Module:
Techniques in Neuroscience

Week 2
Electrophysiology: Looking at live neurons in action

Topic 1
An introduction to electrophysiology – Part 3 of 3

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Now we come on to intracellular recordings. Here I'm going to compare two things: firstly, current-clamp with voltage-clamp, which is the nature of the recording that is occurring; secondly, I'll be talking about sharp electrodes versus patch-clamp electrodes as the nature of the recording electrode used in the different techniques.

First of all, I'm going to talk about using sharp electrodes. Here, a biological preparation is a creature called *Ciona intestinalis*, which is a sea squirt, and we isolate the oocytes from it, which we can then put an electrode into, which is actually penetrating the plasma membrane, so is intracellular recording using a sharp electrode. So, here is a comparison of sharp electrodes in the two different recording setups - either in current-clamp on the left or voltage-clamp on the right. Top left, A, shows a single action potential. So, what we've done here is stimulated the oocyte with a small brief pulse that has caused an action potential to occur. As you can see, this is in the order of 100 mV. Below that, I have done some different intensities of stimulation. You can see the red line, the intensity was not big enough to stimulate an action potential, so it's not present. The green one shows a large action potential and the blue one shows one of the same size, but of slightly longer duration.

These are recordings in current-clamp, where we're injecting currents and recording the voltage. On the right-hand side, we have the same cell and the same electrode. And in C, we are in voltage-clamp mode. Here, you can see a single voltage step and the current that results from it, and this voltage step induces the current that we see when it generates the action potential, but instead of looking the voltage, we're now looking at the current flow through the membrane that generates that voltage pulse: the action potential.

Similarly, we can apply different voltages as shown in D, each of different colours. If the voltage is too low, as we saw in B, the green one shows no current is evoked because the voltage was not strong enough. However, as you can see in the dark blue one, a large current is generated when we use a larger voltage. As you can see, the current-clamp and voltage-clamp are related to each other. One in current-clamp mode, we are recording the voltage but changing the current, and in voltage-clamp mode, we are stimulating with voltage and recording the current.

Now, we use patch-clamp electrodes. The patch-clamp electrode does not go into the cell, it goes to the cell's surface and interacts with the membrane and forms what we call a gigaseal and you can see that occurring in

A. This is known as an on-cell patch, where the glass electrode has made a gigaseal with the cell membrane. If we're very lucky, we may find a few channels in that action membrane that we've sealed to, and we would be able to record single channel activity, known as cell-attached patch recording. If we pull the electrode away at this point after we make the gigaseal, this pulls the patch of membrane off the cell and we can then have access to the intracellular side of the patch. We can apply drugs there and still record the single channel activity in B.

Another alternative shown in C is where once we've got cell-attached patch, we actually break through the membrane with the electrode still attached to the whole cell and this goes to a version called whole-cell clamp, where we have access to the inside of the cell which enables us to record from all the ion channels in the membrane of that particular cell - this is called whole-cell recording. If we pull the electrode away at this point, we would then develop an outside-out patch, where we'd pull off a small patch of membrane - if we're lucky, with one or two channel in it - and there, the outside of the membrane would be exposed to the outside environment, as would be normal for the cell.

One final variation of the patch-clap technique is a little bit more sophisticated and this involves filling the electrode with a solution that contains a pore-forming antibiotic. As before, we make a gigaseal as in A, but instead of moving the electrode or breaking the membrane we just wait until the antibiotic diffuses to the membrane and forms pores in it by itself. This is called perforated patch. This has a big advantage over whole-cell voltage clamp under some conditions in that the contents of the cell are not dialysed by the electrode solution and you don't lose things from inside the cell because the holes formed by the antibiotics are small enough to prevent this happening- but we still get electrical activity recordings.

In this slide, we see using whole-cell or patch-clamp recording electrodes, on the left, a current clamp recording where action potentials generated in rat retinal ganglion cells are recorded. As before, we're using current ejection to record the voltage. On the right, we have the voltage-clamp situation where I'm using voltages to generate the currents that evoke voltage change. This time, I'm using a stimulus that is acid or pH change, where we're evoking acid-sensing ion channels – ASICs - to generate a current when they open. At the top, on the right (number A), you can see that as we change the membrane potential, the current size changes and at the bottom (B1 and 2), as we increase the acidity of the application of the compounds, that increases the size of the current. So, as with the oocytes, we can use whole-cell or patch-clamp recording electrodes to record in current-clamp and voltage-clamp mode.

Here again is whole-cell patch clamp in current clamp mode. Here's an example showing that you can use electrophysiology with another biological technique. In this case, simultaneously, we are recording the intracellular calcium levels in this neuron.

So, we have, at the top of each section, a trace that records the concentration of intracellular calcium, and at the bottom, the action potentials that that particular neuron is generating. So, if we take the top left section, you can see that the action potentials evoked number 17 and that produced a relatively small rise in intracellular calcium inside the same cell. Top right, we've increased the number of action potentials to 28, the calcium concentration increases because more calcium channels are opened each time an action potential occurs. And then, bottom left and bottom right, we are sequentially increasing to 36 and 48 - the number of action potential generated - and this correlates with the increase in calcium rise that we get, showing that calcium entry during an action potential is related to the number of action potentials that are opened or activated in that cell.

And the final technique I'm going to talk about, is single channel recording. In this example, this is a cell-attached patch where we have two single channels in the patch. The line on the bottom of each of these traces, C, is the

line at which both channels are closed. O1 is the level at which one channel is open, and O2 is the level at which both channels are open. As you see, it looks very step-like. So, no channels can be open at C, one channel can be opened at O1 and two channels can be opened at O2.

It's probably easier if I start with a lower trace first. You'll notice on the left I have a sign saying 'Vp- 10', and this means we've just depolarised the cell by a small amount, 10 millivolts. As you can see, the single channel conductance is quite low and the time the channel is open is also low. Indeed, towards the right of that trace, the channel almost closed most of the time. If we move up to the middle trace, Vp -20, you can see now, most of the time, one or other or both of the channels are open, so the activity of the channel has increased but so has the single-channel current- which has got larger.

The top trace, Vp -30, is even more depolarised. Here you can see, the channel is open even more often, either one or both of them, and the size of the current has got bigger. This means you can record two important things about the channel: you can calculate its single channel conductance and you can measure its voltage dependence due to the change in voltage that the channels are recorded at.

Now, we're going into a situation where we can use patch-clamp in vivo. This is a very recent technique or application of the technique. So here, we have an animal that is held in a stereotactic and anaesthetised to keep it still. Again, we put the electrode into the brain, but this time, as it shows in A in search mode, we are applying a positive pressure to the back of electrode- we're actually forcing air into the back of the electrode. This pushes out the fluid out the front and this means it keeps the path clear so the electrode can be moved through the tissue by a manipulator. Once the electrode hits a cell, we see a change in the pulse and it becomes irregular due to the heartbeat frequencies that you pick up when you touch the cell, and we turn the pressure off so it goes to neutral.

We then allow the cell to go to cell attach mode, as before, and the electrode seal, giga-ohm seal, is formed. After a small period, in D, we then apply a very short sharp suction - this time, a negative one - so we're sucking the electrode solution up which destroys the patch of membrane that's made under the seal, and this allows us to go into the whole-cell mode as before. So, we're basically recreating that previous slide but in the brain of a living animal. This enables us to record quite sophisticated responses shown in the section on the right, where the whiskers of the mouse have been stimulated by moving them, and you can see it evokes action potentials and EPSPs in the cells in the brain that are associated with that whisker response.

Here, I've given you a table on the applications of the different techniques. I've separated them into the columns: which one can be used in humans in vivo, which ones can be used in non-humans in vivo, and finally, which techniques can be used in vitro.

The only technique that's used in humans in vivo is extracellular recording, but only part of an existing treatment. For example, if somebody was going in for deep brain stimulation electrode implantation, you could then - with the patient’s permission - do some recordings as the electrodes were being implanted.

In non-humans, as are shown, both extracellular recordings can be used, either implanted in live awake animals or in anaesthetised animals. Intracellular recordings can only really be done with anaesthetised animals because they need to be kept still. And finally, single channel recording can again only be done in anaesthetised animals.

In vitro, all three techniques can be used, and sometimes, in some cases, human tissue can be used under these conditions as well, but obviously with the correct permissions from the patients involved.

Here, we have a summary slide for all the intracellular recording techniques that I've described, giving you both the advantages and some of the disadvantages of each type. So hopefully, what I've shown you is that electrophysiology can record the electrical activity of whole brain tissue, a single neuron or even a single ion channel in the brain.

Electrophysiology is a dynamic, functional, SI unit-based, real-time, hi-fidelity and high temporal resolution approach. Many electrophysiological approaches can be used vivo. Electrophysiology can be used simultaneously or in conjunction with optical, molecular, biochemical and pharmacological techniques. I think we're not going to understand the nervous system without it.