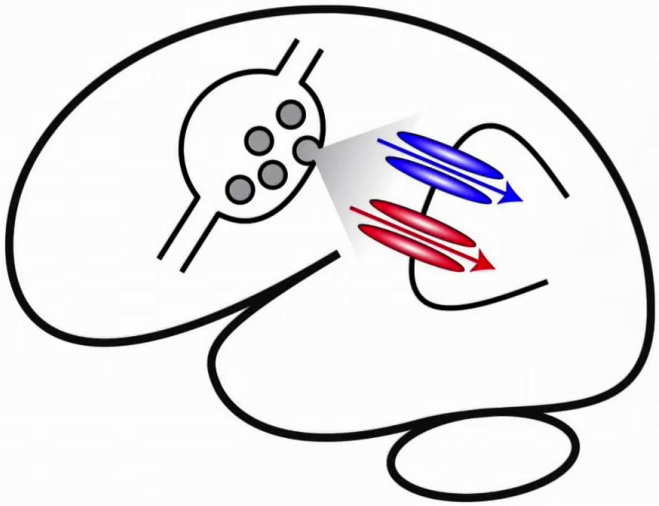

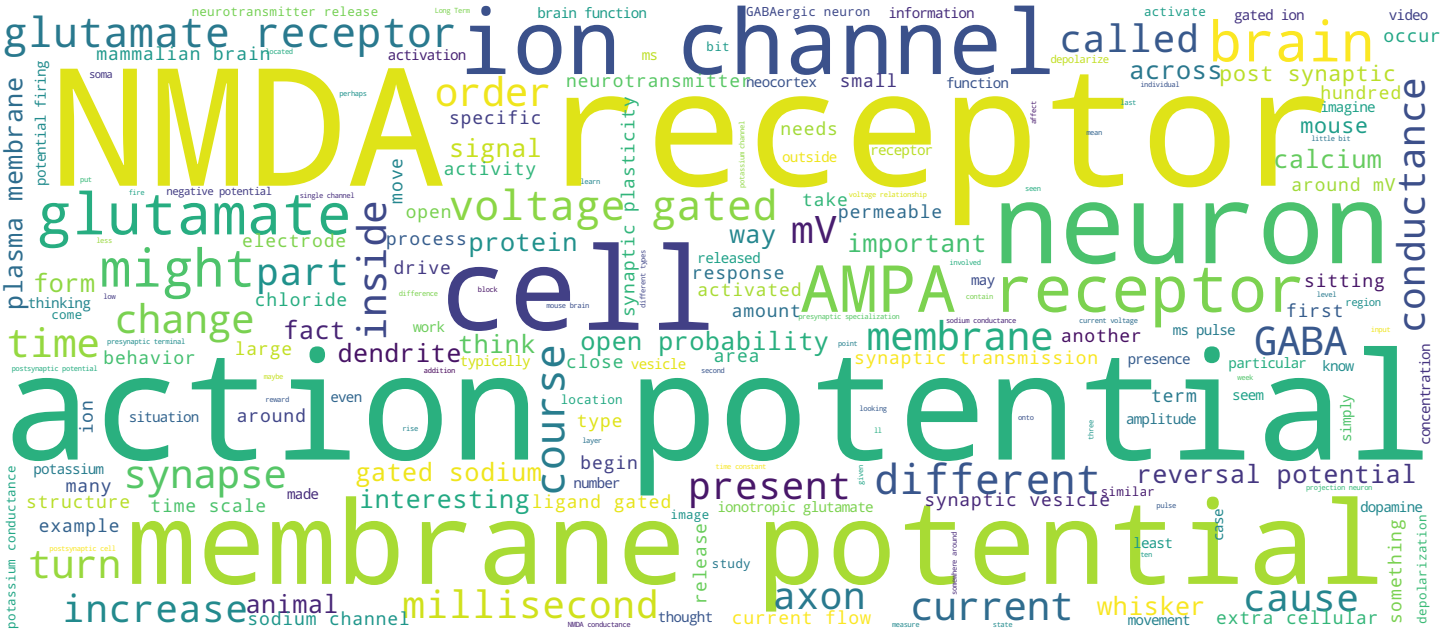

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

4.1 Glutamate receptors

Cellular Mechanisms of Brain Function

Prof. Carl Petersen

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Video

Glutamatergic excitatory synaptic transmission

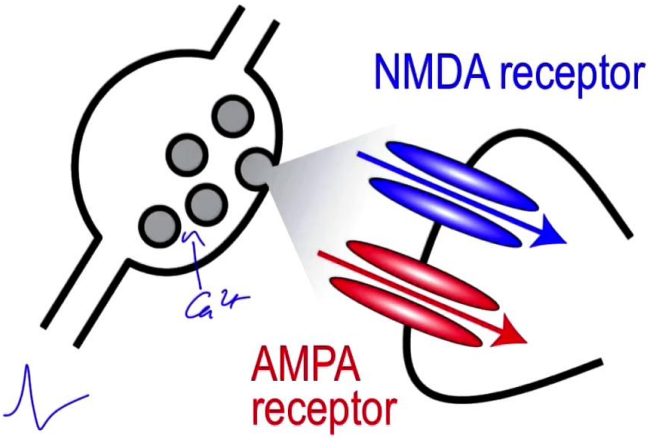
Welcome back to week four of cellular mechanisms of brain function. This week, we're going to talk about glutamatergic excitatory synaptic transmission. We've already studied the basics of membrane biophysics and excitability and we've talked about synaptic transmission as the most important way in which neurons can communicate with each other. Now, neurons, or most neurons from the mammalian brain, are not spontaneously active. That is in the absence of synaptic input, they are hyperpolarized in terms of their membrane potential, maybe being at -70 mV, so there are many tens of millivolts hyperpolarized relative to the action potential threshold, which is at around -40 mV. In order for the nerve cells to begin to become active to find action potentials and to participate in brain function and the processing of information, it's essential that these neurons become depolarized, that they become excited, and begin to find action potentials. The way in which neurons become excited is through excitatory synaptic transmission, and by far, the most important excitatory neurotransmitter in the mammalian brain is glutamate.

Notes

Summary

0m 05s

Ionotropic glutamate receptors



Cellular Mechanisms of Brain Function

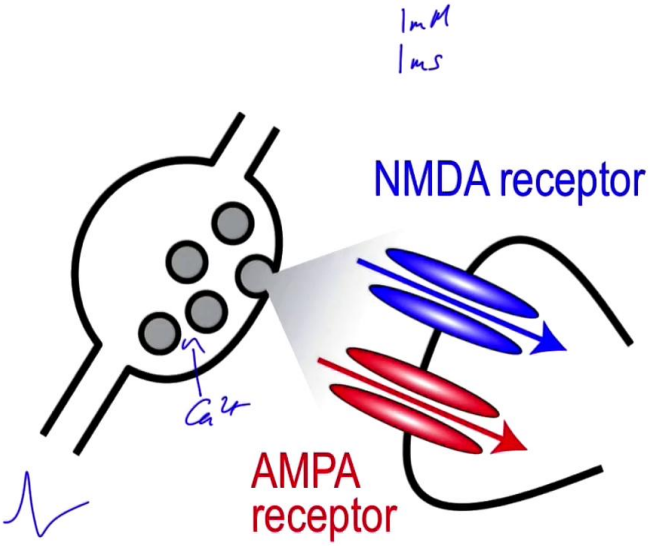
Something like 80% of all the synapses in the mammalian brain rely upon glutamate and so glutamatergic synaptic transmission is a fundamental importance and that's what we'll study during this week. If an action potential occurs in a glutamatergic neuron, that action potential will propagate down the axon and it'll reach presynaptic specializations, boutons, which are filled with synaptic vesicles, each of which is packaged with glutamate, probably at a concentration of around 100 mM of glutamate in each vesicle and that glutamate has been put in there specifically by the vesicular glutamate transporter that's present on every synaptic vesicle. Some of these synaptic vesicles will be docked and release-ready and when the action potential invades, the bouton calcium influx occurs does then the exocytosis of the contents of that synaptic vesicle. The glutamate diffuses across the synaptic cleft and there, it can bind onto postsynaptic receptors causing excitatory postsynaptic potentials. The fusion of a single vesicle is for it to increase the concentration of glutamate in the synaptic cleft to about 1 mM for a period of about 1 ms.

Notes

Summary

1m 24s

Ionotropic glutamate receptors



Cellular Mechanisms of Brain Function

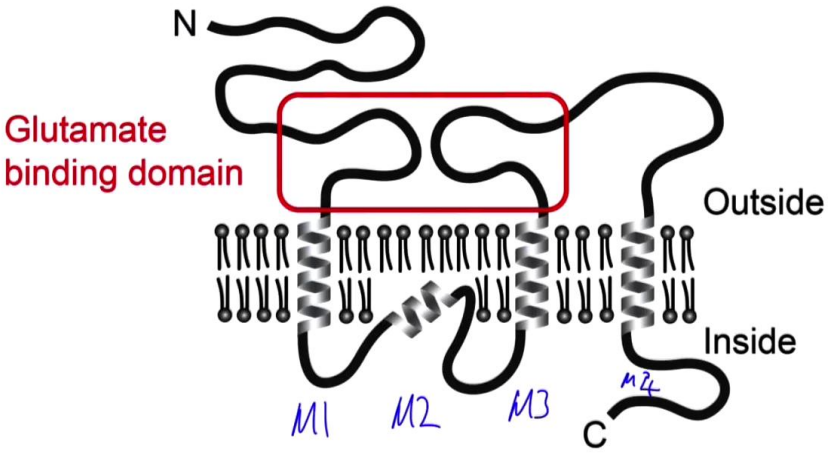
So we're thinking about brief, transient pulses of glutamate as a major way in which neurons communicate with each other. And that's what the postsynaptic cell then needs to be sensitive to. A 1 mM puff of glutamate that then needs to act on ligand-gated neurotransmitter receptors, glutamate receptors, and for their glutamate receptors, we have two major subtypes. We have the AMPA receptor and NMDA receptor, and we'll discuss more details about the similarities and differences of these two ligand-gated ionotropic glutamate receptors.

Notes

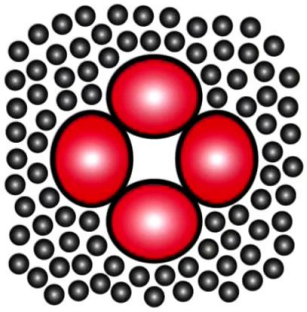
Summary

2m 58s

Ionotropic glutamate receptors: structure



Glutamate receptors have 4 subunits.



Cellular Mechanisms of Brain Function

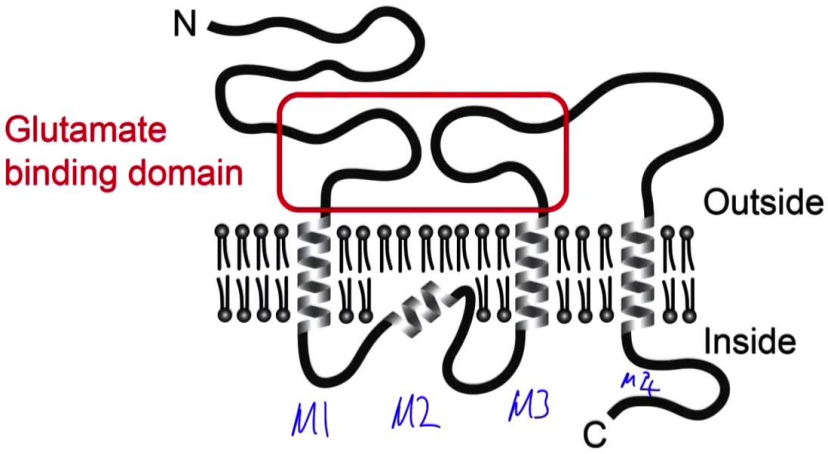
Both the AMPA and NMDA receptors are so-called ionotopic glutamate receptors. That means that they're intrinsically ion channels and the open probability of the ion channel depends upon the binding of the neurotransmitter glutamate. There are of course, transmembrane proteins and of course, made of a sequence of amino acids, starting from an N terminal, running through transmembrane alpha helixes regions, as in first transmembrane alpha helix, there's a second alpha helix that doesn't cross the membrane all the way, there's a third, and a fourth transmembrane helix. Because there are only three alpha helixes that completely cross the plasma membrane from outside to inside, the N terminal and the C terminal domains are on different sides of the membrane and the N terminal domain has a large extra cellular region and that is also where the glutamate binding domain is encoded, somewhere in this area circled here in red. So the binding of glutamate changes the structure of the ion channel and causes an increase in the open probability of the ion channel and the ion channel itself is made from four subunits that come together and look a bit like this.

Notes

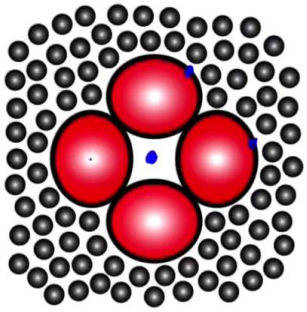
Summary

3m 37s

Ionotropic glutamate receptors: structure



Glutamate receptors have 4 subunits.



Cellular Mechanisms of Brain Function

So there are four different subunits-- come together, are sitting in the plasma membrane, this is now a top view of the plasma membrane, and down the middle we have the pore-forming ion channel which then gets activated as glutamate binds to a number of these different subunits that increases the open probability, and we then have a cation channel that's permeable to sodium, potassium, and in some cases, also to calcium.

Notes

Summary

5m 07s

Ionotropic glutamate receptors: ion permeability

AMPA receptors

Na^+ and K^+
Reversal potential ~0 mV

Single channel
conductance ~ 5 pS

NMDA receptors

Na^+, K^+ and Ca^{2+}
Reversal potential ~0 mV

Single channel
conductance ~ 50 pS

There are these two major subtypes of ionotropic glutamate receptors, the AMPA receptors and the NMDA receptors, and they are encoded by different gene families and so there are four genes that are responsible for coding AMPA receptors and there are seven genes that are responsible for coding NMDA receptors. When glutamate binds to an AMPA receptor, that increases it's open probability and the AMPA receptor is permeable to both sodium and potassium ions, a little bit more permeable to sodium, and it has a reversal potential of somewhere around 0 mV. The single channel conductance is relatively small, at around 5 pS, but that depends heavily upon the precise molecular details of which subtypes of AMPA receptors are part of that ion channel. NMDA receptors are also activated by glutamate. So glutamate binds the NMDA receptor and that increases the open probability of the NMDA receptor, but only if co-agonists are present in the extra cellular fluid and so either glycine or d-serine need to be present in the extra cellular space in order for glutamate to activate the NMDA receptor.

Notes

Summary

5m 36s

Ionotropic glutamate receptors: ion permeability

AMPA receptors	NMDA receptors
Na^+ and K^+	Na^+, K^+ and Ca^{2+}
Reversal potential ~0 mV	Reversal potential ~0 mV
Single channel conductance ~ 5 pS	Single channel conductance ~ 50 pS

Glycine
D-serine

And usually they are, and so usually this is not thought to be an important regulator, but there may well be circumstances where this really works as a co-agonist together with glutamate in order to activate the NMDA conductance. When activated, the NMDA receptor is permeable to both sodium, potassium, and importantly, is also permeable to calcium, something that most of the AMPA receptors aren't able to do. Calcium permeability is extremely important because postsynaptically, it'll turn out that calcium has important sickling properties just as it does in the presynaptic terminal where calcium is a final signal that causes neurotransmitter release. Calcium postsynaptically will turn out to have an important role in governing synaptic plasticity. The overall reversal potential of the NMDA receptor is also around 0 mV. So both of the ionotropic glutamate receptors in the end, try to bring the membrane potential close to 0 mV, that's clearly depolarized relative to action potential threshold and so both the AMPA and the NMDA receptors are excitatory and they form the major excitatory neurotransmitter receptors in the mammalian brain. NMDA receptors have a considerably larger single channel conductance compared to AMPA receptors.

Notes

Summary

6m 56s

Ionotropic glutamate receptors: ion permeability

AMPA receptors	NMDA receptors
Na^+ and K^+ Reversal potential ~0 mV	Na^+, K^+ and Ca^{2+} Reversal potential ~0 mV
Single channel conductance ~ 5 pS	Single channel conductance ~ 50 pS

Glycine
D-serine

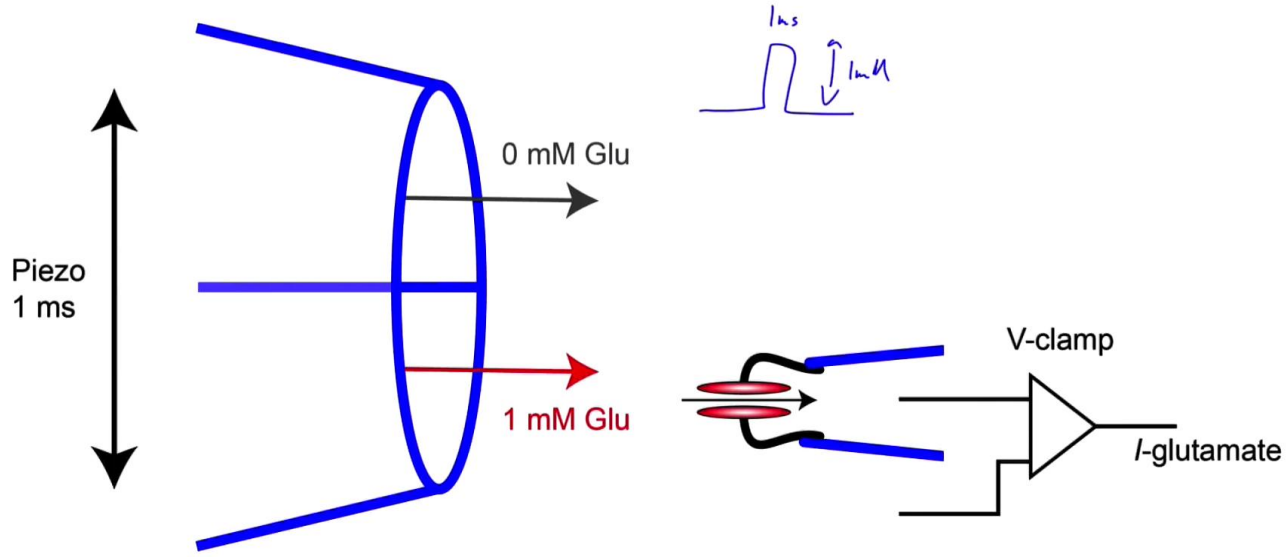
And so they're distinguishable upon many, many features, their ion channel permeability, single channel conductance, and molecularly, they're of course encoded by distinct gene families.

Notes

Summary

8m 19s

Measuring kinetics of ligand-gated ion channels



Cellular Mechanisms of Brain Function

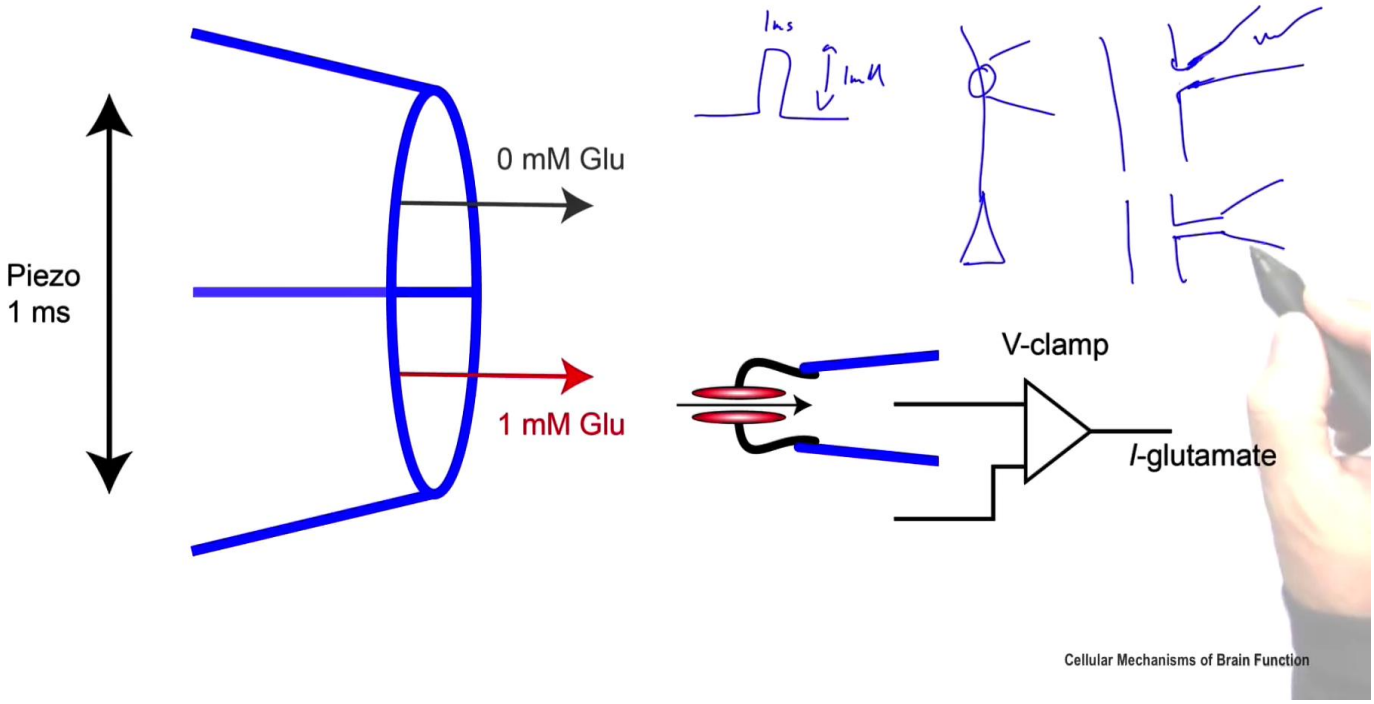
There are also very important differences in the kinetics of the currents that are activated. And it's important to study the kinetics of the AMPA and NMDA receptors under as close as possible physiological conditions. And so, what we think might be interesting, in terms of thinking about synaptic transmission, are brief pulses of glutamate that last about 1 ms and have an amplitude of around 1 mM. Now in order to make brief pulses like this, of glutamate, experimenters have designed an interesting way where one has a laminar flow of two different fluids: one fluid that contains 0 mM of glutamate, and another one that contains 1 mM of glutamate, otherwise containing identical concentrations of the other components of the extra cellular fluid. By having these two laminar flows on a piezo actuator, one can then rapidly move this laminar flow and then cause different concentrations to be hitting upon a given region of space. And so one can imagine moving this piezo actuator and giving a 1 ms pulse of 1mM of glutamate, to a small patch of membrane that's sitting on a patch clamp electrode. And this membrane here, then should preferably have glutamate receptors on it.

Notes

Summary

8m 33s

Measuring kinetics of ligand-gated ion channels



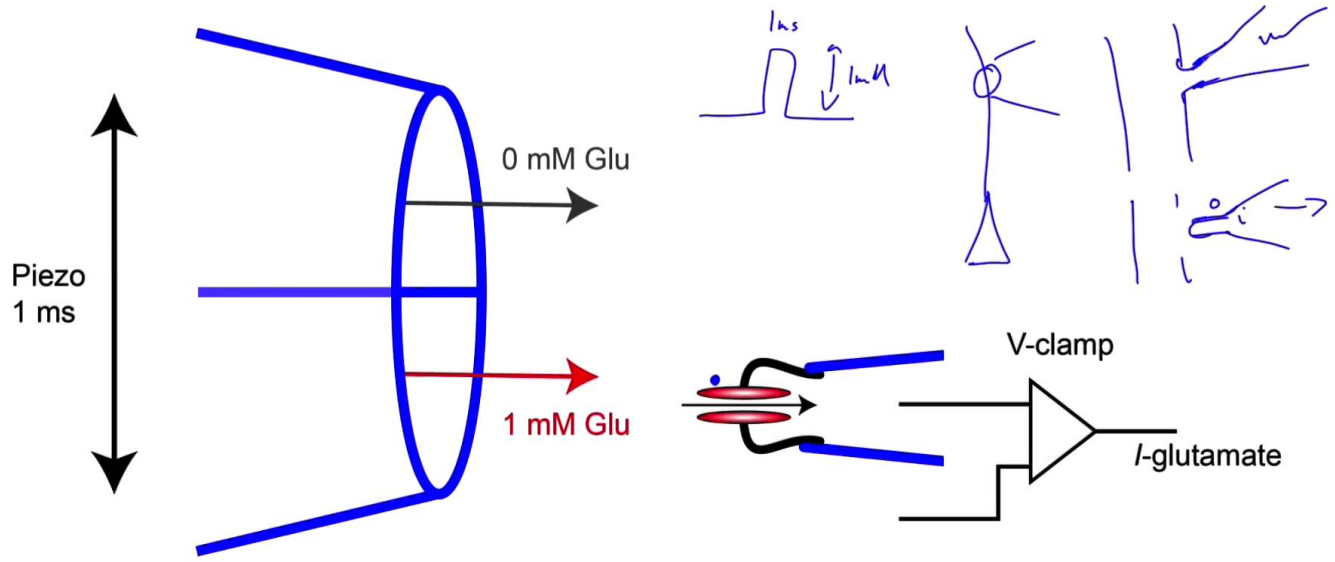
And glutamate receptors, we know, of course are sitting on dendrites of neurons, and so if we imagine a dendrite of a neuron here, there might be glutamate receptors and synapses present on one part of this membrane and so let's zoom in and look at this in some more detail and so here's the plasma membrane. We bring in the patch electrode onto this piece of dendrite, we can then suck a bit of the membrane into the glass electrode. This is our standard patch clamp recording method invented by Neher and Sakmann, we get the gigaohm electrical seal of the patch of membrane around the electrode, we can then rupture this membrane, in the so-called whole-cell recording method by a brief pulse of pressure, so we suck the membrane and that ruptures this membrane. We now have access to the inside of the membrane through the recording electrode, and if you now retract this electrode a little bit, the dendrite stays in the same location, but a bit of the membrane gets pulled out towards the membrane. And if you keep pulling on the electrode, eventually, this breaks and you're left with a small patch of membrane attached to your electrode with the outside of this membrane being on the outside relative to your patch electrode.

Notes

Summary

10m 06s

Measuring kinetics of ligand-gated ion channels



Cellular Mechanisms of Brain Function

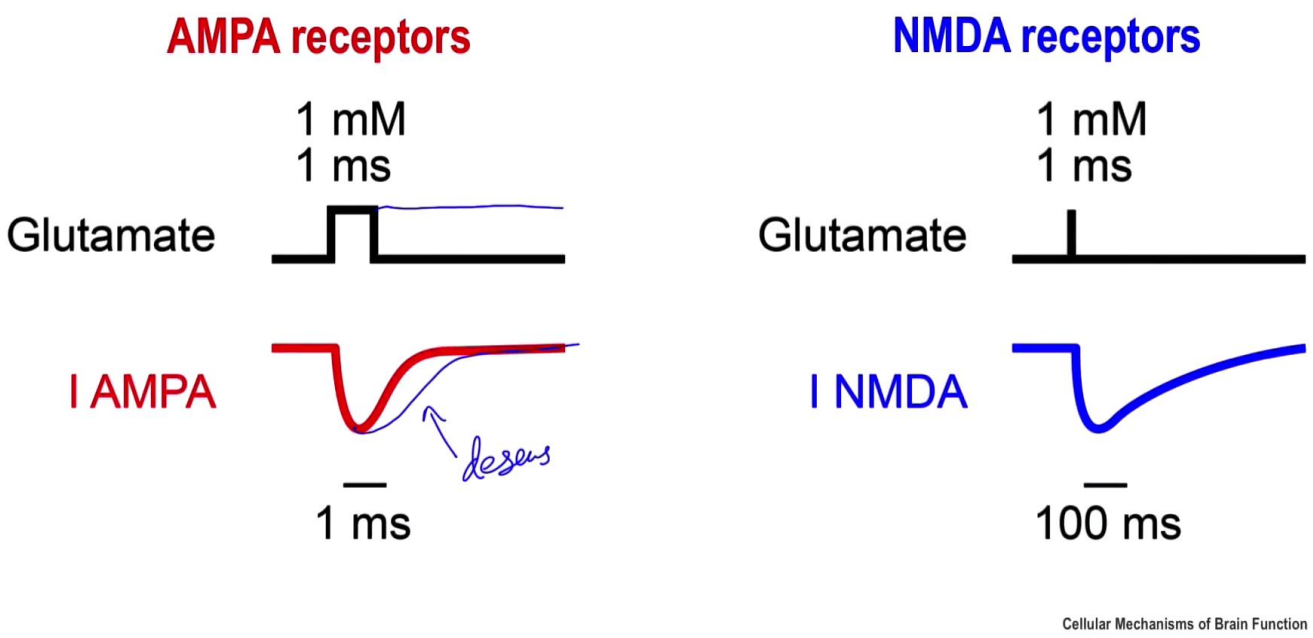
And so here, if there are glutamate receptors present, then they have the glutamate binding site in the correct orientation. You can give the 1 ms pulse of glutamate through the piezo transducer and we can then measure on your patch electrode the AMPA receptor or the NMDA receptors here, we voltage clamp the electrode, and we can then measure the currents flowing through these glutamate gated-- ligand-gated ion channels.

Notes

Summary

11m 29s

Ionotropic glutamate receptors: kinetics



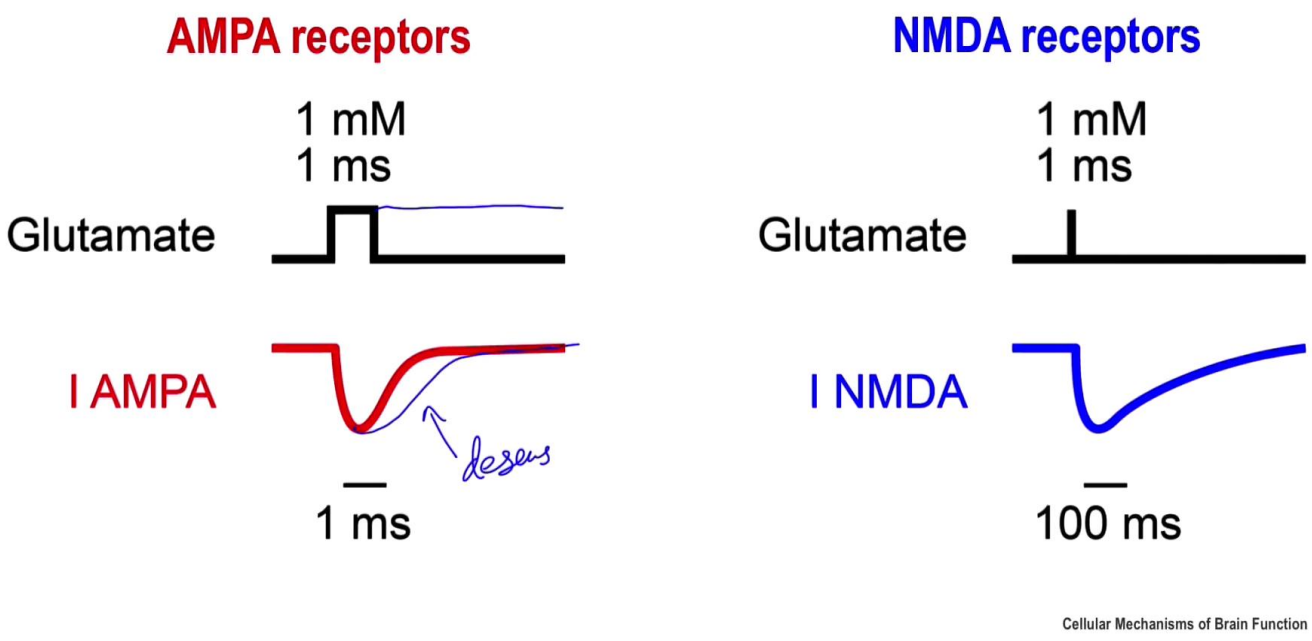
So, if we do this for patches of membrane that contain AMPA receptors and we give this a 1 ms pulse of glutamate with an amplitude of 1 mM, we find that the AMPA receptor responds very rapidly. We get rapid activation of a glutamate gated current that then also rapidly turns off in response to this 1 ms pulse of glutamate. We get, maybe, a couple of milliseconds of AMPA current. So AMPA receptors are activated extremely quickly and they also turn off extremely quickly. And in fact, it turns out that it's very difficult to have long lasting AMPA receptor currents because even if the glutamate is prolonged, it turns out that the AMPA current doesn't extend in time. And there's a very strong desensitization that takes place where, in the presence of glutamate, the AMPA receptors desensitize and are only able to give transient currents lasting for a few milliseconds. The situation for NMDA receptors is rather different. Again, we give the same 1 mM, 1 ms pulse of glutamate, and we activate NMDA currents, but as you will see here, the time scale is very different here, we're looking at 100 ms, here we were looking at 1 ms and in fact, it takes some time before the NMDA current maximizes.

Notes

Summary

11m 56s

Ionotropic glutamate receptors: kinetics



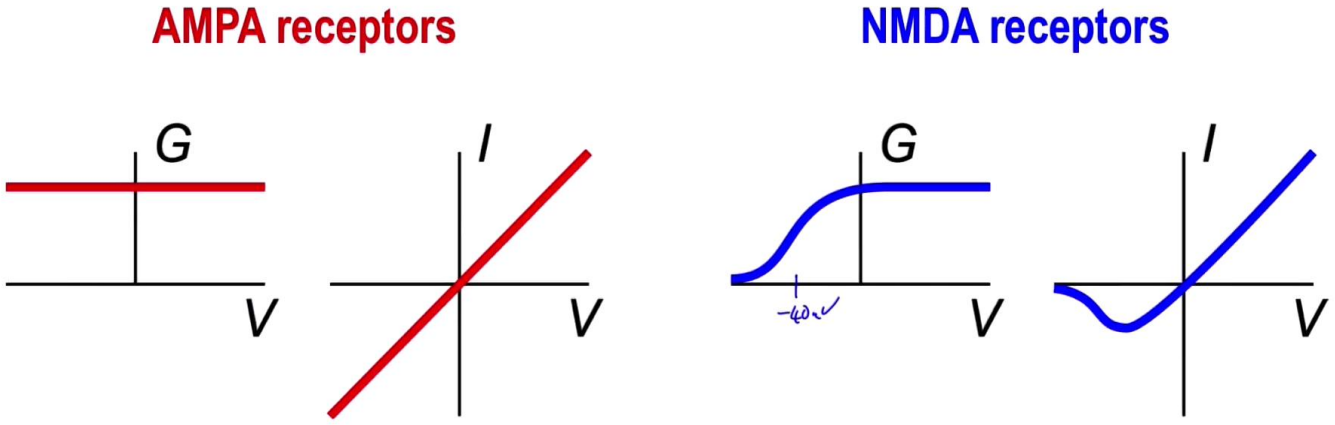
It maybe takes some 20 ms before the NMDA conductance is maximally opened, long after the glutamate has already gone. And the NMDA receptor currents remain active for hundreds of milliseconds after the glutamate pulse. And so it's clear that the temporal kinetics of AMPA conductance and NMDA conductance are extremely different, with one or two orders of magnitudes slower activity in NMDA receptors, compared to the extremely fast AMPA receptors.

Notes

Summary

13m 26s

Ionotropic glutamate receptors: *I–V* relationship



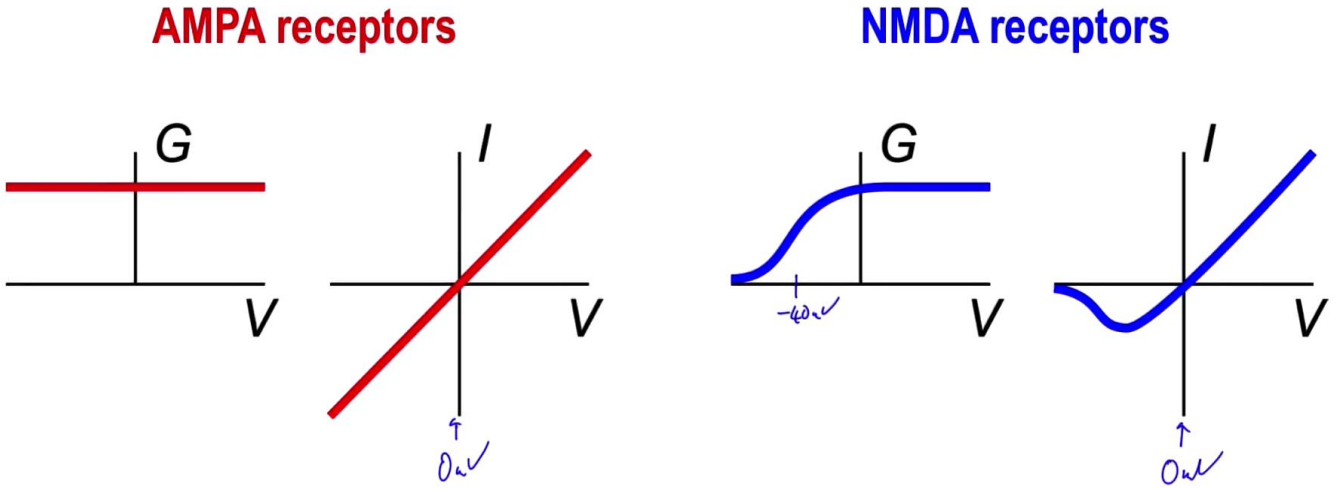
There are also important differences in the voltage dependence of the currents generated by AMPA and NMDA receptors. AMPA receptors are basically voltage insensitive, in at least, most of the forms of AMPA receptors. And so if we think about the conductance activated by a pulse of glutamate, this is independent to voltage, and that then gives rise to a linear current voltage relationship. For NMDA receptors, the situation's rather different. At hyperpolarized potentials, and that's something hyperpolarize theta - 40 mV, the conductance of the NMDA receptor is strongly reduced. The open probability that the amount of current flow is blocked here at negative potentials, and at positive potentials, we come here with a full activation. And so we get these current-voltage relationships that look very similar to what we saw before for voltage-gated sodium, and voltage-gated potassium, and calcium conductances, but here, the reversal potential here is close to 0 mV, of course in both cases, for the AMPA and the NMDA receptor, whereas for the voltage-gated sodium conductors, we of course, had reversal and voltage-gated at the sodium reversal potential.

Notes

Summary

14m 00s

Ionotropic glutamate receptors: *I–V* relationship



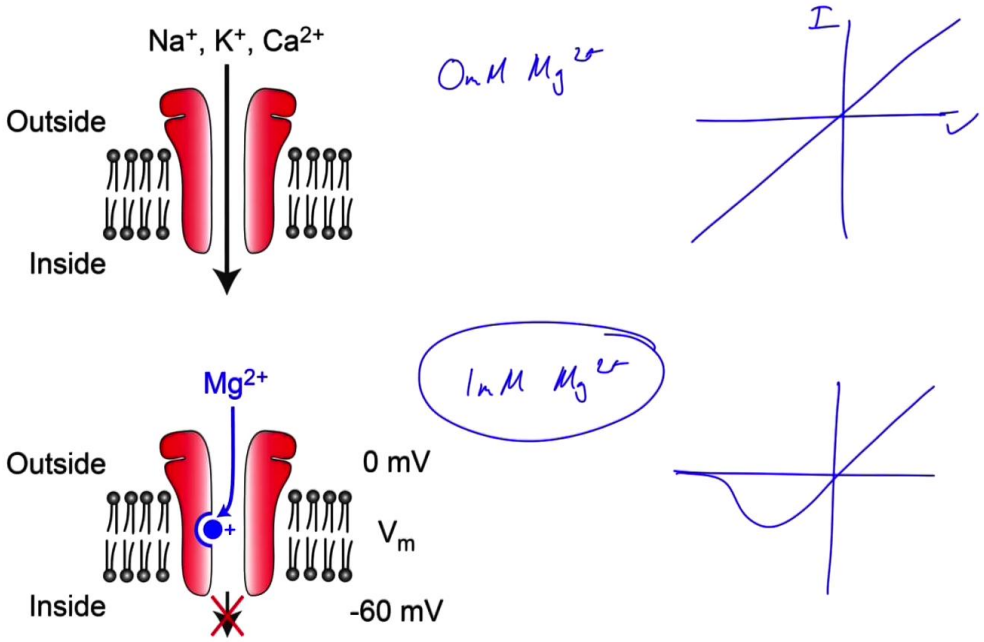
So there's an obvious difference here under physiological conditions between the non-voltage dependence of AMPA receptors and a strong voltage dependence for NMDA receptors. And although this looks very similar to the phenomenology of voltage-gated sodium channels, in fact, the physical basis is very different.

Notes

Summary

15m 18s

Voltage-dependent Mg^{2+} block of NMDA receptors



Cellular Mechanisms of Brain Function

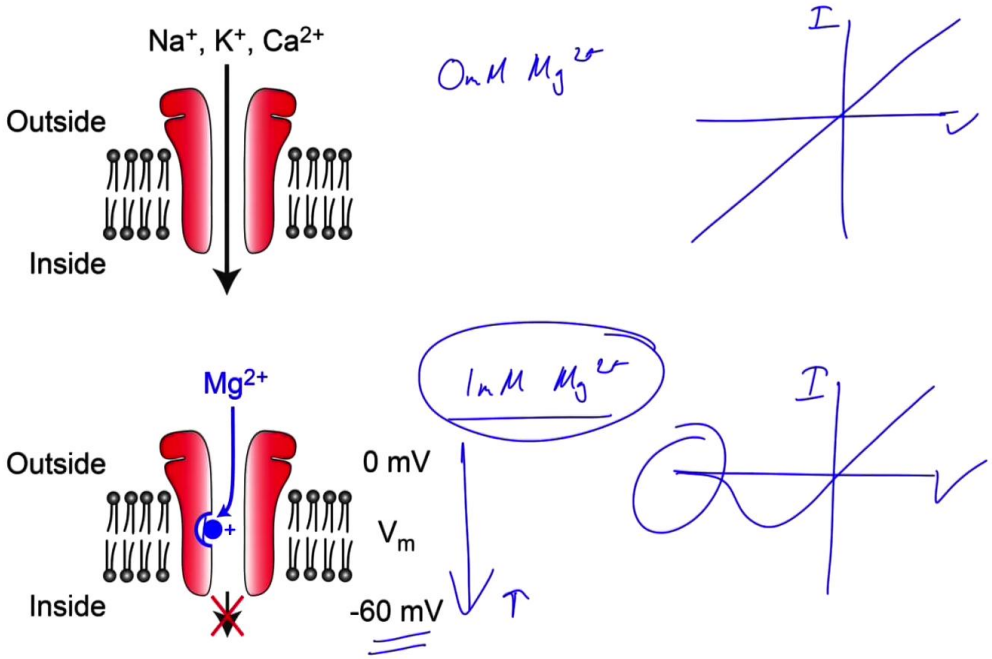
In fact, the NMDA receptors aren't intrinsically voltage sensitive, but they gain that attribute because of a magnesium block. And so in the presence of zero magnesium in the extra cellular space, in fact, the NMDA receptor is linear-- voltage and current-- and is only in the presence of extra cellular magnesium that we have a strong dependence upon voltage for the NMDA receptor. Now, physiologically, magnesium is always present in the extra cellular solution. And so the voltage dependent magnesium block, or the NMDA receptors is extremely relevant, functionally to the properties of the NMDA receptor. The fact that the block only occurs at negative potentials is because that's when there's an electric field that tries to suck magnesium inside the ion channel and the magnesium is in fact, blocking the ion channel by sitting in the ion channel, binding to a relatively high affinity site and preventing the permeation of other ions.

Notes

Summary

15m 41s

Voltage-dependent Mg^{2+} block of NMDA receptors



Cellular Mechanisms of Brain Function

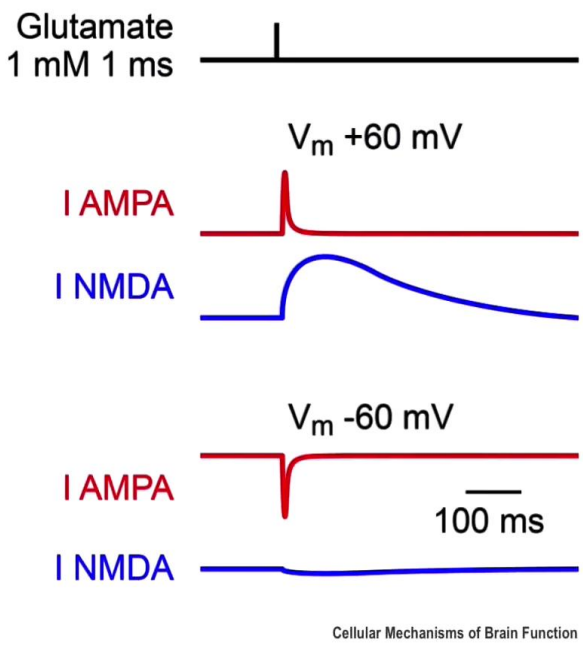
And so the magnesium pops in and out in a so-called fast, flickering block where it directly blocks the permeation of the other ions and doesn't affect the open probability of the ion channel, but is just simply being blocked by the presence or absence of magnesium and normally with 1 mM magnesium present in the extra cellular space and negative membrane potentials that confers a very strong block on the conduction through the NMDA receptor, and that's why we have this interesting shape where at negative potentials, magnesium comes in, blocks current flow through the NMDA receptor, and as we get to more and more positive potentials, then the magnesium gets pushed out from this internal binding site and we get the full current flow of the other ions through the NMDA receptor. So there's not a voltage sensing domain of the NMDA receptor, but rather a voltage dependent block by magnesium in the pore of the ion channel.

Notes

Summary

16m 55s

AMPA and NMDA receptors



So we've seen that there are two very different ionotropic glutamate receptors that are present in the mammalian nervous system. We have the AMPA receptors that are extremely fast, giving rise to millisecond duration currents in response to a 1 ms pulse of glutamate and the AMPA receptors don't depend upon the membrane potential. So at -60 mV or +60 mV, the amount of current flow is the same and the reversal potential is somewhere around 0 mV. For the NMDA receptor, the situation's really, very different. We have long lasting, slow currents in response to just millisecond pulses of glutamate. It takes tens of milliseconds for the NMDA receptor to open and once the glutamate is bound, it remains bound and their ion conductance can remain active for hundreds of milliseconds after the glutamate has already long gone from the synaptic cleft. The NMDA currents are also interesting because they're actually blocked at resting membrane potentials. So at the resting membrane potential of a neuron, at around -70 or -60 mV, if the glutamate is puffed onto NMDA receptors, there's actually very little current flow because magnesium is blocking that ion channel. So, NMDA receptors depend upon both postsynaptic depolarization and the presence of glutamate, whereas AMPA receptors are always active if glutamate is present.

Notes

Summary

18m 01s

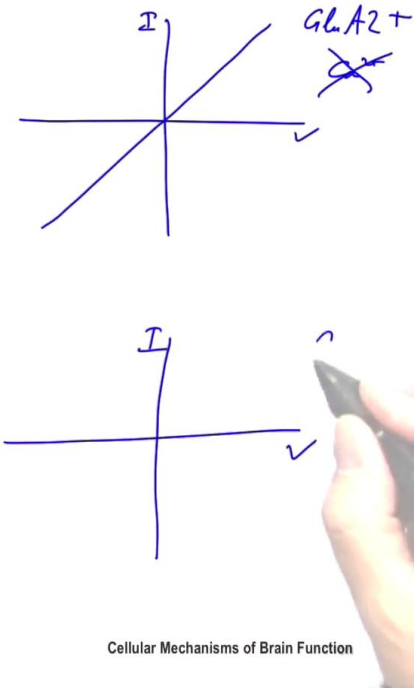
AMPA receptor diversity

GluA1-4 *gria1-4* (GluR1-4; GluRA-D)

GluA2 is a subunit of most AMPA receptors.

AMPA receptors containing the GluA2 subunit have linear IV relationships and lack calcium permeability.

AMPA receptors lacking the GluA2 subunit are inwardly rectifying and are calcium permeable.



Cellular Mechanisms of Brain Function

There's considerable diversity amongst the different types of AMPA receptors, and there are in fact, four genes that encode AMPA receptors in the mammalian genome, and these are then labeled in their gene names as glutamate receptor ionotropic ampa and then subtypes 1, 2, 3, and 4. So that's the gene names that then give rise to the different types of subunits that are typically labeled GluA1, GluA2, 3, and 4. And in older papers, you will read a different nomenclature, whether it's called GluR1-4 or GluRA-D. So there's a variety of different nomenclatures and this is the most modern one that should be used from now on. Now, most AMPA receptors contain the so-called GluA2 subunit. And if the AMPA receptor contains the GluA2 subunit, then as described, we have linear current-voltage relationships, and there's no calcium permeability. Now, some AMPA receptors don't have the GluA2 subunit. And under those conditions, the current-voltage relationship is really quite different. So now we're thinking about GluA2-lacking AMPA receptors and they have a so-called inward rectification where they like current to go into the cell and depolarize it, but they don't like current to go out of the cell, in fact, there's an intracellular polyamine block at positive membrane potentials.

Notes

Summary

19m 37s

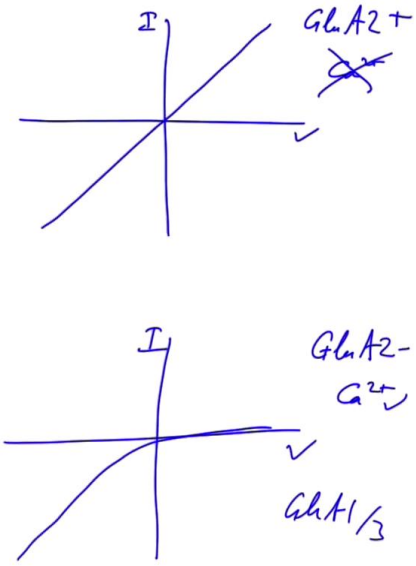
AMPA receptor diversity

GluA1-4 *gria1-4* (GluR1-4; GluRA-D)

GluA2 is a subunit of most AMPA receptors.

AMPA receptors containing the GluA2 subunit have linear IV relationships and lack calcium permeability.

AMPA receptors lacking the GluA2 subunit are inwardly rectifying and are calcium permeable.



In addition, GluA2-lacking AMPA receptors are permeable to calcium, and so have interesting sickling roles. And so whereas most AMPA receptors are composed of combinations of GluA2 with GluA1 or GluA3 heteromers, there are some which don't contain the GluA2, and are simply GluA1 or 3, for example, as a subunit they lack the GluA2 and then they have these interesting voltage dependencies that again, depend upon intracellular components like polyamines that block the ion channel from the inside. So there's some diversity in the AMPA receptors and this is then further regulated and involved in different types of synaptic plasticity and other sickling features.

Notes

Summary

21m 21s

Kainate receptors

GluK1-5 *grik1-5* (GluR5-7; KA1,2) ?

In addition to AMPA receptors, there are also Kainate receptors that bear some resemblance to the AMPA receptors, but they have a much less defined function. And there are five genes that are encoded by the so-called kainate receptor family-- glutamate receptor ionotropic kainate 1-5, GluK1-5, they used to be labeled GluR5-7; KA1, 2. They are ionotropic ligand-gated glutamate receptors just like the AMPA receptors to which they're more similar compared to, at least, the NMDA receptors. They seem to have smaller currents, they seem to be slower, in some cases, they may also be located on presynaptic specializations rather than postsynaptically, but on the whole, much less is known about the function of the kainate receptors.

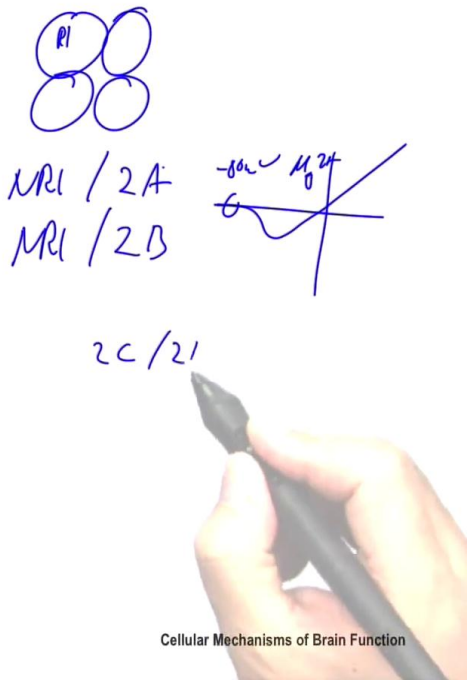
Notes

Summary

22m 18s

NMDA receptor diversity

GluN1	*grin1*	(NR1) ✳
GluN2A-D	*grin2A-D*	(NR2A-D)
GluN3A,B	*grin3A,B*	(NR3A,B)
GluN2A,B	strong Mg^{2+} block	
GluN2C,D	weak Mg^{2+} block	
GluN2A	fast (~100 ms)	
GluN2B,C	medium (~300 ms)	
GluN2D	slow (~1 s)	
GluN3		



The NMDA receptors form the other major family of ligand-gated glutamate receptors, and they're composed of seven different genes. There's the grin1 gene, grin2A-D, and grin3A, and B. And what's important about the NMDA receptors is that the NR1 subunit, the GluN1 subunit, is absolutely required for function. So all NMDA receptors are thought to have the GluN1 subunit as part of the four subunits. So one of these will be the NR1 subunit, at least. This will then combine with other subunits, and the most common combination is with NR1 with 2A or NR1 with 2B. And that's most of the glutamate receptors in the mammalian brain are encoded by either the NR1 2A or the NR1 2B. Both of these combinations are very strongly blocked by magnesium as we've already seen, they have an almost complete block at, let's say, at around -80 mV, the NMDA receptor's completely blocked by the presence of magnesium. However, two other subunits, the 2C and the 2D, have a much weaker block with respect to magnesium. And so in comparison, they would have something this, where there's a substantial amount of unblocked NMDA receptor current even at these very hyperpolarized membrane potentials.

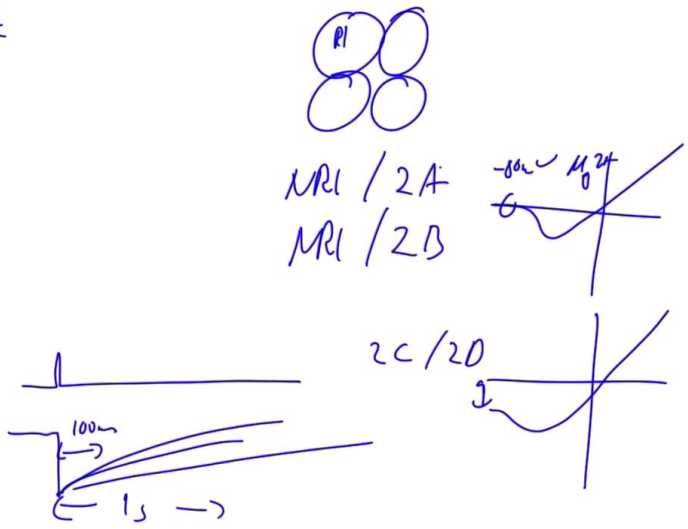
Notes

Summary

23m 13s

NMDA receptor diversity

GluN1	*grin1*	(NR1) ✳
GluN2A-D	*grin2A-D*	(NR2A-D)
GluN3A,B	*grin3A,B*	(NR3A,B)
GluN2A,B	strong Mg^{2+} block	
GluN2C,D	weak Mg^{2+} block	
GluN2A	fast (~100 ms)	
GluN2B,C	medium (~300 ms)	
GluN2D	slow (~1 s)	
GluN3		



Cellular Mechanisms of Brain Function

So there's differences in the degree of magnesium block comparing across the different subunits of the NMDA receptor. We'll also discuss the very long time constants where in response to a 1 ms pulse of glutamate, the 2A subunit might have a time course of something like 100 ms, whereas the slowest of the subunits might have times of around 1 s for it's sort of half decay time, and the 2B and 2C are somewhere in the middle. And so the time constants of the conductance activated by glutamate is extremely different comparing across these different subunit combinations. And so you can get a great deal of diversity in NMDA receptor function by having different combinations of these different NMDA receptor subunits present at the synapse, and indeed different cells and different synapses have very different features. Finally the GluN3, subunits A and B, have less known functions, but might function presynaptically or play other roles in sickling and synaptic plasticity.

Notes

Summary

24m 52s

Metabotropic glutamate receptors

mGluR1-8 *grm1-8*

7 transmembrane, G-protein coupled receptors (GPCRs)

Group 1 (mGluR1,5) – couple to PLC, Ca^{2+} signalling
Group 2 (mGluR2,3) and group 3 (mGluR4,6,7,8) – inhibit AC

Finally, of course, all though we're primarily interested in the fastest forms of communication between neurons mediated by the ionotropic glutamate receptors that generate postsynaptic potentials on the millisecond time scale, we mustn't forget about the metabotropic glutamate receptors. These are 7 transmembrane receptors, they couple through g-proteins, activate [inaudible] through sickling pathways, like phospholipase or they inhibit adenylate cyclase in track of calcium sickling. They probably have multiple, diverse roles, but are yet to be fully explored. And there's eight genes that encode these metabotropic glutamate receptors and these are likely to have slower sickling functions than the ionotropic glutamate receptors that are working on the millisecond time scale.

Notes

Summary

26m 02s

AMPA and NMDA receptors

There two main types of ionotropic glutamate receptors:

AMPA receptors

- fast
- Na^+/K^+ permeable

NMDA receptors

- slow
- $Na^+/K^+/Ca^{2+}$ permeable
- voltage-dependent Mg^{2+} block

Cellular Mechanisms of Brain Function

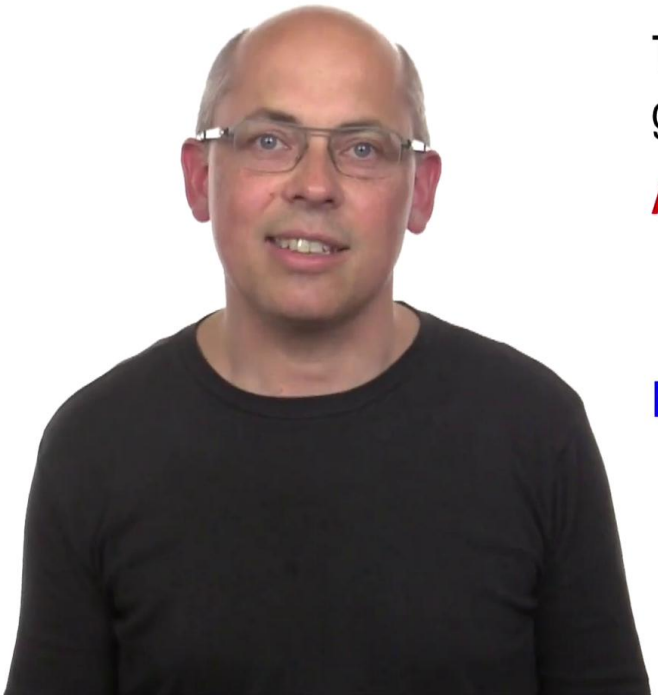
So the take home message from today's lesson, is that there are two major subtypes of ionotropic glutamate receptors. There are the AMPA receptors, that are responsible for the millisecond activation of a synaptic conductance in response to glutamate, and there are the NMDA receptors, that are working on the tens of milliseconds or hundreds of milliseconds time scale. They're activated more slowly, they last for hundreds of milliseconds, long outlasting that pulse of glutamate from the presynaptic specialization. The conductances that are opened, are both excitatory for AMPA and NMDA receptors, they reverse at 0 mV, the AMPA conductance being permeable to sodium and potassium, and the NMDA conductance, in addition, allowing calcium entry into the postsynaptic specialization, where it'll turn out it has an important role in controlling synaptic plasticity. In addition, the NMDA receptor has a very important dependence upon the postsynaptic membrane potential. At negative potentials, the extra cellular magnesium enters inside the pore of the NMDA receptor and blocks it, making the other ions impossible for them to permeate.

Notes

Summary

26m 51s

AMPA and NMDA receptors



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Cellular Mechanisms of Brain Function

And so through this voltage dependent magnesium block, the NMDA receptor is both sensitive to the state of the postsynaptic cell, it needs to be depolarized in order to unblock the magnesium, and it, of course, also needs to have glutamate release from it's presynaptic terminal. So the NMDA receptor has an interesting coincidence role, where it's both sensitive to the activity of the presynaptic cell, as well as a postsynaptic cell in which it's embedded. And we're going to learn more about the importance of that coincidence detection as we consider synaptic plasticity in the next lesson.

Notes

Summary

28m 13s