γ-aminobutyric acid (GABA)
GABA

• GABA-mediated regulation occurs in nearly all developmental stages
• Found in everything from bacteria to mammals
• First identified in mammalian brain in 1950. In 1959 GABA was shown to be the active substance in a brain extract termed “Factor I”. The “I” was used to designate the inhibitory action of the extract on neuronal activity in a crayfish stretch receptor preparation, which was being used to detect inhibitory and excitatory substances present in the mammalian brain.
**GABA Synthesis**

- Derived from glucose metabolism via the Krebs cycle.
- \(\alpha\)-ketoglutarate is transaminated to glutamate by GABA-T (GABA \(\alpha\)-ketoglutarate transaminase).
- Glutamate is metabolized to GABA by GAD (glutamic acid decarboxylase)
GAD

• GAD is expressed only in cells that use GABA as a neurotransmitter.
• GAD is not present in glia
• Two forms of GAD ($\text{GAD}_{65}$ and $\text{GAD}_{67}$) have been identified in adults; encoded by separate genes.
GAD\textsubscript{67}

- GAD67 is distributed throughout the cell. It may be involved in maintaining basal synaptic levels of GABA and appears to synthesize GABA that is used for functions other than neurotransmission (e.g., GABA acts as a trophic factor).
- Ablation of GAD67 results in >90% reduction in basal GABA levels in the brain, a cleft palate, and neonatal lethality.
GAD$_{65}$

- GAD65 is localized to nerve endings, where its associates with synaptic vesicles. The catalytic activity of GAD65 is regulated by neuronal activity and plays a role in fast modulation of inhibitory neurotransmission in response to an increase in demand.
- GABA synthesized by GAD65 is not required for development and early survival but is critical for fast modulation of neurotransmission in response to an increase in demand.
- GAD65$^{-/-}$ mice show no obvious developmental abnormalities but are prone to epileptic seizures, display increased anxiety, and have defects in handling of environmental stimuli, including light and stress.
VGAT

• 10 TM domains
• VGAT activity depends on the pH gradient (vesicle lumen vs. cell cytosol) and membrane potential.
• VGAT is a co-transporter (symporter) of Cl\(^-\) and GABA (or glycine). During vesicle refilling, two Cl\(^-\) ions are transported with GABA into the synaptic vesicle.
• VGAT\(^{-/-}\) mice are not viable and die between embryonic day (E)18.5 and birth.
GAT

• Na⁺/Cl⁻-dependent transporter; driving force for transport is Na⁺ movement down its concentration gradient (2Na⁺ and 1 Cl⁻ co-transported with GABA)
• 12 TM segments
• Present on both neurons and glia
• terminate GABA-ergic transmission, maintain low ambient extracellular concentrations of GABA and recycle GABA for reuse by neurone
Conditions for a reversed action of the GABA transporter. (a) The direction of GABA transport is dependent on the respective Na⁺ and GABA concentrations in the intracellular and extracellular space and on the membrane potential of the neuron. (b) High firing rates, which result in high intracellular sodium in combination with vesicle depletion (low extracellular GABA), induce reversal of the GABA transporter at the presynapse. The induction mechanism for the reversal of the GABA transporter in glial cells is still unknown. However, GABA release from glial cells will preferentially activate extrasynaptic GABAₐ and GABAₐ receptors and lead to tonic inhibition.
GABA Receptors

$\text{GABA}_A$ - fast; blocked by bicuculline; direct activation of anion channel; targets for anticonvulsants, anxiolytics and sedative-hypnotics.

$\text{GABA}_B$ - slower; bicuculline-insensitive; G protein linked- targets for antispasmodics.

$\text{GABA}_C$ - moderately fast; anion channel; sensitive to picrotoxin but not baclofen, benzos, & bicuculline; predominately retinal; also called $\text{GABA}_A\rho$ receptors
GABA\textsubscript{A} structure

Heteropentamer composed of homologous subunits that share a common structure:
- a large amino-terminal extracellular domain
- four transmembrane domains (TMs)
- a large intracellular domain between TM3 and TM4
$\text{GABA}_A$ structure

GABA binds at the $\alpha$-$\beta$ interface and benzodiazepines bind at the $\alpha$-$\gamma$ interface.
GABA$_A$ Subunits:

- Confer distinct pharmacological and electrophysiological properties
- Have an expression pattern that is specific for certain neurons
- Have distinct cellular localizations
- Likely to have specific functions
- Subunits are all ~55kDa and have 20-30% sequence identity between classes and ~70% identity within a class
**GABA_A receptor subtype distribution**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1β2γ2</td>
<td>HPC-int, CTX , CRB-pyramidal</td>
</tr>
<tr>
<td>α2β2/3γ2</td>
<td>spinal, HPC-pyramidal</td>
</tr>
<tr>
<td>α3βnγ2/γ3</td>
<td>ACh, NE, DA, 5HT neurons</td>
</tr>
<tr>
<td>α2βnγ1</td>
<td><strong>Bergmann glia, limbic nuclei</strong></td>
</tr>
<tr>
<td>α5β3γ2/γ3</td>
<td>HPC-pyramidal</td>
</tr>
<tr>
<td>α6βγ2</td>
<td>CRB-granule</td>
</tr>
<tr>
<td>α6γδ</td>
<td>CRB-granule</td>
</tr>
<tr>
<td>α4βδ</td>
<td>HPC-dentate Thalamus</td>
</tr>
<tr>
<td>others</td>
<td>throughout</td>
</tr>
</tbody>
</table>

more than 80% of all mammalian GABA_A receptors are of the α1β2γ2 type. Clustering of genes can account for some of the expression patterns.
Five subunits from seven subunit subfamilies (α, β, γ, δ, ε, θ and ρ) assemble to form a heteropentameric Cl\(^{-}\)-permeable channel.

Receptors composed of α1, α2, α3 or α5 subunits together with β and γ subunits are benzodiazepine-sensitive, are largely synaptically located and mediate most phasic inhibition in the brain (with the notable exception of extrasynaptically localized α5-containing receptors) (FIG. c). By contrast, those composed of α4 or α6 subunits together with β and δ subunits make up a specialized population of predominantly extrasynaptic receptor subtypes that mediate tonic inhibition and are insensitive to benzodiazepine modulation.
Why extrasynaptic receptors?

• A remnant from ontogeny?
• Early in development little or no synaptic contact.
• Tonic conductance sets the inhibitory gain of neuronal input and output and changes in the gain will profoundly affect neuronal excitability
• δ expression alters with ovarian cycle and with epilepsies. Gaboxadol- δ agonist enhances slow wave sleep
• α5 containing receptors play a role in cognition and learning and memory.
• GABA transport inhibitors: GAT-1 antagonist tiagabine used in epilepsy.
Tonic inhibition and extrasynaptic receptors

- Diffusional inhibitory neurotransmission mediated by GABA\textsubscript{A}Rs located outside the synapses activated by GABA levels in extrasynaptic space.
- Tonic conductance seen in principal neurons and interneurons in CRBLM, CTX, HPC, Thal., Spinal cord. On CRBLM granule cells they are found far from the synapse, but on granule cells of DG they are perisynaptic. They mediate tonic currents in CA1, CA3 CTX layer 5.
- Regulated at \(\mu M\) [GABA] = high affinity sites. Synaptic Rs which react to [GABA] at 1.5-3.0 mM (lower affinity) and decay within a few hundred milliseconds. GABA uptake is the most important regulator of tonic conductance.
- Some Rs can be tonically active without GABA present.
- Tonic Receptors have limited desensitization and contain either \(\delta\) or \(\alpha5\) or \(\epsilon\) or only \(\alpha\beta\) containing receptors.
Relative distribution of GABA receptors
How many GABA\textsubscript{A} receptor subtypes are there?

19. Each is encoded by a separate gene but many in clusters of genes. \(\alpha\) and \(\beta\) subunits, typically share only 30–40\% sequence identity. \(\epsilon\) and \(\theta\) subunits display 50\% identity to \(\gamma\) and \(\beta\) subunits, respectively
Trafficking of $\text{GABA}_A$ receptors.

GABA subunits are synthesized and assembled into pentameric structures in the endoplasmic reticulum (ER). PLIC1 causes accumulation at the synapse by blocking ubiquitylation in the ER.

Trafficking through the Golgi network and to the plasma membrane are facilitated by N-ethylmaleimide sensitive factor (NSF) and brefeldin A inhibited GDP/GTP exchange factor 2 (BIG2), which bind $\beta$ subunits, and GABAA R associated protein (GABARAP), which binds $\gamma_2$ subunits.

Golgi specific DHHC zinc finger domain protein (GODZ) palmitolates gamma subunits to deliver the receptor to the membrane.
<table>
<thead>
<tr>
<th>Protein</th>
<th>Interacting GABA&lt;sub&gt;A&lt;/sub&gt; subunits</th>
<th>Subcellular localization</th>
<th>Putative functions</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP2</td>
<td>β and γ</td>
<td>Clathrin-coated pits</td>
<td>Receptor endocytosis</td>
<td>87,89,92,93</td>
</tr>
<tr>
<td>BIG2</td>
<td>β1–3</td>
<td>Golgi, trafficking vesicles, plasma membrane</td>
<td>Receptor trafficking</td>
<td>49</td>
</tr>
<tr>
<td>GABARAP</td>
<td>γ2</td>
<td>Mainly in Golgi</td>
<td>Receptor trafficking</td>
<td>22–24, 26–33</td>
</tr>
<tr>
<td>Gephyrin</td>
<td>α2</td>
<td>Synaptic sites</td>
<td>Receptor clustering and stabilization at synaptic sites</td>
<td>55,69–71, 75–77,79</td>
</tr>
<tr>
<td>GODZ</td>
<td>γ</td>
<td>Mainly in Golgi</td>
<td>Palmitoylation of γ subunits and receptor trafficking</td>
<td>46,48</td>
</tr>
<tr>
<td>GRIF1 and 2 (TRAK1 and 2)</td>
<td>β2</td>
<td>Intracellular compartments</td>
<td>Receptor trafficking</td>
<td>51,54</td>
</tr>
<tr>
<td>HAP1</td>
<td>β1–3</td>
<td>Endosomes</td>
<td>Post-endocytic sorting of GABA&lt;sub&gt;A&lt;/sub&gt;Rs</td>
<td>88</td>
</tr>
<tr>
<td>NSF</td>
<td>β1–3</td>
<td>Golgi and plasma membrane</td>
<td>Receptor trafficking</td>
<td>34</td>
</tr>
<tr>
<td>PLIC1</td>
<td>α and β</td>
<td>Intracellular compartments</td>
<td>Modulates receptor cell-surface expression</td>
<td>18</td>
</tr>
<tr>
<td>PRIP1 and 2</td>
<td>β1–3 and γ2</td>
<td>Intracellular compartments</td>
<td>Regulation of receptor phosphorylation/trafficking</td>
<td>39,40,42,44</td>
</tr>
<tr>
<td>Radixin</td>
<td>α5</td>
<td>Plasma membrane</td>
<td>Receptor clustering and binding to actin cytoskeleton</td>
<td>84</td>
</tr>
</tbody>
</table>

AP2, clathrin-adaptor protein 2; BIG2, brefeldin-A-inhibited GDP/GTP exchange factor 2; GABA<sub>A</sub>, γ-aminobutyric acid; GABA<sub>A</sub> R, GABA type A receptor; GABARAP, GABA<sub>A</sub> receptor-associated protein; GODZ, Golgi-specific DHHC zinc-finger-domain protein; GRIF, GABA<sub>A</sub>R-interacting factor; HAP1, Huntington-associated protein 1; NSF, N-ethylmaleimide-sensitive factor; PLIC1, protein linking IAP to the cytoskeleton; PRIP, phospholipase-C-related catalytically inactive protein.
Regulation of GABAA receptor endocytosis and post-endocytic sorting.
Dysregulation of GABAA receptor trafficking in neurological disease.
GABA during development

- 65% of neocortical GABA neurons arise from the neocortical proliferative zone and are deposited in the developing cortical plate.
- GABA responsive precursor cells are neurogenic radial glia. These have a higher apparent affinity for GABA and are insensitive to receptor desensitization and slower decay kinetics.
- The first subunits expressed are α4 β1 and γ1. There is a clear shift in subunit expression between proliferative and post mitotic zones. Post mitotic neurons are almost exclusively α3 β2/3 and γ3. Perinatal cortical plate expresses α1,2,5 δ and γ2. γ2-subunit results in increased desensitization rates and is necessary for postsynaptic clustering. α1-subunit expression is low perinatally and increases postnatally, it is associated with an increase in decay kinetics of GABA mediated synaptic currents.
A General Principle

- Developmental changes in channel kinetics are a general phenomenon for ligand gated ion channels. Immature GABAA, nAhRs, Glu, Gly all have slower synaptic decay kinetics and/or longer channel open times than seen in mature receptors.
Development of GABA actions

If there is a mismatch between the strength of GABA and glutamate, then it could result in prevention of growth and synapse formation, if GABA prevails, or toxicity, if glutamate prevails.

The solution is:

1) GABA is initially excitatory due to high intracellular Cl⁻ gradient
2) GABA-releasing and glutamate synapses are formed sequentially
3) A network driven pattern of electrical activity in all developing circuits generated by GABA-mediated giant depolarizing potentials, permitting oscillations of intracellular calcium
4) When the density of glutamate and GABA synapses is sufficient, a Cl⁻ extruding system is activated to convert GABA to an inhibitory
GABA on Development

• GABA is excitatory early in development and then becomes inhibitory, transition results from a shift in the equilibrium potential.
• The timing of this shift is necessary for total dendritic branch length and numbers of branches.
• Eliminating the excitatory action of GABA by premature KCC2 expression during perinatal development impaired the morphological maturation of cortical neurons, without affecting their radial migration to layer II/III of the somatosensory cortex. (Cancedda et al., 2007).
Allosteric Modulation

1909 Paul Ehrlich “Substances can only be anchored at any particular organism if they fit into the molecule of the recipient complex like a piece of mosaic finds its place in a pattern.”

1937 A.J. Clark “It is necessary to postulate a complex receptor with which two drugs could unite without displacing the other drug.”

The way in which two drugs could bind to and interact on a receptor surface without physical contact is by allostery.
GABA-A Positive allosteric modulators

barbiturates

? β

γ

α

GABA

neurosteroids

GABA benzodiazepines

Alcohol, Other anesthetics
Allosteric Modulators

Neurosteroids- are brain derived metabolites of ovarian steroids and corticosteroids. Low doses modulate high dose has direct effects without GABA.

Barbiturates-increase channel open duration-no change in frequency of conductance. Can act directly w/o GABA at high concentrations.

Benzodiazepines-increase channel opening frequency requires GABA binding. Needs a $\gamma$ subunit.

When there is a low GABA efficacy = more effective neuromodulation. But regional distributions can obscure a positive modulation effect overall.
Spectrum of benzodiazepine receptor ligands

- Agonists
  - midazolam
- Partial Agonists
  - bretazenil (Ro 16-6028)
  - flumazenil (Ro 15-1788)
- Antagonists
  - Ro 15-4513
- Partial Inverse Agonists
  - Ro 19-4603
- Inverse Agonists

Intrinsic efficacy

Benzodiazepine receptor
- GABA\textsubscript{A} receptor affinity
- Chloride channel gating
Distribution of diazepam sensitive GABA-A receptor subtypes (subunits \(\alpha1,2,3,5\)). White = highest, blue = no expression.
Benzodiazepine pharmacology of GABAA receptor subtypes

<table>
<thead>
<tr>
<th>Pharmacological effect</th>
<th>Receptor involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiolysis</td>
<td>α 2- containing</td>
</tr>
<tr>
<td>Sedation</td>
<td>α1- containing</td>
</tr>
<tr>
<td>Anticonvulsive</td>
<td>α1-containing and those not containing α1</td>
</tr>
<tr>
<td>Anterograde amnesia</td>
<td>α1-containing</td>
</tr>
</tbody>
</table>
GABA-C Receptors

GABA-C

- homooligomers
- retinal bipolar cells, Sup. Colliculus and hpc
- microtubule assoc. protein.
- chromosome 6
- not modulated
- Activates cAMP-protein dep. kinase
GABA A responses blocked competitively by bicuculline and noncompetitively by picrotoxin.

GABA C homo-oligomeric or pseudo-homo-oligomeric assembly selectively activated by cis-4-aminocrotonic acid blocked by 1,2,5,6-tetrahydropyridine 4-yl methylphosphinic acid.
GABA-B Receptors

- 7TM heterodimer (first demonstrated)
- pain epilepsy, spasticity
- G-protein coupled -adenyl cyclase
- inward rectifying K+ and N, P/Q Ca++ channels
- Coil-coil C-terminal domains
- baclofen, hydroxysaclofen
- CTX, HPC, CRBLLM,Thal., DRG
- R1 Ser246  R2 Pro246 binding site globular domains.
GABA B functions

- Plays a role in presynaptic release and activates $K^+$ channels postsynaptically.
- Presynaptic GABAB-Rs regulate the probability of neurotransmitter release and shape the synaptic glutamate signal.
- Postsynaptic GABAB-Rs activate inwardly rectifying K channels to generate a shunting conductance or hyperpolarization.
- GABAB-Rs also directly modulate postsynaptic NMDA-R Ca signals in spines (via a PKA pathway) and does not affect NMDA or AMPA synaptic currents.
- Thus, in addition to modulating the electrical properties of neurons, GABAB-Rs also have the capability to influence the biochemical signaling cascades generated at synapses.
Development of GABA-B synapses

• Functional GABA-B receptor mediated postsynaptic responses do not occur in neocortex until after the second postnatal week but presynaptic actions are active perinatally.

• Pre-synaptically localize R1a subunit is expressed earlier than the R1b subunit.

• GABA-B activation can influence movement and migration of immature cortical neurons suggesting that expression and activation of GABA-B receptors occurs during embryological period.