Computational Neuroscience

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Week – 01

Lecture - 05

Lecture 05 : Methods of Recording Neural Activity

Welcome. So we are at the introduction to neurons. This is the first week of the course and so we discussed our basic brain structures. We discussed about the different sensory region, the frontal region which is the decision making region. We also looked at the midbrain, the brain stem and so on. So now that you have a fair idea of what the neural structures are in the brain, we will now go into the methods of recording neural activity from the brain.

So as you can imagine when we discussed about the sensory systems and about executive systems where decision making is made or if we think of the memory or the limbic system which is the hippocampus and so on, we need to gain access to the activity of neurons in order to understand what computation is being performed by the neurons. And when we mean gain access to the activity, we mean that we have some measure of the membrane potential or the spiking in the neurons. Let me remind you again that these action potentials are the spikes and are the currency of computation and communication between neurons through synapses on the right hand side. That is one neuron produces action potentials and transmits it to the axon terminal here and then with neurotransmitter release goes on to the next stage with the current injection which we will look at more detail later on.

And it is being able to record this activity from the neurons in the brain from the required structure based on the question we have that is what is required in order to understand the computation being performed. So how can we record this activity? That is the action potentials. Of course, we need to first decide on which location that we want to record from. Then there are a few considerations that we have that is what is the resolution of the analysis that we are doing that is required and similarly what is the resolution in terms of spatial resolution and in terms of temporal resolution. So if we look at some of the methods that is applied in humans, if we look at the left hand side on the top is fMRI and at the bottom it is EEG.

So fMRI refers to functional magnetic resonance imaging and EEG is electroencephalography. Both of these are applied in humans and also done in animals during experiments and what these provide are activity in different brain regions at different temporal resolutions and different spatial resolutions in the different structures in the different brain regions. So these are the first one is an indirect method of measuring activity and this is more direct method but it is not of the resolution of the signal neurons. So let us see how we measure activity through fMRI. It is essentially that oxygen is required when action potentials are produced in one brain region.

So the oxygenation level of the hemoglobin at those regions that decides the magnetic properties of the tissue there and what fMRI allows us is to measure the magnetic field radiance that are created by application of an external magnetic field because when the hemoglobin is deoxygenated it becomes paramagnetic while it is oxygenated it is diamagnetic. So because of the change in magnetic properties of the hemoglobin oxygenated and deoxygenated hemoglobin that indirectly tells us how much activity is going on in that area or location of the brain. So it is a very indirect measure of spiking activity going on in the brain. So we can based on comparisons with some baseline we can look at where activity is reduced let us say those are the regions that are marked in blue and where activity is increased which is the region that are marked in the hot colors or red. So this kind of method allows us to ask many different questions.

However, we are limited in the sense of the temporal resolution that is fMRI signals the bold signals or blood oxygenation level dependent contrast signal. These bold signals have a peak that occurs that is the time scale at which it occurs is of the order of few seconds 5 to 6 seconds. And so while we say that action potentials are very fast or brief excursions in membrane potential and that is the currency of computation. So we are nowhere close to that level of temporal resolution. So it is activity over a lot of duration I mean over many seconds that this method gives us.

So and also in terms of the region over which we can look at the activity is of the scale of few millimeters. So in fMRI usually we talk of voxels or volume pixels in volume that is 3 millimeter by 3 millimeter by 3 millimeter and you can imagine with the neuron being 10 to 20 microns in diameter how many neurons are present within each voxel. So it is of the order of hundreds even thousands. So that says that we are limited by both temporal and spatial resolution. But then why do we use it? Well the usage is very important because it is used for diagnostics.

It allows us to look at the entire brain simultaneously like each section simultaneously and we can decipher answers to questions like which region of the brain is connected to

which region during what task which regions are getting activated and so on. So while it is not at the level of action potentials and single neurons it provides us the advantage as we spoke of looking at the entire brain and at least simultaneously almost in the entire section at least each frame and is also used for diagnostics and detection of activity changes and so on. So if we go ahead further then we if we use electrodes to get to a more direct measure we can have scalp electrodes which allows us to record electrical activity directly from the underneath the scalp region in the brain. So over here are different bands frequency bands of analysis that has been done on EEG signals. So this is so if we collect the EEG signal it is based on many different electrodes that are located on the skull.

There are systems by which we decide on which location to put the electrode and so on and from based on the signals we decide from which region the signals are coming but these regions turn out to be extremely large. So the total some total activity in the neural tissue of a very large volume underneath the electrode is what is reflected in these EEG signals. However over the many years doing correlational studies and now even more direct causational studies we have a fair bit of idea of what is underlying each of the different frequency regions particularly beta, alpha, theta, gamma and delta. So as we know the most important among them is possibly the gamma activity or the gamma band which is in the range of 30 to 80 hertz around there and it is associated with cognitive processing and particularly a certain kind of inhibitory interneurons are connected to that activity. So again EEG also allows us for diagnosis and can be used for a number of decoding techniques that is EEGs have been the basis of designing brain computer interfaces often or if possible if we can go intracranial with ECOG then we get a more cleaner signal more large signal and that can be used further for brain computer interfaces.

So by training or through the learning of an algorithm based on the EEG signals we can decode what a person is trying to do let us say from the motor cortex if we have the EEG or ECOG signals from those signals depending on the changes in the different frequencies and so on we can actually decode what the motor cortex is trying to do and so in fact this kind of decoding methodologies have been applied in order to help paralytic patients to move a robotic arm by thinking that they are moving their own arm and maybe feed themselves. And there are multiple such applications that have come about through these signals plus these are also useful in many kind of diagnostics especially epilepsy and so on. So again this has a very good temporal resolution in the sense that at least we can sample these signals at hundreds of hertz and their variations can be seen over the scales of tens of milliseconds or less and but the spatial resolution again is extremely poor and even poorer if I may than fMRI because it is hard to localize the signal to particular structures in the brain very difficult. So in order to get into more

fundamental questions in terms of computation being performed in the brain we need access to the spiking activity at single neuron resolution and if possible to sub threshold activity in single neuron resolution. So that is using metal electrodes here so this is an example of a metal electrode that has been inserted into the brain region and here let us say this is the neuron cell body from which we happen to occur.

So the region of interest is decided and this is an invasive method where we can insert the electrode into the brain region of interest and blindly sort of like a fishing expedition and waiting for an action potential to occur from the electrode signal. So we do not know where exactly we are so it we only go by what is observed by the signal that is obtained by the electrode and through amplifiers are recorded on a computer after analog to digital conversion. So if we happen to be close to neuron we may extracellularly so this electrode tip is outside the neuron and in the extracellular medium very close to the neuron cell body there is a signature of the spiking activity and that is what is reflected in the spike train that is observed here. So this allows us to understand when spikes are occurring if in from the extracellular signal of probably a neuron or part of a neuron. So usually that is why we call it single unit recording and as opposed to single neuron recording because we cannot be absolutely sure whether we are recording from the neuron cell body or maybe from a piece of the large piece of dendrite or so on.

However even though it is blind this has been the mainstay of recording and understanding and applying computational neuroscience to understanding brain function, coding of sensory stimuli, decoding of different events from the spiking and so on. So this is probably the most used methodology and has been has provided the maximum amount of data in terms of understanding the neurons and the brain structures as a system or networks of neurons. And so this single electrode method as you can see is serial in nature that is we go in with an electrode record from one unit and then after finishing recording that is whatever stimulus we want to provide to the neuron in vivo we provide that finish the recording move on and wait for if we can get signals from another neuron and so on. So this is essentially serial in nature that is somewhat parallelized by multi electrode arrays of this kind where we have multiple shanks of electrodes that can be inserted into the brain and each of these electrodes can provide signals from single unit. And there are recent technologies have provided even more extensive microelectrode arrays where there are thousands of contact points where we can record from thousands of neurons simultaneously.

However, all this is blind that is we do not know what type of neuron we are recording from and it is also hard to say always in fact what exact region we are recording from. So and the further the other thing which is true for many methods is that we are not gaining access to the sub threshold activity that is the membrane potential that is the voltage

inside minus outside the neuron how that is changing what is going on in the process to build up to an action potential. So those things are missing in these metal electrode recordings. So the other method from that to gain access to information about the membrane potential is patch clamp recordings which is done which is shown on the right hand side and is done with glass micropipette. So here we are showing that this is a glass micropipette which has an opening at its one end so the glass capillary is pulled and made into and broken to make this hole and these glass capillaries have a metal inside the lining of the glass and that metal provides the electrical connection from the electrode tip and so by either by looking when we are able to look at the neurons through a microscopy and if we are recording from brain slices then we can use these methods or by blindly going into the brain also we can do perform this method and gain access to the membrane potential.

So how is that done? So essentially the glass micropipette goes in and you sit on the neuron we are making a sort of an indentation and with a gradual suction pull up the cell membrane into the opening of the electrode. Now this region has a solution that is the same in terms of the ionic concentrations of the different materials as the inside of a neuron so that when with further suction this membrane is opened up there is direct connection between the two regions so and the internal solution of the electrode and the inside of the neuron requires to be the same and they are also electrically isopotential. So what this now does is allows us to record the membrane potential that is inside minus outside by having a reference ground outside. Now with the kind of amplifier that we have that we will talk about later what is allowed in this is that we can now measure the membrane potential by having the total current across the membrane of the neuron in our control which is method of recording in whole cell mode which is called current clamp and similarly we can actually measure the amount of current that is flowing across the membrane of the neuron by holding the membrane potential that is V in minus V out at a particular value or a particular profile and measure the current during that period. So for example on the right hand side it is shown with current injections of variety of steps so here it is zero current then a hyperpolarizing current then in steps up to a depolarizing current and you can see the membrane potential is reduced first and then gradually it goes up until it reaches threshold and action potentials are produced at the highest intensities.

So this methodology also again is providing us a direct method direct access to the action potential and in fact the membrane potential but we are blind when we are applying this methodology to collect activity from neurons in an intact animal. Also these methodologies the three shown here are essentially applied in experimental animals by performing required surgeries. It is extremely difficult to do these in humans and because of ethical reasons and it is allowed only with patients who require electrode implantation for some dysfunction where they agree to the experimentation and it is

ethically approved then we can actually piggyback their surgeries of electrode implantation to with experiments where we record from the electrodes. So that kind of methodology in the recent past has provided a wealth of data from humans and provided us insights about processing in brain structures by humans as while most of the data that we will talk about is based on what we learn in animal experiments. So here a big question that remains is we are still missing the kind of neurons that we are recording from.

So in order to do that in the recent past the methodologies that have developed are the recording of action potential indirectly through the calcium changes in neurons. So there is a method called two photon imaging where you use IR laser that is pulsed and through scan mirrors we can direct the light onto the into the brain. There are reasons that allow us to go deeper into the tissue with this method with this kind of light and we can excite fluorophores that are present within the brain tissue. So this is an example so here is the IR laser that we are talking about let us say it is a 800 nanometer wavelength light that is falling. So these scan mirrors which are controlled by galvanometers can actually then excite different pixels on this region where we are interested to image and the fluorescence that is somehow tagged to the activity which we will discuss in a little bit is then collected back through the same objective and directed to amplifiers for in this case photomultiplier tubes which collect signals that the fluorescent signals that can be red or green depending on the kind of fluorophore we have.

So how is this applicable so this is sort of the surface of the brain showing the different blood vessels that are present. Now here are two examples one from a larger area and one from a closer area where the neurons are visible with a particular dye which is a calcium indicator that is this particular dye binds to calcium and becomes fluorescent when it is internalized by the neurons. Similarly, nowadays which we will see these dyes have been replaced by actually calcium dependent proteins like GCaMP of different versions which essentially perform in the same way but are more cleaner in the sense that we do not have to inject the dye into the brain but they are already expressing in the transgenic mice or they are injected and the neurons are transfected by virus that allows us to express these proteins in the neurons. So how does this all work so here is an example where a whole cell recording is being performed so here is the pipette with the dye this is a neuron that is filled up with the dye and parallelly the imaging as well as a voltage recording is being done in the current clamp mode and so with a current pulse at this location an action potential is produced in the neuron. So at the same time the fluorescence which is measured as delta F over F that is the change from as a fraction of the fluorescence in baseline that shows a clear calcium transit.

So this spike corresponds to this calcium transient however it must be noted that the

time scale of this transients are longer much longer than the spikes which are of milliseconds. However with fast scanning we can decode where the spiking events are occurring. So this is again an indirect way but we can now because of the genetics we can now express the protein the calcium indicator protein in specific neuronal types or we can express some other color tag fluorescence tag on a particular type of neuron and record simultaneously from different kinds of neurons. So here is an example where we are going to look at a video where so here we will see an example of how the imaging occurs. So here is an example of how the imaging looks in terms of activity.

So what you will see in this video are neurons particular types of neurons in this case particular inhibitory types of neurons that are expressing the calcium indicator protein and you will see that the neurons get brighter their fluorescence increases whenever there are action potentials in these neurons. So if you look at this movie again so that you can actually now see more clearly so here is a neuron that is producing an action potential here is a neuron that will produce an action potential and so on. So by monitoring the activity of the neurons throughout our experimental duration we can now so by monitoring the activity of the action potentials in many different neurons of a particular type we can get intricate detailed information about activity in specific types of neurons. Similarly by here are examples where a particular type of neuron is expressing a red fluorescent protein and the green signal is for the calcium indicator and so as you can see that we can simultaneously gain information about those red neurons which indicate one kind of neuron and also the other neurons that are marked in green which is showing the calcium signals. So here the examples are that here we have excitatory neurons only in green here we have only particular types of inhibitory neurons in green and in this case simultaneously we are imaging activity in inhibitory neuron and excitatory neuron simultaneously.

The yellow ones are the ones that are expressing red as well as the green for the calcium indicator and the only green ones are the basically the non-red ones. So these happen to be or by design are particular inhibitory neurons and these would be excitatory neurons. Similarly on the left we have a similar sort of approach. So with this methodology we gain access to much more detailed information about the neural activity. So now based on this we will go on to gradually understand how these spiking events occur and to understand that we have to look into the properties of the membrane or the excitable nature of the membrane which we will start in the next week.