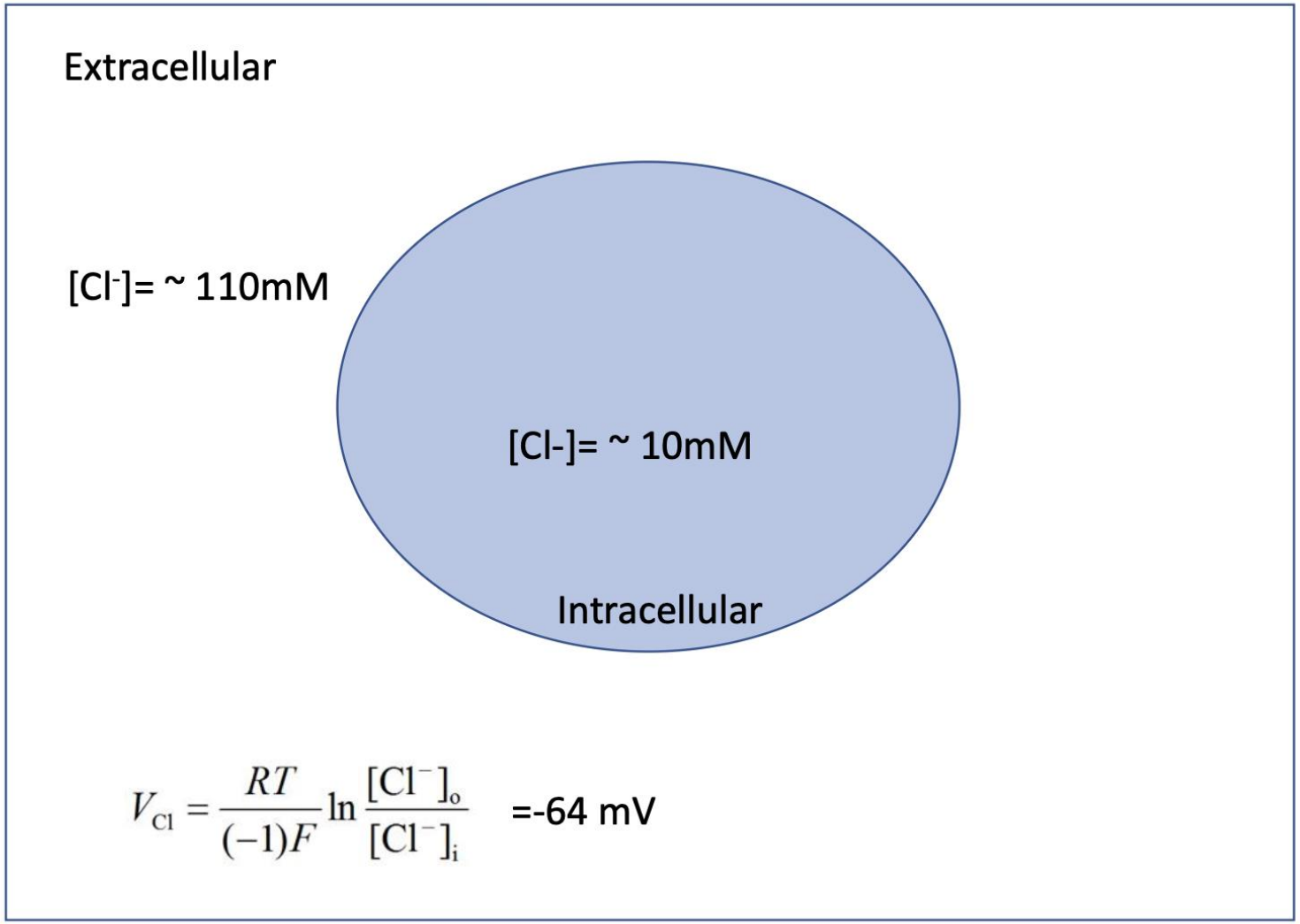
Chloride homeostasis: basic mechanisms in physiological and pathological conditions

- ✓ Chloride is the main physiological anion, serving as the principal compensatory ion for the movement of major cations such as Na^+, K^+ and Ca^{2+}

- ✓ A fine regulation of chloride homeostasis is necessary in order to maintain a proper cellular functions.

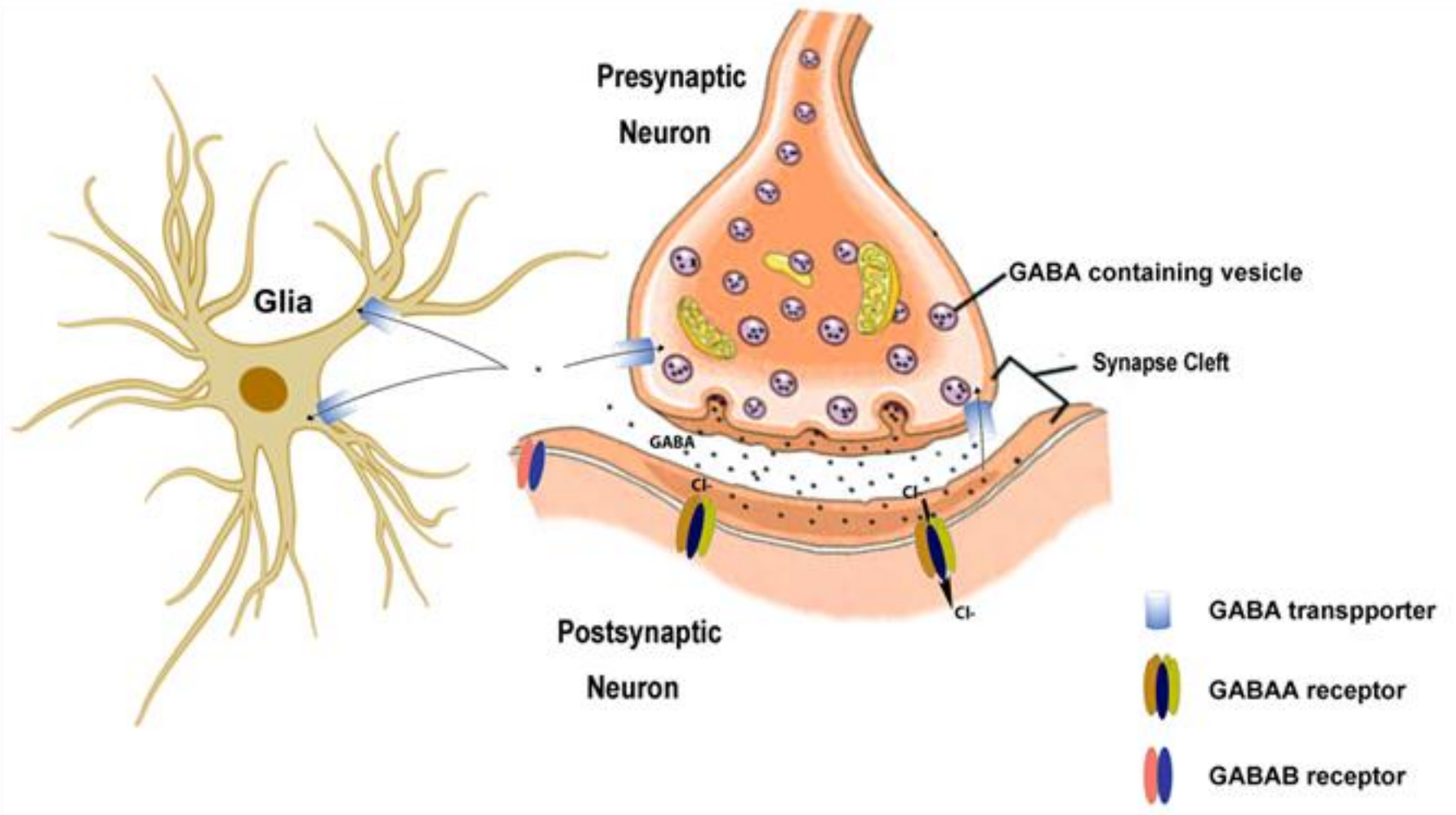
- ✓ Functions attributed to chloride channels include the control of membrane potential, cell volume homeostasis and regulation of cell proliferation and apoptosis

Chloride Distribution Across the Neuronal Membrane



$$V_{Cl} = \frac{RT}{(-1)F} \ln \frac{[Cl^-]_o}{[Cl^-]_i}$$ =-64 mV

But let's see what happens in neurons...



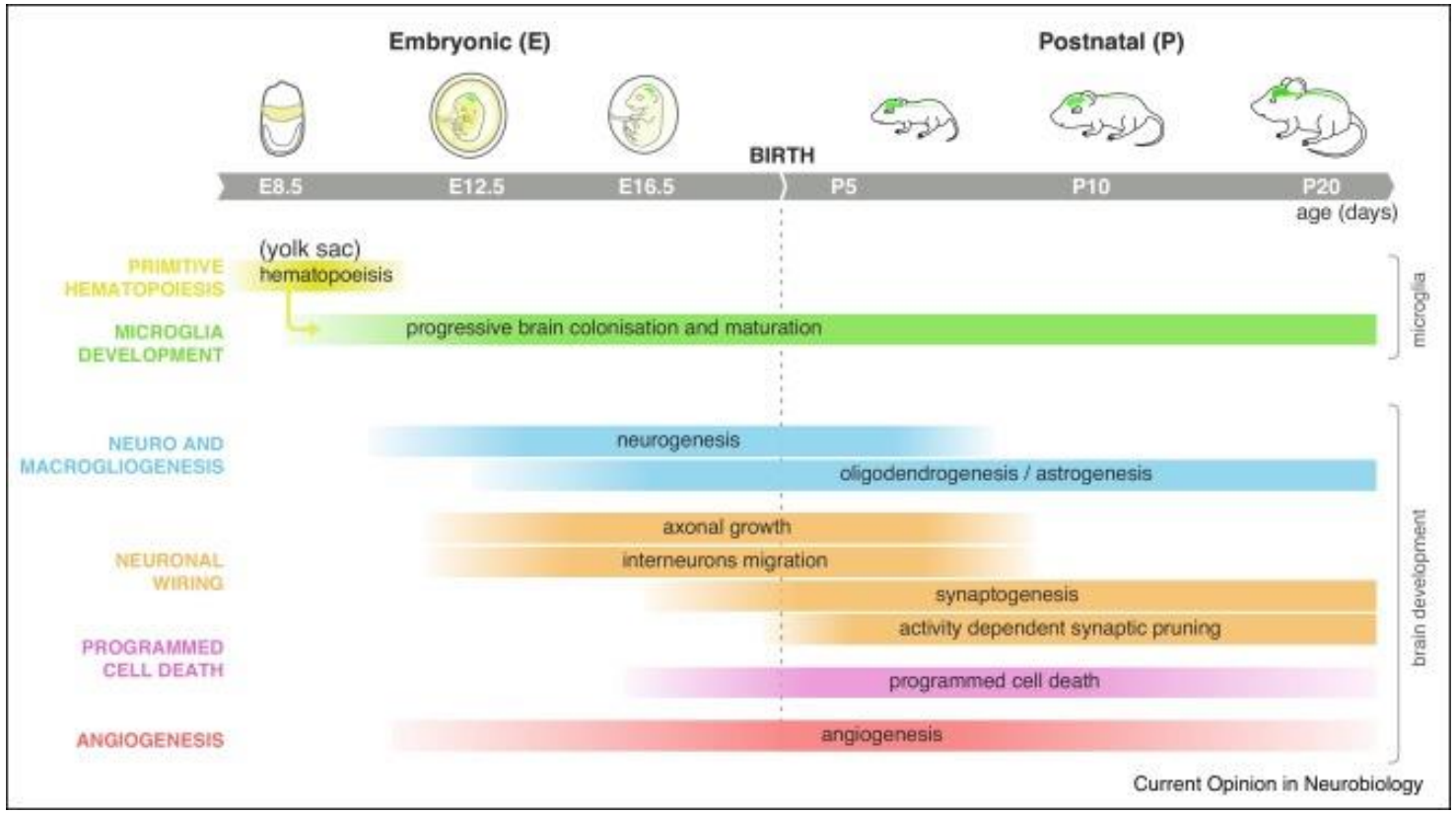
Developmental Regulation of Intracellular Chloride in Neurons

In rodents….

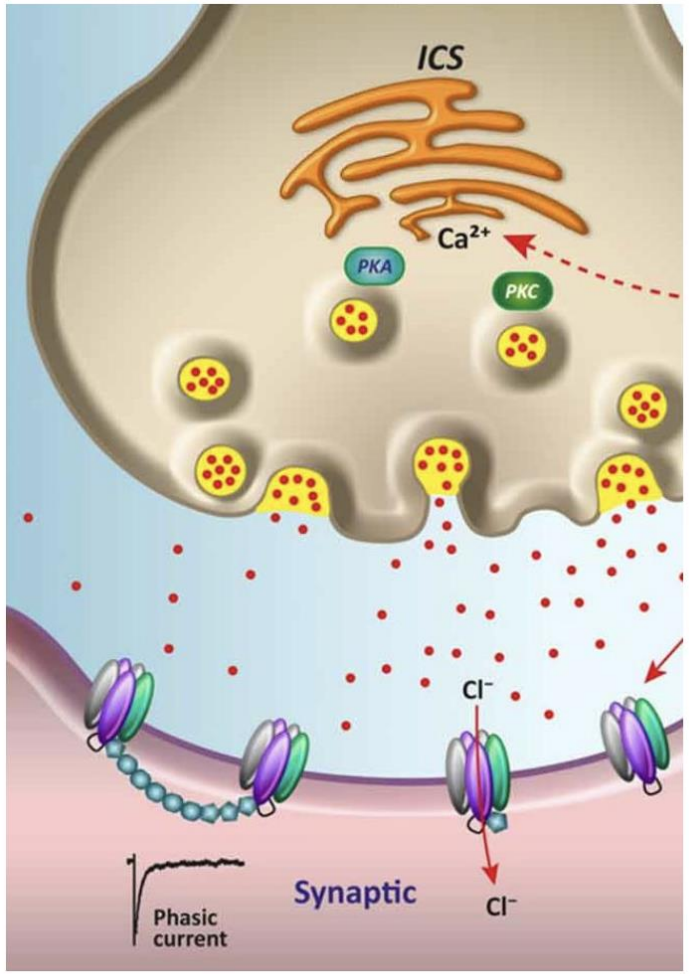
During the first postnatal week, intracellular [Cl^-] levels are higher than those typically found in mature neurons (approximately 25 mM).

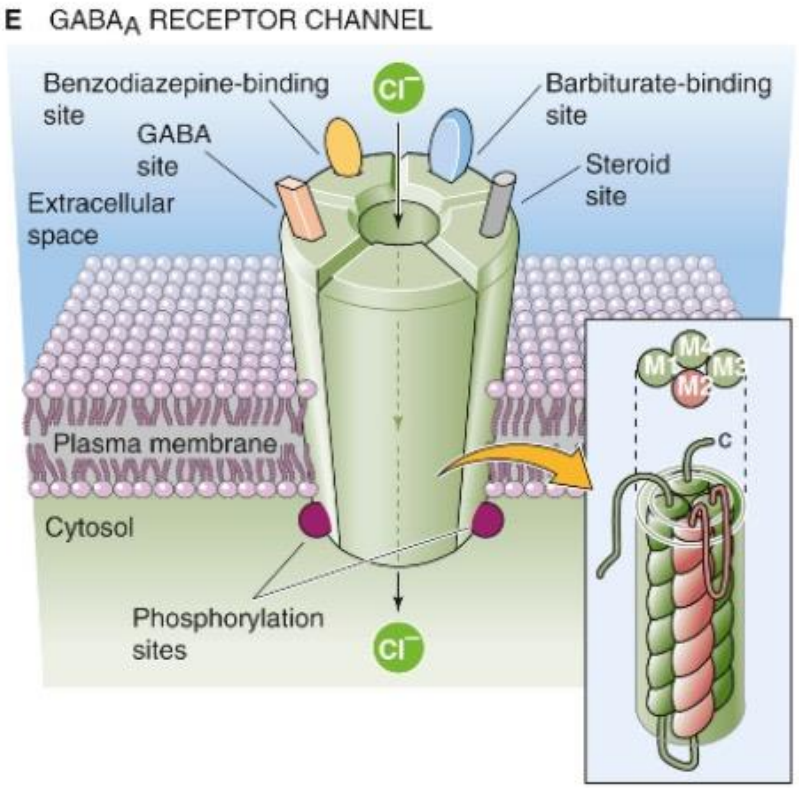
This elevated chloride concentration has a distinctive impact on neuronal physiology.

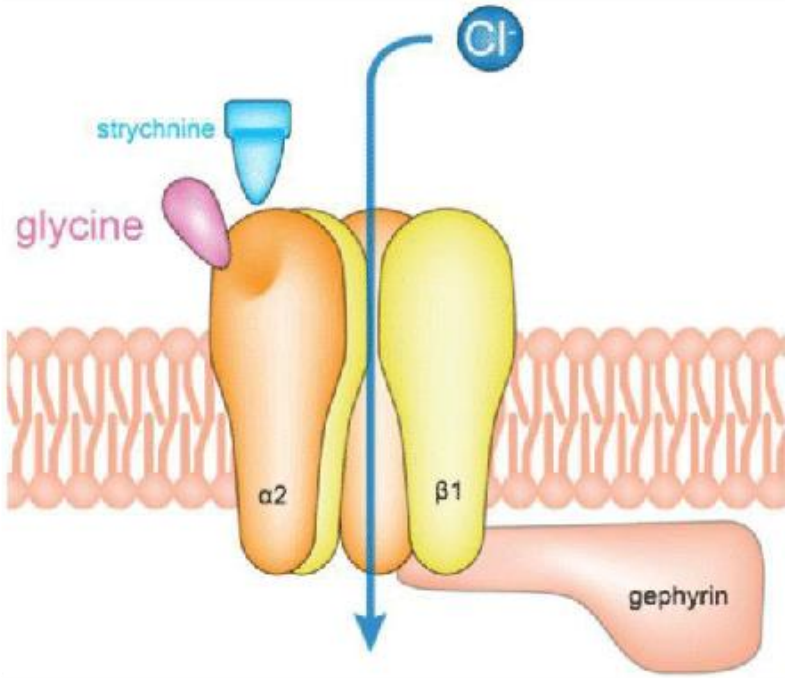
Starting from the second postnatal week, [Cl^-] decreases to around 5 mM, reaching the levels characteristic of mature neurons.



GABAA and Glycine receptors are Cl^- permeable channels







GABAA and Glycine receptors are Cl^- permeable channels

The binding of GABA or Glycine to the receptor opens a central pore, thus enabling Cl^- to move through the inner channel

Cl^- electrochemical gradient determines the direction of its flux

$$V_{DF} = V_m - V_{eq}$$

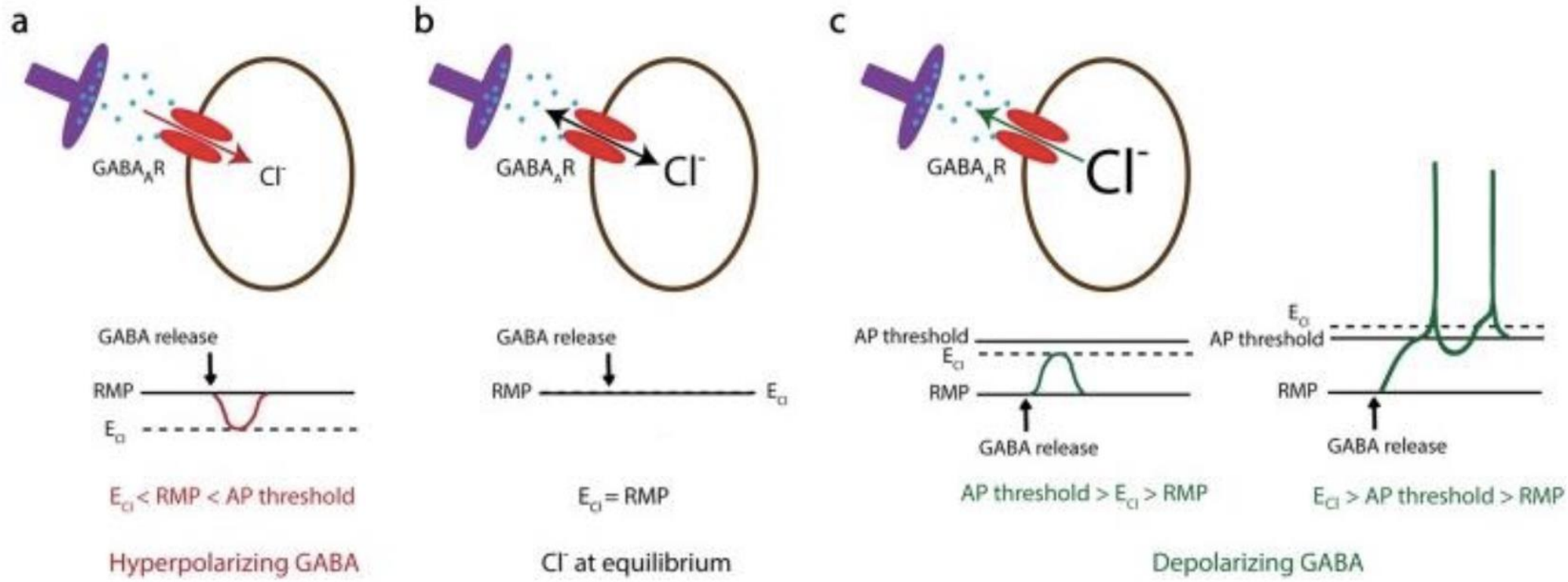
V_{DF}=electrochemical driving force

Vm= membrane potential

Veq= equilibrium potential for the ion of interest

Equilibrium potential (E_Cl) ≈ –78 mV

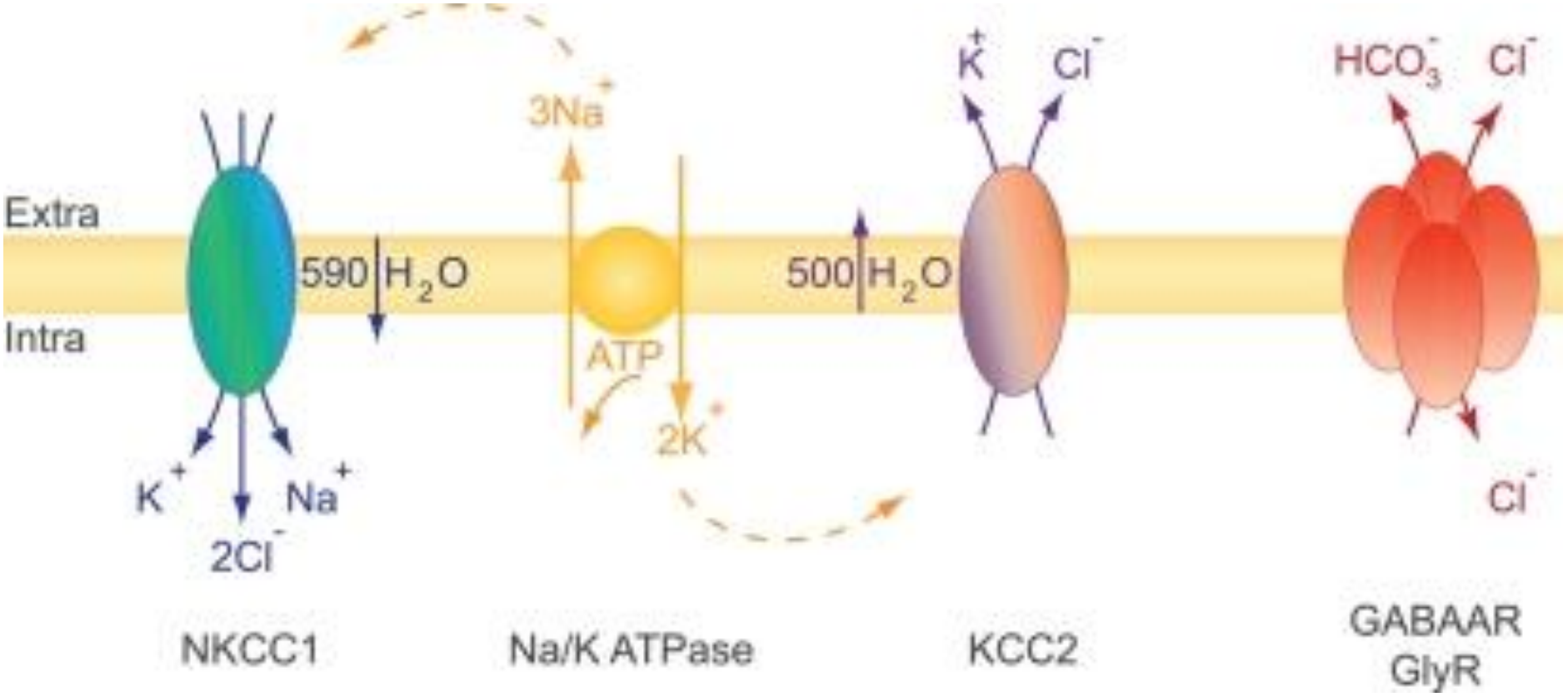
The [Cl-]i dictates the polarity of the current through GABAA and Glycine receptors



Modified from Rahmati et al.,2018

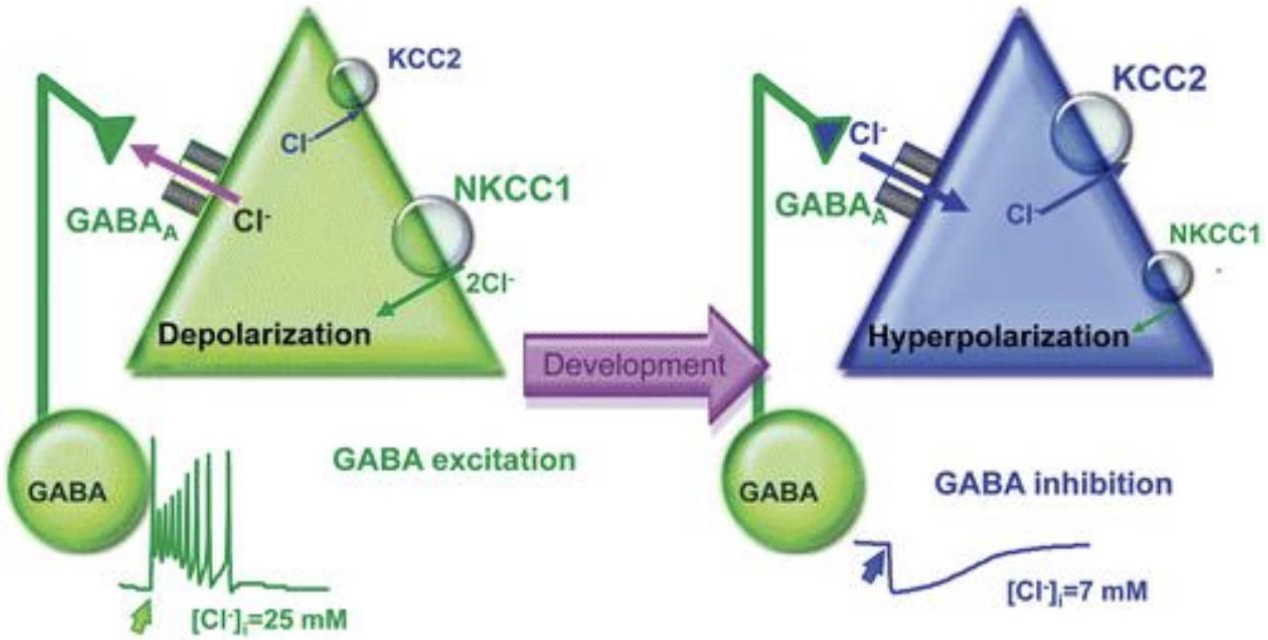
Regulation of Neuronal Chloride Levels: How Is [Cl^-] Regulated in Neurons?

- Chloride balance is maintained by cotransporters.
- **NKCC1:** imports Cl^- (in immature neurons).
- **KCC2:** exports Cl^- (in mature neurons).



Developmental Regulation of NKCC1 and KCC2

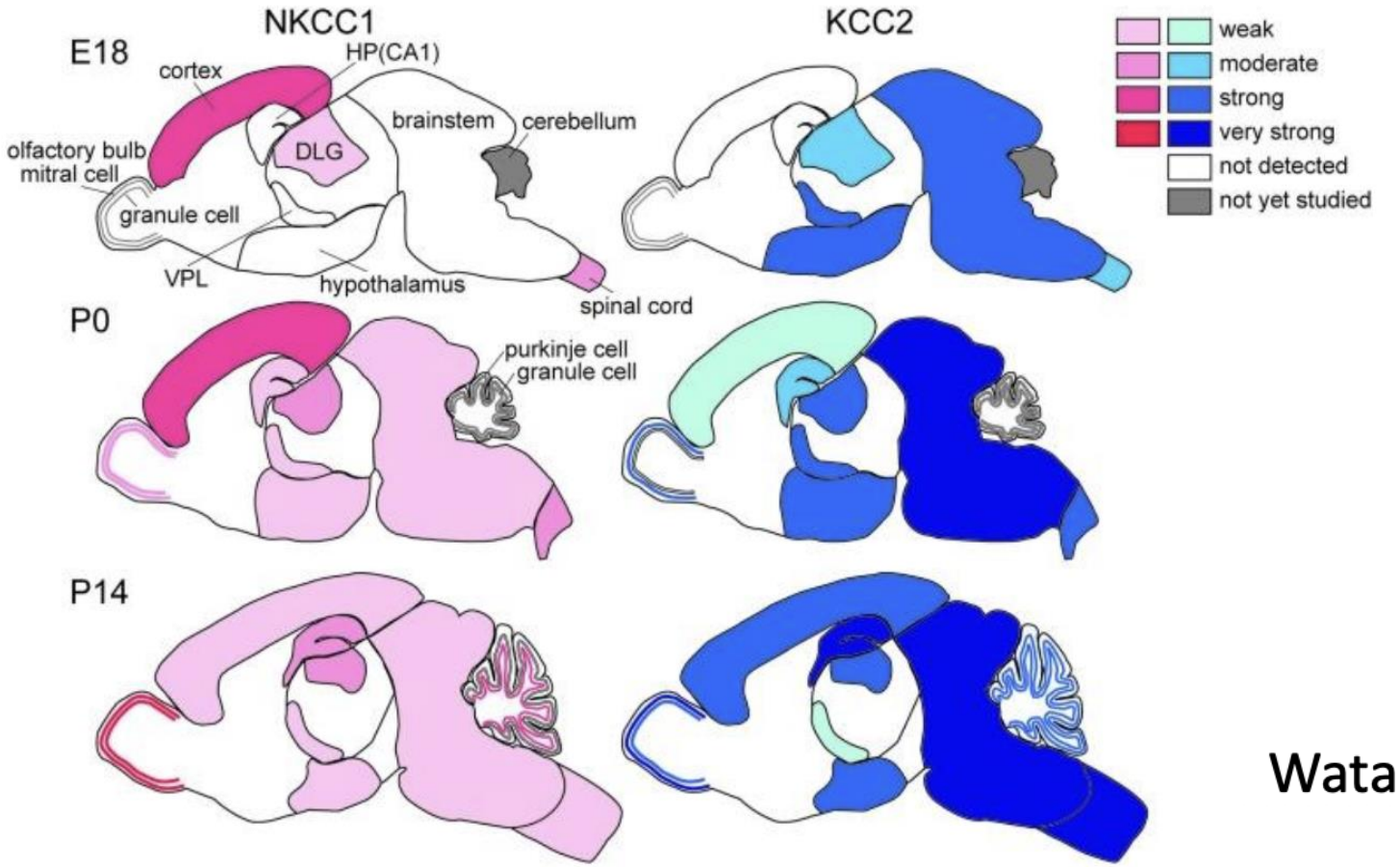
- **NKCC1** is highly expressed early postnatally.
- **KCC2** expression increases during neuronal maturation.
- The switch determines the GABA shift from depolarizing to hyperpolarizing.



Ben-Ari et al.,2012

Developmental Regulation of NKCC1 and KCC2

NKCC1 and KCC2 levels during development

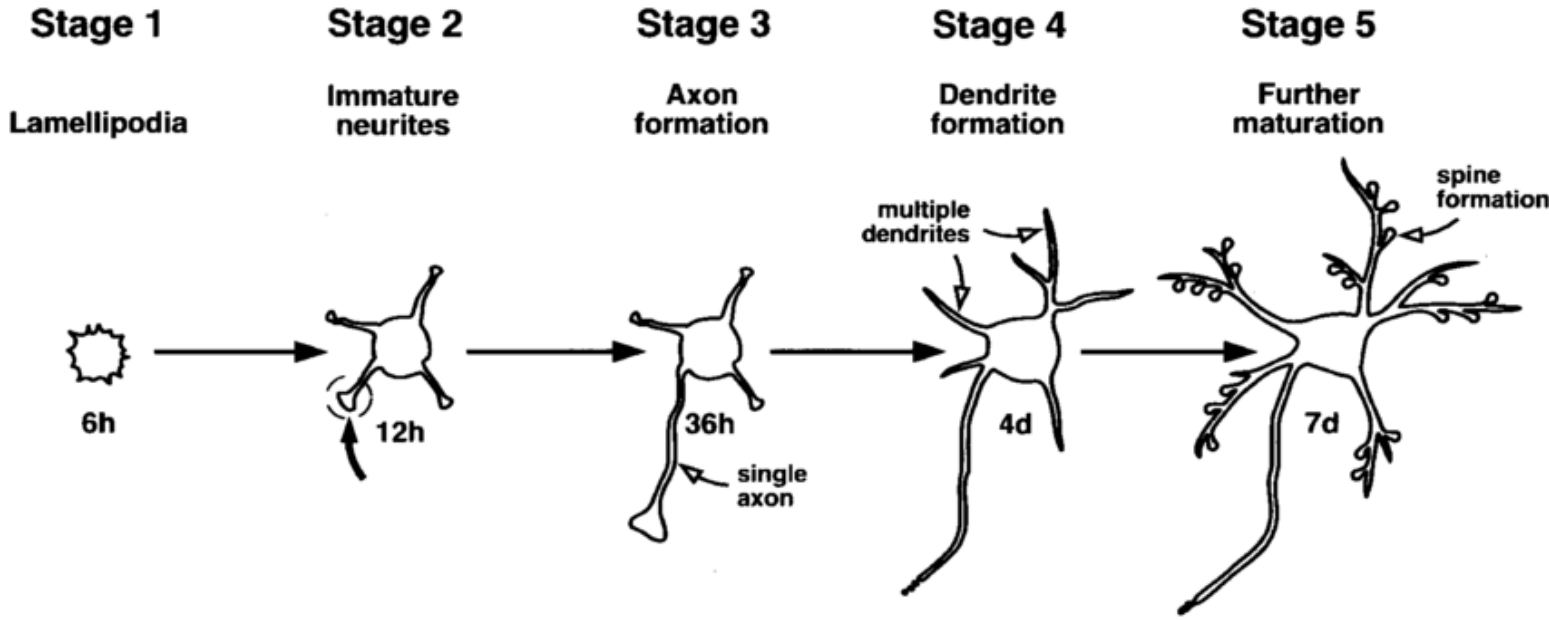


Watanabe & Fukuda,2015

Functional Role of Depolarizing GABA in Development

Functions of Depolarizing GABA in the Developing Brain

- Promotes neuronal growth and differentiation.
- Guides migration and circuit formation.
- Regulates synaptogenesis and activity-dependent refinement.
- Modulates proliferation.

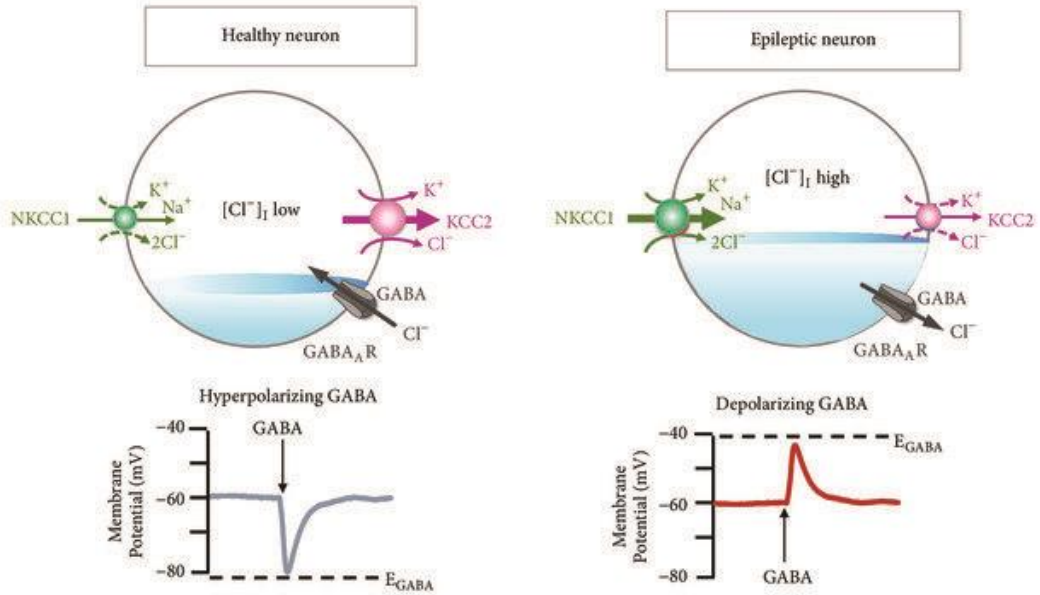


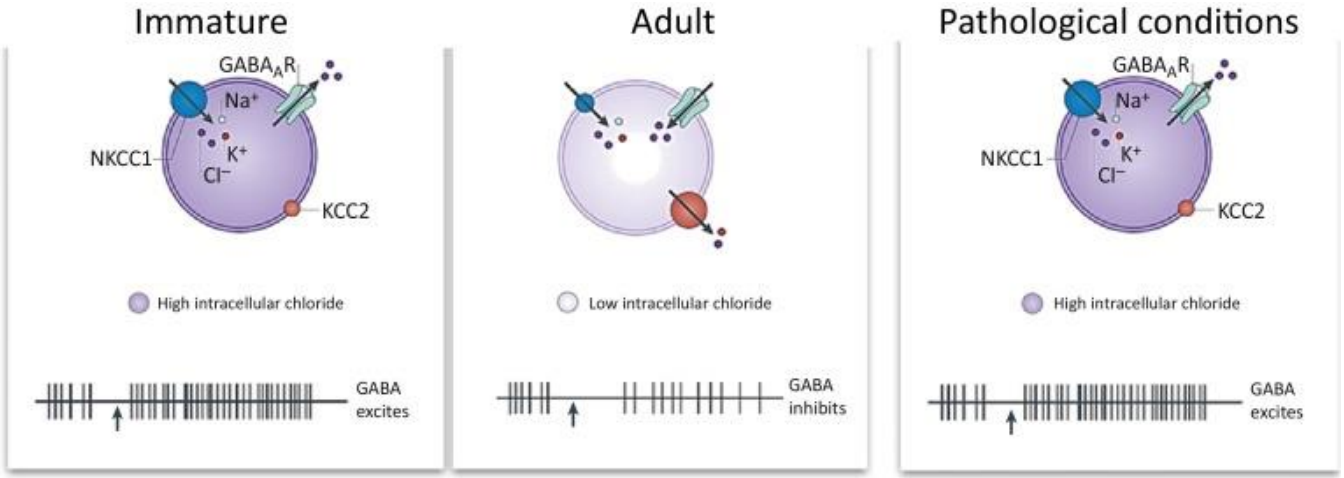
Consequences of Altered Chloride Homeostasis

Disrupted Chloride Regulation in Disease

Altered [Cl^-] gradients contribute to:

- **Epilepsy**
- **Autism spectrum disorders**
- **Schizophrenia**
- **Ischemic stroke**
- **Bipolar disorder**





Trends in Neurosciences

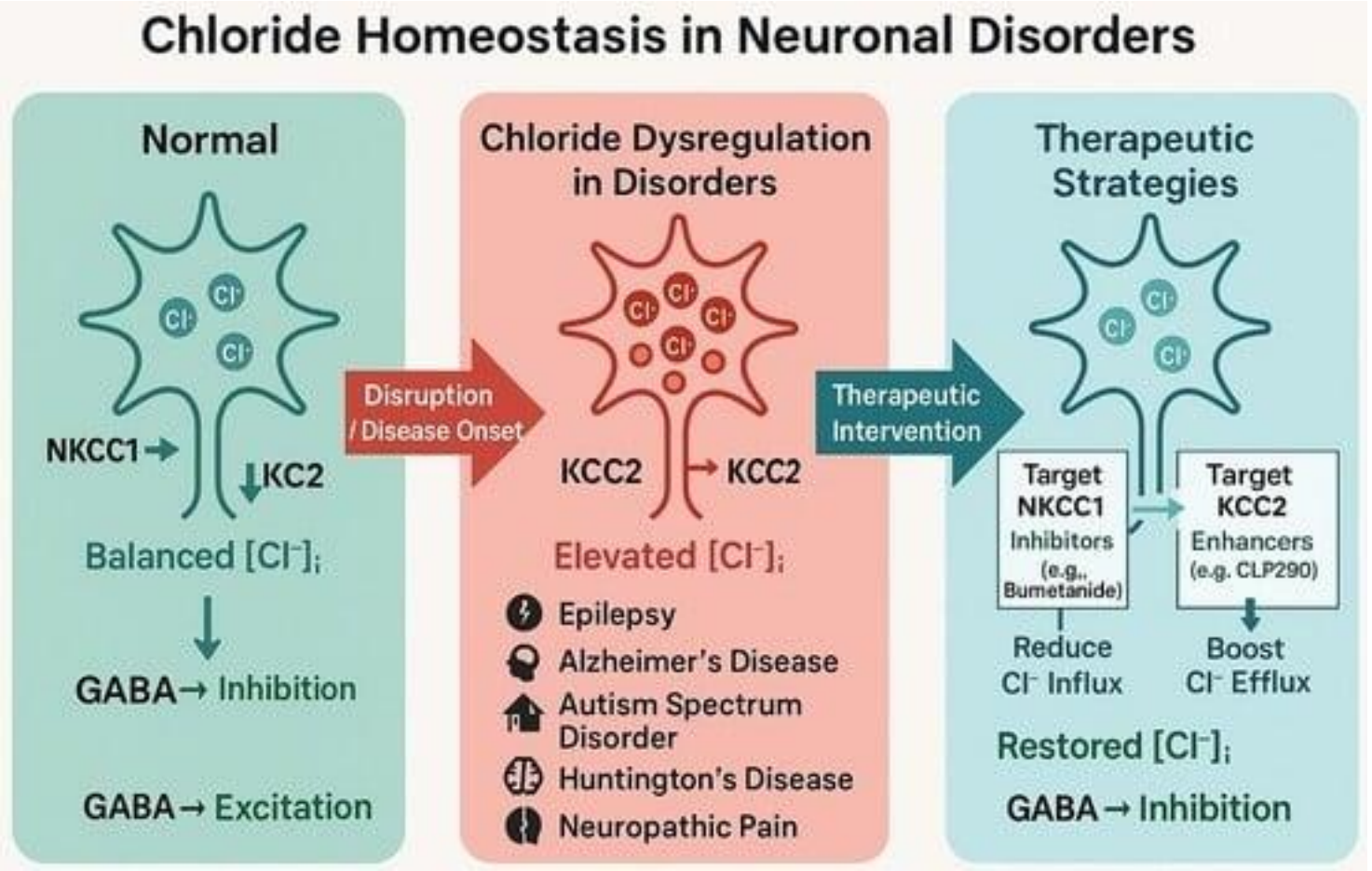
Chloride Homeostasis and Drug Resistance in Epilepsy

Altered Chloride Regulation as a Mechanism of Drug-Resistant Epilepsy

In many forms of refractory epilepsy, GABAergic inhibition becomes ineffective or excitatory.

This results from impaired KCC2 function or increased NKCC1 activity, leading to elevated intracellular [Cl^-].

Reduced Cl^- extrusion shifts EGABA to more depolarized values → GABAA receptor activation no longer inhibits neurons.



Chloride Homeostasis and Drug Resistance in Epilepsy

Altered Chloride Regulation as a Mechanism of Drug-Resistant Epilepsy

Loop diuretic bumetanide, an NKCC1 inhibitor, has been explored to restore Cl^- balance and enhance GABAergic inhibition.

Targeting Cl^- homeostasis represents a promising therapeutic strategy in drug-resistant epilepsy.