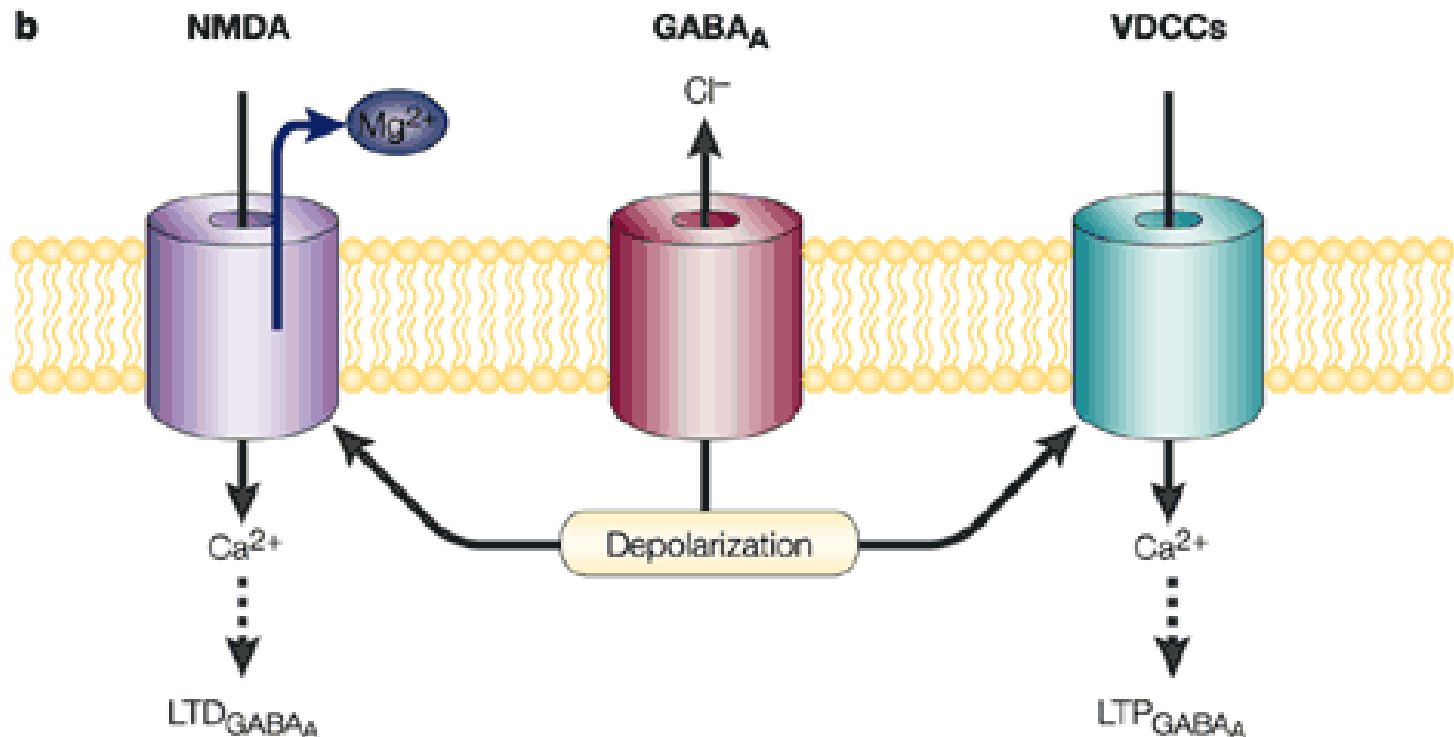
Consequences: GABA receptors can relieve the Mg^{++} block on NMDA receptors in the absence of AMPA receptor currents.



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Young neurons can be synchronized via gap junctions. Therefore, GABA_A receptors can generate giant depolarizing potentials.

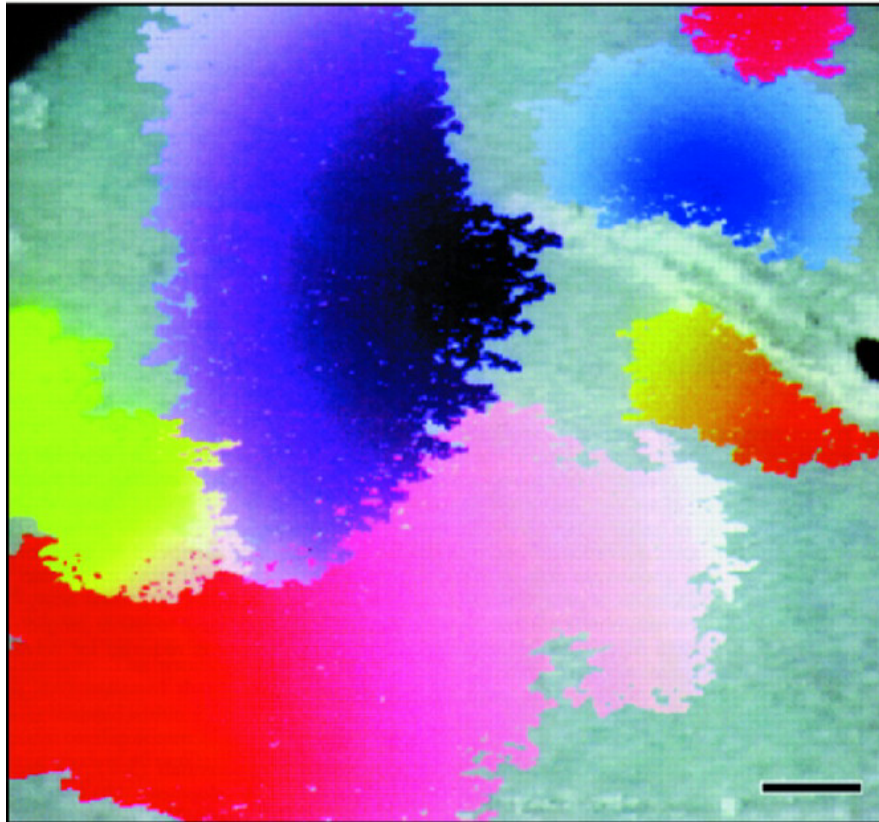
But: Depolarizing GABA currents can produce either LTD or LTP in hippocampal interneurons. Large depolarizations minimize depressing effects of depolarizing GABA currents.



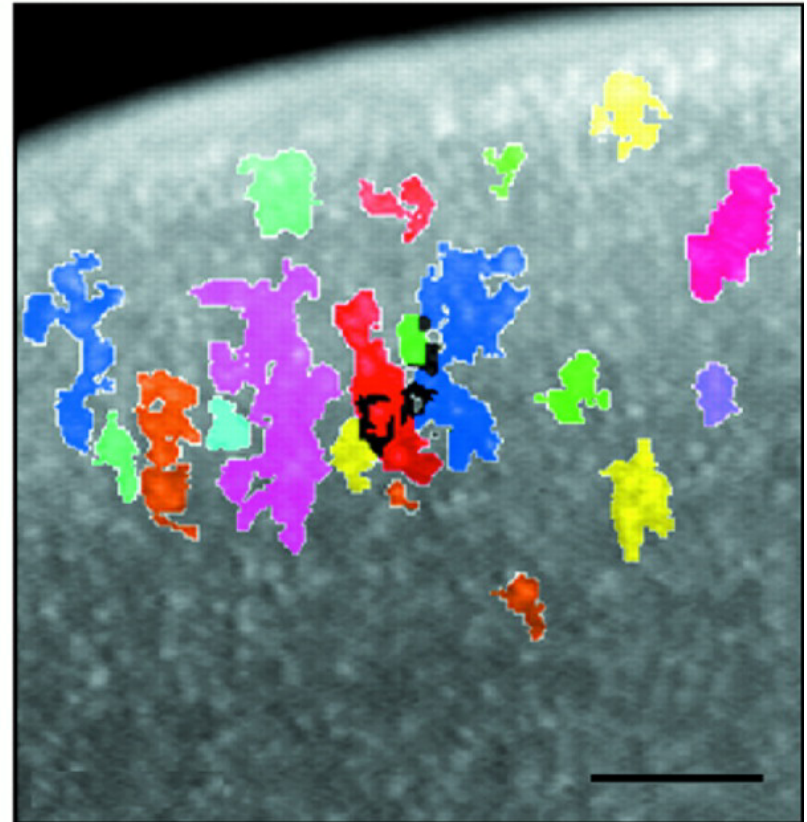
From: Ben Ari, 2002

In the late fetal or early postnatal nervous system of all vertebrates examined, groups of cells show large synchronous Ca^{++} influx. Some Ca^{++} waves are initiated by acetylcholine. Many are initiated by GABA neurons producing excitatory post-synaptic depolarizing currents and firing together due to gap junctions.

Retina

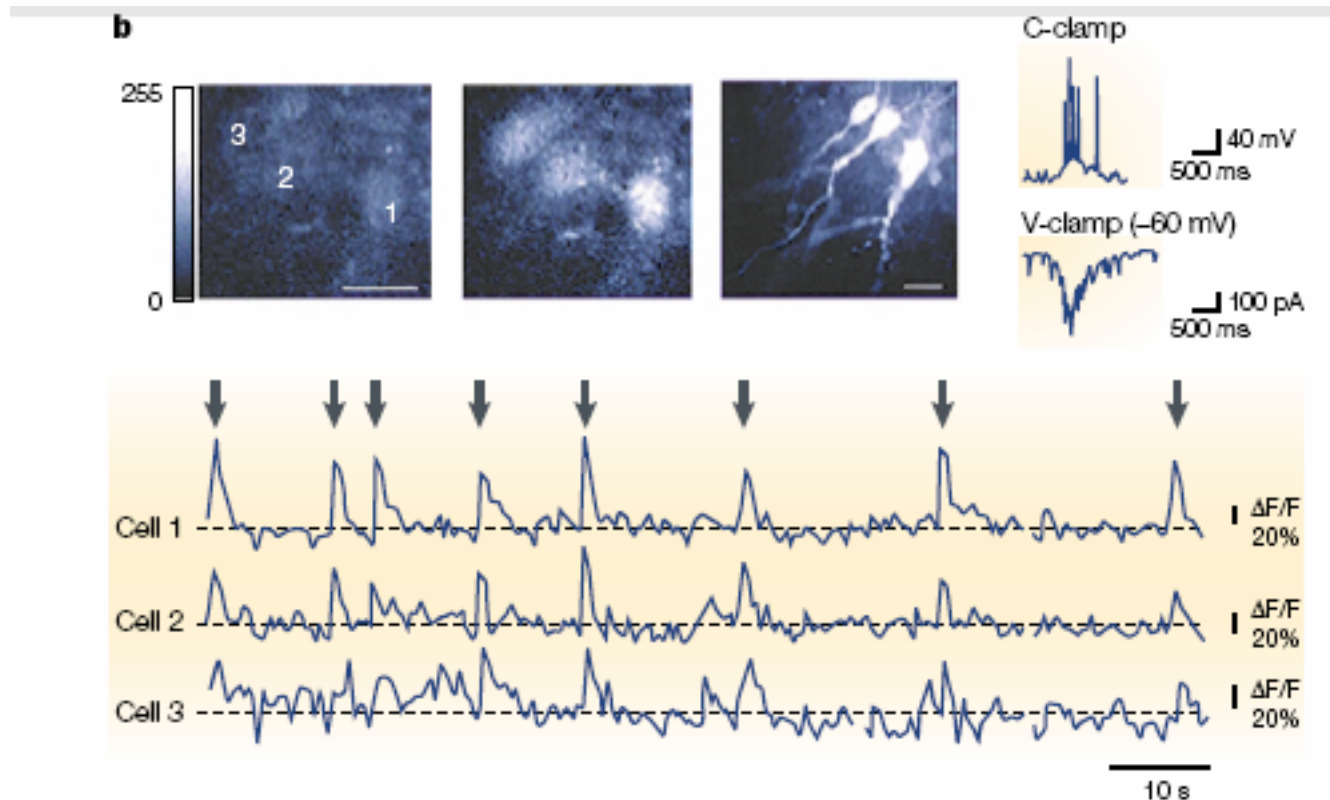


Cortex



Scale bars = 100um

Giant Depolarizing Potentials: Calcium Imaging of Single neurons loaded with the calcium indicator Fluo-3AM

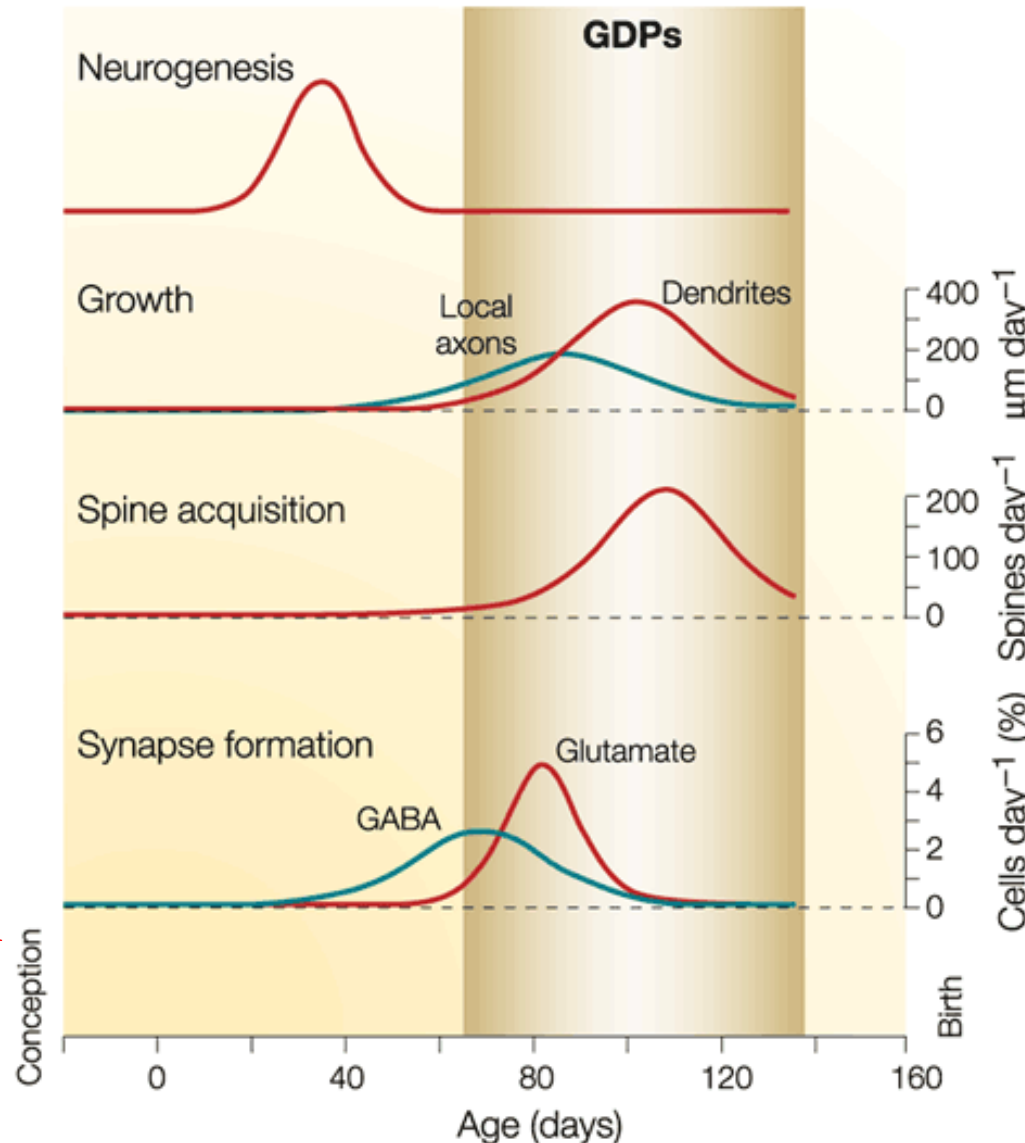


Ben-Ari, Y., (2002) Nature Reviews Neurosci.

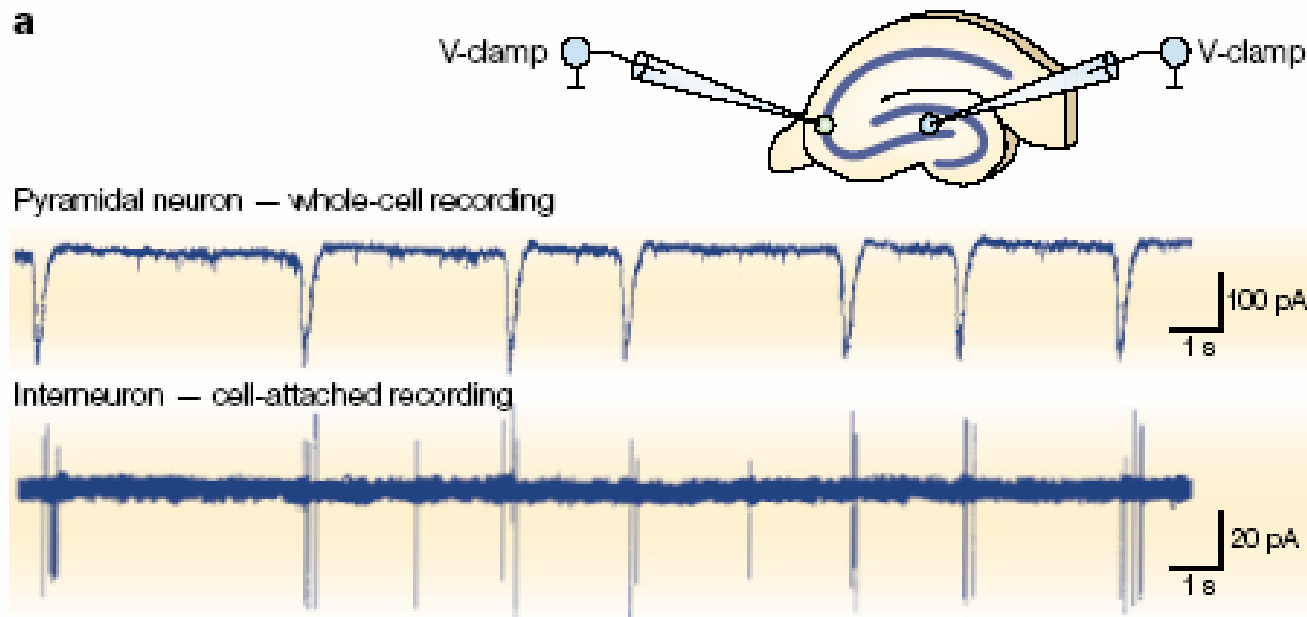
In the late embryo nacent hippocampal circuits are cyclically exposed to waves of depolarization generated by giant depolarizing potentials (GDPs) driven by GABA_A receptors and coordinated by gap junctions between GABAergic neurons.

These GDPs are present during the early period of synaptogenesis and their importance is not completely understood.

However, GDPs in young neurons through their activation of VDCC channels, lead to the expression of cFos, BDNF, KCC2, and a functional shift in GABA action.



Electrophysiological recording of coordinate effects of Giant Depolarizing Potentials recorded from distant neurons in a neonatal hippocampus

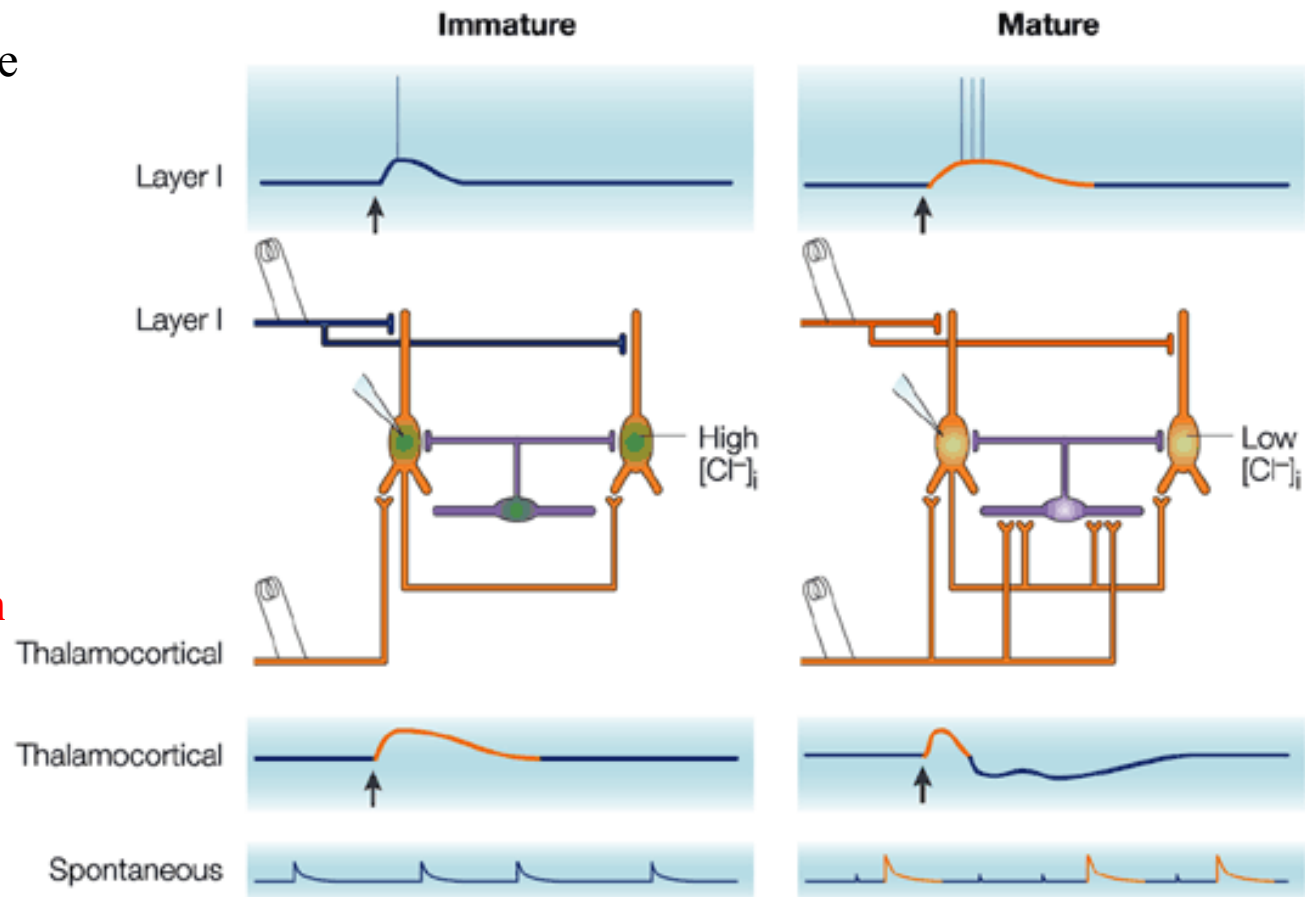


Ben-Ari, Y., (2002) Nature Reviews Neurosci.

Several factors change the function of cortical circuitry during maturation.

Layer I afferents arise from outside of the cortex and many are withdrawn in the early neonate period in rodents. Also early transient neurons (Cajal Retzius Cells) are GABAergic and have axons that run tangentially in cortex. Between late fetal/neonate brain and more mature stages the pyramids with apical dendrites in layer I change their E_{rev} for Cl^- . Thus both thalamocortical and layer I stimuli are excitatory. In more mature cortex layer I stimulation evokes glutamatergic excitation while thalamocortical stimulation evokes short latency excitation and longer latency inhibition through both $GABA_A$ and $GABA_B$ receptors.

1. Transient GABA inputs
2. Changing Cl^- currents in pyramids
3. Development of connections to and from GABA interneurons



Growing evidence that not all “synaptic” interactions occur at the morphological synapse

- early GABA release occurs prior to synapse formation does not depend on the snare complex or Ca^{++} Demarque, M et al., (2002)Neuron 36:1051-1061.
- many NMDA receptors are “extra-synaptic”
- even when a single input is stimulated there is “overflow” to extrasynaptic NMDARs see: Diamond, J., 2002
- the scaffolding and the signaling systems underlying extra-synaptic NMDAR receptor functioning may differ from NMDARs positioned under the synapse.
(see: Hardingham et al., 2002)