Neurotransmitter receptors

- Neurotransmitter receptors
  1. **ionotropic**, a 'ligand-gated' ion channels
  2. **metabotropic**, a G-protein coupled receptors that modulate separate ion channels
- Neurotransmitter receptor activation following ligand (neurotransmitter) binding results in the opening of ion channels and ionic flux. This ion flux is the postsynaptic current (or 'end plate' current for a muscle cell)
- These postsynaptic currents result in depolarization or hyperpolarization of the membrane potential (postsynaptic potential or 'end plate' potential) depending on the **types of ions** flowing through the channel pores and the ions' respective **electro-chemical driving forces**

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Speaker notes

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nAChR

- Pentamer- 5 subunits to make a pore. Selective for cations
  - Pore diameter 10x greater than voltage-gated Na^+ channels (3 nm vs 0.3 nm)
- Nicotine mimics ACh to stimulate receptor, an agonist
- nicotinic ACh receptors (nAChR) produce excitatory postsynaptic potentials (EPSPs or EPPs)
- Many toxins specifically bind and block nAChR; these are antagonists
  - alpha-bungarotoxin (snake venom)– binds to alpha subunit of nAChR very tightly and prevents ACh from activating it

Speaker notes

As we've shown in our examples earlier the nAChR receptor is a non-selective cation channel. Or another way to think of it is that it is selective for cations.

5 subunits

nAChR permeable to Na+, K+, and Ca2+

In physiological solution, calcium flux estimated to be 2% of total current through nAchR. For comparison calcium flux is estimated to be 7% of the current in the voltage gated L-type calcium ion channel. But with high density clustering of many nAchRs at muscle end plate synapses, total calcium flux through these channels could influence the local environment significantly https://doi.org/10.1523/JNEUROSCI.10-10-03413.1990

This Ca^2+^ permeability depends on subunit composition of the nAchR pentamer. mammalian α9α10 receptors receptors show higher calcium ion selectivity (important function in cochlear hair cells) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4245820/

from [^Picciotto:2000]:

> *some subtypes of nAChR in the brain (those containing the b2 subunit) are located diffusely throughout the membrane of the neuron, with no obvious concentration at the synaptic junction (Hill et al. 1993). https://us02web.zoom.us/j/86077044985?pwd=USt1SGJ6Wm1VSitncWlmTjgzSmZrQT09 a number of alpha and beta subunits have expression throughout brain (medulla, superior colliculus, cortex, beta2 subunit expression 'very high' in thalamus). Only alpha3 KO mice have high mortality [#Picciotto:2000].*

[^Picciotto:2000]: Picciotto, M. R., Caldarone, B. J., King, S. L., and Zachariou, V. (2000). Nicotinic receptors in the brain. Links between molecular biology and behavior, Neuropsychopharmacology, 22(5), 451-65. PMID 10731620

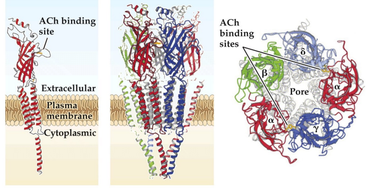
Low (nM) concentrations of nicotine are found in the blood of moderate smokers (Henningfield et al. 1983). These are sufficient to enhance excitatory transmission in cultures of neurons from the medial habenula or the hippocampus (Gray et al. 1996; McGehee et al. 1995) [#Picciotto:2000]

Many effects of nicotine probably through presynaptic or preterminal nAChRs instead of through postsynaptic AChRs (Léna et al. 1993; Marshall et al. 1997; McGe- hee et al. 1995; Summers and Giacobini 1995; Vidal and Changeux 1993; Wonnacott et al. 1990; Yang et al. 1996) [#Picciotto:2000]

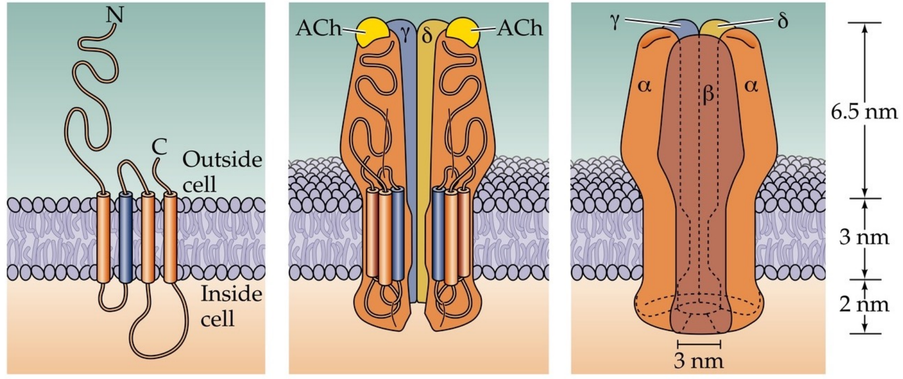
Most effects of nicotine go through nAChR

Structure of the nACh receptor

- 5 subunits come together to make a pore
- Each subunit has 3-4 membrane spanning domains
- In muscles the receptor has 2α, β, δ, γ, ε subunits. The α subunits bind ACh, both need to be bound for channel to open. α subunits also binds bungarotoxin and nicotine



Neuroscience 5e Fig. 6.3



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Speaker notes

The alpha subunits bind ACh.

Muscle nAChR

- Pentamers of 2α1, β1, γ, δ in fetal mammals vs. 2α1, β1, δ, ε in adult mammal
- ACh, nicotine, curare, and bungarotoxin binding sites are on the α1 subunits
- Multiple isoforms for each subunit, depending on which isoform is in channel get different properties

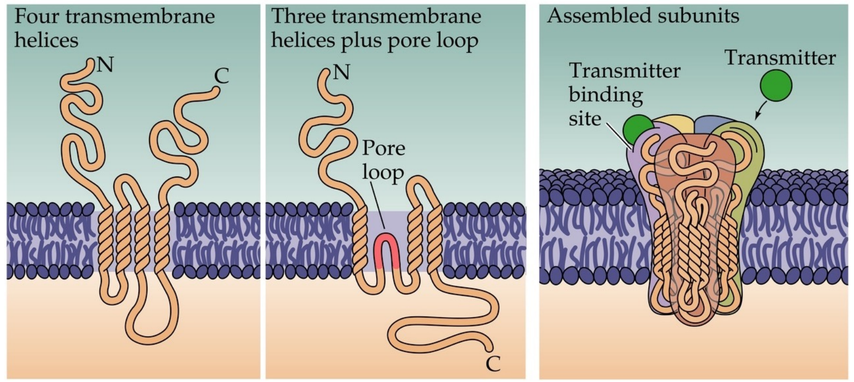
In neurons its slightly different. 5 subunits 3α:2β. Bungarotoxin only inhibits muscle nACh receptors

Changes in subunit composition during development.

curare is a competitive antagonist.

Ligand gated ion channels

- Built up of 4 or 5 monomers
- Each monomer spans the membrane 3 or 4 times
- Each monomer contributes properties
- Mixing and matching from a large pool of monomer isoforms creates receptors with different properties



Neuroscience 2e 2001

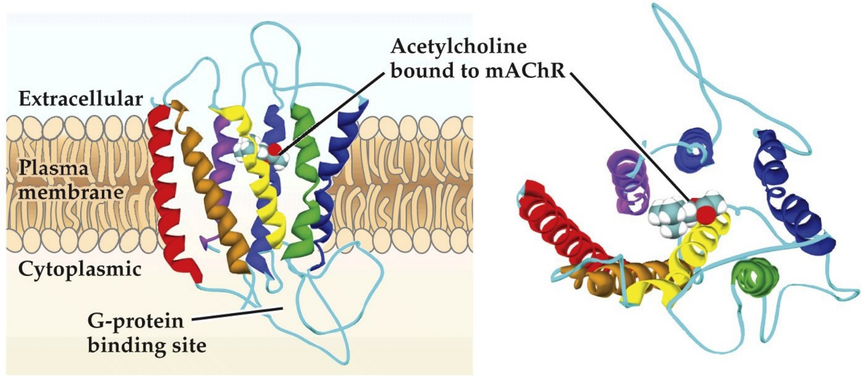
Speaker notes

Ligand gated channels in general are made up of 4 or 5 subunit monomers.

Muscarinic ACh receptors

- Muscarine, a poisonous mushroom alkaloid, is an agonist
- Metabotropic (G-protein coupled receptors), mediates most ACh effects in the brain
  - typically linked to K^+ channel opening that results in inhibitory postsynaptic potentials (IPSPs)
- 5 or so isoforms
- mAChR blockers are used for pupil dilation (atropine), motion sickness (scopolamine) and asthma treatment (ipratropium)

Amanita muscaria, Onderwijsgek, CC BY-SA 3.0 nl



Neuroscience 5e Fig. 6.4

Speaker notes

- seven transmembrane spanning domains
- coupled to G proteins
- causes variety of slow postsynatpic responses
- highly expr in striatum and varous forebrain regions
- activate inward rectifier K^+ channels (allow more K current at hyperpolarized potentials)
- or Ca^{2+} activated K^+ channels
- exert inhibitory influence on dopamine mediated motor effects
- though in hippocampus mAChRs are excitatory, acting by closing KCNQ type K^+ channels

Also found in ganglia of PNS. Mediate peripheral cholinergic responses of autonomic effector organs like heart, smooth muscle, exocrine glands. Inhibition of heart rate by vagus nerve.

- KCNQ...
- mutations in four out of five KCNQ genes underlie diseases including cardiac arrhythmias, deafness and epilepsy.
- http://www.ncbi.nlm.nih.gov/pubmed/11252765
- KCNQ/M (Kv7) very slow voltage-gated K channels, suppress repetitive firing
- Inhibited by ACh and many neurotransmitters, but enhanced by others
- http://physiolgenomics.physiology.org/content/22/3/269

atropine : from deadly nightshade family : dilate pupils, treat slow heart rate : anticholinergic, muscarinic antagonist : inhibits parasympathetic nervous system : WHO essential medicine

scopolamine : colorless, odorless alkaloid drug : competitive antagonist, antimuscarinic : motion sickness, postoperative nausea and vomiting : WHO essential medicine : from flowering plant genus *Scopolia*

ipratropium : opens up medium and large airways of lungs by causing smooth muscles to relax : anticholinergic and muscarinic antagonist : treats obstructive pulmonary disease and asthma : WHO essential medicine

Clitocybe dealbata : muscarine can occur in this species sufficient concentrations to be deadly : commonly found growing in lawns in North America an Europe : white flat topped

Amanita muscaria, Onderwijsgek, CC BY-SA 3.0 nl : red mushroom with white speckles : muscarine first isolated from this species in 1869 : muscarine actually only in trace amounts in this species : muscimol is a predominent compound from this mushroom though

Glutamate receptors

- Both ionotropic and metabotropic
- Ionotropic– AMPA/Kainate receptors and NMDA receptors (named after the agonists that stimulate them)
  - All are non-selective ion channels with E_{rev} close to 0 (above threshold therefore excitatory)
  - Formed from an association of 4 subunits. There are a variety of possible subunits which can combine to create many receptor isoforms

Speaker notes

- form tetramers
- Kainate receptors, or KARs, are ionotropic receptors that respond to the neurotransmitter glutamate.
- Kainic acid (kainate) is a natural marine acid present in some seaweed. Kainic acid is a potent neuroexcitatory amino acid that acts by activating receptors for glutamate
- Domoic acid is a structural analog of kainic acid and proline.
- Domoic acid (DA) is a kainic acid analog neurotoxin that causes amnesic shellfish poisoning

Glutamate receptor subunit types

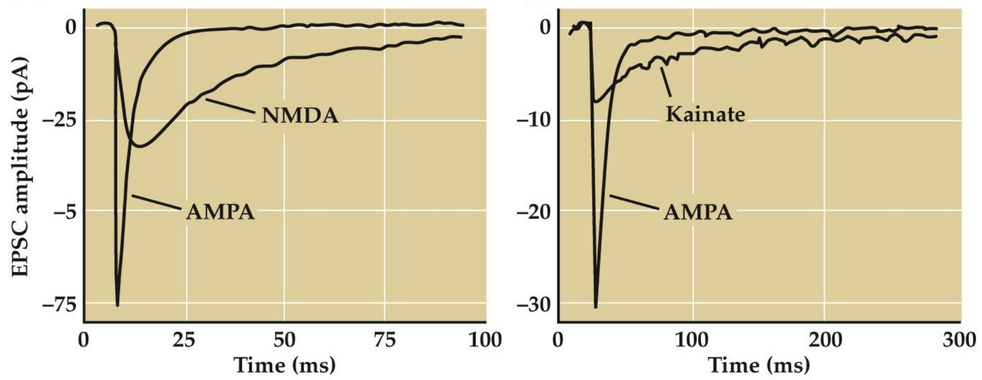
AMPA	Kainate	NMDA	Metabotropic
GluR1	GluR5	NR1	mGluR1
GluR2	GluR6	NR2A	mGluR5
GluR3	GluR7	NR2B	mGluR2
GluR4	KA1	NR2C	mGluR3
GluR5	KA2	NR2D	mGluR4
		NR2D	mGluR6
		NR3A	mGluR7
		NR3B	mGluR8

Speaker notes

No notes on this slide.

AMPA/Kainate receptors

- ionotropic glutamate receptors that allow Na^+ or K^+ ion flow
- multi-subunit channels (typically as heterotetramers from a pair of GluR2 plus a pair of GluR1, GluR3, or GluR4)
- evoke EPSPs that are large and fast
- AMPA receptors are more common than Kainate receptors



Neuroscience 5e Fig. 6.6

Speaker notes

- Each AMPAR is composed of 4 subunits and has four sites to an agonist like glutamate can bind (one per subunit)
- alternative splicing of each of the 4 subunit genes can result in a number of more isoforms
- GluR1 and GluR2 especially important in synaptic plasticity by being upregulated
- Non-selective cation channel like nAChr, but tetramer and less calcium permeability. mRNA editing of a intramembrane domain of the GluR2 subunit switches a glutamine to an arginine. This post translational modification results in AMPA receptors that have resistance to calcium permeability. Though AMPA-R usually contain one or more GluR2 subunits, ones that are missing GluR2 subunits do have more calcium permeability and may be important in developing neurons and early forms of synaptic plasticity in some neurons https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3092818/

NMDA receptor

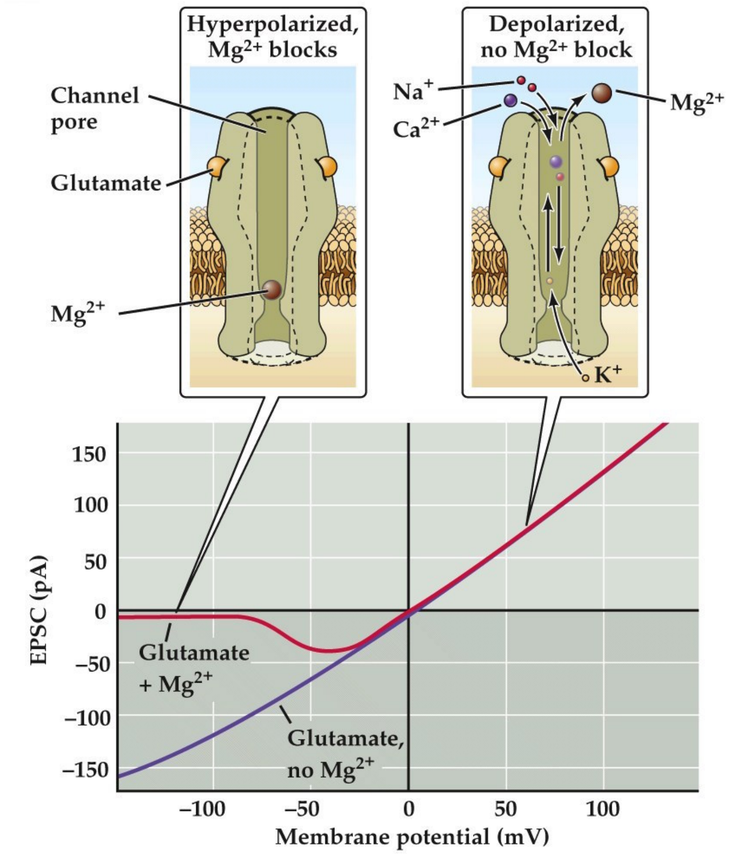
- Glutamate receptors that allow flow of Ca^{2+} as well as Na^{+} and K^{+}. As a result EPSPs produced by NMDA receptors can increase the Ca^{2+} concentration in the neuron. Acts as a second messenger to activate cellular processes
- Formed as a heterotetramer of 4 subunits (typically 2 NR1 and 2 NR2 subunits)
- Needs a co-agonist, glycine to open channel
- Blocked by Mg^{2+} in the pore during hyperpolarizing conditions. Depolarization can remove block. Needs either a bunch of presynaptic cells to fire at the same time or repeated firing of presynaptic cell to open channel
- Key component of a model for learning
- Evoke EPSPs that are slow and long lasting
- PCP “angel dust” binds and clogs channel. Get symptoms similar to schizophrenia

Speaker notes

- NR1 has the glycine agonist binding site
- NR2 has the glutamate binding site
- NR2B predominant in developing brain before switching to NR2B being predominant in adults
- PCP “angel dust” binds and clogs channel. Get symptoms similar to schizophrenia. Some hypothesize NMDA receptor is involved in this disease.

NMDA receptors require removal of a voltage-dependent Mg^{2+} block

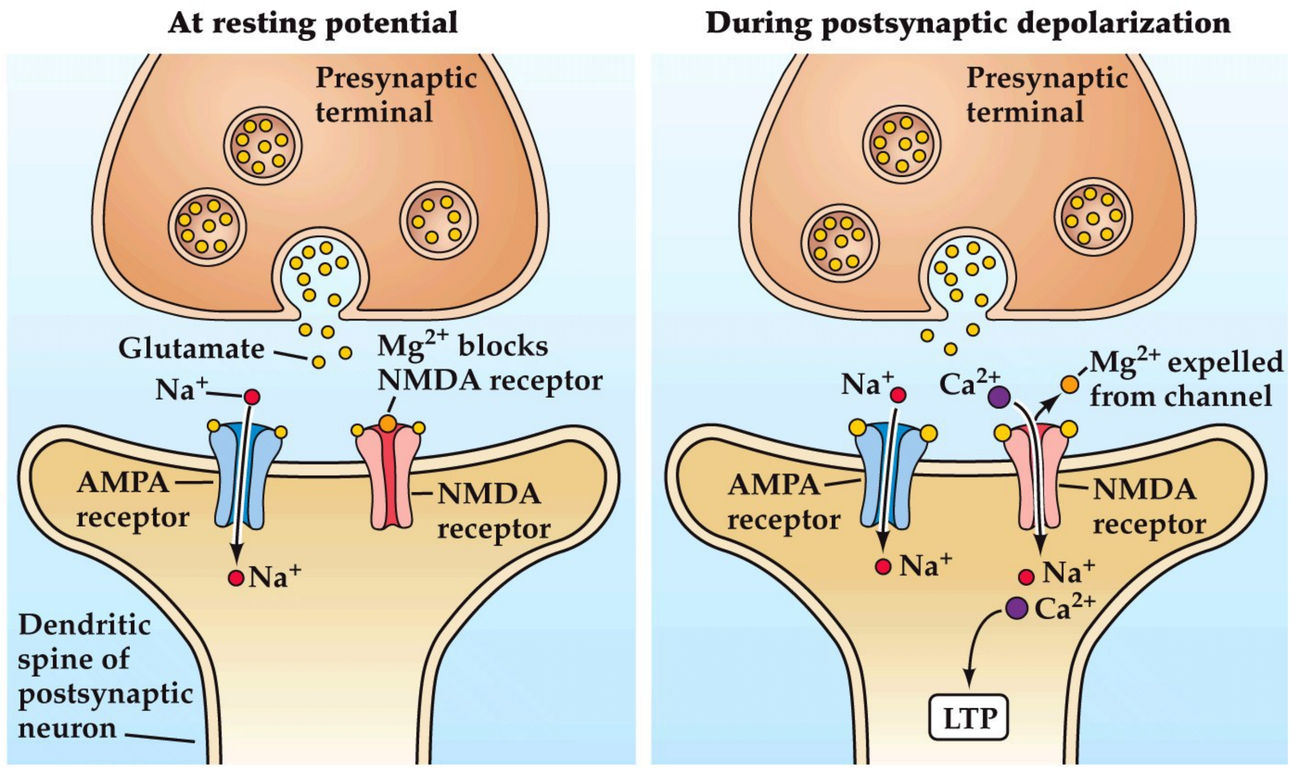
- Mg^{2+} blocks pore– removed by depolarization
- This is possible because AMPA and NMDA receptors are often at the same synapse



Neuroscience 5e Fig. 6.6

Speaker notes

NMDA receptors can open only during depolarization



Neuroscience 5e Fig. 8.10

Speaker notes

chp 8 more on NMDA-R mediated mechanisms involved in learning and memory, adv neuroscience.

Metabotropic glutamate receptors (mGluRs)

- Large class of receptor subtypes
- G-protein coupled
- Often leads to inhibition of postsynaptic Ca^{2+} and Na^{+} channels
- But sometimes inhibitory sometimes excitatory

Speaker notes

- group I (mGluR1, mGluR5) associated with IP3 signaling and ER Ca2+ channel opening. Also associated with Na+ and K+ channels. Can result in EPSPs but can also result in IPSPs.
  - activated selectively by 3,5-dihydroxyphenylglycine (DHPG) (but not other groups)
- group II mGluRs 2 and 3 prevent formation of cAMP (by activating Gi that inhibits adenylyl cyclase) and result in presynaptic inhibition (not apparently affecting PSPs directly)
- group III, including mGluRs 4, 6, 7, and 8 prevent formation of cAMP and have similar functional pathway and consequences as group II

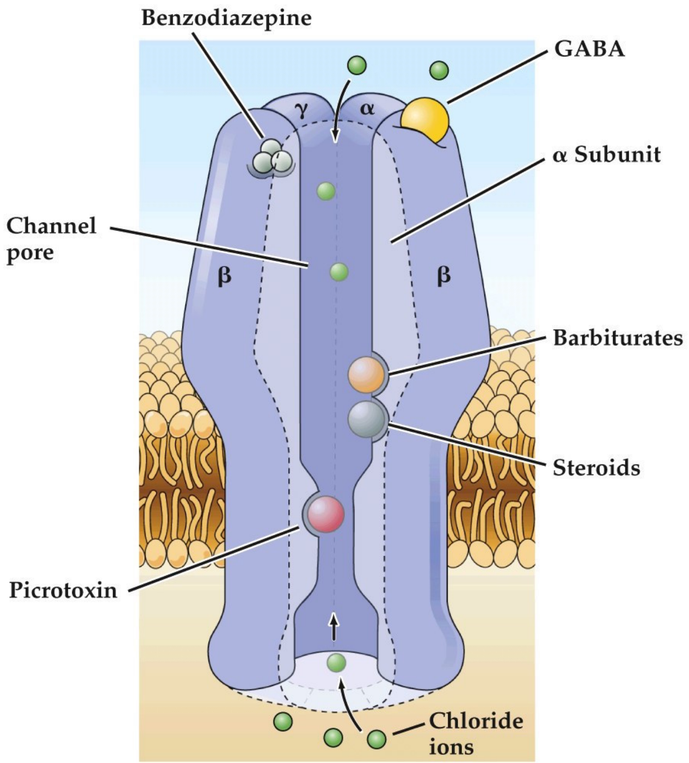
GABA receptors

- Three types of GABA receptors: A, B and C
- A and C are ionotropic, B is metabotropic
- A and C are inhibitory because their channels are permeable to Cl^-. The flow of Cl^- into the cell lowers the potential. E_{rev} is less than the threshold potential
- Pentamers, subunit diversity as well as variable stoichiometry, allows for variable functions of GABA receptors
- Glycine receptors generally have the same properties as GABA receptors

Speaker notes

- pentameric
- GABAB metabotropic receptors always inhibitory. Coupled indirectly to K+ channels and can decreased Ca2+ conductance resulting in less cAMP production. Baclofen is a potent and selective GABAB agonist. GABA responses that are insensitive to bicuculline and baclofen are termed GABAC responses.
- GABAA: muscimol potent agonist from mushrooms. Bicuculline classical antagonist and convulsant.

Ionotrophic GABA Receptors



Neuroscience 5e Fig. 6.9

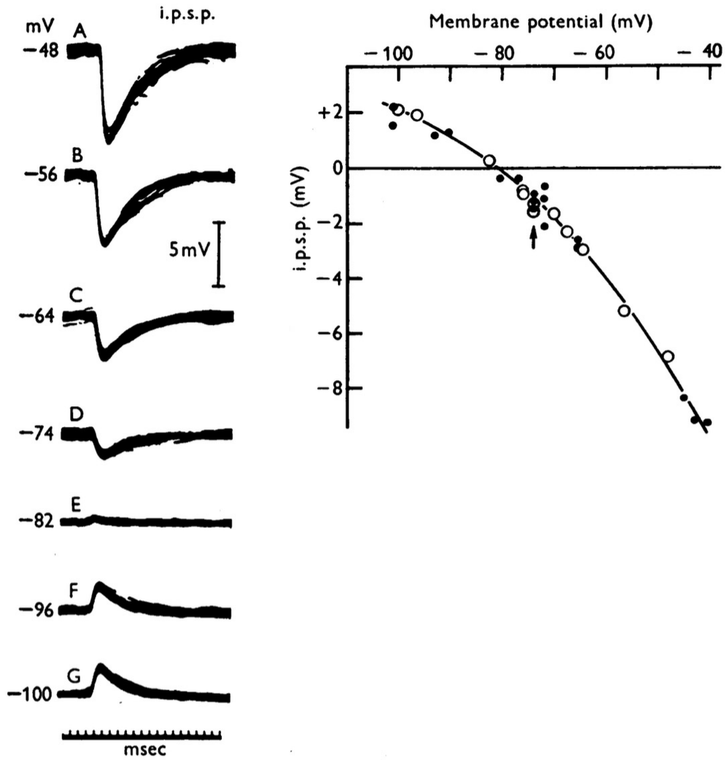
Speaker notes

picrotoxin

> *Found primarily in the fruit of the climbing plant Anamirta cocculus, it has a strong physiological action. It acts as a non-competitive channel blocker for the GABAA receptor chloride channels.[3] It is therefore a channel blocker rather than a receptor antagonist.*

Examples of IPSPs recorded at different membrane potentials

Erev is at the Nernst potential for Cl^- (e.g. –80 mV)



Coombs et al., J Physiol 1955 Fig. 1

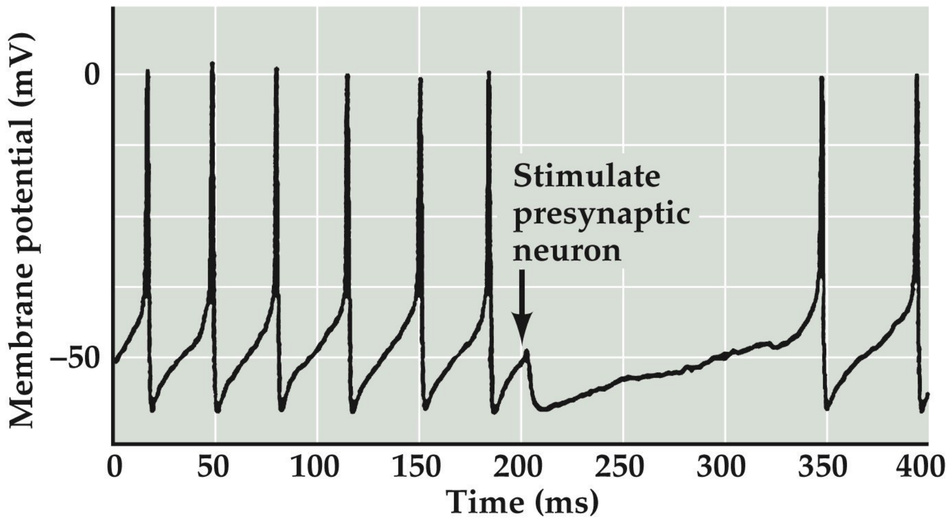
Speaker notes

Coombs, Eccles, Fatt 1955: double barreled pipete, inject small currents through one barrel (for voltage clamp) in biceps motorneuron (crustacean) to hold Vm while stimulating afferent nerve inputs to get IPSPs. Erev was found to be close to ECl. Notice hyperpolarization when Vm was above -78 mV, small depolarizations when Vm below -80mV. They found that messing with Cl- concentrations would correspondingly alter the IPSPs but not when messing with Na or K concentrations. Thus Cl- ion flux is necessary for the IPSPs.

[#Coombs:1955]: Coombs, J. S., Eccles, J. C., and Fatt, P. (1955). The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential, J Physiol, 130(2), 326-74. PMID 13278905

Ionotropic GABA receptor mediated IPSPs

Stimulate GABA producing interneuron, record from post-synaptic neuron



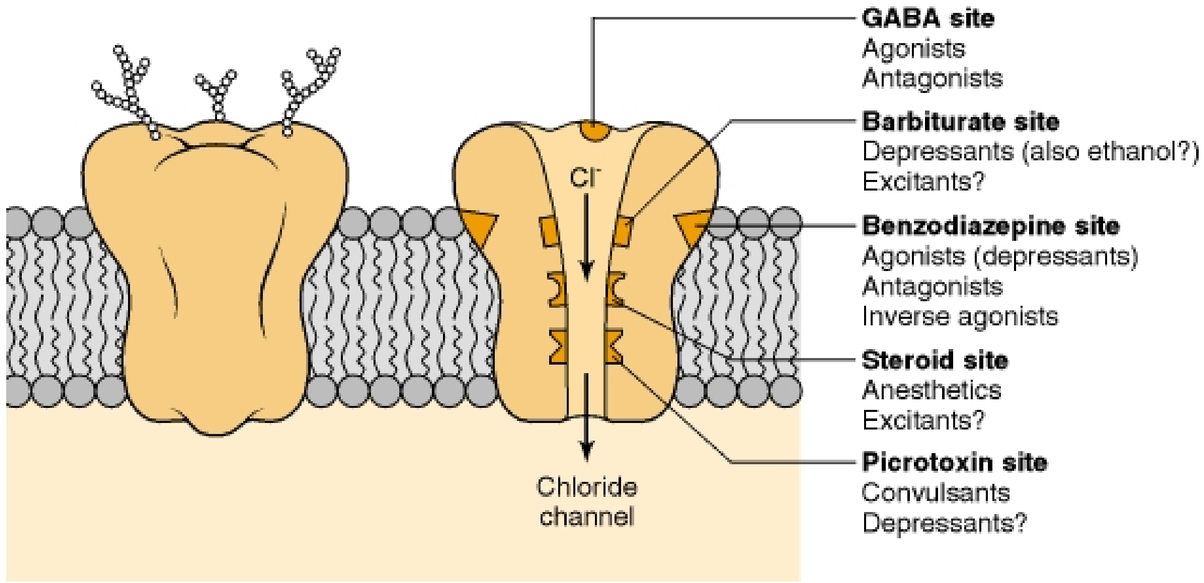
Neuroscience 5e Fig. 6.9

Speaker notes

Chavas and Marty performed Gramacidin perforated patch recordings from young rat cerebellum interneurons and purkinje cells. *Interneurons had more depolarized GABAA reversal potentials than purkinje cells at matched ages (e.g. P12, likely from higher [Cl-]intra for interneurons compared to purkinje cells).*

[#Chavas:2003]: Chavas, J. and Marty, A. (2003). Coexistence of excitatory and inhibitory GABA synapses in the cerebellar interneuron network, J Neurosci, 23(6), 2019-31. PMID 12657660

GABA receptors bind many interesting things



Basic Neurochemistry 6e Fig. 16.2

Speaker notes

from: https://en.wikipedia.org/wiki/Barbiturate#Mechanism_of_action

> *Barbiturates act as positive allosteric modulators, and at higher doses, as agonists of GABAA receptors.*

from: https://en.wikipedia.org/wiki/Benzodiazepine#Pharmacology

> *Benzodiazepines work by increasing the efficiency of a natural brain chemical, GABA, to decrease the excitability of neurons.*

from: http://thebrain.mcgill.ca/flash/i/i_03/i_03_m/i_03_m_par/i_03_m_par_a

> *GABA's effect is to reduce neural activity by allowing chloride ions to enter the post-synaptic neuron. These ions have a negative electrical charge, which helps to make the neuron less excitable. This physiological effect is amplified when alcohol binds to the GABA receptor, probably because it enables the ion channel to stay open longer and thus let more Cl^- ions into the cell.*

> *Still other substances block a natural neuromediator. Alcohol, for example, blocks the NMDA receptors.*

> *It has now been established that all substances that trigger dependencies in human beings increase the release of a neuromediator, dopamine, in a specific area of the brain: the nucleus accumbens.*

Serotonin receptors

- Large family of receptors called 5-HT 1-7
- 5-HT3 is a ligand-gated non-selective cation channel, thus it is excitatory
  - Same basic structure as nACh receptor
- All others are metabotropic– likely that perturbations in these receptors are involved in many neural disorders
  - excitatory: 5-HT2,4,6,7
  - inhibitory: 5-HT1,5

Speaker notes

most receptors are metabotropic

- 7 members of family
- 5HT1, 5HT5 are Gi: inhibitory (decr cAMP)
- 5HT2,4,6,7 are Gs or Gq: excitatory (incr cAMP or incr IP3/DAG)
- 5HT3 is non selective ionotropic cation channel (excitatory)

Catecholamine receptors

- Act exclusively by activating G-protein coupled receptors. Contribute to complex behaviors
- Norepinephrine and epinephrine each act on α and β adrenergic receptors
- Mostly used to control smooth muscles, especially cardiovascular
- B-blockers are used to treat hypertension, anxiety, and panic

Speaker notes

- more examples of catecholamine metabotropic receptors in action later in course (e.g. dopamine D1, D1 types in basal ganglia function)

Peptide receptors

- Virtually all mediate their effects by activating G-protein coupled receptors
- Neuropeptide-Y receptor important in food intake/obesity
- Opiate receptors have been identified and shown to be important in addiction (e.g. μ-opioid receptor)

Speaker notes

Opioid peptides distributed throughout the brain. Colocalize with GABA and 5-HT. Tend to be depressants. They act like analgesics when injected intracerebrally. Initiate effects through GPCRs. Activate at low concentrations (nM to uM). mu, delta, kappa opioid receptor subtypes play role in reward and addiction. mu-receptor is primary site for opiate drugs.

ATP and other purines (adenosine)

- ATP is contained in all synaptic vesicles
- Has specific receptors on post-synaptic cells
  - P2X– ionotropic non-selective cation channel
  - A2A– adenosine receptor (blocked by caffeine)
- Used in spinal cord, motor neurons, and other ganglia

Speaker notes

Another neurotransmitter that we didn't talk much about last time is ATP.

Receptors for ATP and adenosine are widely distributed through the nervous system as well as other tissues.

One class of purinergic receptors for ATP and adenosine are P2X-receptors which are ionotropic non-selective cation receptors.

Other purinergic receptors are metabotrobic GPCRs like A2A adenosine receptor throughout brain and heart, adipose tissue, and kidney. Xanthines (e.g. caffeine and theophylline) block adenosine receptors. This is thought to be the cause of its stimulant effects.

Summary

- Two types o neurotransmitter receptors– ionotropic (ligand-gated ion channel) and metabotropic (G-protein coupled receptor)
- Both lead to opening or closing of ion channels. Ionic currents then either increase or decrease the probability of firing an action potential
- Postsynaptic neurons are usually innervated by many different inputs– it is the combination of EPSP and IPSPs that determines if an action potential occurs

Speaker notes

No notes on this slide.