Cognition and its Computation

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Lecture - 10
Single Neuron Imaging and Manipulation of Neural Activity

Welcome. So, we have been talking about general electro physiological methods, where, we record activity of single neurons, with either metal electrodes or we can do experiments where we can measure the membrane potential or potential between the inside and outside of the neuron, using Patch-Clamp techniques. As we mentioned, all these procedures are mainly serial in nature, although with multiple channels of electrodes.

We can get information about multiple neurons simultaneously. However, it is still blind, in the sense that we do not know what type of neuron that we are recording from, which neuron we are recording from and so on. So, as new knowledge, about the brain shows, that we have lots and lots of types of neurons and they are of different morphology, of different functional properties and generally there is a high amount of specificity in terms of how they connect with one another and their functional types.

So, gradually it becomes more important, to understand the activity of particular types of neurons simultaneously. For example, we will be talking about excitatory neurons, inhibitory neurons and so on or neurons with particular types of markers in them, which are molecular markers that identifies a particular disease type or a particular neuron that is involved in a particular kind of processing and so on.

So, we can actually with recent technology, we can actually tag individual types of neurons, with a particular marker and be able to record activity simultaneously, with from a particular type and another particular type simultaneously and we able to gain more information about the details of the functioning of brain and how these aspects can be connected to behavior and cognition.

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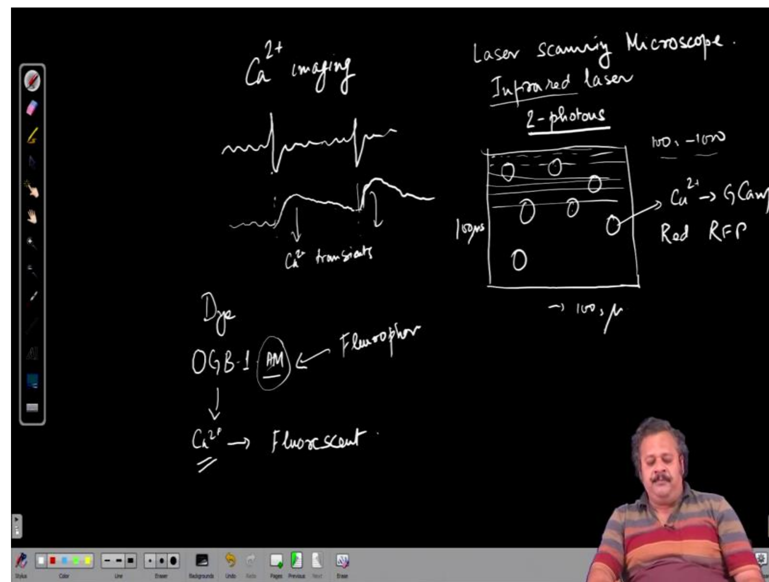

So, in order to do this over the last 2 decades, a technique that has come about is what we know as calcium imaging or calcium 2 plus ion imaging. If we know the that the amount of calcium that is inside a neuron. So, among many ions, calcium is one of the ions that is also involved in terms of activity of neurons, that is most neurons. So, we have been talking about action potentials or spikes in individual neurons.

And we know that when a neuron produces an action potential, then the calcium concentration inside the neuron also increases and then decays over some period of time, and so, like this. So, if we were able to measure this calcium signal or the calcium concentration, inside the neuron, we would indirectly be able to infer, the times at which spiking has occurred.

What these are called are calcium transients and they are associated with spikes. They are there are inherent problems also, but what is advantageous as we will gradually see is that, while we are indirectly measuring the activity of neurons from this calcium signal, because of the way we will measure the calcium activity we will be able to gain access to information of activity of many many 100s to even a 1000 neurons simultaneously.

Of course, this cannot be done in humans, yet. And, but a lot of knowledge in the past 2 decades have been gained from this particular method or using this principle that action potentials are associated with calcium transients. So, in the recent in the beginning, what

people have done is that, by introducing a particular dye in neurons. So, a particular dye that is let us say OGB-1, Oregon Green BAPTA-1 AM dye, acetone methyl ester dye.

The this particular part is that the dye is like this in the outside or external medium, and when the dye goes into the neuron this breaks off and this OGB-1, actually becomes fluorescent. And, so, it is also binding to calcium which allows it to be fluorescent. So, if we were able to measure this fluorescence signal from a neuron, we would indirectly be able to measure the amount of calcium in the neuron.

And if we were able to measure this at time intervals, sufficiently close, we would be able to understand how the calcium concentration or an indirectly the calcium concentration is changing inside a neuron and this can be done simultaneously from multiple neurons using a laser scanning microscope. So, as you may be aware, for fluorescence, we need an excitation light.

And in this case this particular case, an infrared laser is used for particular reasons about how we can penetrate the tissue, how the amount of absorption by the tissue is lower at these frequency ranges or wavelength ranges. And also, the ability to generate very fast pulses of these laser that allow us to penetrate deep and also allows what we call simultaneous absorption of 2 photons, at the same time in terms of exciting this fluorophore or fluorophore is excited by 2 photons simultaneously.

And, that provides us the fluorescent signal, from a very small volume of tissue about a femtometer and we can if we scan this excitation light, along region of the brain tissue at a particular plane of focus, which can be done very fast, we can actually get an idea of how the calcium in single neurons that are present within that particular plane that we are imaging and get the fluorescence signal from all of them.

So, in this method, what we can get is information from multiple neurons. So, from 100s to a 1000 neurons or sometimes more if we can go very fast in terms of scanning, this excitation light and collecting the emitted light then we can gain access for information from a very close circuitry very very in a in a scale of few 100 microns, we can gain access of information from all of them.

So, typically this is of the order of 100s of microns and 100s of microns and this region can contain up to in order of 100s to 1000s of neurons. So, here we can actually identify

the neurons that we are recording from at least the morphology, directly from the filling up of the dye in the neuron. Similarly, we can in more recent times over the last decade, people have been using genetically encoded calcium indicators, that are present within the neuron initially, from the very beginning only in mice.

And. So, most of these experiments have been done in mice, which can be easily genetically manipulated to provide such fluorescent markers within them. So, now, give given the ability to manipulate the types of neurons to contain a particular type of protein let us say, we can express the calcium indicator or calcium indicator protein that is G-Camp in particular types of neurons.

Because, now we can get a gain access at the molecular level, and can express the calcium indicator only in that type of neuron or we can have the calcium indicator in all the neurons, and may be have a particular red coloured protein RFP, Red fluorescent protein, in a particular type of neurons. So, we can have differential information from one particular type of neuron and the other neuron simultaneously.

So, this has become a very powerful technique, but; obviously, there are disadvantages which is that we are limited to having the animal the experimental animal to be fixed at a location, because as you can see this kind of imaging is at a micron resolution and any kind of movement is going to cause real problems in terms of gaining signals from the neurons and the surrounding regions.

So, in that sense, it can be done only in static animals, very recently people have developed methods. In fact, in this week we have come across a new method where, we can have an implantable two-photon microscope. So, to speak with attached fibers to carry the light and collect the light from the neurons as well. However, those are very very recent advancements.

But, we can see the disadvantage here is that, since it is based on light penetration of the tissue, we can only image from certain depths, up to certain depths that too in very good conditions. So, most of the brain is much deeper than possible axis, of the two-photon microscope microscopy, this has its own drawbacks. However, the plethora or the amount of information that is available with this method, itself is also an important aspect.

And also, the ability to have information from different types of neurons knowing the particular types of neurons in the network and how they behave differently from the other neurons, again adds a lot of knowledge in terms of our understanding of behavior, in terms of learning, in terms of how we recollect information and so on. So, this kind of methodology is an important hallmark of gaining information.

So, the next part is how we can actually manipulate activity of neurons. So, when we discuss later on, about neuronal activity and how neuronal activity represents information how neuronal activity is related to a particular behavior, how neural activity adds up to provide particular percept and so on. In order to establish, how important that as that those particular neuronal types are in terms of the behavior that we are trying to understand or the aspects of cognition we are trying to understand, from let us say an animal experiment point of view at least so far.

We need to be able to do experiments, where we can actually change the activity of neurons reversibly, that is we want to be able to turn on some neurons or a group of neurons that is make them fire, the spikes that we were observing or we can also try to stop them from firing action potentials and reversibly if possible, to understand the exact role those neurons are playing by understanding how the behavior is changing with the manipulation.

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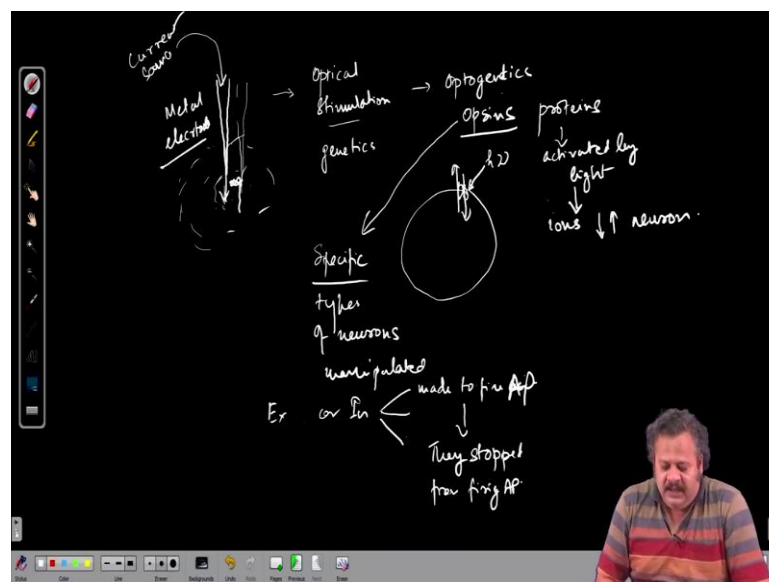

And so, to this effect what people have been doing and there are lot of experiments, about manipulation have been done with the same metal electrode that is used for recording, by injecting current into the brain tissue into that volume, around the tissue. So, we can injects through using a current source, a small amount of current or stimulation can be done and stimulate the neurons in a particular region.

So, these are very small currents in the order of micro amps and like you may have heard about deep brain stimulators, particularly in reference to Parkinson's disease the same kind of technique is used in order to stimulate neurons. But, this is going to stimulate all the tissue; all the tissue around the tip of the electrode. So, be it axons there, be it neurons there, everything is going to be stimulated.

So, in spite of this drawback, that it is good it may might be stimulating all the tissue brain tissue around there, such stimulation has led to lot of understanding about a how a particular region is related to a particular behavior and we will have discussions on this when we talk about attention and so on how such manipulation of activity of neurons can allow us to understand the role of a particular region or particular types of neurons, in terms of behavior.

So, the same stimulation experiment, that can be done in animals as well as humans, in experimental animals and as well as humans, with the disadvantage that it is going to stimulate everything, the newer ways of doing the same experiment uses optical stimulation and with the use of genetics. And the pioneer of optogenetics is Karl Deisseroth and also his mentee Ed Boyden who developed this technique and is now widely used in terms of manipulation of neural activity, reversible manipulation of neural activity.

So, the technique is now being also used in other animals like in the primate for further more important questions that cannot be studied in mice. But it started off in mice, because of the ease of being able to do the genetic manipulations of introducing opsins or proteins that are activated by light and then they allow ions to flow into or out of the neuron.

So, in the electrical stimulation here, what we see is that, the current in this region that flows from one tip usually it is done with a bipolar electrode that is there are two tips the current flowing from one tip to the other in the process injects the current in the neurons.

And that stimulates the neuron to fire the spikes or action potentials. Here with light, they are activating they are able to activate opsins that are they are present on the membrane of the neuron.

So, if you have the neuron there are opsins that are present in the membrane of the neuron, where by light, the protein behaves like I mean with the light falling on to the protein or after the absorption of the light the protein allows the flow of ions into or out of the neuron, just like something that we will call ion channels later on. And this in a way allows is like a stimulation of the neuron, that is current flow into the neuron or out of the neuron can change its activity.

And, so with light, we can target specific types of neurons, by expressing this opsins in specific types of neurons. Since this is genetic manipulation, specific types of neurons can be targeted or manipulated. For example, as we said excitatory neurons or inhibitory neurons or different classes of inhibitory neurons can also be activated. Activated in the sense, they can be made to fire action potentials or they may be stopped from firing action potentials.

So, this provides us a very precise way to control the activity of particular neurons in a particular region, by the use of light stimulation.

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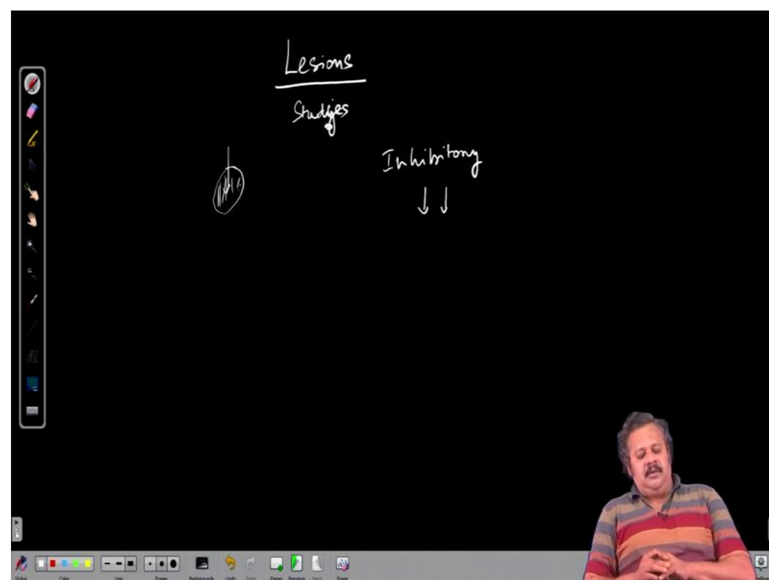

Or then, with this kind of manipulation of the activity, we can ask a variety of questions that were initially studied mainly with lesions. So, in our lectures later on, we will be mentioning many studies where, particular brain region was lesion or accidentally from patients with lesions in a particular region hence they having some behavioural deficits, we have correlated that knowledge and concluded the role of particular regions of the brain based on such lesion studies.

So, you can imagine that if a particular region of the brain has been lesion, that is there is a stroke in a particular region of the brain, we understand that from other methods and we see that the person has a deficit in a particular behavior and then we with the correlation we establish that or not established we tend to think that that particular region is involved in that behavior.

However, a much stronger evidence can be built, if we can actually instead of lesioning, a particular brain structure, if we can actually change its activity in the normal intact person or intact animal, experimental animal and see if we can manipulate the particular behavior. And if we can stop the simultaneously in the next set of experiments if we can shut-off that particular region from being able to perform its normal function and see the deficits.

So, we can have more confidence on to how that particular type of that region, how that particular type the region plays a role in a particular behavior. So, and with the optogenetics, we can further go deeper into the questions of involvement of even specific types of neurons in that process and make conclusions about what let us say, particular classes of inhibitory neurons do in certain learning behavior or some cognitive aspect.

Of course, these are still only methodologies available in for use in animal models. We do not envisage using this yet in humans, although there are certain efforts on the way to be able to do such manipulations in humans, of course barring the ethical considerations, we are no I mean we can technically be able to do it.

So, I with this understanding of the methodologies and the of recording activity of gaining information about activity of neurons and of manipulating activity of neurons we will go into the understanding of more into how computation is performed in the brain by understanding how does action potentials are generated which is the currency of computation in the brain.

Thank you.