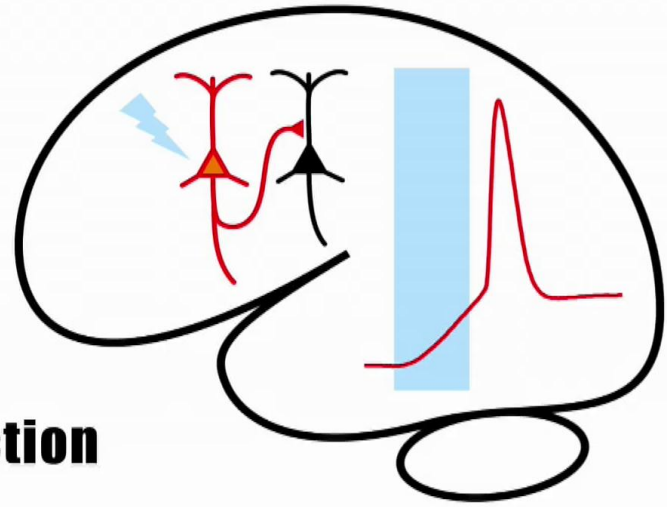
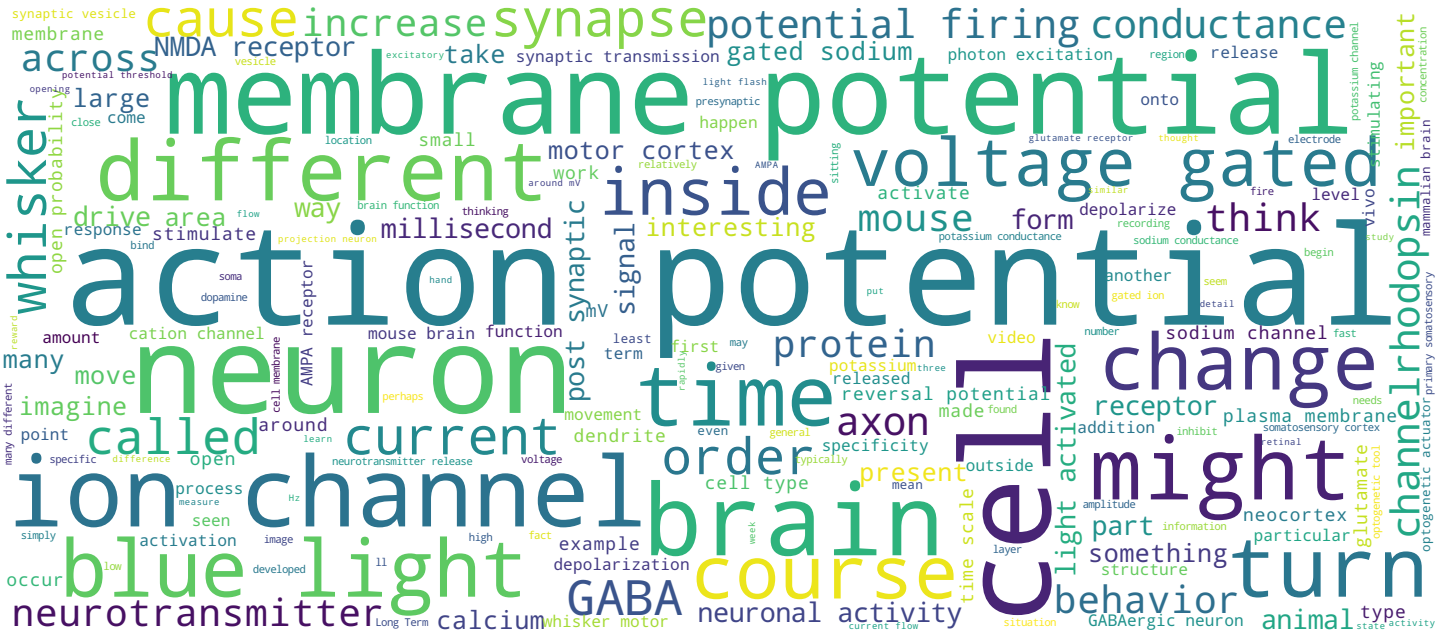


6.5 Controlling brain function

Cellular Mechanisms of Brain Function

Prof. Carl Petersen



Search MOOC

Video

EPFL

Controlling brain function

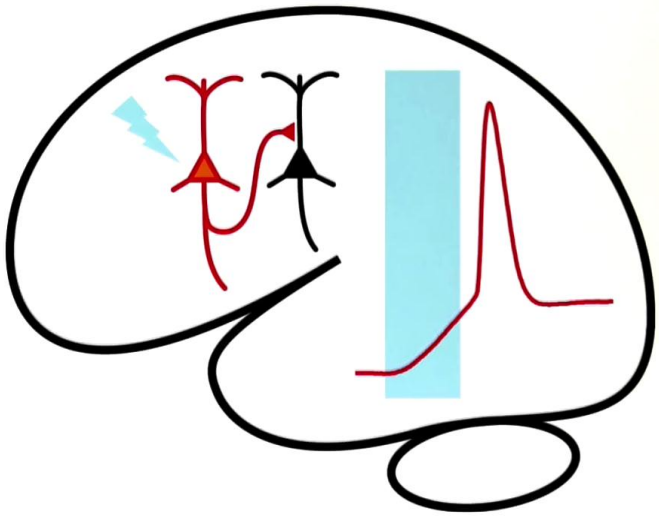
In the last two videos we've seen that electrophysiological recordings, and optical imaging techniques are extremely useful in measuring neuronal activity in an awake behaving mouse. These measuring techniques allow us to correlate neuronal activity with behavior. But in order to get a causal argument linking that neuronal activity to a specific behavior, we need to be able to manipulate those neurons, we need to be able to stimulate them, and inhibit them with high specificity in terms of time and space and see how that impacts the other network effects and the behavior that we're interested in. Until recently, to stimulate neuronal activity, the best tool that neuroscientists have had is to put stimulation electrodes into the brain, and by driving brief current pulses through those stimulation electrodes, one could activate the neurons close to that stimulation electrode. However, axons of passage, or neurons in connected brain areas, would also be stimulated, limiting the specificity of electrical microstimulation. On the other hand, to inhibit neuronal activity, neuroscientists have had even poorer control.

Notes

Summary

0m 05s

Optogenetics



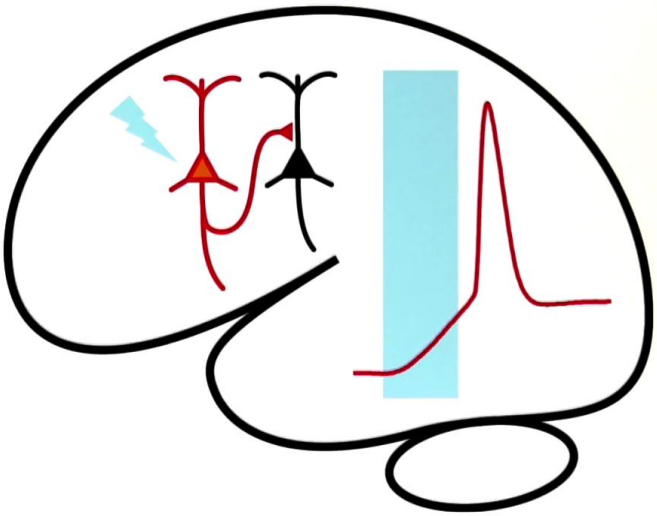
Cellular Mechanisms of Brain Function

Typically they've lesioned brain areas in animal models, or they've injected pharmacological agents, like tetrodotoxin, to block action potential firing, or CNQX and APV, to block glutamatergic synaptic transmission. These reagents certainly block activity near to the injection site, but they do so on long time scales, typically involving many minutes, and of course they don't have any cellular specificity. They inhibit everything nearby, and typically many different types of neurons are intermingled. And so, again, there's a lack of specificity. Over the last decade, neuroscience has gone through a revolution, allowing us to deal with causal interventions in the mammalian brain through genetically expressing actuators that control neuronal activity. And so that allows us the specificity of mouse genetics to decide which cells we're stimulating or inhibiting, and to have these actuators under optical control. And that means we can point light at specific cells that might express our optogenetic actuator, and then take control of that cell, for example, making it depolarize and fire an action potential.

Notes

Summary

1m 22s



Cellular Mechanisms of Brain Function

And we can then utilize the specificity of light, being able to turn light on and off with millisecond temporization, and being able to point it at specific parts of the brain in order to take a highly specific control of the mouse brain during behavior. Let's see how that works.

Notes

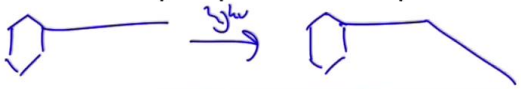
Summary

2m 39s

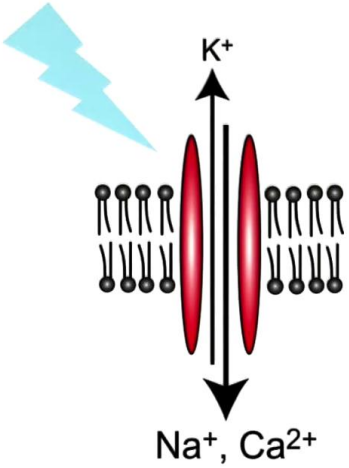
A light-activated cation channel

Channelrhodopsin-2 (ChR2) is a light-activated cation channel, cloned from the green algae *Chlamydomonas reinhardtii*.

Retinal is bound to ChR2 and changes conformation from all-*trans* to 13-*cis* upon photon absorption.



Nagel, Szellas, Huhn, Kateriya, Adeishvili, Berthold, Ollig, Hegemann, Bamberg (2003) *Channelrhodopsin-2, a directly light-gated cation-selective membrane channel.* Proc Natl Acad Sci USA 100: 13940-13945.



Cellular Mechanisms of Brain Function

The optogenetic revolution begins with the cloning and identification of light-activated cation channels, and in particular the identification of channelrhodopsin-2 as a light-activated cation channel in this seminal paper here from Nagel et al. in 2003 began the optogenetic revolution for the neuroscientists. Channelrhodopsin-2 is a transmembrane protein that was cloned from the green algae *Chlamydomonas reinhardtii*. It's a plasma membrane ion channel, and bound to it is the molecule retinal, which is an aldehyde derived from Vitamin A, and that's covalently bound to the channelrhodopsin protein. And it's the retinal itself that interacts with light. And so retinal is a relatively small molecule. It absorbs a photon, and that then drives a conformational change in the retinal, changing it from a *trans* to a *cis* motif, so it changes its structure, the retinal. The retinal is bound into the channelrhodopsin channel, and causes a conformational change of the channel protein, and increases its open probability. And so in that way, light directly gates the cation channel, encoded by channelrhodopsin-2.

Notes

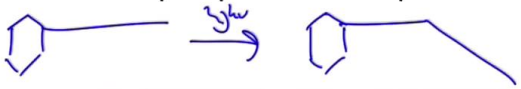
Summary

2m 58s

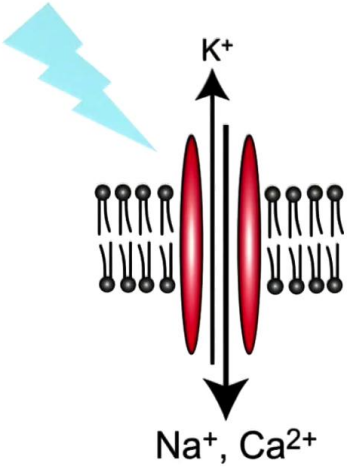
A light-activated cation channel

Channelrhodopsin-2 (ChR2) is a light-activated cation channel, cloned from the green algae *Chlamydomonas reinhardtii*.

Retinal is bound to ChR2 and changes conformation from all-*trans* to 13-*cis* upon photon absorption.



Nagel, Szellas, Huhn, Kateriya, Adeishvili, Berthold, Ollig, Hegemann, Bamberg (2003) *Channelrhodopsin-2, a directly light-gated cation-selective membrane channel.* Proc Natl Acad Sci USA 100: 13940-13945.



Cellular Mechanisms of Brain Function

So we've already seen other ion channels that are gated, for example, by neurotransmitter, like the glutamate receptors, or by voltage, like the voltage-gated sodium channels. Here we now have a light-activated channel where absorption of a photon on the middle-second time scale causes an increase in open probability. And it's a relatively non-selective cation channel, so it's permeable to potassium, sodium, calcium and also protons, and under physiological conditions the major conductance is an inward sodium and calcium current, that then has a reversal potential of somewhere around 0 mV. So in terms of putting it inside neurons, this is an excitatory light-activated channel.

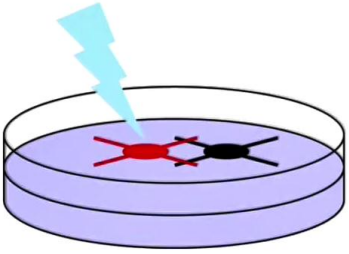
Notes

Summary

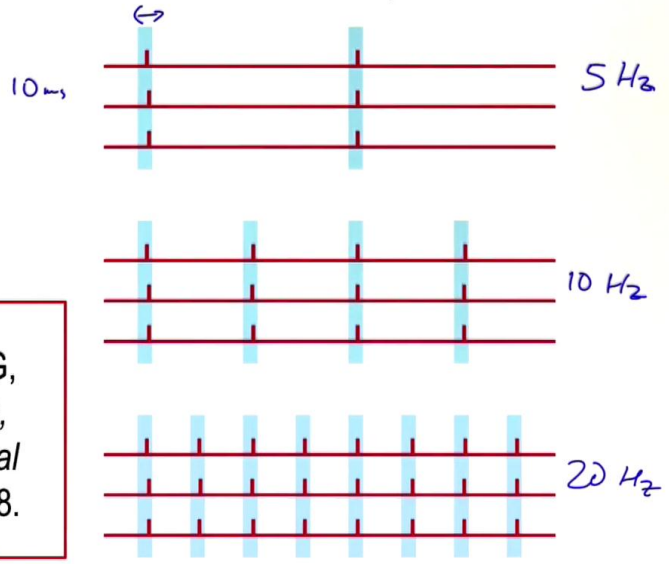
4m 30s

Neuronal stimulation with channelrhodopsin-2

ChR2 can be used to stimulate neurons with millisecond precision.



Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2006) *Millisecond-timescale, genetically targeted optical control of neural activity.* Nature Neuroscience 8: 1263-1268.



Cellular Mechanisms of Brain Function

The next important step in the optogenetic revolution was to put channelrhodopsin into neurons, and that was successfully done by Ed Boyden, when he was in Karl Deisseroth's laboratory, in the seminal paper from 2006. What they found was that they could express channelrhodopsin in cultured hippocampal neurons in a dish, and the cells that expressed channelrhodopsin turned out to be light-sensitive, and brief pulses of blue light would drive action potential firing with millisecond temporal precision. So they gave 10 ms light pulses, and action potential firing, in cells that were expressing channelrhodopsin occurred with very low jitter and highly reproducible, every time there's a blue light flash, an action potential was driven in these neurons. And they found that they could drive the neurons at different frequencies, so they could stimulate at 5 Hz, at 10 Hz, and even at 20 Hz, and they could reliably drive action potential firing with every blue light flash. This is remarkable, because there were many things that were uncertain. It was unclear, for example, whether retinal would be sufficient in mammalian neurons, to allow the single expression of channelrhodopsin to be sufficient to drive neuronal action potential firing.

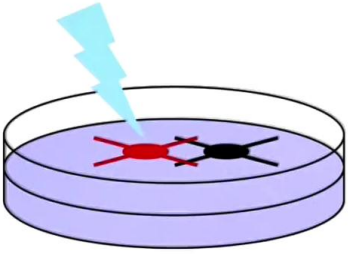
Notes

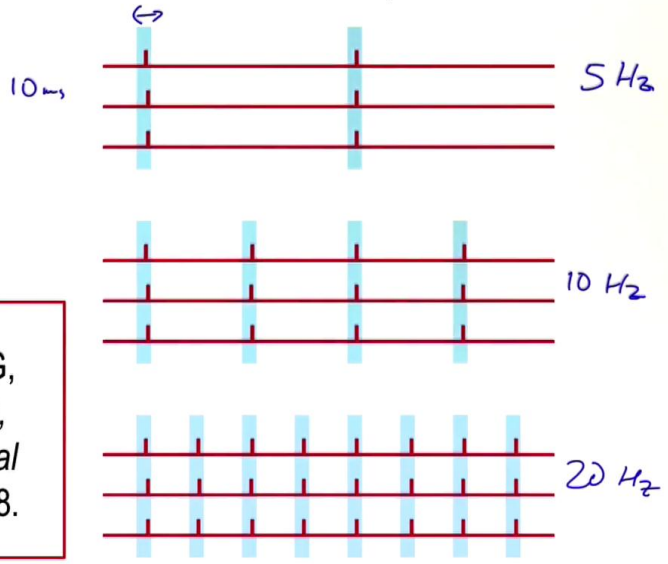
Summary

5m 17s

Neuronal stimulation with channelrhodopsin-2

ChR2 can be used to stimulate neurons with millisecond precision.





Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2006) *Millisecond-timescale, genetically targeted optical control of neural activity.* Nature Neuroscience 8: 1263-1268.

Cellular Mechanisms of Brain Function

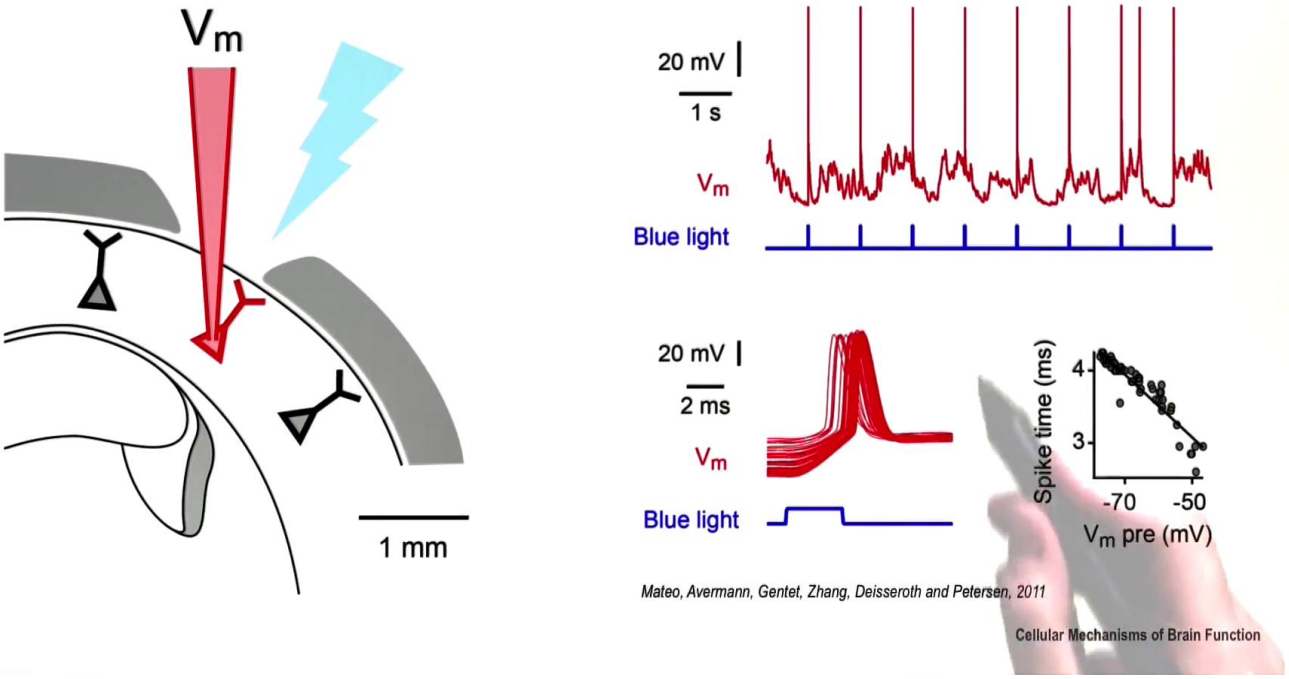
And so this caused the beginning of a revolution, and it's really the robustness of the channelrhodopsin that's made it used widely, across the neuroscience community.

Notes

Summary

6m 40s

Channelrhodopsin-2 *in vivo*



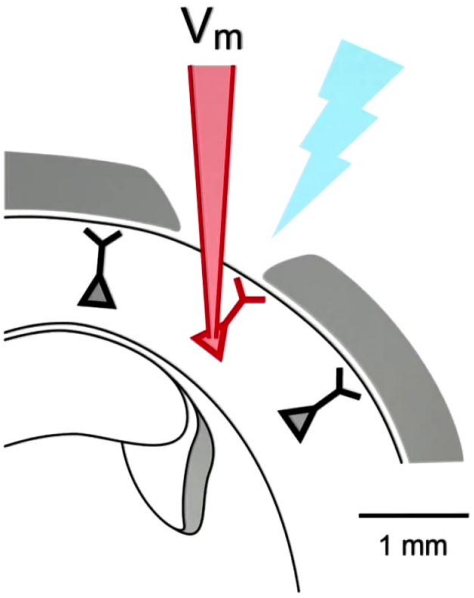
An important question, of course, was how well channelrhodopsin-2 works *in vivo*, in the living mammalian brain. And so here we've expressed channelrhodopsin-2 in the primary somatosensory cortex. We come in with a whole-cell membrane potential recording from one of the neurons that's expressing channelrhodopsin, and *in vivo* there are membrane potential fluctuations that are large in amplitude, and occurring on a variety of time scales, and so it's interesting to see to what extent we can get reliable control of action potential firing under these conditions of high variability *in vivo*. And it turns out that, at least under some experimental conditions, brief blue light flashes applied to cells *in vivo* cause extremely reliable action potential firing, just like it had been described in the cultured hippocampal neurons by Ed Boyden. So irrespective of the membrane potential at the time of stimulus, each time a blue light flash, here 3 ms in duration, was delivered to the cell, it would cause a depolarization, it would reach action potential threshold, and an action potential would be evoked.

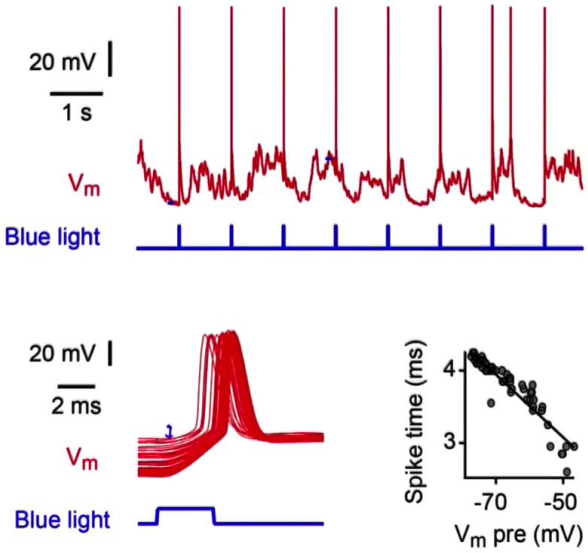
Notes

Summary

6m 52s

Channelrhodopsin-2 *in vivo*





Mateo, Avermann, Gentet, Zhang, Deisseroth and Petersen, 2011

Cellular Mechanisms of Brain Function

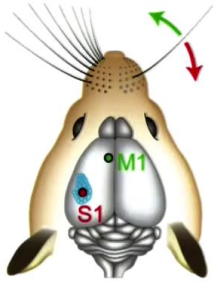
This occurred in response to every blue light flash, but there was some variability in the timing of the action potential with respect to the onset of the blue light. As you'll see, in the membrane potential there are spontaneous fluctuations in the pre-stimulus membrane potential. So sometimes the blue light is delivered when the neuron is hyperpolarized, and at other times the blue light is delivered when the neuron is spontaneously depolarized. And it turns out that that makes a difference to the precise timing of the action potential. If the neuron is spontaneously hyperpolarized, the blue light comes on, we need to depolarize by a long distance, and that takes some time before we reach action potential threshold. On the other hand, if we're spontaneously depolarized, we turn the blue light on, the depolarization begins at the same point, but now there's less depolarization that needs to happen before we hit AP threshold, and the action potential, therefore, occurs earlier. And so every blue light stimulus gives a single action potential output, but the precise timing varies by a millisecond or so, depending upon the exact depolarization, the state of the cell that we're recording from. And so channelrhodopsin-2 turns out to work extremely robustly *in vivo*.

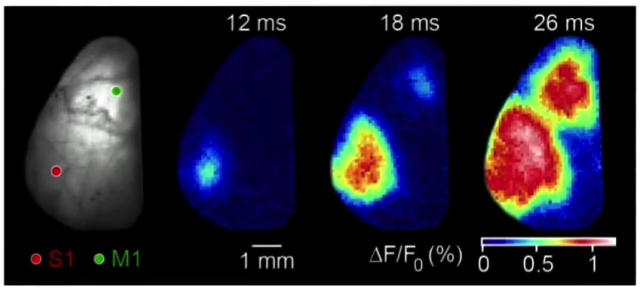
Notes

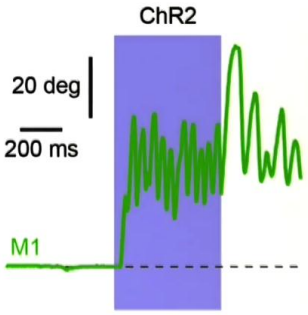
Summary

8m 05s

Channelrhodopsin-2 can drive behavior







Matyas, Sreenivasan, Marbach, Wacongne, Barsy, Mateo, Aronoff and Petersen, 2010

S1 – primary somatosensory cortex
M1 – primary motor cortex

Cellular Mechanisms of Brain Function

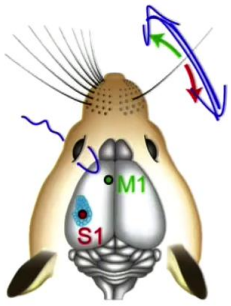
A next question one might ask is whether stimulation of neurons with channelrhodopsin-2 can drive a behavioral output in the mouse. And an obvious place to look, then, for optogenetic influence on behavior is by stimulating the motor cortex. So, we investigated here, in a system that we've already studied in some detail, the somatosensory whisker signaling pathway where if we stimulate a whisker we can image neuronal activity across the dorsal surface of the neocortex with voltage-sensitive dyes. We stimulate the whisker, and the first thing that happens, if we look in the neocortex, is that there's depolarization and sensory processing in the primary somatosensory cortex. Some 5-6 ms later there's an additional hotspot of activity, sensory processing, in the motor cortex, and so we can localize the whisker motor cortex in the mouse through voltage-sensitive dye imaging. We can then express channelrhodopsin, specifically in this area of the brain, by injecting a virus to express channelrhodopsin in the whisker motor cortex, and then in our head-restrained mice we can then turn on the blue light and see what happens when we stimulate the whisker motor cortex.

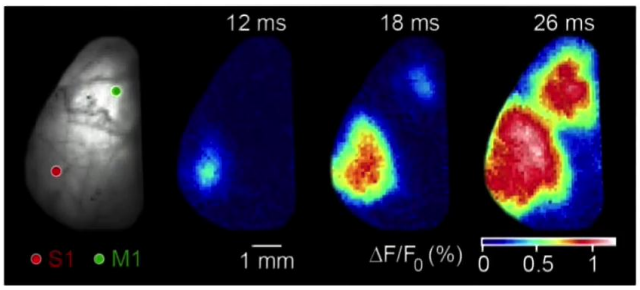
Notes

Summary

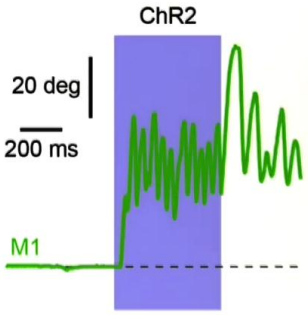
9m 27s

Channelrhodopsin-2 can drive behavior





Matyas, Sreenivasan, Marbach, Wacongne, Barsy, Mateo, Aronoff and Petersen, 2010



S1 – primary somatosensory cortex
M1 – primary motor cortex

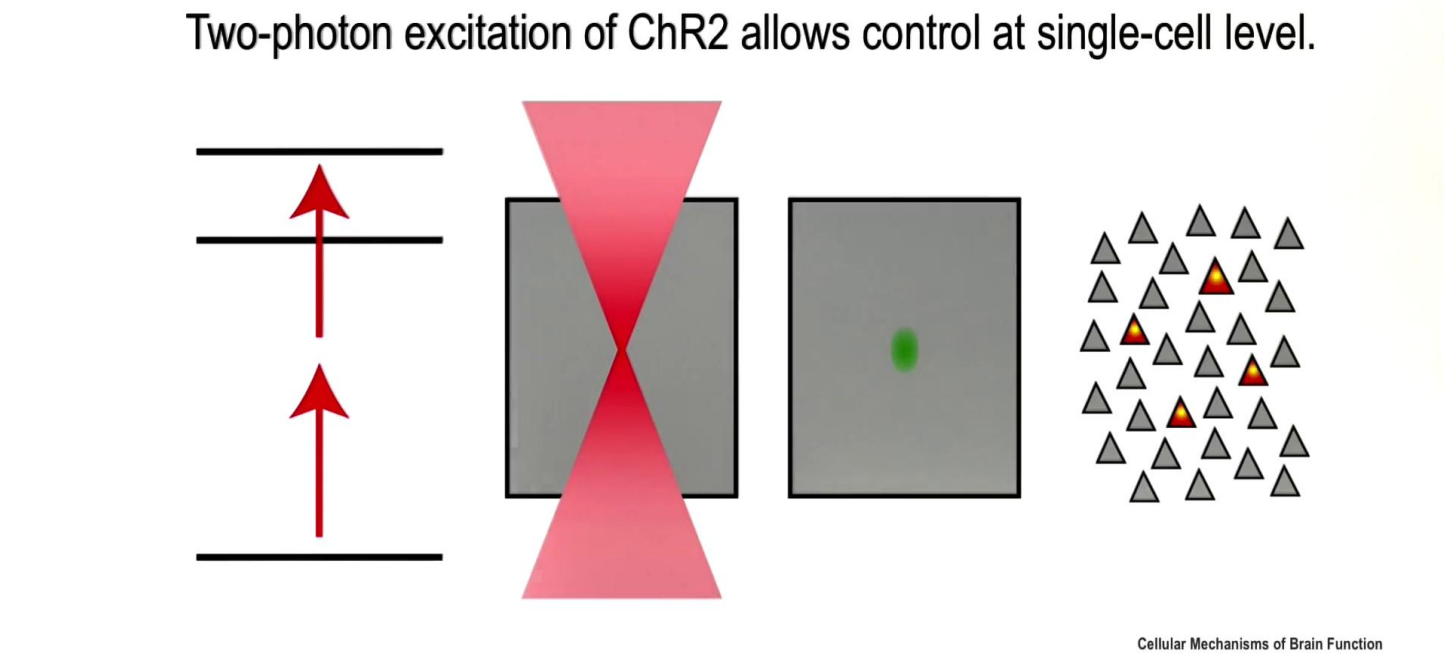
And perhaps not surprisingly, this causes the whisker to move, and we get into this what appears to be something like the exploratory movement of the mouse, as it's exploring its immediate environment, the whisker going backwards and forwards rhythmically, as we maintain the blue light stimulus here on the motor cortex area. This occurs with very short latencies, and is extremely robust. Every time you turn the blue light on, about 20-30 ms later, the animal begins to move its whiskers in this exploratory way, searching for something in its immediate environment. And so channelrhodopsin-2 works *in vivo*, it can drive action potential firing, and it can also drive behavior, here visualized in the whisker motor system.

Notes

Summary

10m 47s

Single-cell stimulation



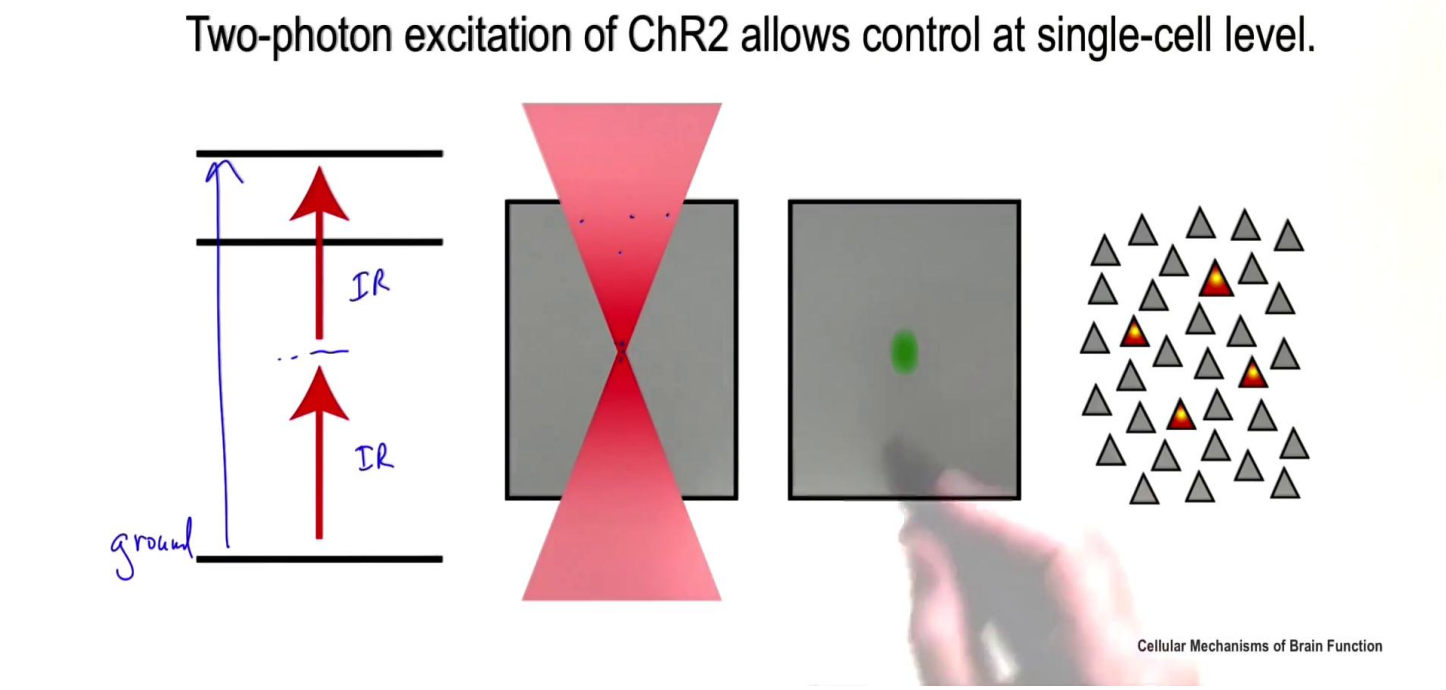
A major drawback of the way in which we were stimulating the whisker motor cortex with channelrhodopsin, was that we don't know exactly how many cells, or exactly which cell types it is that we're stimulating. We're simply applying blue light to the whisker motor cortex, and many neurons, thousands of neurons, were expressing channelrhodopsin, and probably subsets of those were firing action potentials. In order to get to that level of detail, where we can stimulate individual neurons, we then need to start putting light beams on specific cells, and stimulating those. We've already seen that it's difficult to focus blue light into the brain, because the brain is a highly scattering medium. We also saw, when we are thinking about high-resolution imaging, that two-photon excitation offered an interesting way out for fluorescence imaging, and we can use exactly the same phenomonology of two-photon excitation of channelrhodopsin to get optical Z-sectioning, and get around the scattering problems of the brain. So if we imagine that this is the energy diagram of the channelrhodopsin, and so we have a ground state, and normally we would give a single blue photon to activate the channelrhodopsin.

Notes

Summary

11m 37s

Single-cell stimulation



We can, instead of the blue photon, deliver two infrared photons, go through the virtual states, and now we have two low-energy photons, and that's good in itself, they scatter less inside the brain, so we've got a better chance of focusing our infrared beam into the brain. And in addition we have this nice phenomenon, this non-linearity of two-photon excitation, where in order for us to go through this, these two photons need to arrive at the fluorophore within a femtosecond of each other, so we need a very high density of photons in order to get the two-photon activation, and that's only present here at the center of the focal spot, and these other areas, as we try to focus this beam of light into a small area here, has a low-photon density, and that's then insufficient to be able to drive two-photon excitation. And so we can confine the excitation of channelrhodopsin through two-photon phenomena, and we can imagine that we express channelrhodopsin in, say, all the cells of a given brain region, and then we simply point our laser beam at different neurons, and we excite them through two-photon excitation, the specific neurons in the specific temporal sequence that we want, and we can then gain control, at a single-cell level, with millisecond temporal resolution.

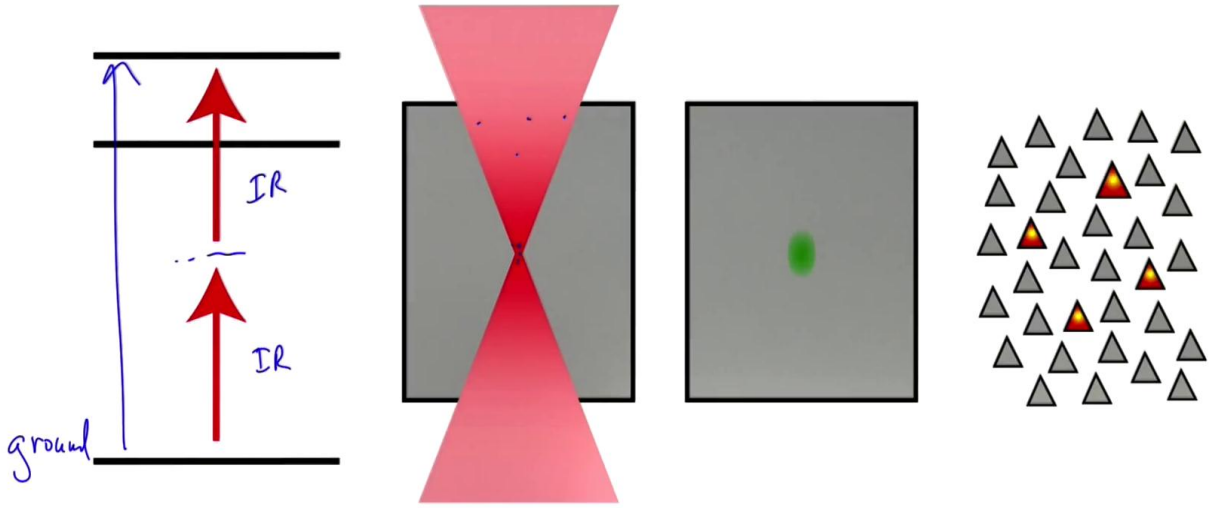
Notes

Summary

12m 55s

Single-cell stimulation

Two-photon excitation of ChR2 allows control at single-cell level.



Cellular Mechanisms of Brain Function

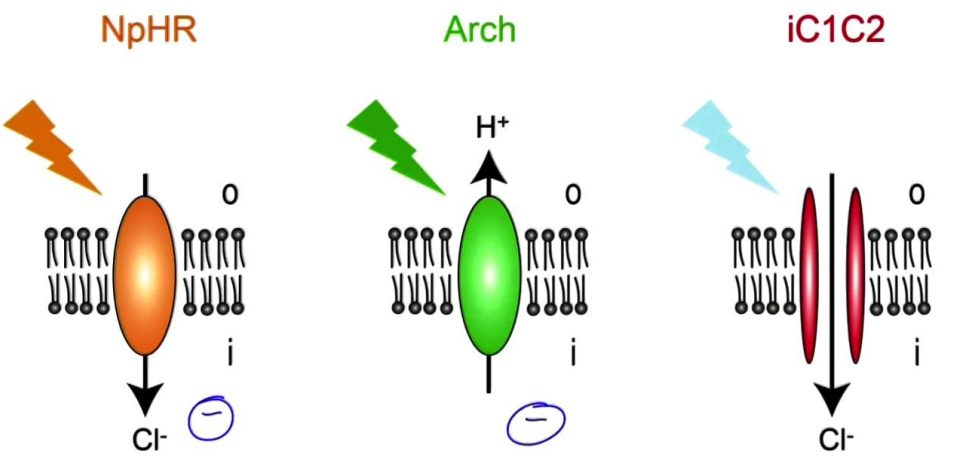
This is still largely being developed, but it's clear that this can work if the conditions are sufficiently well controlled. And so there's a great deal of hope for the future, that this type of methodology, two-photon excitation, will allow us to take control of neurons and neuronal networks at the required level of single cells and millisecond temporal precision.

Notes

Summary

14m 15s

Optogenetic inhibition



Zhang et al. (2007) Chow et al. (2010) Berndt et al. (2014)
Wietek et al. (2014)

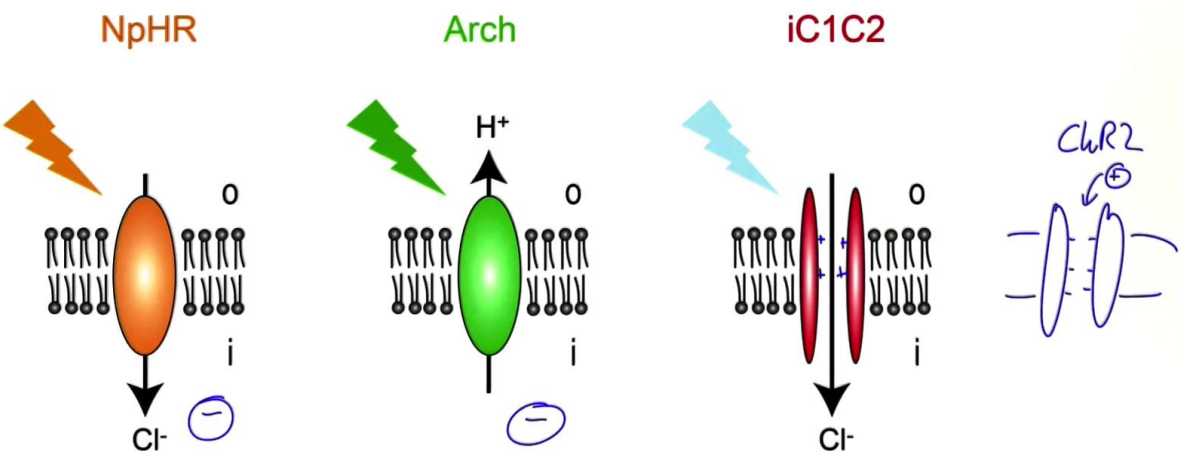
Remarkably, in addition to the optogenetic tools for stimulating neuronal activity, optogenetic tools for inhibiting neuronal activity have also been developed over the last decade. The first protein that was identified is the so-called *halorhodopsin* molecule, which encodes a light-driven chloride pump. Absorption of a photon into halorhodopsin moves one chloride ion from the outside to the inside of a cell, and so that causes a hyperpolarization of the neuron. Another molecule, called *archaerhodopsin* or *Arch*, is a proton pump, and absorption of a green photon, by Arch, removes a proton from the inside of a cell and moves it to the outside, again causing hyperpolarization. For each yellow or green photon absorbed by these pumps, one ion is moved. And so it's clear that this is a much less efficient process than the opening of an ion channel, where you can imagine that a single photon, causing the opening of an ion channel, lets many ions through, and so there's a drop in efficiency, and in general, of course, pumps are known to be much slower than ion channels. And so there's considerable excitement about the recent development of a light-activated chloride channel through genetic engineering of the channelrhodopsin ion channel.

Notes

Summary

14m 39s

Optogenetic inhibition



Zhang et al. (2007)

Chow et al. (2010)

Berndt et al. (2014)
Wietek et al. (2014)

Cellular Mechanisms of Brain Function

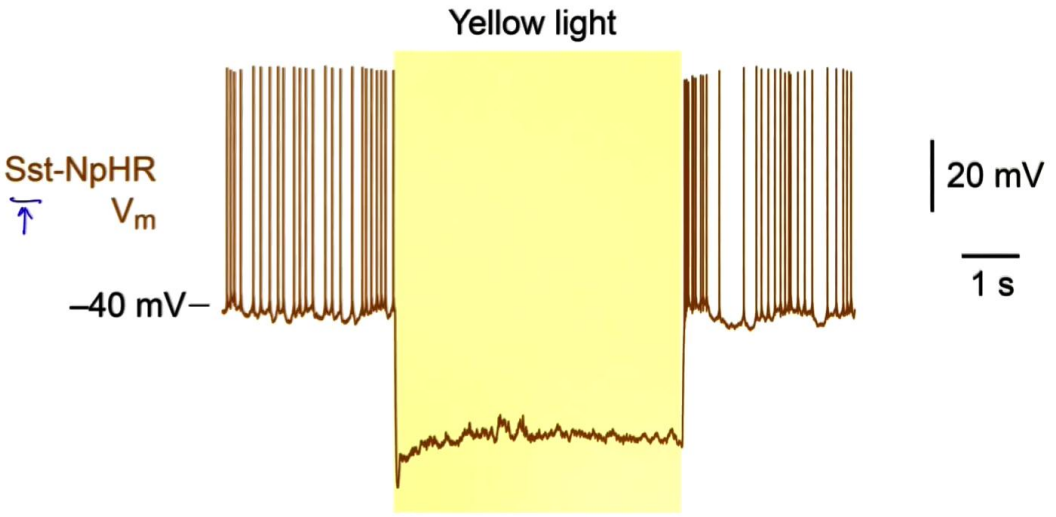
The normal channelrhodopsin-2 ion channel has some negative glutamate residues sitting here along the pore-forming region, and that's then presumably the reason that it's a cation-selective channel. The cations like to interact with the negative charges. What these two papers managed to do was to convert the cation channel of channelrhodopsin into a chloride channel, and in part this was done by putting positive charges here in the pore-forming region, and that then allows chloride ions to permeate, and they then made a light-activated chloride channel. And it's possible that this is going to be a much more efficient way of inhibiting neurons, compared to the light-activated pumps.

Notes

Summary

16m 11s

Cell-type specific optogenetic inhibition



Gentet, Kremer, Taniguchi, Huang, Staiger and Petersen, 2012

Cellular Mechanisms of Brain Function

The light-activated pumps do, nonetheless, work, to some extent, at least in some cell types. And here's an experiment in which halorhodopsin, the light-activated chloride pump, is expressed in a specific type of GABAergic neuron in the neocortex, one that we've already talked about. This is the somatostatin expressing GABAergic neuron, and what's special about these cells is that they have a very high input resistance, something like 200 megohm, and that means that small currents in these cells create a big difference in membrane potential. And so even though the halorhodopsin may not be the most efficient way of delivering an optogenetic inhibition, it nonetheless worked very well in the cell type. And so here in an awake behaving mouse the somatostatin cell is firing away during normal quiet wakefulness. We turn the yellow light on, that activates the light-activated chloride pump, and it causes an important hyperpolarization of the membrane potential, and a complete shutdown of action potential firing. The light goes off, and action potential firing returns. The inhibition can last many seconds, and for this particular cell type, it's complete. However, it has to be said that halorhodopsin hasn't worked equally efficiently in all cell types, and so it's good that further genetic engineering goes on, to improve the efficacy of the optogenetic inhibitory molecules.

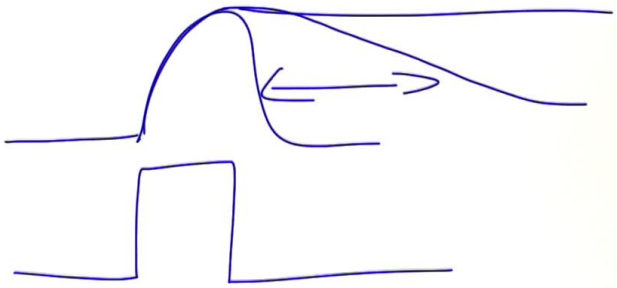
Notes

Summary

17m 01s

Optogenetic tool development

Higher conductance, ion selectivity
Faster, slower, switching
Spectral variants
Dendrite, soma, axon



+ optogenetic tools are being developed for controlling many other cellular and molecular processes.

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Indeed, optogenetic tool development is something that's ongoing in many neuroscience laboratories. Researchers are working on making higher conductance ion channels that would allow greater ion permeability, and thus a more potent optogenetic actuator, or they're trying to change the ion selectivity, making calcium-permeable light-activated channels, or potassium-selective ones, in addition to improving the chloride selectivity of the newly developed ion channels. Changing the kinetics is also extremely interesting, making the channelrhodopsin faster would be something extremely helpful. Right now, if one gives a 1 ms pulse of light, the channelrhodopsin turns on rapidly, but it also stays on for considerably longer than one might want. And so it might be nice to have optogenetic actuators that followed the light pulses more accurately. And so faster off variants of channelrhodopsin would be extremely welcome. It's also interesting to think about slower variants that would stay on for longer periods of time, where one pulse of light would leave it on for a long period of time. And, indeed, switchable channelrhodopsins have already been developed, where one color of light would turn the channelrhodopsin on, and a different wavelength of light would turn the molecule off.

Notes

Summary

18m 29s

Optogenetic tool development

Higher conductance, ion selectivity
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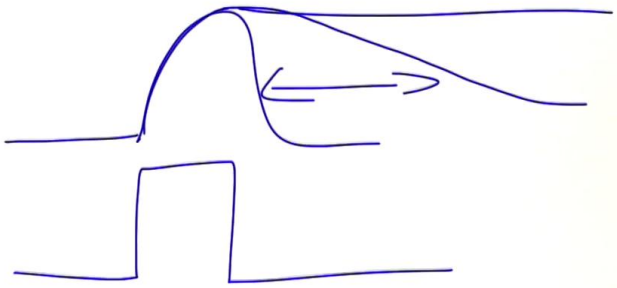
Spectral variants
Dendrite, soma, axon



+ optogenetic tools are being developed for controlling many other cellular and molecular processes.

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Further spectral variants are being developed so that channelrhodopsins are not just activated by blue light, but by red light, infrared light, ultraviolet light, blue light, and you can imagine a day when there will be different types of channelrhodopsins expressed in many different genetically defined neurons, and we'll be able to separately control their activities through different spectral variants of channelrhodopsins. One could also imagine targeting the channelrhodopsins, or other optogenetic actuators to different neuronal compartments or sub-cellular compartments, that might all provide interesting information about how neurons function. Finally, we've been discussing optogenetic tools for changing membrane potential, but there are many other cellular and molecular processes that are open to optogenetic actuators, including gene expression, synaptic transmission, and a whole variety of other signaling pathways within neurons and cells in general.

Notes

Summary

19m 53s

Optical control of neuronal activity

- Channelrhodopsin-2 (ChR2) encodes a light-activated cation channel, that can be used to drive action potential firing in genetically-defined neurons with millisecond precision.
- Optogenetic actuators are being developed to inhibit neurons and to control many other processes.

Cellular Mechanisms of Brain Function

So in this video we've seen that channelrhodopsin-2 is a powerful tool for the neuroscientist. Channelrhodopsin-2 encodes an intrinsic light-activated cation channel that when expressed in neurons allows us to control their firing with millisecond precision. This then allows us to ask causal questions about what the stimulation or activity of a specific group of neurons, that are genetically defined, contributes to a given behavior. We've also seen that new optogenetic actuators are being developed to inhibit neurons with high temporal precision and specificity, and further optogenetic tools have been made to inhibit neurotransmission, or to affect different signaling pathways. So altogether, this week we've seen that there are interesting and useful ways in which we can measure and manipulate the mouse brain activity in awake behaving animals, and what we'll look at next week is how we can use these tools to address specific questions relating to sensory perception and sensory motor integration.

Notes

Summary

20m 48s