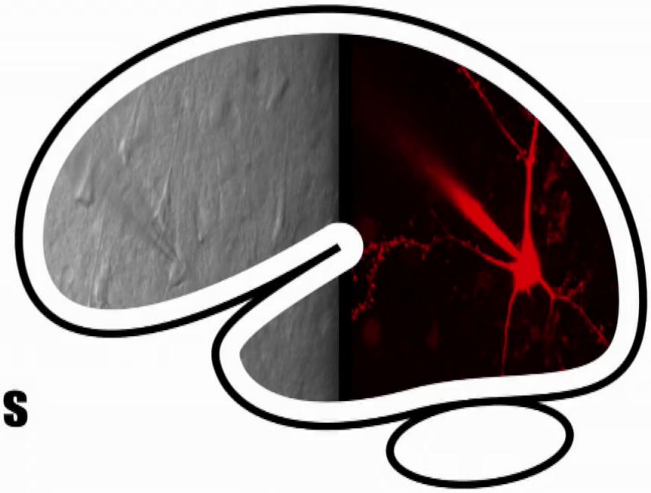
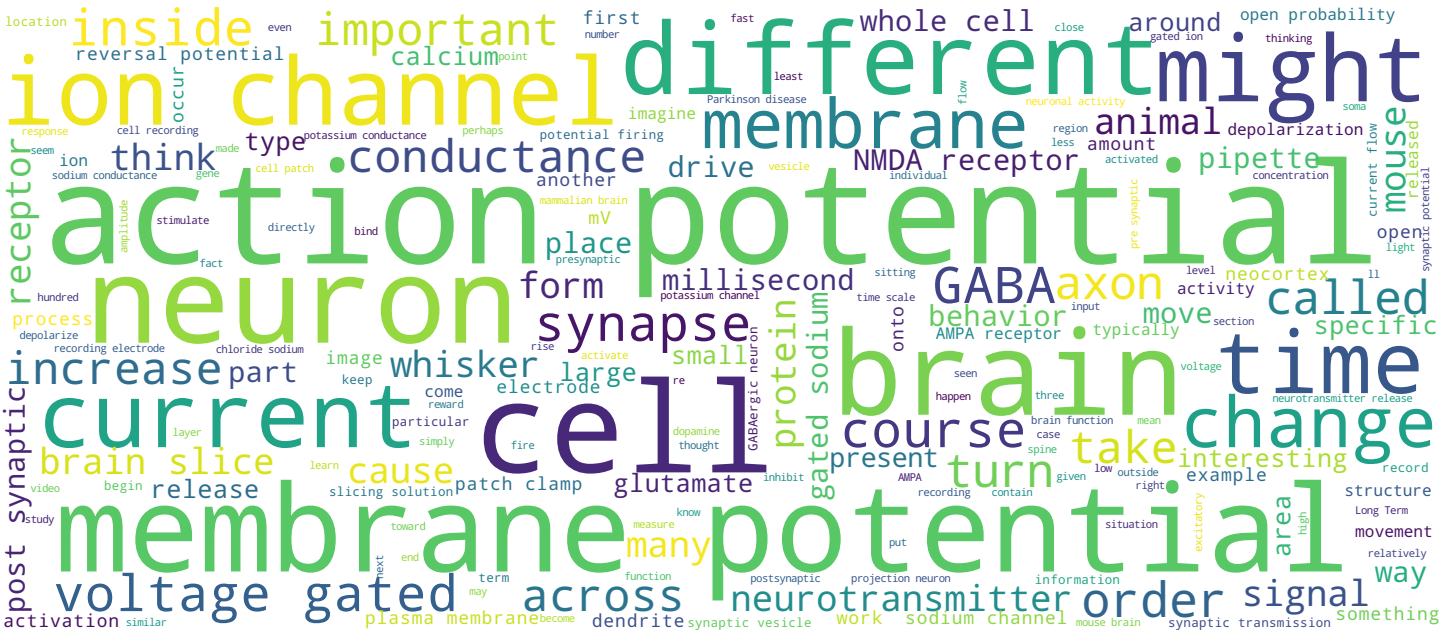
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE



2.5 Whole-cell recordings

Cellular Mechanisms of Brain Function

Prof. Carl Petersen



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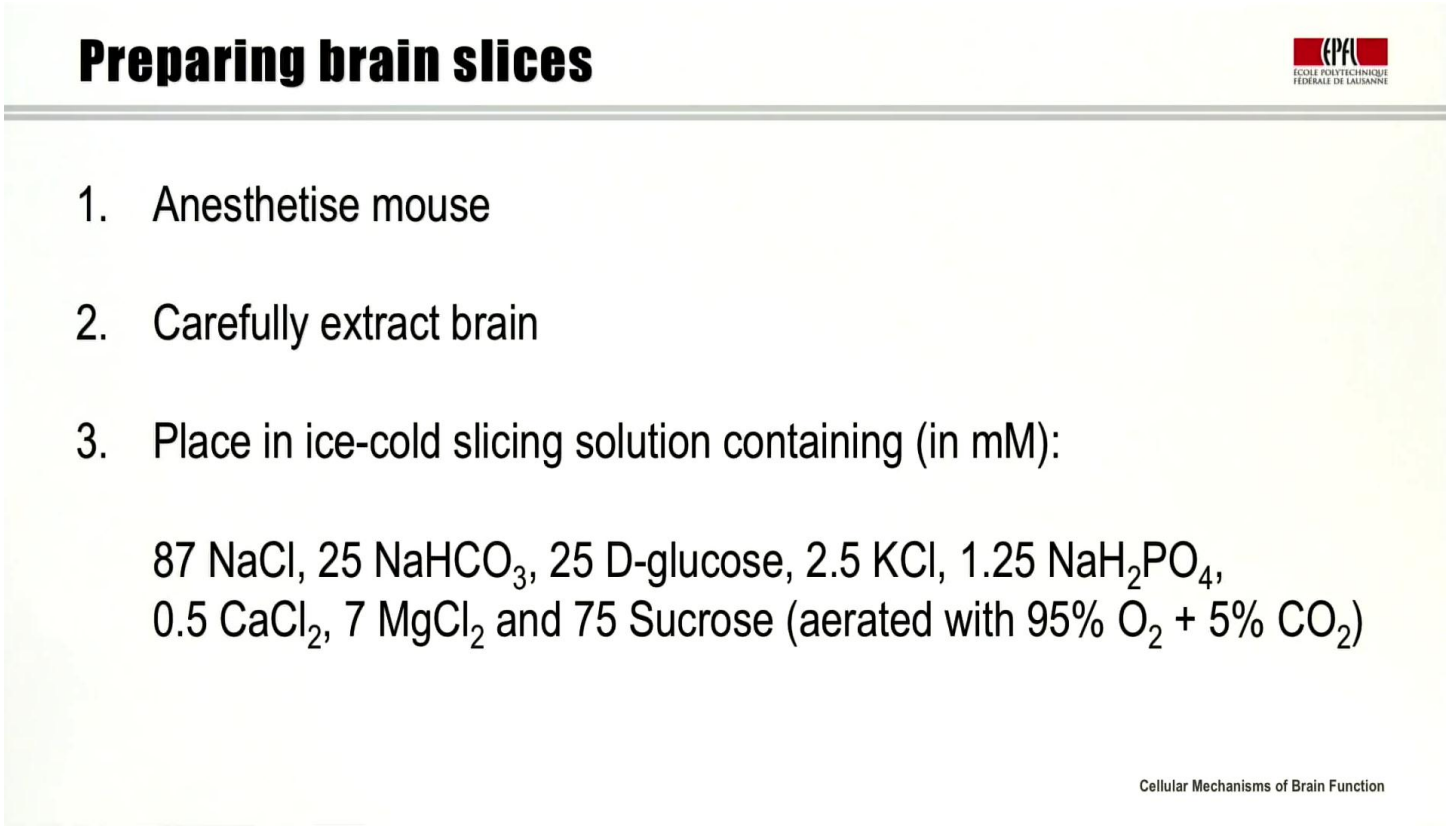
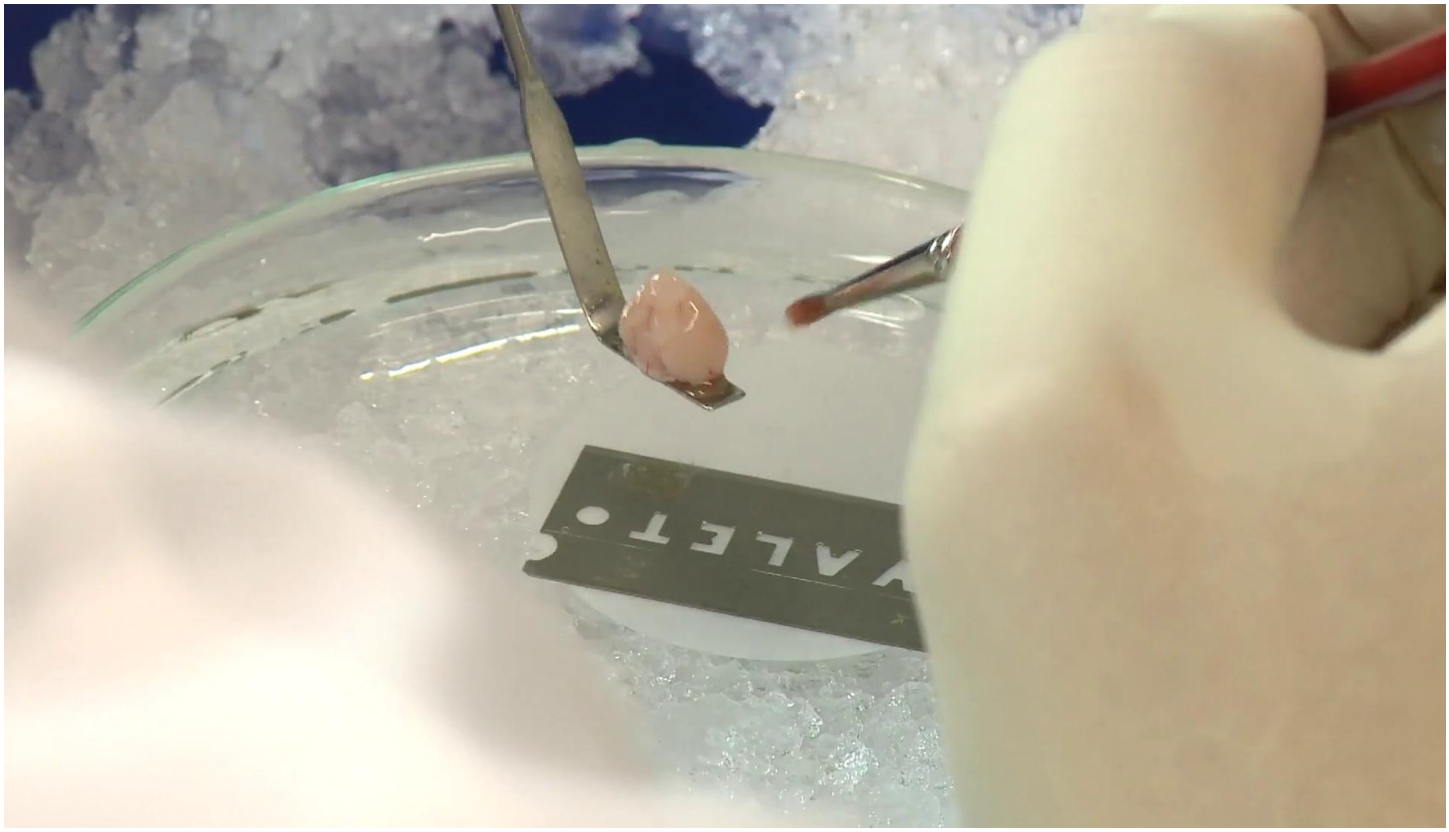


In this video, we're going to make a lab excursion and we're going to visit Semihcan Sermet, who is a Ph.D. student in my laboratory. He's going to show you how to make whole-cell recordings from brain slices. Hi, my name is Semihcan Sermet. In this video I will explain how to make whole-cell recordings from brain slices. I will explain the process in four parts. First we will prepare the brain slices, then we image the neurons, we prepare our recording electrodes, and finally we perform whole-cell patch-clamp recordings. Our first step is preparing the brain slices. We first anesthetize the mouse, carefully extract the brain, and then place it in ice-cold slicing solution containing, in millimolars, 87 sodium chloride, 25 sodium bicarbonate, 25 D-glucose, 2.5 potassium chloride, 1.25 sodium phosphate, 0.5 calcium chloride, 7 magnesium chloride, and 75 sucrose which is aerated with 95% oxygen and 5% carbon dioxide. This solution is not a normal artificial cerebrospinal fluid. It is modified to keep the cells healthy during the cutting process.

Notes

Summary

0m 05s

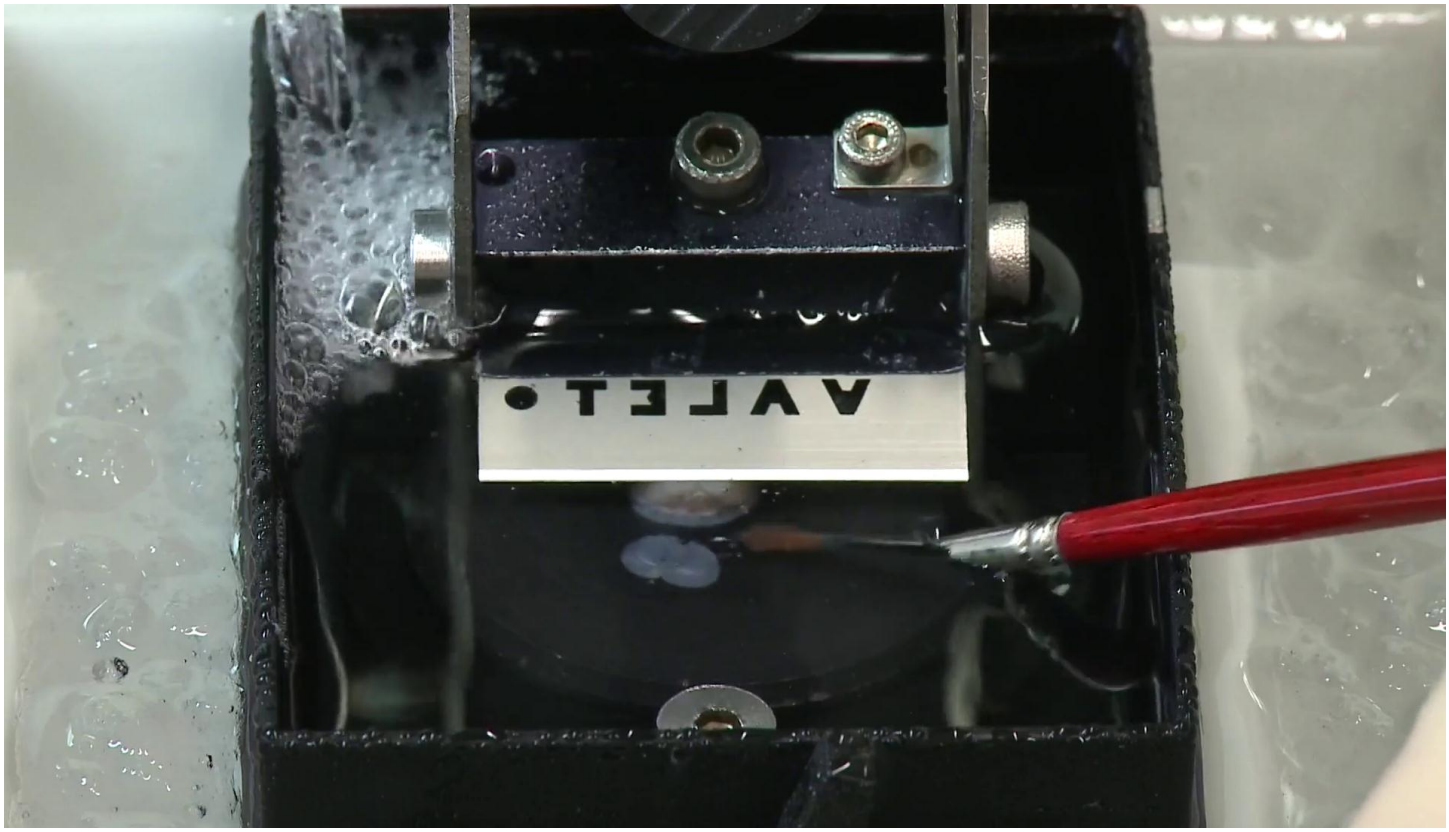


First, we extract the brain from the mouse and place it in the ice-cold sucrose-based slicing solution. This solutions helps to keep neurons in a good condition during the slicing process. The we chop off the cerebellum. Since we will be recording [in] neocortex, we do not need [that brain] region.

Notes

Summary

1m 19s

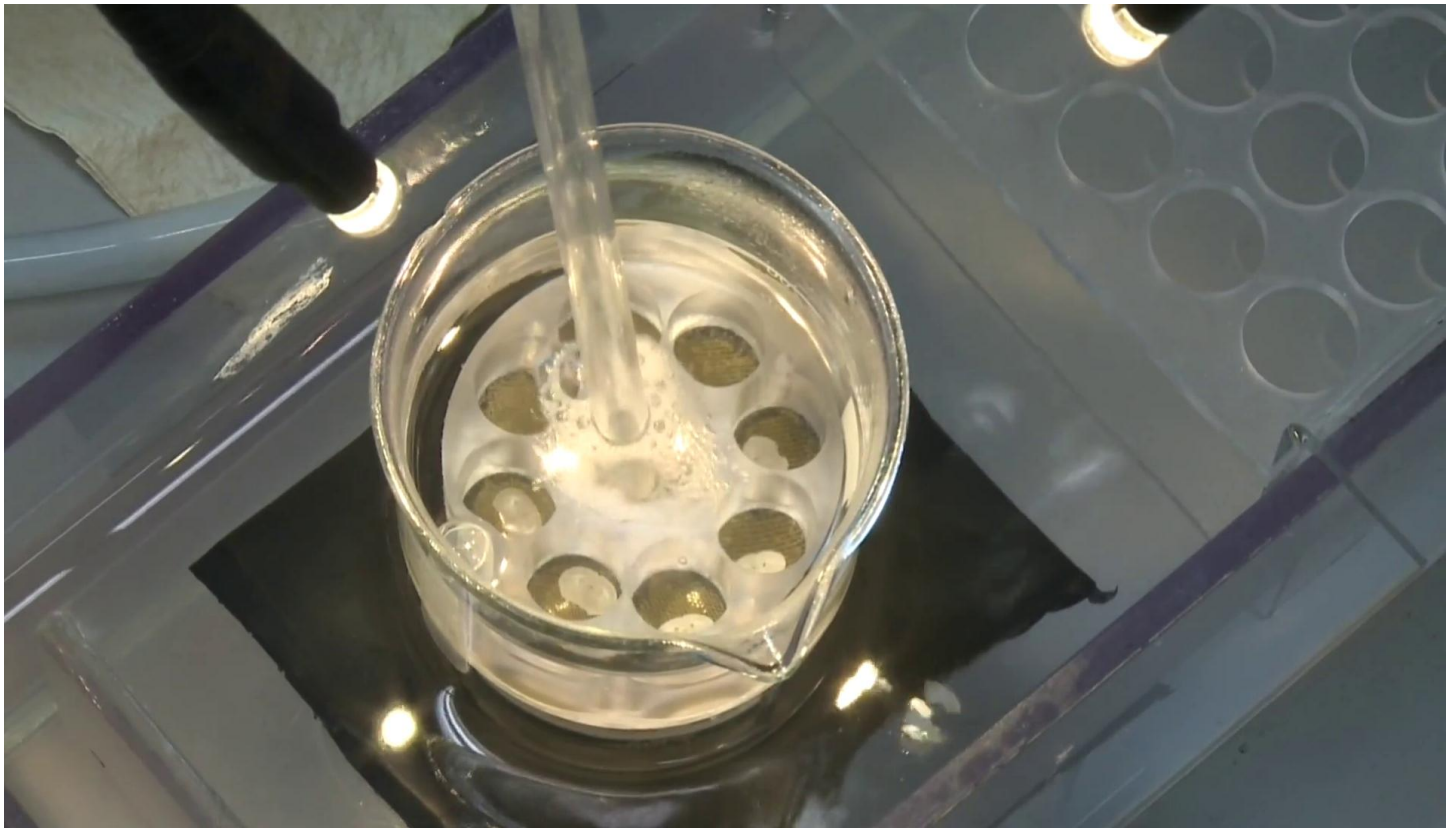


Take the brain and carefully transfer it onto the platform which will be going into the slicing chamber. The brain is fixed onto the platform with glue. Then we take the platform with the brain and place it into the chamber filled with cold slicing solution. The solution is bubbling with 95% oxygen and 5% carbon dioxide to keep a balanced pH, which is 7.3. We slice the brain with the vibrotome. It has a sharp blade which vibrates at 85 Hertz and advances with a speed of 0.09 millimeters per second. Slices are 300 micrometers thick. When the blade reaches the end of the brain tissue, we gently push the slice towards the blade and separate it from the rest of the brain.

Notes

Summary

1m 37s



Then we take the brain slice with the Pasteur pipette and transfer it to a chamber filled with 35° Celsius warm sucrose slicing solution. We keep the slices here about 30 minutes. During the slicing, the vibrating blade damages cells in the superficial parts of the slices, the parts next to the blade. In the warm sucrose slicing solution, the damaged cells tend to float away from the slice, leaving healthy tissue behind.

Notes

Summary

2m 25s

Visualising neurons in brain slices

1. Slices incubated at 35 Celsius for ~30 minutes in slicing solution

2. Slices are transferred to room temperature artificial cerebrospinal fluid (ACSF) containing (in mM):

   125 NaCl, 25 $NaHCO_3$, 25 D-glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$ and 1 $MgCl_2$ (aerated with 95% O_2 + 5% CO_2)

3. A selected slice is placed in the microscope and superfused with artificial cerebrospinal fluid (ACSF) at 35 Celsius.

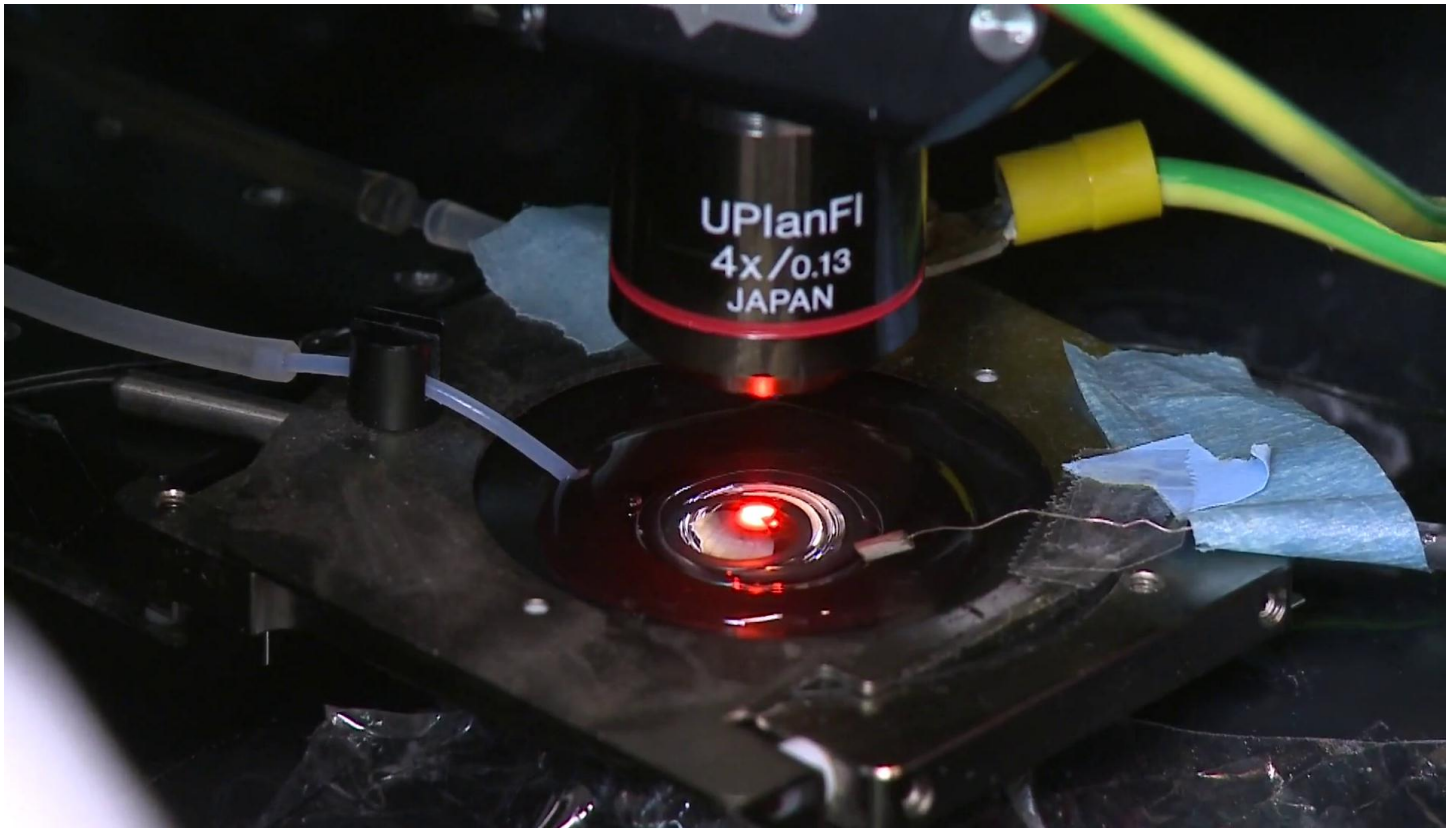
Cellular Mechanisms of Brain Function

We keep the slices incubated at 35° Celsius for 30 minutes in slicing solution, and then we transfer them to the room-temperature artificial cerebrospinal fluid, containing, in millimolars, 125 sodium chloride, 25 sodium bicarbonate, 25 D-glucose, 2.5 potassium chloride, 1.25 sodium phosphate, 2 calcium chloride and one magnesium chloride, which is aerated with 95% oxygen and 5% carbon dioxide. Then we select the slice and place it in the microscope, superfused with artificial cerebrospinal fluid at 35 Celsius.

Notes

Summary

2m 52s



We take our brain slice from the chamber filled with ACSF, bubbling with carbogen, and place it into the recording chamber. The chamber is superfused with ACSF.

Notes

Summary

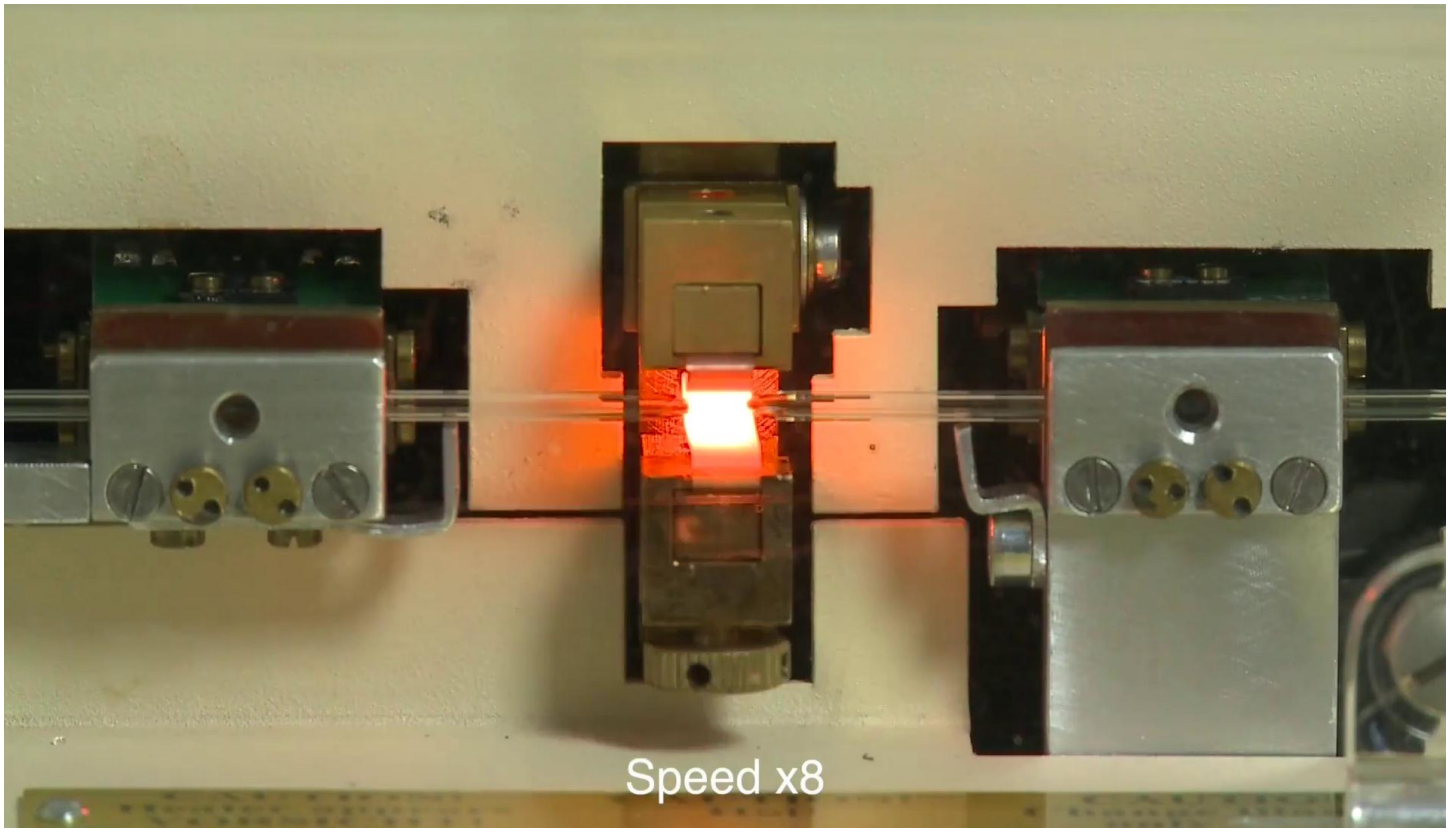
3m 32s

Then we fix the brain slice with the grid and then start visualizing with the microscope. First we look at the lower magnification, 4X, to see the brain region we would like to record from. Then we switch to the higher magnification, 60X, to visualize the cells. In this case, we use a high-contrast infrared video microscopy. It is important to find a region with healthy cells. In order to do that, we [look at] the slice vial, focusing up and down, until we find a good cell to record. Now we prepare our patch-clamp recording electrodes. We take a borosilicate glass capillary and place it into a pipette puller. The electric [element covers] the middle of the pipette and delivers heat to melt the glass.

Notes

Summary

3m 42s

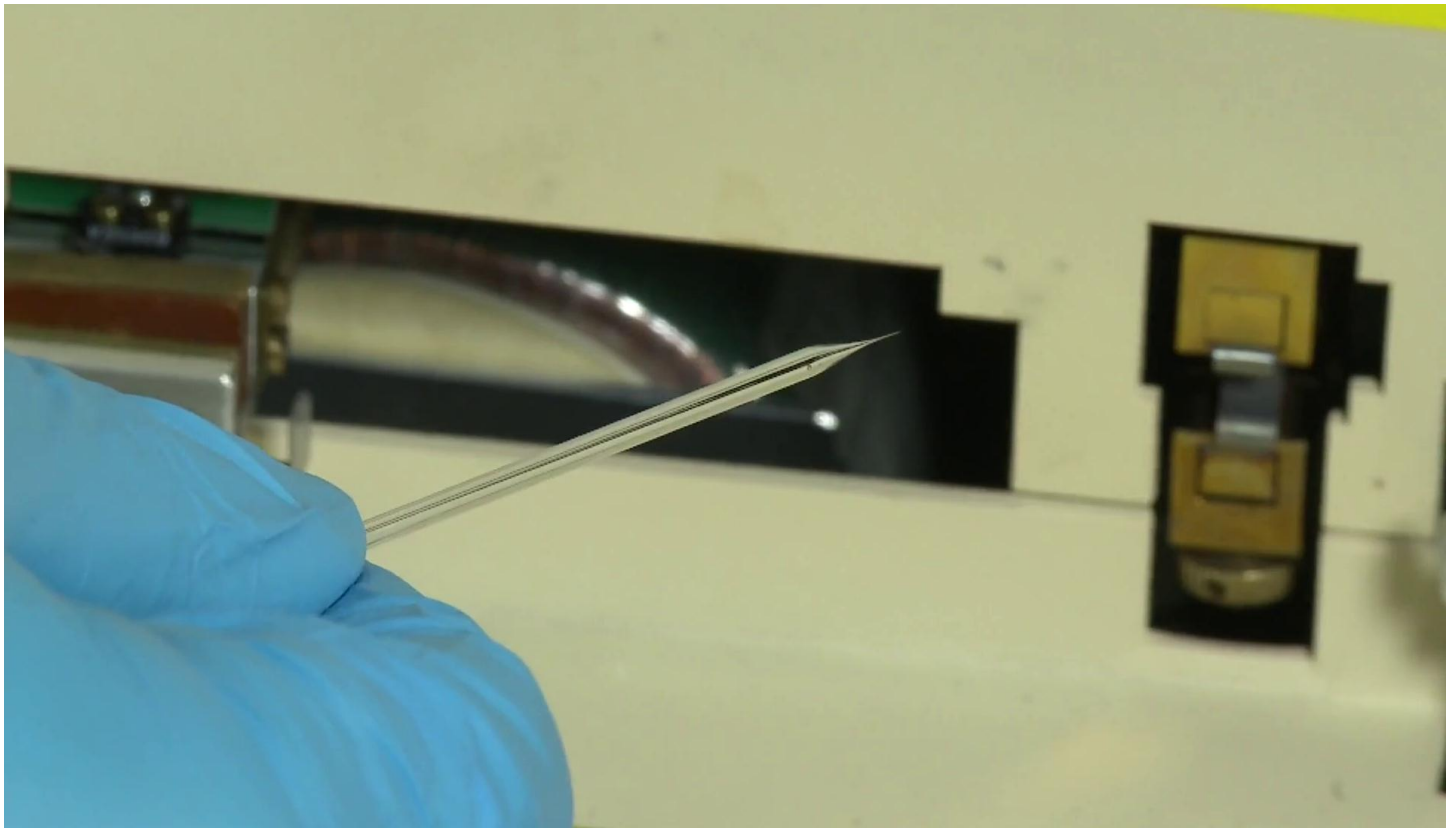


The pipette is fixed at both ends and force is applied to pull the electrode. With enough heat, the middle of the pipette begins to melt and the pulling force causes lengthening and thinning of the glass capillary.

Notes

Summary

4m 22s



After several rounds of heating and pulling, we obtain pipettes with a tip size of approximately one micrometer.

Notes

Summary

4m 34s

Whole-cell recording

The recording electrode is filled with intracellular solution containing (in mM):

135 K-gluconate, 4 KCl, 4 Mg-ATP, 10 Na_2-phosphocreatine, 0.3 Na-GTP and 10 HEPES (pH 7.3, 280 mOsmol/l).

Alexa-594 dye (10 µM) was added to the intracellular solution.

Cellular Mechanisms of Brain Function

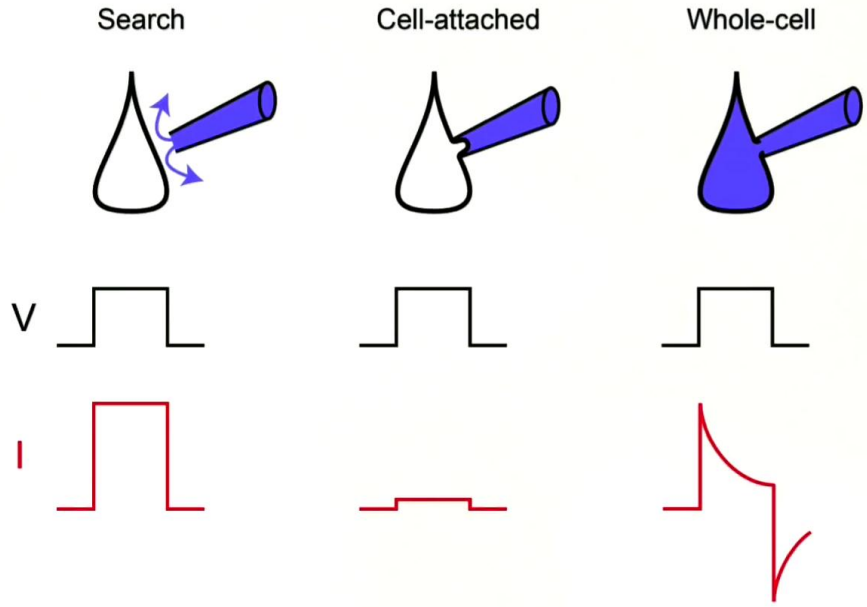
Before we start the whole-cell recording, we fill our glass pipette with the intracellular solution, which contains, in millimolars, 135 potassium gluconate, four potassium chloride, four magnesium ATP, ten sodium phosphocreatine, 0.3 sodium GTP and ten HEPES, which has a pH of 7.3 and osmolarity of 280 milliosmol per liter. We also add Alexa-594, red fluorophore for further imaging.

Notes

Summary

4m 42s

Whole-cell recording



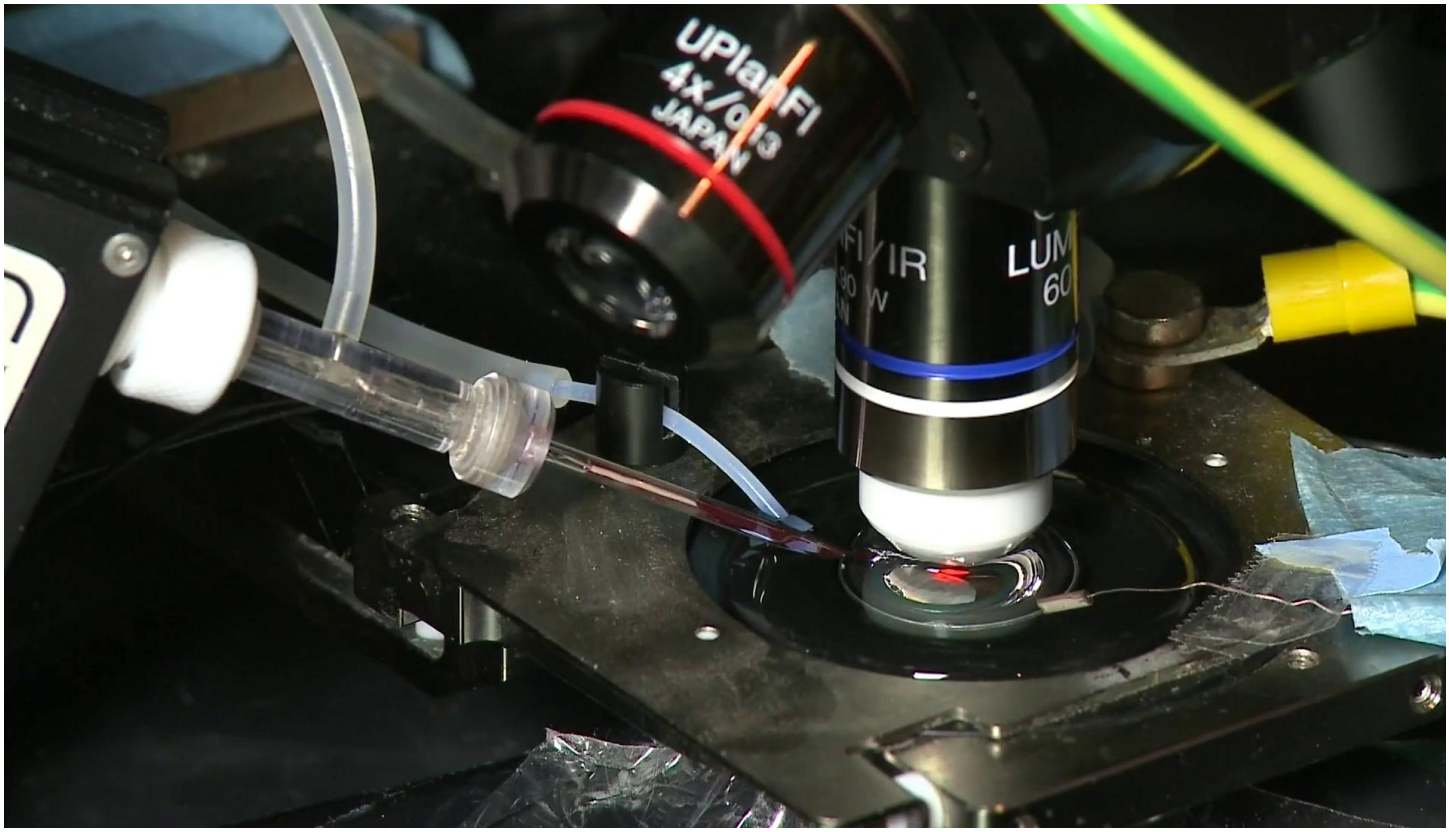
Cellular Mechanisms of Brain Function

In order to have a better understanding of the whole-cell recording process, we can first look at these schematic examples. First, before putting our pipette into the bath [and the tissue], we apply positive pressure in our pipette so the intracellular solution, shown as blue, can flow out and then the tip stays clean. We apply a test voltage and observe the current flow through the pipette. Then when we touch the cell, we remove the pressure and suck gently. Then a membrane attaches to the pipette tip and then forms a tight electrical seal. At this time, only a very small amount of current passes through the pipette tip. This is now a so-called cell-attached patch-clamp configuration. Then, we do further brief suction pulses to break the membrane in order to establish the whole-cell patch-clamp configuration. Then we break the membrane and we see a current flow like this charging the membrane because of the cell capacitance.

Notes

Summary

5m 13s

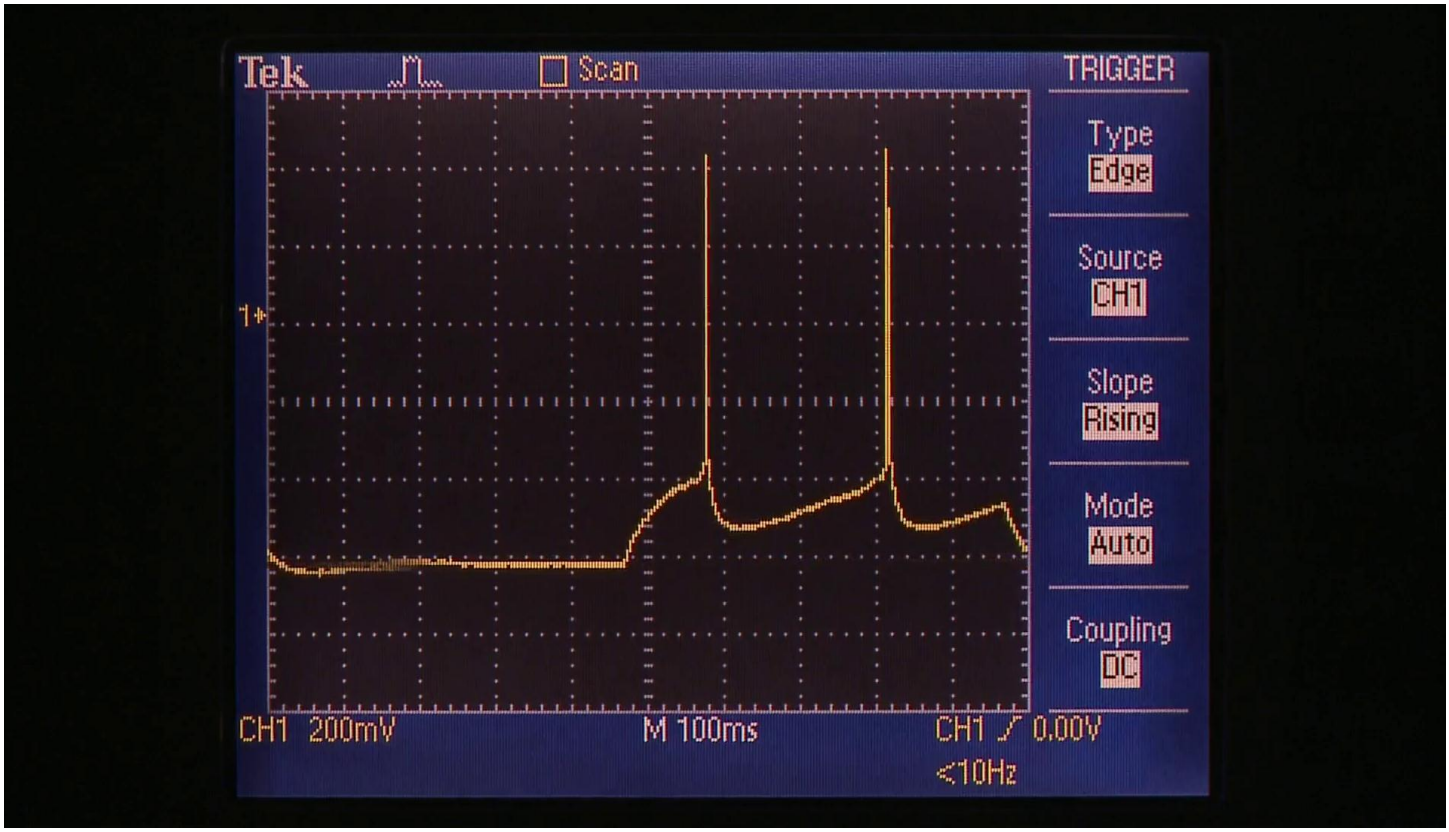


We fill our glass pipette with internal solution and place it on the head stage, which has the silver chloride recording [electrode] on it. The reason for the internal solution looking purple is that it contains a red fluorophore called Alexa-594 to allow fluorescence imaging. Before we move the pipette into the ACSF bath solution we apply positive pressure on it to keep the tip clean by always having an outward flow of solution. First we find our pipette under low magnification and then we switch to the higher magnification which we were using to visualize the cells. It is important to move the pipette around with great care, making sure not to damage the brain slice.

Notes

Summary

6m 15s

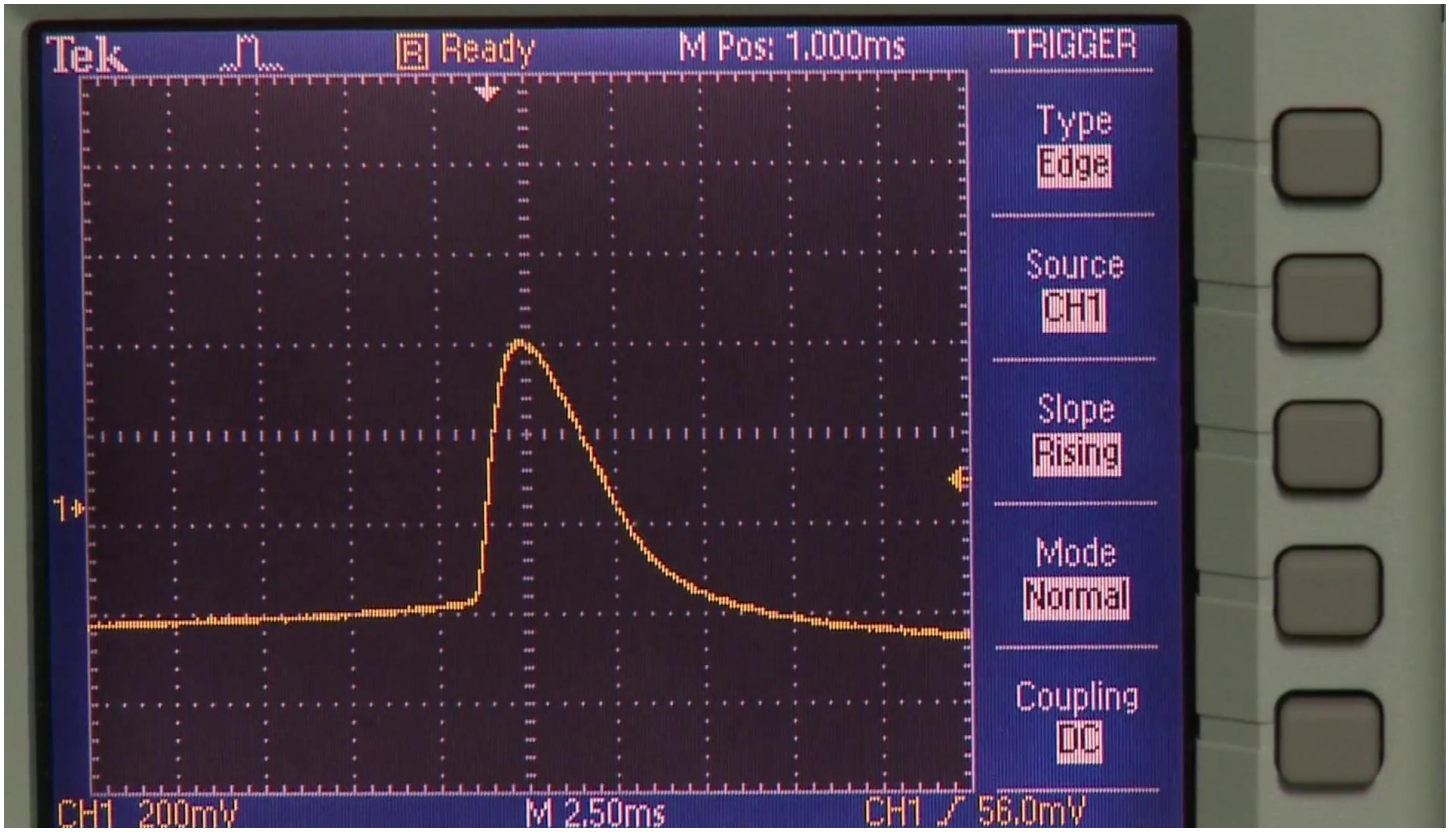


We bring the pipette down towards the brain slice, near to the location of the neuron we would like to record. When the pipette touches the brain slice surface, we can see the tissue getting pushed away from the tip. That is because of the positive pressure around 15 millibars we apply to the inside of the pipette, causing an outward flow from the pipette tip. Then we reach our cell step by step. First we focus deeper and then we go down with our pipette. When we reach the cell, we touch the cell carefully and move the pipette slightly towards the cell body. Now we see a small dimple where the tip of the pipette touches the cell body. Then we remove the pressure and suck gently to establish a gigaseal. When we have a gigaseal, we do further brief suction pulses to break the membrane in order to establish the whole-cell patch-clamp configuration. Sometimes we have to continue the suction pulses to improve the opening, reducing the [access] resistance between the electrode and the inside of the cell. Then we switch to the current clamp mode to measure the membrane potential. We can then start injecting current into the cell. Here we apply currents for 500 milliseconds.

Notes

Summary

6m 55s



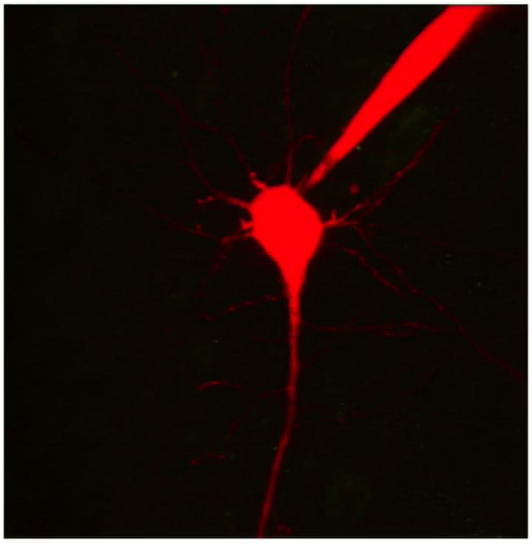
First we apply hyperpolarizing current, then we increase the current and depolarize the cell and see action potentials. The more we increase the current, the more action potentials we see. In this case, we are recording from a neocortical layer V pyramidal cell. We can zoom in and look at the action potential waveform. We can see that action potential [half-life] in this cell is around two milliseconds.

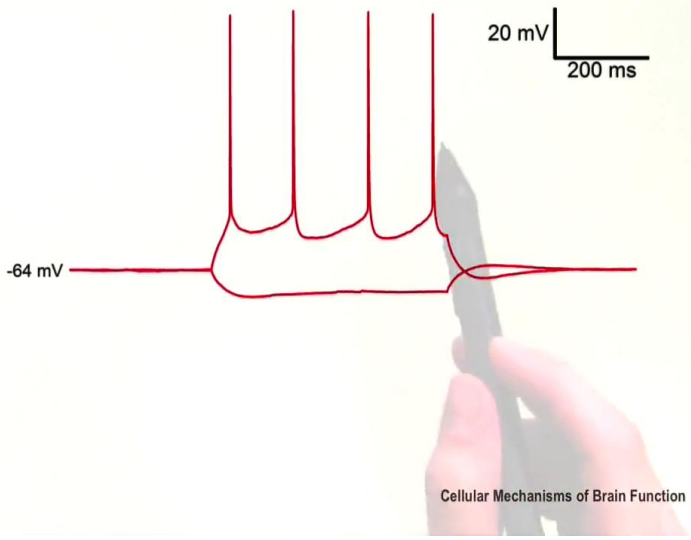
Notes

Summary

8m 07s

Structure and function





Here on the left we see the two photo-microscopic image of the cell we were recording from. On the right, we see the membrane potential traces of the same cell responding to the injected currents. In the bottom trace, which is here, we see that the cell hyperpolarizes and in the top trace, we can see here, the cell depolarizes and fires action potentials.

Notes

Summary

8m 33s

Whole-cell recordings from brain slices *in vitro*

- The mammalian brain can be cut into thin (300 µm) slices, and the neurons remain viable.
- Neurons can be visualised through high-contrast infrared video microscopy, allowing *in vitro* whole-cell patch-clamp recordings of membrane potential.

Cellular Mechanisms of Brain Function

So we've seen that the mammalian brain can be cut into thin slices and the neurons remain viable. We can visualize the neurons through a high-contrast infrared video microscopy, allowing in vitro whole-cell patch-clamp recordings of membrane potential.

Notes

Summary

8m 56s