Neurobiology

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Week - 01

Lecture 1.4: Methods in Neuroscience

In this video, we are going to look at the different types of techniques and approaches that are used to study the brain. The brain is a very complex organ and a lot of different approaches are required to study different aspects of it. And a lot of techniques have been invented over the years to study it, some of which are still being in use. Each technique has different advantages and disadvantages and they together tell us a comprehensive picture of the brain. And even as we speak, many new techniques are being invented in the field. So the repertoire of techniques is growing every year.

Some of the classical technologies like electrophysiology have been around for over a century. While there are other techniques that are more recent, such as optogenetics, which has been around only in the 21st century. And it allows us to manipulate the activity of specific neurons and then see the effect on the brain or the behavior. And also there are some techniques that have become outdated over the years.

So they were useful at some point of time, but either they were too cumbersome or they were found out to be not reliable enough and therefore they are discontinued now. An example of such a technique that has become outdated now is phrenology. Phrenology was being used about 200 years ago and it was basically trying to answer this question, what is each part of the brain doing? Now this technique was based on two types of observations. The first observation is that if you look at these skulls of different people, you find that their shapes and sizes are slightly different. So a skull may have a bump on the left side or some bumps on the right side or on the top of the brain or in the backside of the brain and so on.

And the second observation was that if you look at the personalities of different people, they are quite different. Some people are more loving while some are not. Some are more hopeful, some are not. Some are more spiritual, some are more content and so on. And people thought that maybe these different bumps are indicating what features or personalities that people will have.

Based on these kinds of studies, the phrenologists were able to create maps such as the one shown on this slide. This figure is slightly fuzzy, but if you look carefully, it's saying that this part of the brain is corresponding to hope. So people who were more hopeful tended to have bumps in this part of the skull. This part of the skull is associated with consciousness. This part is associated with self-esteem and so on.

Now these maps were based on a small number of subjects and perhaps they looked fine or reasonable at the time that they were made. But over time, we now know that these maps do not really hold true. So it is not really the case that people who are hopeful tend to have a bump in this part of the skull, in most of these people. So this idea that you can find aspects of personalities in different bumps over the skull does not really hold true now. And therefore, we do not use phrenology as a science and it's now considered a pseudoscience.

But the basic idea that different parts of the brain are doing specific functions is still a reasonable idea. And later in the course, we will see how we can find what different parts of the brain are doing. Let us take a look at the most common techniques that are used for studying the brain. In this slide, we have put these techniques on two axes. On the x-axis here, we have time on the log scale.

So zero indicates one second. And then this is tens of seconds, then hundreds of seconds, thousands of seconds, and so on. And on this side, it is one tenth of a second, one hundredth of a second, and this is one thousandth of a second or a millisecond. So this is our temporal axis or temporal resolution. And on the y-axis, we have size.

So zero here indicates one millimeter. And then as we go up, we go to 10 millimeter, 100 millimeter, 1000 millimeter. So this would cover the whole brain. And as we go down, then we are looking at one tenth of a millimeter, one hundredth, one thousandth, and one ten thousandth. So at the very small size scale, we'll have the synapses.

And then as you go higher, we'll have dendrites and neurons, then layers of neurons, columns of neurons, and then large maps formed by these neurons and then the whole brain. So we'll try to see for each technique, what is the temporal resolution, at what time scale we get the information from the technique, and what size scale we can see with that technique. One more feature that is shown in this slide is the invasiveness of the technique, as is shown by this legend here. So techniques that are shown in hotter colors are more invasive, and techniques that are shown in shades of blue, the cooler colors are less invasive. So let me start by zooming into this part of the slide.

So on this top right corner, you see this technique of lesions. So when we are using lesions, basically we are looking at the brain and seeing what part of the brain is damaged or removed.

And if we find such a lesion in the brain, and we correlate it with what functional abilities the person has lost, then we will know what is that part of the brain, what function that part of the brain is involved in. So these lesions can be observed on the scales of months or years. I mean, they happen very slowly.

And if someone has a lesion, then it can be observed. And then also the size of the lesion would be on the order of tens of centimeters or tens of millimeters. So it will cover a large part of the brain. We can say that it has low temporal and low spatial resolution. So the spatial size is large, so the resolution is poor.

And on the temporal side also, we are seeing changes that are happening on the orders of months or years. So the temporal resolution is also poor. And it is also very invasive. If one tries to create a lesion, let's say in an experimental animal, then it would be a very invasive, damaging experiment for the animal. And even if these occur naturally, then they are quite damaging to the brain.

So this is a very invasive technique. Next on the left side of it, we see PET, positron emission tomography. The idea in PET is that some kind of a radio labeled dye is given to the person. They drink it and the dye goes to the brain. And then if a part of the brain is active, it tends to absorb the dye.

And then we can measure the gamma rays that are coming out of the brain using an instrument. So it's an indirect way of knowing which parts of the brain are active. And then based on what the person is doing at that time, the activity of the brain can be correlated with the function. And that can tell us something about what is that part of the brain involved in. Similar technique is 2-Deoxy glucose.

In this case, a labeled form of glucose is given to the person and then to the person or to an animal in the case of biological experiments. And then depending on when the brain is active, the glucose is consumed more. And therefore, the parts of the brain that are labeled with this radio labeled glucose will indicate which parts of the brain are active at a particular time. Another technique here is micro lesions. This is also an invasive technique.

This is similar to lesions. So these micro lesions can occur naturally. There are also some approaches now by which some small lesions can be created temporarily in the brain. For example, with this approach called transcranial magnetic stimulation. So you apply some strong magnetic pulses to a small part of the brain repetitively. And then for a short time, that part of the brain becomes inactive.

This part of the brain may be on the order of sizes of millimeters. And this experiment can be done on the time scale of minutes or hours. And therefore, using these micro lesions, one can see what is that part of the brain doing by seeing what is the effect on the person. Now, another technique here in this large blue region is fMRI, Functional Magnetic Resonance Imaging. This is a very commonly used technique in human neuroscience.

This allows us to know in the human brain what is each part doing. The technique is very similar to the MRI imaging approach that is commonly used in the field of medicine. So it's a large scanner, magnetic resonance scanner. And the person goes in the scanner. And the scanner applies strong magnetic fields.

And these fields activate certain nuclei in the brain. And then when these nuclei return to their normal state, they release some radiation that is imaged. And based on that, we get a picture of the brain. One difference in normal MRI and functional MRI is that in the normal MRI, we are trying to image the structure of the brain. Whereas in functional MRI, our focus is on understanding the blood flow to different parts of the brain.

The parts of the brain that are more active tend to have more blood flow. And depending on the blood flow, the level of oxygenated blood changes. And the oxygenated and non-oxygenated bloods respond differently to magnetic pulses. So by looking at this contrast between oxygenated and deoxygenated blood, we are able to get a proxy for neural activity. This approach allows us to see structures that are on the scale of one millimeter to several centimeters.

And the changes that are occurring in the brain are on the order of one second or more. So the temporal resolution is about a second. And the spatial resolution is about one millimeter or higher. Another technique that is commonly used to study the functioning of the brain in humans is electroencephalography or EEG. And if the EEG signal is measured after some specific events in the brain, then it's called event-related potential, ERP.

So in this technique, basically electrodes are placed over the skull, which are measuring the electrical activity that can be sensed over the skull. At the skull, the activity that reaches comes from many different parts of the brain. So our spatial resolution is on the order of several centimeters. But the temporal resolution is quite good. So we can see activity that is happening at the level of milliseconds.

A related technique is magnetoencephalography or MEG, which measures the magnetic fields that are being generated by electrical activity in the brain. So this is a magnetic scanner, which measures the magnetic field coming from different parts of the brain. And then it tries to localize the source of that magnetic field and then infer what electrical activity must be present in different parts of the brain. So usually EEG and MEG are used when the person is doing a task.

And based on the task and what signals are being generated, one can try to infer the correlation between the activity in certain parts of the brain with the functional aspects.

Another technique is the use of optical dyes. One of the most commonly used dyes is a protein called GCaM, which fluoresces when a neuron is active. So by imaging these dyes, one can figure out which neurons in the brain are active. The fluorescence changes on the order of tens of milliseconds to hundreds of milliseconds.

So that's the temporal resolution. And the spatial resolution can go from the individual neurons to groups of neurons. The other commonly used technique is electrophysiology, which is shown in two parts here. So one type of electrophysiology is called patch clamp, in which a very sharp electrode is basically patched or it connects to an individual neuron or a part of a neuron. So it has very good spatial resolution. And then it's able to measure the electrical activity inside that neuron or a dendrite at very high temporal resolution, up to a millisecond or even low.

Another type of electrophysiology is called extracellular electrophysiology, in which the electrodes, a single electrode or multiple electrodes are put inside the brain, not inside a neuron, but outside neurons. And then they get the electrical signals from neurons in the nearby areas. And then using some data processing, it is possible to isolate the signals that are coming from different neurons. So one can break down that composite signal into different signals that may be coming from different neurons, even though they are not being directly observed. So such inferred neurons can be called single units.

And with this technique also, the temporal resolution is quite good. We can get signals at the level of milliseconds or even better. And the spatial resolution is up to individual neurons. And then the last technique shown in this slide is light microscopy, which is the normal microscopy in which you look at a brain under a microscope. It allows us to see the components of the brain in great detail.

So the spatial resolution is quite good. But the temporal resolution is poor. It's just structural imaging of the brain. What would be an ideal technique to study the brain? What would be the most powerful method if we can have our wish? Maybe we can list down what are the desirable characteristics of such a method. Well, it should have very good temporal resolution because you know that the activity in the brain happens at very fast timescale at the level of milliseconds or even less. So our technique should be able to provide us the millisecond or even microsecond level temporal resolution.

The second desirable characteristic is very good spatial resolution. The neurons and the components of neurons, different dendrites or axons of the neurons, are on the order of microns.

So our technique should have at least micron level spatial resolution. It should also have very high bandwidth. We should be able to get a lot of data at once out from the technique.

And finally, it should be non-invasive so that it can be used on humans as well as a variety of animals without causing much problem. But as of today, we do not have a technique that satisfies all of these desirable features. In fact, in neuroscience, one of the major limitations has been in terms of the techniques. So all the techniques that we saw on the last slide are very useful, but each one has certain limitations in one of these four parameters. Now that we understand the different methods that are used to study the brain, let us look at different aspects or different subfields within the field of neurobiology or neuroscience.

Overall, the field tries to understand how the brain functions, but we can approach it from different angles. One angle is to study how the brain forms or the development of the brain. And this field is called developmental neurobiology. So developmental neurobiologists study the whole process by which, as the fetus develops inside the womb, how the cells that ultimately form the brain, how they are dividing and how they are getting organized into circuits and how the brain comes about and how the connections between these different types of cells are formed. And that can give us some idea about how the brain is organized and how it functions eventually.

Another subfield is called cellular and molecular neurobiology. As the name indicates, this subfield is concerned with what is happening inside the cells of the brain at the cellular and the molecular level. So what are the different types of proteins or different metabolites that are present in the brain and what functions they are doing. Then one can go at a level higher and try to understand how the neurons are interacting with each other to form circuits or brain regions and what functions are being performed by these circuits or networks of neurons.

So this comes under the field of systems neuroscience. Then at the next level, one can try to understand how different parts of the brain are working together to result in various cognitive abilities such as thinking or emotions or decisions making. And this comes under the field of cognitive neuroscience or psychology. One may also approach studying the brain from the angle of diseases. There are several neurodegenerative diseases in which parts of the brains or certain cell types in the brain become defective or they start to die. For example, Alzheimer's disease or Parkinson's disease or a stroke.

And this comes under the field of neurology. So this is a medical specialty and neurologists try to study the disorders that involve damage to different parts of the brain. Another medically related discipline in the field of neuroscience is psychiatry. Neurologists are also doctors like neurologists, but they focus on mental illnesses. These are illnesses like depression or anxiety or schizophrenia in which there is no visible damage to a structure in the brain. There is certainly some changes in the brain that are resulting in the psychological illnesses such as depression and anxiety.

The tools and the approaches used by neurologists and psychiatrists can be very different. Another field of subfield of neurobiology or neuroscience is to understand the general computational principles, how the brain functions. And this comes under the fields of theoretical neuroscience or cognitive science where the focus is not so much on specific neurons or circuits, but more at an abstract level in terms of computations that are happening in the brain. And finally, one can look at the really big questions like consciousness or free will. They are also related to the brain, but they come under the fields of philosophy.

These different disciplines may study the same phenomena at different levels. So to take an example, let's think of the brain as a machine which is receiving sensory stimulus and ultimately is deciding the behavior. Now the field of behaviorism or behavioral psychology, which was quite common say 50 years ago, they thought of the brain as a black box and the goal was to find a function or a mapping between the stimulus and the behavior. So basically some stimulus comes and some behavior goes out. And they did not think at all about the subjective experience or the emotions or anything.

They just wanted to find a mapping between the stimulus and the behavior. That is one approach. Now the same phenomenon of stimulus to behavior mapping was studied by cognitive psychologists by explicitly considering the mind. And they made models of the mind with different attributes, different features. So the mapping depends not only between what stimulus is coming, but it also depends on the current state of the mind and how the mind functions.

And the field of experimental neuroscience tries to look at the whole circuit that is involved in processing the stimulus and generating the behavior. So there may be a lot of processing steps here that one can try to study experimentally. So let's say I look at a cup and after looking at the cup, I raise my hand to lift the cup. Let's see what is going on in the brain during this simple process.

The light from the cup falls on my eye, on my retina. And the processing within the retina can take 20 to 40 milliseconds. Then the signal from the retina goes to the thalamus, a part of the thalamus called the lateral geniculate nucleus, LGN. Then the signal goes to the back of the brain in the visual cortex where different layers of the visual cortex. So the signal is first processed in V1. So by this time it's already 40 to 60 milliseconds have passed since the light came on the retina.

And then it goes to V2, then V4, then inferior temporal cortex, the posterior part, and then the interior part. So by now about 100 milliseconds have passed. And then the signal goes to prefrontal cortex. And then it goes to premotor cortex. And then ultimately to the motor cortex where a motor command is generated and then the signal is passed down through the motor neurons in the spinal cord.

And then by the time it reaches the finger muscle from the spinal cord, 200 to 250 milliseconds may have passed. And then the finger moves or the hand moves. At each of these stages, different levels of processing are happening. So retina is basically processing the pixel level image.

The visual cortex is identifying simple shapes in these images. And then in the prefrontal cortex, decisions are being made whether one should reach towards the object or one should move away from the object and so on. Now, how did we arrive at all this information? How did we figure out that these different parts of the brain are involved in these functions? This has been done using the various techniques that we have seen previously in this video, the electrophysiological approaches or the imaging-based approaches, fMRI, EEG, and so on. And these circuits involve a lot of different cell types. And for understanding what are the different cell types that make up the brain, let's go to the next video. Thank you.