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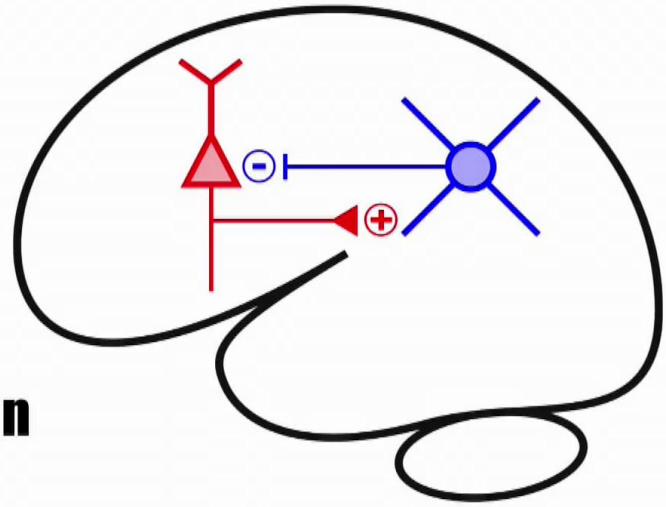
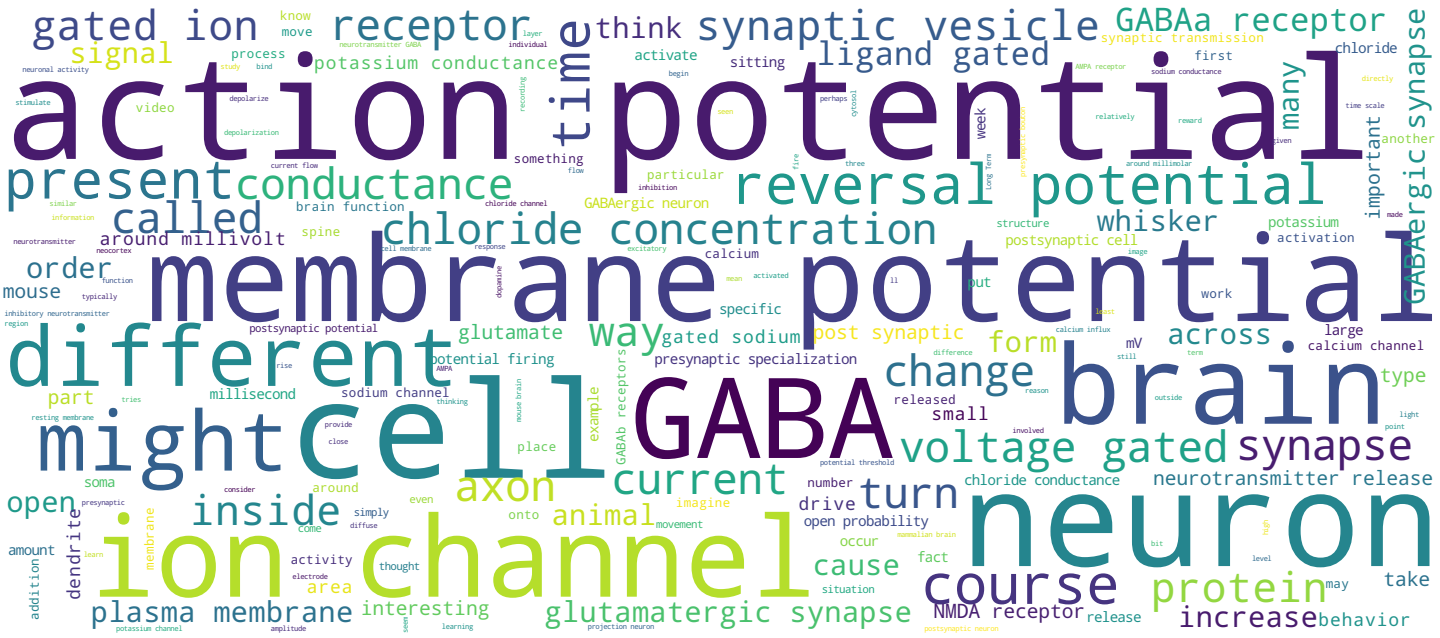


5.1 GABAergic inhibition

Cellular Mechanisms of Brain Function

Prof. Carl Petersen



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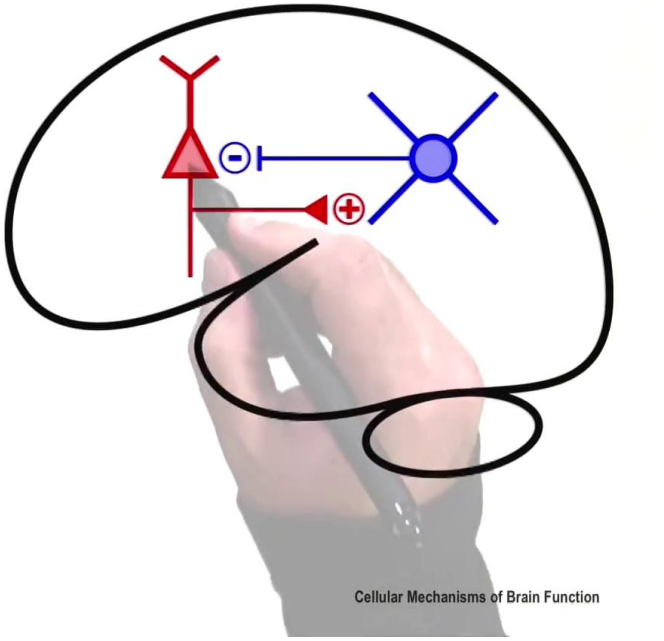
Video

EPFL

GABAergic inhibitory synaptic transmission



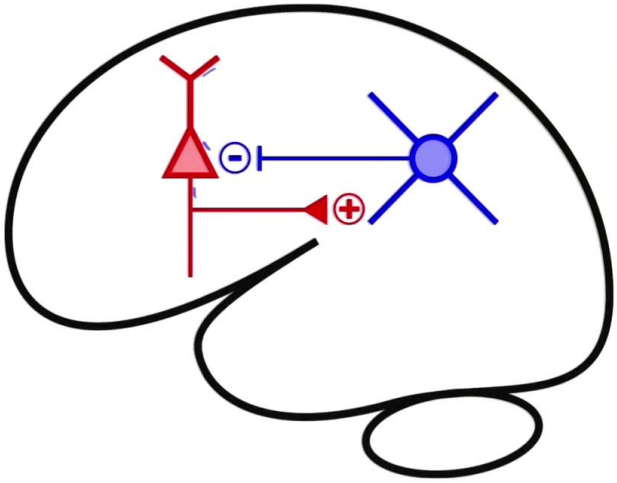
Welcome to Week 5 of Cellullar Mechanisms of Brain Function. We've now learned quite a bit about excitatory glutamatergic synapses in the mammalian brain. Glutamatergic synapses account for about 80% of the synapses and they provide an absolutely fundamental role in brain function. Most neurons are electrically silent and they need some excitatory drive in order to drive action potential firing and create processing and signals across the brain. But if there were only glutamatergic excitation in the neuronal networks of the brain, then there would be explosive activity and all the cells would be continuously active. And there's, therefore, a need for inhibition to balance the excitation. And it's that inhibition that we're going to be considering this week. Most of the inhibition in the mammalian brain comes about for the neurotransmitter gamma-Aminobutyric acid, known as GABA for short. Most of the neurons in the brain are glutamatergic neurons when they fire action potentials. That action potential propagates down the axon and from presynaptic specializations, glutamate is released, and that glutamate has an excitatory effect on the postsynaptic cells.

Notes

Summary

0m 05s

GABAergic inhibitory synaptic transmission



Cellular Mechanisms of Brain Function

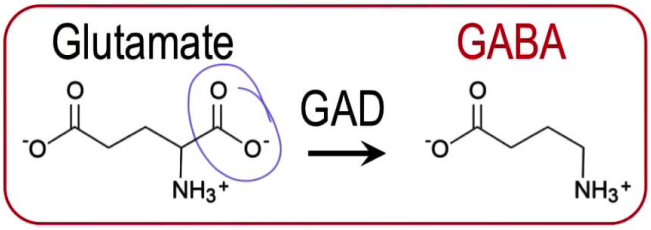
It tries to increase the amount of action potential firing of the postsynaptic cell. For a GABAergic neuron, the action potential again propagates down the axon, but at the presynaptic specialization, the neurotransmitter that's released is called GABA and that GABA has an inhibitory influence upon the postsynaptic neurons. It tries to prevent the postsynaptic neuron from firing action potentials. And in the same way that there are many types of excitatory neurons there are also many types of GABAergic neurons. The synapses that are formed by these GABAergic neurons can have very high specificity. They can target specific compartments like distal dendrites, the axon initial segment, the soma, and those synapses are created at very specific locations and presumably have very specific functions. During the course of the videos of this week, we'll consider some of the specificity and general features of GABAergic synaptic transmission.

Notes

Summary

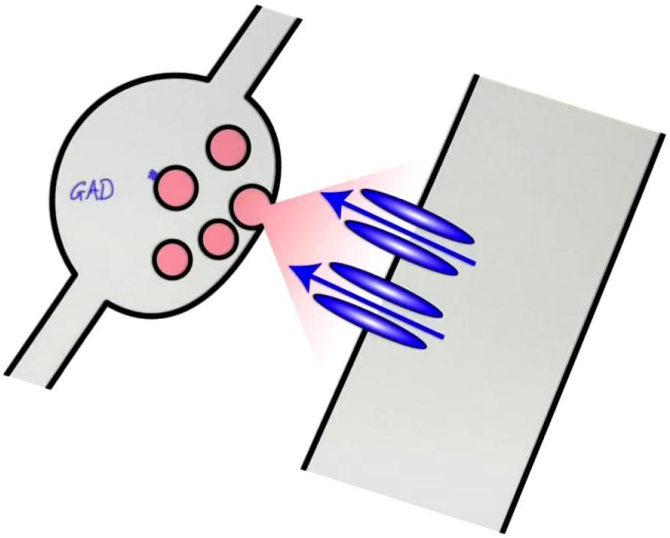
1m 29s

GABAergic synapses



Vesicular GABA transporter
VGAT

Ionotropic GABA receptors
Outward postsynaptic current



Cellular Mechanisms of Brain Function

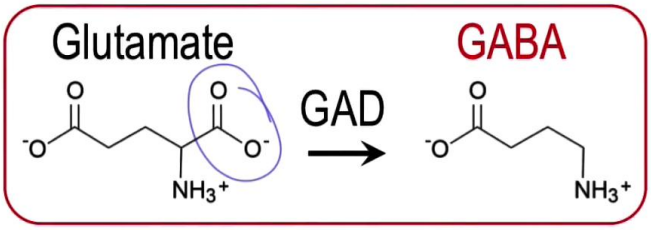
Let's consider how a GABAergic synapse is put together. We have the axon and presynaptic boutons, that contain synaptic vesicles, just like before for the glutamatergic vesicles. But the synaptic vesicles now need to be packaged with a different neurotransmitter. Before we're putting glutamate inside these synaptic vesicles and now we need to put the neurotransmitter GABA inside these vesicles. Interestingly, the neurotransmitter GABA is synthesized in a single enzymatic step from glutamate so the main excitatory neurotransmitter Glutamate, also an amino acid and present in the cytosol of all cells, is treated by a specific enzyme Glutamic Acid Decarboxylase, or GAD for short, and that removes this CO2 group from Glutamate and creates the neurotransmitter GABA. This enzyme GAD is present in the cytosol even of the presynaptic boutons and so GABA is produced right at the place where it's needed and even some of the GAD enzyme seems to be present directly on the synaptic vesicle membrane. So, there's a local production of the inhibitory neurotransmitter GABA right where we need it.

Notes

Summary

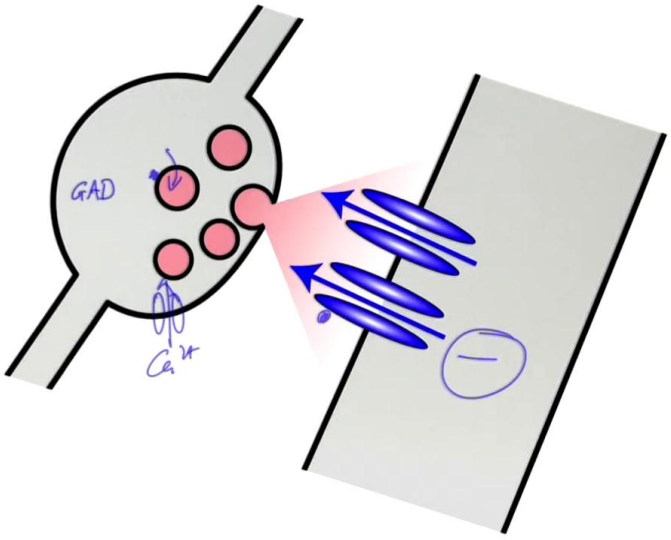
2m 34s

GABAergic synapses



Vesicular GABA transporter
VGAT

Ionotropic GABA receptors
Outward postsynaptic current



Cellular Mechanisms of Brain Function

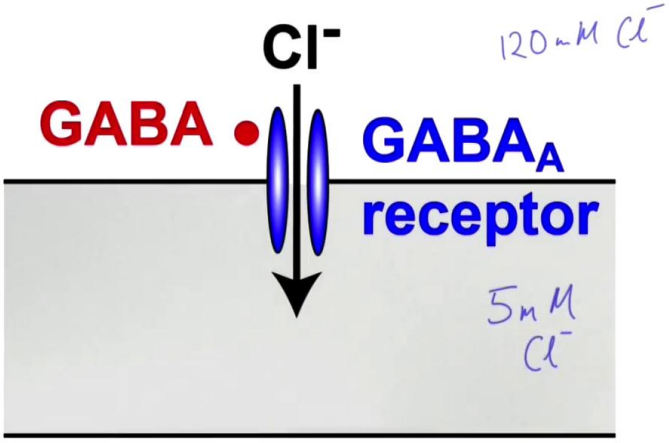
That GABA is then packaged into synaptic vesicles through a protein, the vesicular GABA transporter or VGAT that's present on the synaptic vesicle membrane it utilizes a proton, an electrical gradient, across the synaptic vesicle membrane and from that energy it sucks GABA inside the synaptic vesicle and concentrates it to a concentration of, probably, around 100 millimolar. The action potential then can invade the nerve terminal, it has a glutamatergic synapse that drives calcium influx and we have the standard exocytotic release machinery that then drives vesicle fusion and release of the GABA into the synaptic cleft. Then it diffuses some nanometers and binds to specific receptors that are present in the postsynaptic specialization. Similar to the way that we had ligand-gated ion channels for glutamatergic synapses, the fast inhibition that occurs at GABAergic synapses is also mediated directly by ligand-gated ion channels. So there are GABA binding ion chanels and the GABA acts directly on that protein increases the open probability and for a GABA receptor that causes an outward current. So, the electrical current flow is outwards, and that causes negative charge inside the postsynaptic cell, and that then is hyperpolarizing, it's a outward postsynaptic current that inhibits the postsynaptic neuron. That's how inhibition works.

Notes

Summary

4m 05s

$GABA_A$ receptors



Nernst equilibrium potential

$$E_{Cl} = \frac{RT}{zF} \ln \frac{[Cl^-]_i}{[Cl^-]_o}$$

$$E_{Cl} = 61.5 \log_{10} \frac{5}{120}$$

$$E_{Cl} = \sim -85 \text{ mV}$$

Cellular Mechanisms of Brain Function

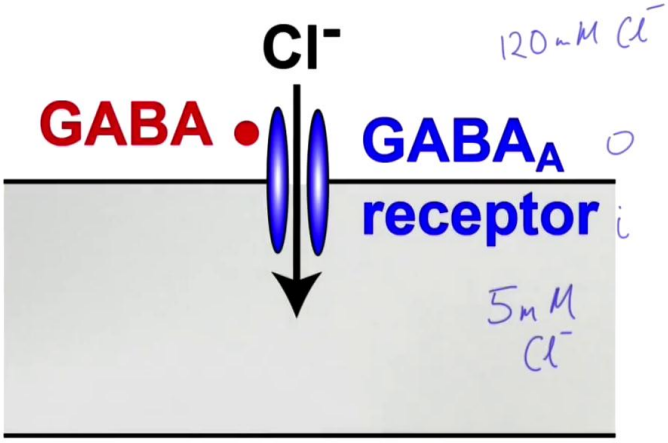
Let's look in a bit more detail to the ionic conductance that's mediated by GABA binding to its ligand-gated ion channel. The GABAa receptor, as it's called, is the site where GABA released directly from the presynaptic specialization, binds to the protein, increases the open probability and the GABAa receptor is permeable to anions, and in particular, chloride is its major permeability. So GABA opens a chloride conductance which is formed by the GABAa receptor. This, of course, is a fast action of GABA, it's a direct action on a ligand-gated ion channel, so those conductances open with millisecond timing and mediates fast inhibition in the mammalian nervous system. Why is a chloride conductance inhibitory? We know that the chloride concentrations are distributed in an uneven way across the plasma membrane. Intracellularly, we have around 5 millimolar of chloride and extracellularly we have around 120 millimolar of chloride. So, there's a strong gradient that wants to put chloride into cells if you open a chloride selective conductance. We can look at the Nernst equation and drive quantitatively what the reversal potential for chloride is.

Notes

Summary

5m 53s

$GABA_A$ receptors



Nernst equilibrium potential

$$E_{Cl} = \frac{RT}{zF} \ln \frac{[Cl^-]_i}{[Cl^-]_o}$$

$$E_{Cl} = 61.5 \log_{10} \frac{5}{120}$$

$$E_{Cl} = \sim -85 \text{ mV}$$

Cellular Mechanisms of Brain Function

We take our equilibrium potential equation, we put in our numbers for 5 millimolar intracellular chloride, 120 millimolar extracellular chloride, we multiply by 61.5 times the log 10, the base 10 logarithm of these numbers, and that gives us a chloride reversal potential of -85 millivolts. That means that, if GABA acts to open the GABA receptor and it's a chloride channel that tries to bring the membrane potential of the postsynaptic cell to -85 millivolts. That's hyperpolarized, compared to the normal resting membrane potential of neurons and it's certainly hyperpolarized compared to the action potential threshold that sits at around -45 millivolts. So, chloride conductances are inhibitory because the reversal potential for chloride is extremely negative and well below action potential threshold So, GABA prevents action potential firing of the postsynaptic cell. The reason that does that is because of the chloride concentrations, the way they're distributed comparing the inside and the outside of cells.

Notes

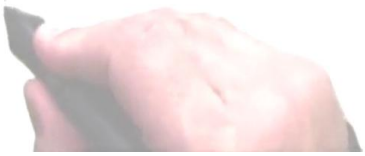
Summary

7m 29s

Cytosolic chloride concentration

$GABA_A$ reversal potential ~ -80 mV. Resting membrane potential ~ -70 mV. The membrane potential helps keep a low cytosolic chloride concentration.

Chloride transporters (notably KCC2) also contribute importantly.



Cellular Mechanisms of Brain Function

A key question that determines the inhibitory properties of the GABA neurotransmitter, is the chloride concentrations. So, this is of course very interesting to find out how chloride is distributed and controlled in neurons. One reason that we have low cytosolic chloride concentrations is because there are leaky chloride channels that are present on all cell membranes. So, there's a permanent chloride conductance that's open on cell membranes. And simply by the fact that the resting membrane potential is sitting at around -70 millivolts, that will then contribute to distributing chloride at around the equilibrium potential of -70 millivolts. So, having negative potentials drives chloride out of cells and gives us negative reversal potentials. But the actual reversal potential for GABA is more negative than the resting membrane potential of most neurons. So, we also need an active process that keeps chloride concentration low. If we now look at the soma of a cell, sitting on its plasma membrane are specific transport proteins, these are 12 transmembrane proteins, so they have multiple regions across the plasma membrane and these are transporters, so they have multiple binding sites and a slow transport rate but they take chloride out of the cell.

Notes

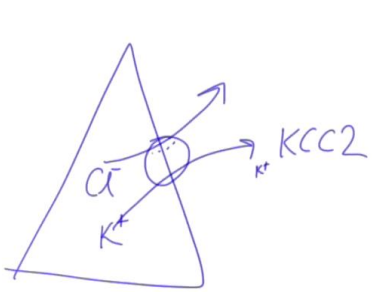
Summary

8m 46s

Cytosolic chloride concentration

$GABA_A$ reversal potential ~ -80 mV. Resting membrane potential ~ -70 mV. The membrane potential helps keep a low cytosolic chloride concentration.

Chloride transporters (notably KCC2) also contribute importantly.



Cellular Mechanisms of Brain Function

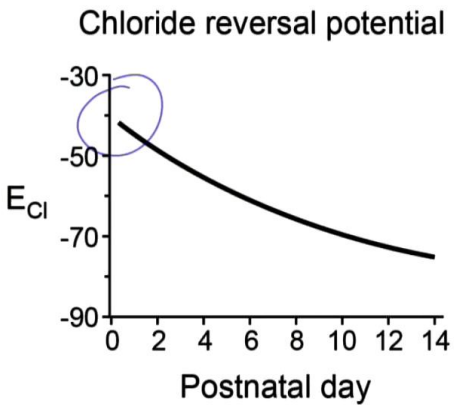
So, that's how chloride concentrations are low. This is the so called transporter, called KCC2, that's neuron-specific so it's only expressed in neurons and it drives low chloride concentrations in neurons in particular. The chloride is, in fact, cotransported by potassium so there's a one to one stoichiometry of potassium and chloride that transport together through the KCC2 protein and it's that potassium that drives the extrusion of chloride. We'll remember that there's a high concentration of potassium inside cells, a low concentration of potassium outside, so there's a strong gradient for potassium to leave and that's what provides the energy to suck the chloride out of neurons and keep chloride concentrations low inside the cytosol of neurons. And it's that low chloride concentration inside neurons that's absolutely critical for making that hyperpolarized reversal potential of GABA that makes GABA an inhibitory neurotransmitter.

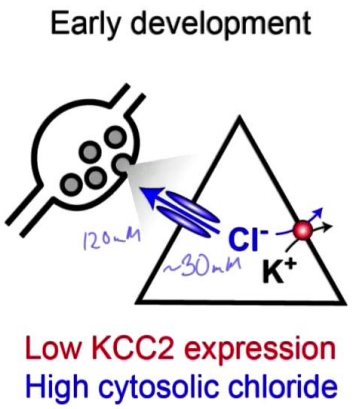
Notes

Summary

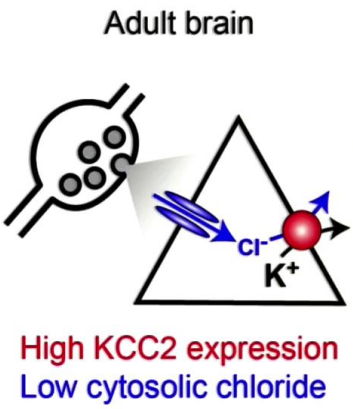
10m 22s

Chloride concentration during early development





Low KCC2 expression
High cytosolic chloride



High KCC2 expression
Low cytosolic chloride

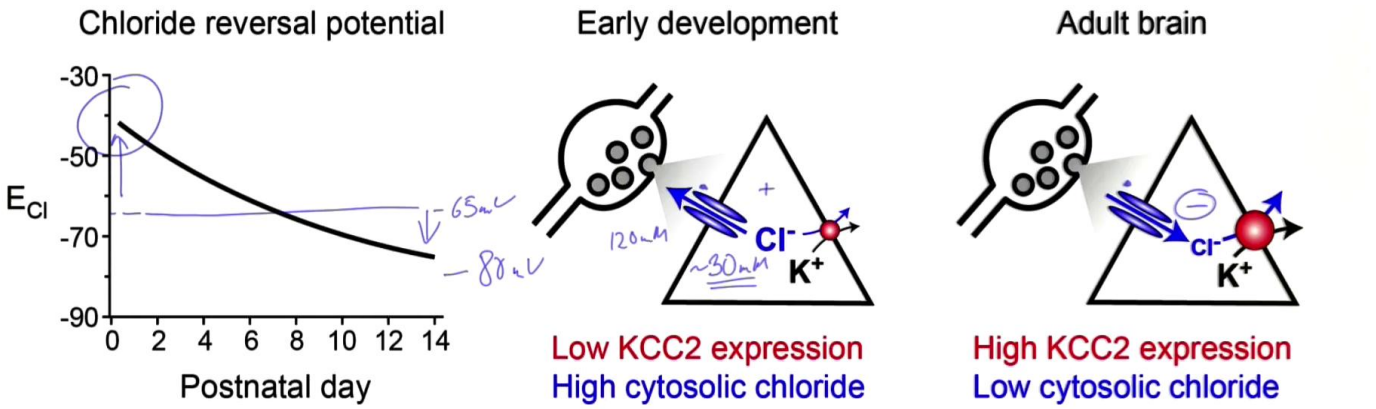
Interestingly, it turns out that GABA isn't always inhibitory. In fact, during very early development, chloride concentrations are radically different from how they are in the adult brain. At very early postnatal stages of rodents that would then correspond to early stages of development for human babies that would be still in the gestation period for a human baby, but rodents are born less developed than humans, so, in the rodent postnatal day 0, there are high chloride concentrations inside the cytosol of neurons, the chloride concentration is thought to be somewhere around 30 millimolar intracellularly, and a high concentration of chloride intracellularly where we leave extracellular chloride to be--- it's normal around 120 millimolar, means that the reversal potential for chloride is quite depolarized compared to the adult condition of -80. So, here at around birth for a rodent, the chloride reversal potential might be sitting at around -40 millivolts. That means that when GABA is released and activates a GABAa receptor chloride actually leaves the cell causing depolarization of the membrane potential.

Notes

Summary

11m 25s

Chloride concentration during early development



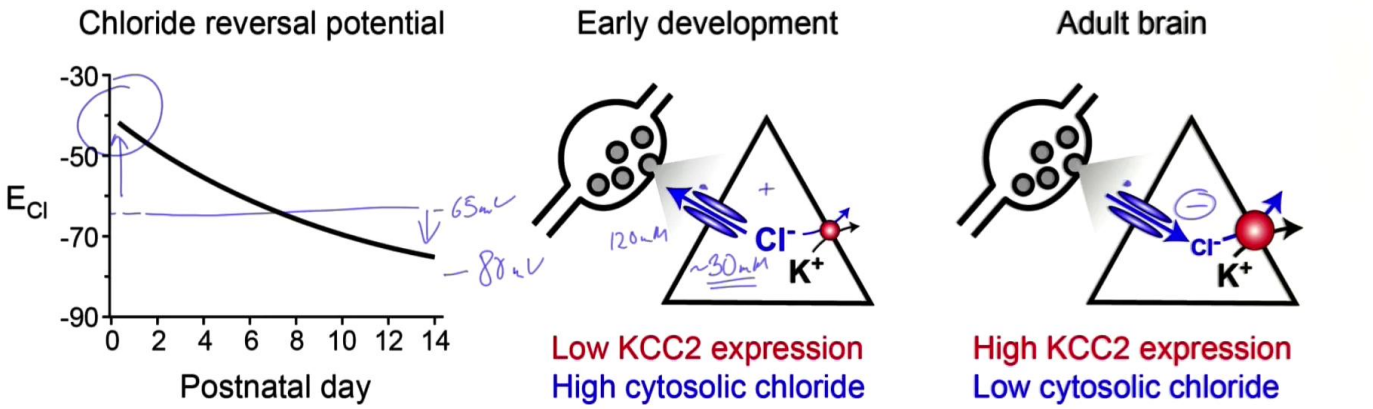
So, if we have a typical neuron at rest here at around -65 millivolts opening of a GABA conductance at early postnatal days causes depolarization and might contribute to exciting the postsynaptic neuron. The reason that chloride concentrations are high during early development, is that the expression of this transport protein, KCC2, is extremely low at early stages of development, so the ability to suck chloride out of the cell hasn't developed yet. So we end up with high chloride concentrations that give rise to depolarized chloride reversal potentials and the excitatory action of GABA. During these first postnatal days, the expression of KCC2 ramps up dramatically and that then sucks chloride out from the cytosol of the cell, the chloride concentration goes down, and now, when we open the GABAa conductance, chloride wants to enter the cell and hyperpolarize it and that's where we get the hyperpolarizing action of GABA, that in the mature state, brings us to around -80 millivolts as the reversal potential. It's interesting to note that chloride concentrations obviously make a big difference to GABA receptor function. Early in development, GABA seems to have an excitatory role it's really only at very, very early postnatal stages.

Notes

Summary

12m 55s

Chloride concentration during early development



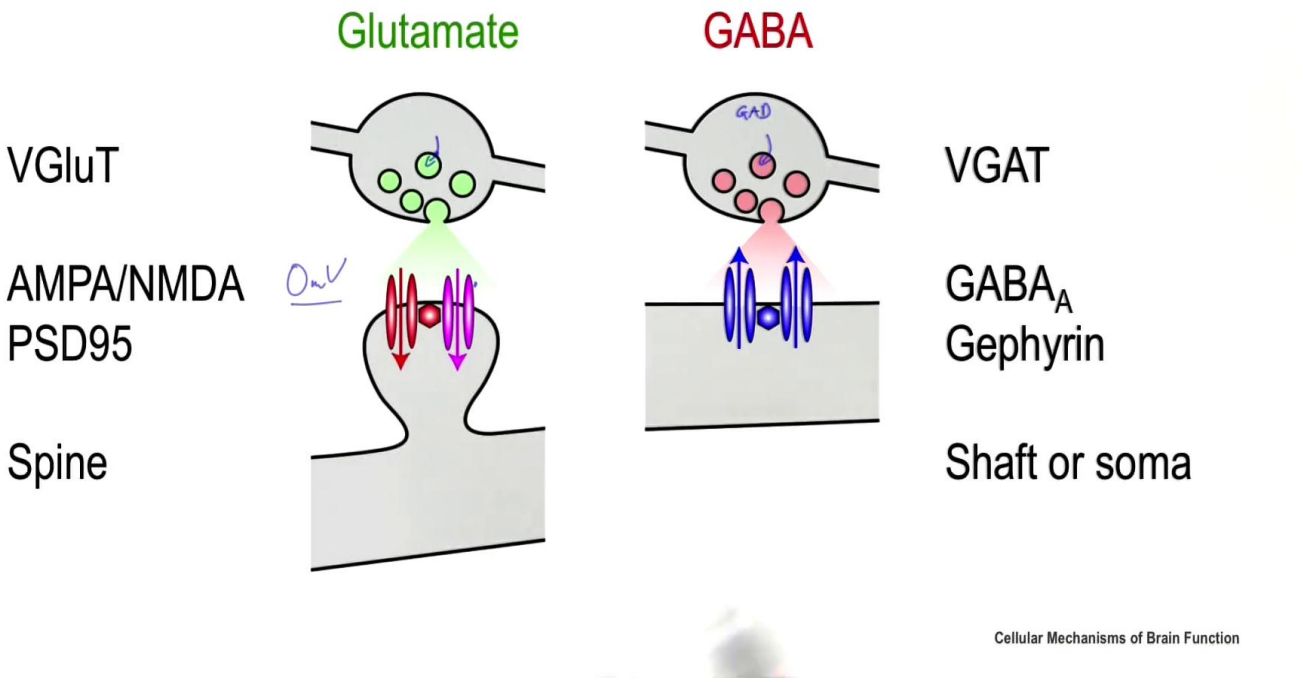
Already in the rodent, one week after birth, it's clear that GABA has an inhibitory function. In addition to developmental changes of chloride, there are also thought to be differences in chloride concentrations in different compartments of neurons. So the KCC2 expression turns out to be different in dendritic and axonal membranes. So it may be, that chloride concentrations might also differ in different neuronal compartments, and that provides additional complexity to thinking about how GABAergic inhibition might work.

Notes

Summary

14m 24s

Glutamatergic vs GABAergic synapses



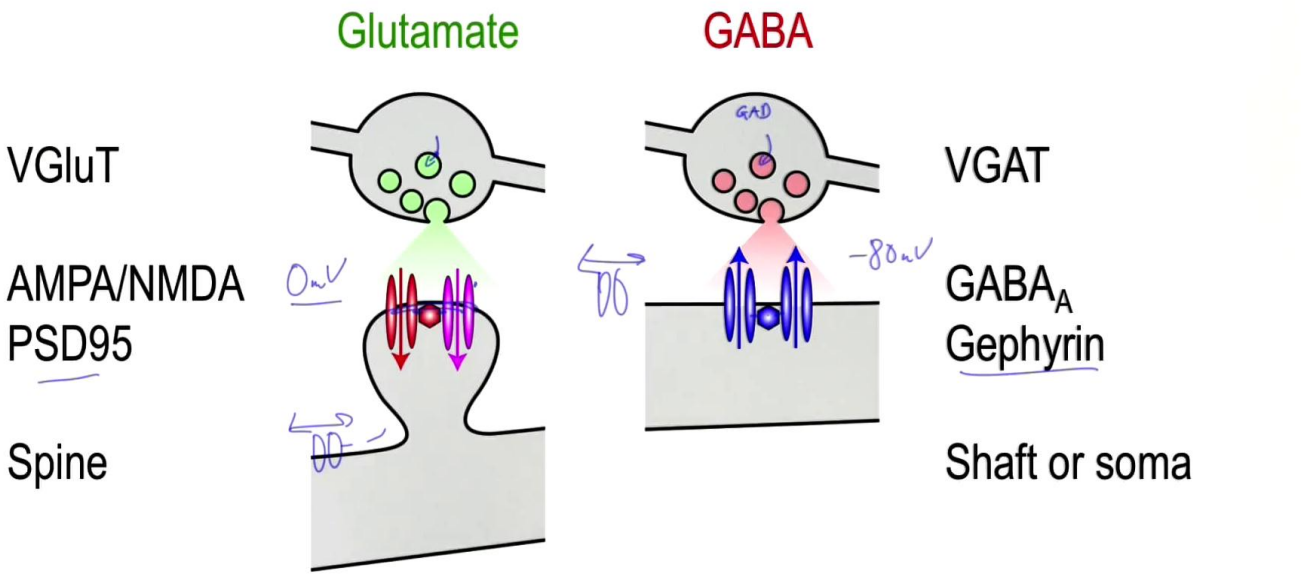
It's now fun to compare what we know about the glutamatergic and the GABAergic synapses. Because they have a great deal of instinct similarities and parallels in the way that they're set up. At the glutamatergic synapse, we obviously want to put glutamate inside the synaptic vesicles. and that glutamate is packaged by a specific vesicular glutamate transporter that's present here, that puts glutamate in the vesicles. In the GABAergic synapse, we need one additional step, we need an enzyme that makes the GABA and then we have a different protein, the vesicular GABA transporter that puts GABA inside the synaptic vesicles. Action potentials, neurotransmitter release machinery are similar at glutamatergic and GABAergic synapses and postsynaptically, of course, we have different receptors. But the fast excitatory and the fast inhibitory neurotransmission, both work the ligand-gated ion channels so the neurotransmitter directly binds to an ion channel, opens a conductance, for glutamate it's AMPA and NMDA, and that has a reversal potential of around 0 millivolts, so it's excitatory and depolarizing. For GABA we have the GABAa receptor, it's a chloride conductance and that tries to bring us to around -80 millivolts so that's an inhibitory neurotransmitter.

Notes

Summary

14m 59s

Glutamatergic vs GABAergic synapses



Cellular Mechanisms of Brain Function

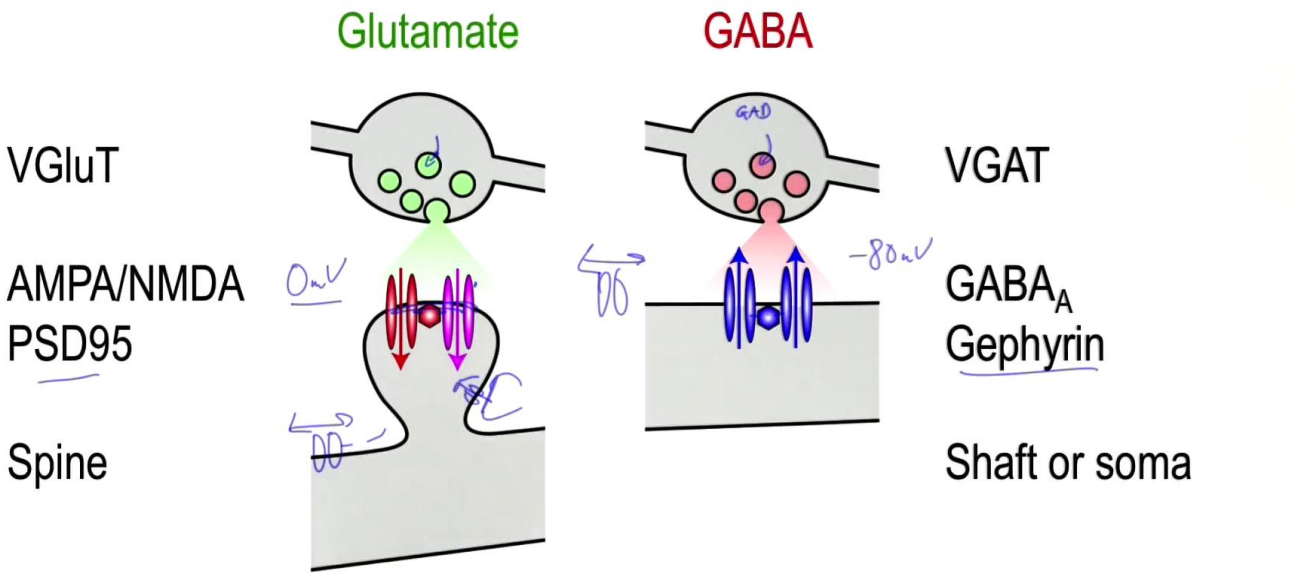
These ligand-gated ion channels are concentrated immediately next to the presynaptic specialization. The way that they're concentrated there is by having binding proteins here that associate tightly with the ligand-gated ion channels and hold them tightly in place right there in apposition to the presynaptic terminal. The ligand-gated receptors are otherwise present in low concentrations extrasynaptically where they can diffuse in the plasma membrane, but when they encounter one of these PSD95 molecules and other molecules in the postsynaptic density they get fixed there, held in place, and that concentrates the ion channels right next to the release site, where they need to be. The same situation is true for the GABAa receptors. They're also present extrasynaptically, they diffuse around on the plasma membrane and they get held in place in a position to the presynaptic specialization also by a scaffolding protein that binds to the receptors and that's called Gephyrin. So PSD95 and Gephyrin seem to play equivalent functions at glutamatergic and GABAergic synapses respectively. For the glutamatergic synapses we noted that there was an interesting structural feature the spine, that tends to be where glutamatergic synapses are made.

Notes

Summary

16m 24s

Glutamatergic vs GABAergic synapses



Cellular Mechanisms of Brain Function

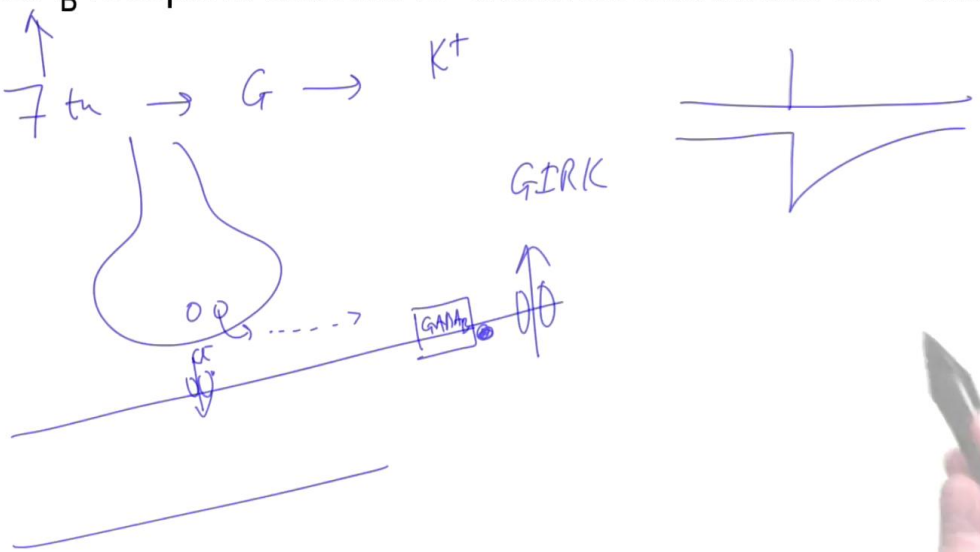
So these spines can grow to specific locations and the fact that they can grow and appear and disappear, appears to make them very suitable for plastic changes and might be involved in the rewiring of the brain during learning and the storage of memories. GABAergic synapses on the other hand are typically made directly on the dendritic shaft or even directly on the soma of the cell, body of the neuron or on the axon initial segment. So the GABAergic synapses are placed in slightly different locations to where the glutamatergic synapses are, although there can be GABAergic synapses present on some spines, and it may be that that provides a specific regulation at the level of individual synapses where we might be able to turn on or off the function of a glutamatergic synapse by putting a GABAergic synapse right here on the spine of a glutamate synapse. Spines always have a glutamatergic input and a small fraction of them also have an inhibitory input, but most of the GABAergic synapses are directly on dendrites or on the cell body.

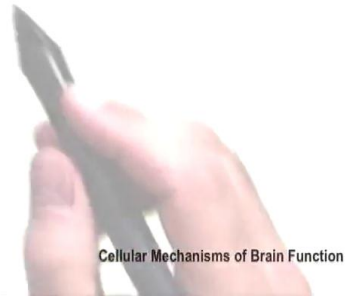
Notes

Summary

17m 49s

GABA$_B$ receptors activate K$^+$ channels and inhibit Ca^{2+} channels.





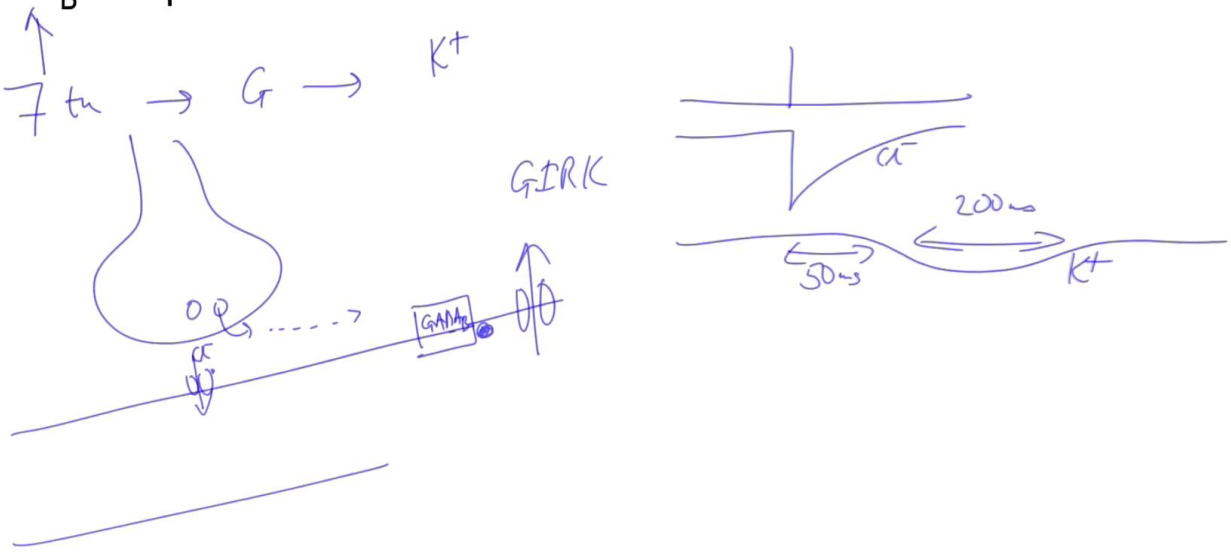
In addition to the fast action of GABA on GABAa receptors, and that's a ligand-gated ion channel that mediates fast GABAergic receptors, there's also a metabotropic receptor, a 7-transmembrane receptor that works via G-proteins that then activate a potassium conductance. So this is a slower way of signaling, so we have to imagine now that we have our presynaptic specialization that releases GABA and postsynapticaly, immediately in apposition to this, we have our chloride channels that bring chloride into the cell when the GABA binds here, and that's a fast IPSP, where if we have an action potential we get a rapid inhibition through this chloride influx into the postsynaptic cell. The GABA can then diffuse some distance, and at some distance to the actual synapse, so at extrasynaptic locations, there are these GABAb receptors and these GABAb receptors work via G-proteins and they activate a potassium conductance, a so-called G-protein inward rectifying potassium conductance, a GIRK channel. That can give rise to much slower postsynaptic potentials that are delayed by perhaps 50 milliseconds before they become activated and they can also last much longer periods of time, several hundred milliseconds, and the delays impart because the GABA has to diffuse and also because there are multiple steps before we can activate this potassium conductance.

Notes

Summary

18m 58s

GABA$_B$ receptors activate K$^+$ channels and inhibit Ca^{2+} channels.



Cellular Mechanisms of Brain Function

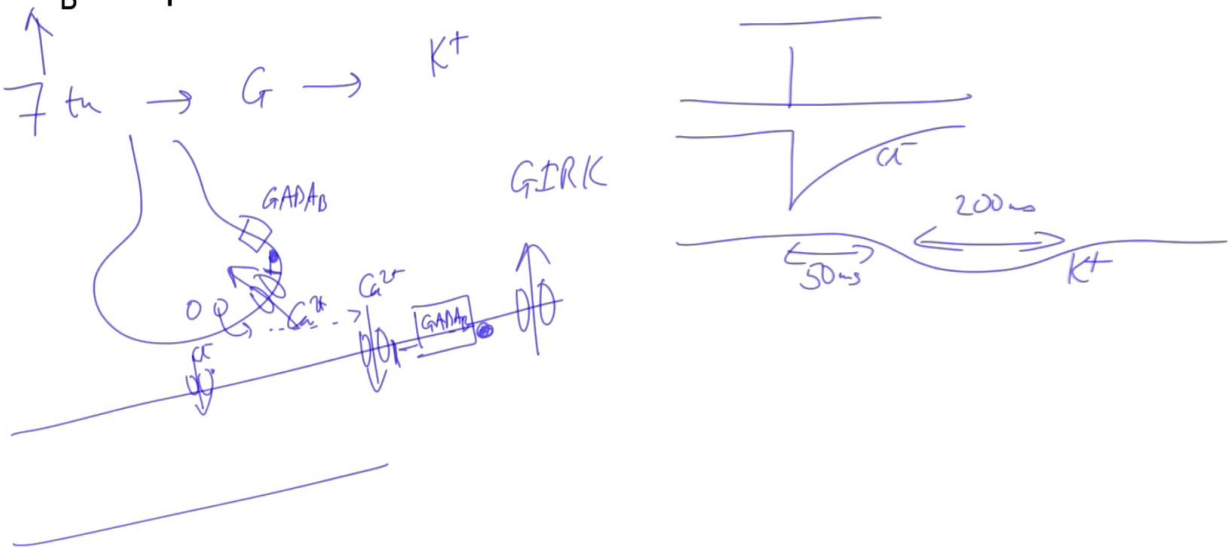
So there's a slow inhibitory postsynaptic potential that's mediated by potassium and there's a fast IPSP that's a GABAa receptor and that's the chloride conductance. In addition to this postsynaptic action on potassium conductances there's also an action of GABAb on calcium conductances so the GABAb receptors inhibit calcium influx that has effects postsynaticaly on excitability, but perhaps more prominently there are also GABAb present on presynaptic specializations, on the nerve terminals themselves there are GABAb receptors again acting through G-proteins and here they also act upon calcium channels, but now they play a very important function because the calcium channels here are responsible directly for neurotransmitter release, and GABAb inhibiting the calcium channels then reduces neurotransmitter release directly and most forms of presynaptic boutons have GABAb receptors present on them where there's a glutamatergic synapse or a GABAergic synapse or any other type of synapse, typically GABAb receptors are present and if you activate GABAb, you inhibit neurotransmitter release and that's another major way in which GABA is inhibitory.

Notes

Summary

20m 40s

GABA$_B$ receptors activate K$^+$ channels and inhibit Ca^{2+} channels.



It has a presynaptic inhibitory action where it reduces neurotransmitter release through inhibiting calcium channels and in addition to the fast GABAa conductance there's also a slow GABAb conductance, this slow potassium-mediated conductance that gives rise to a slow IPSPs.

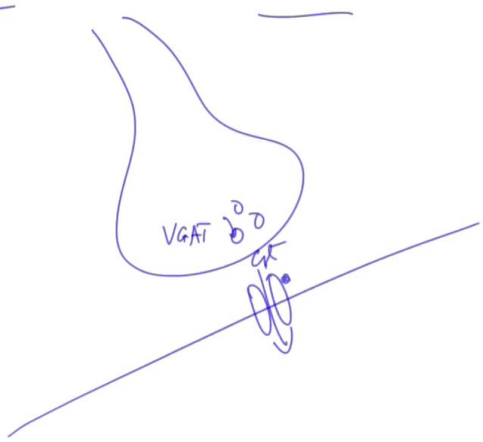
Notes

Summary

22m 02s

Glycine

Glycine is the major inhibitory neurotransmitter in the brainstem and the spinal cord.



Cellular Mechanisms of Brain Function

Finally it's worth pointing out that although GABA is by far the most important inhibitory neurotransmitter in the main brain, if we go to spinal cord or the brainstem, then GABA has a less prominent role and glycine instead takes over as a major inhibitory neurotransmitter. Glycine works in almost the identical way as GABA does at the GABAa receptor. So there are glycinergic presynaptic specializations, glycine is packaged into neurotransmitter vesicles and in fact it's the same vesicular GABA transporter that also packages glycine and postsynaptically we have glycine receptors, that again, are chloride channels that bring chloride in through the action of glycine acting on direct ligand-gated ion channels. So the glycine receptor is very similar to the GABAa receptor and glycine plays an almost identical role in the spinal cord and brainstem as GABA does in the main brain.

Notes

Summary

22m 22s

GABAergic inhibition

- GABA is the main inhibitory neurotransmitter in the brain.
- $GABA_A$ receptors are ligand-gated chloride channels, thus having hyperpolarised reversal potentials in the adult brain.
- $GABA_B$ receptors activate postsynaptic K^+ channels and pre-/post-synaptically inhibit Ca^{2+} channels.

Cellular Mechanisms of Brain Function

So in this video, we've got an overview of GABAergic inhibition. GABA is the main inhibitory neurotransmitter of the brain. It accounts for about 20% of the synapses and the other 80% of the synapses are largely excitatory glutamatergic synapses. GABA exerts its inhibitory action upon two types of receptors: GABAa receptors that are present in the postsynaptic specialization they're held in place by gephyrin that concentrates GABAa receptors and GABA acts on these GABAa receptors to open directly a ligand-gated ion channel, it's a chloride conductance and the reversal potential for chloride makes this inhibitory. Chloride has a reversal potential at around -80 millivolts so the action of GABA is to try to bring the postsynaptic membrane to around -80 millivolts. That's clearly hyperpolarized to action potential threshold so GABA has a fast inhibitory action through the GABAa receptor. GABA also acts upon metabotropic receptors, GABAb receptors, and there it drives slow inhibitory postsynaptic potentials through G-Proteins that activate potassium channels, the GIRK ion channels, and it presynaptically inhibits neurotransmitter release by inhibiting calcium channels that directly are involved in driving neurotransmitter release.

Notes

Summary

23m 26s

GABAergic inhibition

- GABA is the main inhibitory neurotransmitter in the brain.
- $GABA_A$ receptors are ligand-gated chloride channels, thus having hyperpolarised reversal potentials in the adult brain.
- $GABA_B$ receptors activate postsynaptic K^+ channels and pre-/post-synaptically inhibit Ca^{2+} channels.

Cellular Mechanisms of Brain Function

So GABA inhibits at multiple levels in the brain and forms a very important counteraction to the excitatory mechanisms of the glutamatergic synaptic transmission. Over the next videos of this week, we'll explore GABAergic inhibition in more detail.

Notes

Summary

24m 57s