Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
Course Instructor: Dr. Shabari Girishan
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore
Week - 06
Lecture - 22

Hello everyone, welcome back to the course on Rodent Neurosurgical Procedures. So, in the last few sessions, we have gone through neuroanatomy, then we spoke about how to prepare for surgery and we also went through the various surgical steps for rodent cranial window preparation or rodent craniotomy. So, today this is a very important session, this is the central core of the entire neurosurgical module. So, which is stereotactic implantation surgery. So, this is a very broad area and I would say a workhorse of any neurosurgical procedure or any neurological experiment that you would want to do on small animals. So, in this module definitely, we are covering only rodents, mice and rats.

So, let us see what we need to do to get a successful experimental outcome or good neurophysiological data. So, stereotactic implantation surgery forms a very basic procedure to access brain data. How are we going to access it depends on your study objective and the methodology that you would want to follow. If you are trying to use a drug delivery system wherein you are trying to instill a drug and study its effect or the toxicity profile of it, you need to implant a drug delivery cannula.

If you are trying to look at the function of a specific area and the brain, then you can use various tools for the same. For example, you can create a lesion by introducing an excitatory toxic drug or a toxic agent or create a radio frequency ablation of one particular area. I have even done a micro suctioning of that particular area to create lesions and study the effects. So, whatever methodology you would want to follow, the basic surgical steps remain the same. So, that is the beauty of it if you master the stereotactic principles and the basic steps of drilling, and creating an access port.

So, that is more than enough and you can use any number of methodologies, any number of the apparatus, and stereotactic apparatus through the same steps that we are going to discuss today. So, I request and I emphasize again that try and learn the significant steps of drilling using the coordinates, and master the principles behind these stereotactic coordinates on a cadaver dissection. A cadaver is a carcass or rat body preserved in formula. So, practice your incisions, practice drilling, practice fixing the coordinates, try and check where you have reached using the cadaveric dissection, take a brain slice of the cadaveric brain and see whether you have hit the target before you start performing on the

experimental subjects. So, with a brief introduction let us begin today's session on stereotactic implantation surgeries.

So, this is the outline of today's session. We will briefly go through the planning involved just to have a recap on how and what we need to do the planning aspect which is the foremost important step that one needs to be you know one is to have a full idea as to where you are going to target and how are you going to target, what is your entry point, what is the trajectory that needs to be considered, all that needs to be done before you even begin the surgical experiment. And next is again the drilling technique. Most of the drilling has been covered, drilling techniques have been covered in the craniotomy aspect itself. It is just a bit of a modification which is much simpler than the craniotomy aspect.

Now, for that, we will take you through the specific operative steps that are slightly different from each other concerning either the microelectrode implantation, the optogenetic cannula implantation or the drug delivery cannula. So, we will see how these steps are carried out in the next few slides. So, the first and foremost is stereotactic planning. So, when I say stereotactic planning it is not the methodology aspect, the entire methodology is different. When it comes to the planning you need to figure out what is the entry point, where exactly you are going to make the bar hole.

And as I said that is going to have a bigger bearing and unless you plan your entire surgery every single step has to be planned before you execute it for a simple reason that it cannot be undone you know. So, better if you have a mental picture of every single step that you are going to perform and, if possible, have a dry run without the rat. Similarly, I suggest for all the electrical setup a bend stop testing and then checking the electronic apparatus completely before you start the experiment is needed. Apart from that, you need to be ready with the stereotactic coordinate coordinates of the structure that you are going to target. Here is an example of a supplementary motor area which has been outlined in red and below you can see the coordinates that are given.

In this example we have taken 2 millimeters lateral to the midline 4.68 a millimetre anterior to Bregma, this is on the right-hand side of the atlas picture. So, if you know the structure, but do not know the coordinates you use this Paxinos invite atlas to figure out the coordinates. Or if you know the coordinates you can always get back to this atlas and see if it is the structure that you want to target with those coordinates. As I said from species to species it does vary; this is done on the Vista rat.

So, use the coordinates from the atlas or the literature, but confirm it, reconfirms it, triple check where you are going to hit it and what is the centre of the target, what is the dimension of that particular target that you want to study. So, preferably for example, if

you are supposed to target only the eccentric portion of the target unknowingly and you have your coordinates wrong, you will probably get very different data or probably a mix of data or false data that you are going to get if you are going to implant it in the different place. And if you are trying to look at the toxicity of a particular drug you are going to get entirely different behavior altogether if you miss the target. So, this is very important on the coronal slice, the axial slice and the sagittal section. One needs to be aware of the position of the target and then check your coordinates before you begin the procedure. So, this is the sagittal section which we have discussed in detail in the serotactic principles and that is the deeper target.

So, whether it is a cortical target or a deeper target you need to know what is the depth of penetration and then what is the entry point you are taking and then of course, the trajectory. We have discussed in detail various trajectory shifts that can happen. Trajectory refers to the path taken by any probe that you are going to use. So, that trajectory is very very important and you need to make sure that you will not go through any major blood vessels which will lead to a sort of hematoma formation or bleeding which will endanger the life of the experimental subject. So, here is an example. I have already discussed how the trajectory would change, and how the depth of penetration is going to differ based on the tilt that can happen sagitally or in the coronal plane.

Sagitally I said the bregma and lambda have to be on the same plane, it has to be a flat surface and that has to be corrected. That example already has been discussed and you have to make sure that the head is centered before you even begin. Here is an example where there is this venous sinus in the path of the trajectory that you are going to use. So, you have to give the tilt down and make sure you avoid the venous sinus before you even enter. The beauty is that the target shift does not happen much when you are trying to change the entry point, but of course, the depth will change and this calculation of a different depth has been covered in the previous session wherein you use the right angle triangle and use your cos alpha function. Take the hypotenuse and adjacent side and calculate the new you know trajectory length.

So, once you have a new dorsal ventral length you use that and then approach the trajectory. So, this is one example where you will have to avoid a critical structure being damaged during the planning of the trajectory. So, this is what I wanted to emphasize when I wanted to give you a brief recap on the importance of stereotactic planning. So, these are the very crucial steps that are sort of a little more complicated than the actual surgery. The surgery you master once you master the drilling technique later becomes simpler all you have to do is a bar hole puncture the dura and get your probe inside, but what is going to determine your entire experimental outcome is this aspect of stereotactic planning which has to be thorough in terms of coordinates and trajectory and the target

area.

So, another safety layer that I can consider with my experience of course, from the human functional neurosurgery that I practice is intraoperative mapping. So, if your experiment is about the motor area you can take another safety layer to confirm your location all right. This is not possible if it is sensory or visual areas for other not very eloquent areas it is difficult to know where you are during the surgery that is what I meant by saying intraoperative mapping. So, there are ways to figure out where your experimental probe is possible for example, even in the sensory cortex where you can implant the viral cortex and then move the whiskers of the rat and see if you get an event-related potential or evoked potentials all right. Similarly, intraoperative mapping motor mapping is possible. For example, what has been shown here is the rostral forelimb area and that is the caudal forelimb area and these are the coordinates B is for bregma and so many millimetres posterior lateral and anterior to bregma.

This is the left hemisphere of the rodent motor cortex which has been divided using the 1-millimeter chemical area based on the coordinates ok. So, based on these star marks, that is the area that has given responses motor responses you implant your probe micro electrode be it a tungsten electrode or for example, any specialized neural probe you can implant in this area and then stimulate with certain parameters you will see a motor evoked responses. Based on those responses you know where you are for example, if you are if the proximal joints are moving and not the distal you know that you are dealing with the rostral forelimb area similarly to the caudal forelimb area. As I said earlier you are depending entirely on the coordinates. So, we do not mean if your study does not give you or if the infrastructure does not give you a facility of using the image guidance wherein you can do the preoperative MRI and then export it to your stereotactic planning system and tell where exactly you are in the brain.

And if you are going to use only the coordinates without that sort of image guidance then interpretive mapping can come to your rescue and tell you whether you are in the motor cortex or outside the motor cortex. So, that much definitely is possible and this is one of the studies I mean one of the examples from our study where we implanted the tungsten microelectrode as you can see here and the steps we are going to discuss anyway. But before we begin your study make sure that your drug delivery cannula or a fibre optic probe or for that matter even microelectrode has to hit the motor cortex this is one way of doing it. You know before you leave a final probe into it you implant the tungsten microelectrode. We give a current of around 1 milliamps to 2 milliamps and vary the frequency pulse width and all that and then see that you get an adequate response before you leave your permanent in place.

So, we have implanted it in the right motor cortex in the rostle forelimb area and you can see the good nice adduction of the forearm and the proximal arm, but not much distal forelimb all right. So, this is a very important safety layer to confirm that you are dealing with the primary motor cortex. You can even for the experiments involved in the secondary motor area use the primary motor area coordinates which would give you an indirect confirmation of the coordinates of the secondary motor area which is just anterior and medial to the primary motor area. So, this is one good methodology that you can adopt if you want to confirm the coordinates if there is a difference in species or differences in the size of the rat and age of the rat because the Paxinose atlas are based on the adult Vista rats. So, based on that if you want to take the coordinates from that atlas and use it this is one way of doing it all right.

So, coming to the actual surgical steps. This has been discussed in detail in the previous session. So, I will not dwell too much on this, but just to give you a brief recap on the left-hand side you have a dental micro drill on the right-hand side you have the coaxial system of the drill which is a bit heavier than the left and the advantages and disadvantages have been discussed already in the previous session. So, as I said earlier you must consider the diameter of the drill bit and the screws as I discussed last time make sure it is 1 or 2 millimetres bigger in size as compared to your drill bit. So, the purchase is going to be good.

We are going to use similar screws as we discussed in craniotomy as well wherein this is going to give a very good rigid fixation where the implant as well as the screws becomes one a big blob of acrylic dental cement and it is all fused to give a good rigid fixation of your primary implant. So, it is very important to practice this as well as implant the stainless steel screws wherein you leave a gap between the surface of the skull and the top of the screw. So, the dental cement or any matter bonds that adhesive layer that you are going to use and to occupy this area into one you know into one unit along with the primary implant. So, this is another very important step that one needs to practice. So, here is an example of drilling burr holes wherein you use this round bit which is around 0.

8 to 0.9 millimetres and go straight from the outer table to the inner table you need, to recognize when you have reached the dura either by the give way sensation with the tactile feedback. So, once the inner table is breached you will have the tactile feedback of the loss of resistance for that matter you can even use the microscope and with continuous irrigation and suction you can visualize the inner table. If it is mice you can even see the dura which is very thin and transparent and you can see the cortical surface with blood vessels and that is the time you are going to stop. You can even use the needle to puncture the papery inner table. If it is thin enough you can even break it with the

needle, but never puncture it or plunge it with force.

So, that is going to damage your cortical targets which is very detrimental and you will have to probably abandon the experiment if there is a plunge of drill inside the brain. So, that is the reason I kept reemphasizing to use of the cadaveric rat models if you are a beginner to practice these steps. So, on the right-hand side, you can see that there is one big hole and four small burr holes that have been drilled. This is in the frontal bone, these four are in the parietal bones. So, this is for the anchoring skull screw that is going to go in that is for the primary implant.

So, before you implant it you need to put the screws in and then you will do the final implant and then start applying the acrylic or metameter bone adhesives all around to form a single unit for rigid fixation. So, let us see how the different techniques or the usage of these stereotactic surgeries are used in various experimental methodologies. Here is an example where the six soda is the six hydroxydopamine which is an excitotoxic drug used to create a Parkinsonian model which will be discussed in the next few sessions. Various models and how the creation of various models. In the last session, we discussed the creation of a stroke model. Here is an example where a Parkinsonian model can be created by using what is known as a Hamilton syringe.

This is a 1-microlitre syringe that needs to be delivered using the micro-infusion pump. So, that is very very important because the infusion rate is very important so as not to damage the soft deeper targets of the brain alright. So, the drug has to be delivered only to the target that you have chosen, so that you will see the changes in the behaviour and the creation of the proper model. So, that is very very important. For example, if you instil it into the medial forebrain bundle that is the structure in the basal ganglia you will see a complete dopaminergic depletion and give you a full-fledged Parkinsonian model.

Whereas, if you try and insulate into striatum corpus striatum as if you remember the structure from the neuroanatomy session if you instil the six order into the striatum you will get a partial dopaminergic loss and you will have a partial effect of the Parkinsonian model. So, but then the procedure is the same. All you have is drill a burr hole and take the Hamilton syringe tip needle and needle tip through the burr hole into the target as per the coordinates. Similarly, on the right-hand side the example is for the viral vector infusion as part of the optogenetic study. Before you use the optic probe you need to introduce a viral vector that is going to induce the fluorescence which will be triggered using the optogenetic probe. So, we will be discussing it in a little more detail in the next few slides.

So, what I am trying to emphasize is that this is a very important methodology with a

similar technique, but with different coordinates and different drugs and different agents and different apparatus that you are going to use. You need to appreciate the changes that you are going to see in each slide with the adapter and the apparatus and the tool that you are going to use to deliver a particular agent or to implant a particular needle or cannula or your microelectrode. So, please try to focus on the adapter that is being used and the tool that is going to be used or fixed onto these adapters. And of course, the basic step of having a burr hole in these with suitable coordinates. So, these are the examples of drug delivery cannulas and the microelectrode implantations.

And of course, this is another example wherein optogenetic stimulation has been used. And what you are doing here is the fibre optic cannula implantation. Using the similar burr hole head has been fixed and that is the anaesthetic mask or tube for the rodent which is used along with the stereotactic apparatus the head is centred then the burr hole is placed and the cannula is implanted all right. So, those are the few examples. So, going into the details of these I will try to cover the microelectrode implantation first followed by the optogenetic probe and the drug delivery cannulas.

So, to begin with, the exposure of the skull is just the same as you all did for the craniotomy purpose as we discussed for craniotomy. You make you prep the skin close to the eyes, do the painting, do the draping and you know after clipping error the preparation is just the same as for craniotomy. You make a midline incision and then expose the skull, I hope you remember the classical landmarks of bregma and lambda that are very important and are the starting point of any neurosurgical procedure for neural experiments. Once that is done use the coordinates and then mark the burr hole area and then use the drill bit to make the burr hole and expose the dura. So, here is an example where they are implanting these many electrodes to record the electrocorticographic activity.

Electrocorticography is the surface electrical activity from the cortex. You know the cerebral cortex from the surface of the brain. They are going to record electrical signals based on various behaviours that the rat is going to perform. More importantly, when the seizure happens either by instilling the seizure-inducing agents like bicuculline, you are going to see the seizure manifest physically in the rat. It will go in for a seizure and when the seizure happens these implants will record the electrodes. It is very important where you are going to implant these electrodes and these are the coordinates that have been shown concerning the lambda at suture and bregma, bregma point and the lambda points. So, this is how you mark it and then perform the burr holes respectively and then get your implant on the implant holder. So, try and see the difference in the adapter that is being used here.

So, there is an adapter to use the Hamilton syringe, there is an adapter to hold the implants like this adapter to hold the flat PCB circuit boards and then an adapter to hold the drill bit. So, this is the versatility of the same stereotactic apparatus that we have been discussing in the last few sessions. Just by changing the adapters you are going to use can use the various tools using similar steps. So, that is the importance of mastering the basic general neurosurgical steps and that is the whole point of having this session in the first place. These are the general basic neurosurgical steps if one masters, then one can do a number of various methodologies and experimental methodologies on the same models. So, once your burr hole is made, once the implant is mounted onto the adapter you are good to lower it down.

So, this is the zoomed-in view wherein the adapter holding the implant and you can see the electrode in the tip of this particular implant. So, there are EMG wires. These wires are concerned with the EMG; those electrode tips are going to record the electrocorticographic signals which are from the surface. These electrodes which are protruding outside are basically for the recording of EMG, not ECoG. ECoG is electrocorticogram EMG is electromyogram, electromyographic signals from the muscles of the neck. So, that is the reason that they have a long extension of the electrode that is going to be inserted into the neck muscles you understand.

So, that will help us to sort of identify the muscular activity during the seizure or any seizure-inducing activity or any cortical stimulation if you want to perform that EMG is going to give us the feedback. It also tells us what is the artifact that is happening because of the muscle movement where the awakened behaving rats do give a lot of muscular signals. If you have that as a reference point your interpretation of the electrocorticographic signals is going to be very useful. So, once the electrode goes inside the burr hole and touches the dura or if you are puncturing the dura and you want to use the electrodes directly on the surface even that is possible. You puncture the dura using the needle tip and as soon as you puncture there is an egress of CSF the brain fluid that comes out is the confirmation that you have punctured the dura all right.

So, once that is done there is a difference in the depth that is going to happen which you need to account for during the depth calculation. So, around 0.2 to 0.3 millimetres or for example, even 0.5 mm of displacement can happen once the CSF comes out all right.

But by and large, you will be able to implant the electrode either onto the surface or even deeper a little bit deeper to the surface of the brain and you will be able to record decently good electrocorticographic signals all right. So, once that is done then comes the anchoring part that you need to hold the implant in place and cover the area with the skin all right. So, what you are seeing here is the bottom of the implant and these are the skin

edges that are brought close to the midline. The EMG wires are crossed to account for the stress and strain that can happen with neck movements and also to give a good rigid fixation that the entire setup is going to be within the acrylic which is shown here. So, once acrylic is placed into the defect the skin edges are brought together and closed either with suture materials or with staples all right.

So, that is about the microelectrode implantation. Once it is implanted, it is very important to have the cable tether cable planned very well which has to come straight on top of the head of the mice. So, that it does not cause any strain and is out of reach for its 4 powers to prevent damaging the electronic setup that you have implanted. So, that is why you need to give a nice good casing all around the electrodes. So, this will make sure that the rat does not pluck out all the electrodes all right. So, there is a 6-pin header that comes with this, the other option is to have a head stage where what you are seeing here is the simple casing that they have done considering multiple electrodes all right.

But nowadays various digital interface devices come in wherein there is a pre-amplifier and then the analog to digital converter (ADC) that can be added in here to form what is known as head stage. So, which is in line with the final implant that you are going to do and then you can have either a wireless electronic setup here which is the ideal situation that will give the rat much more freedom to walk around and do all sorts of activities if your experiment has a lot of behaviour experiments involved. So, tether cable does affect the movement a little bit, but by and large, you will be able to manage and get good electrical signals all right. So, the next important thing is to put the entire system in the behavioural box.

So, there is a school of thought on using Faraday Cage. If your study involves a lot of interpretation of electrical signals from the brain in different areas and if the surroundings are not electrically silent what is meant is by saying that if there are a lot of electronic apparatus that can add noise to your recordings then it is always a wise to have a Faraday Cage which will cut off all the unwanted signals. So, you need to plan the cage accordingly, have a conduit or a grommet for water supply, have foot pellets, have good bedding and then plan your electrical cable as per the requirement of freedom of movement within the cage. So, all these are pretty important. If your experiment has any sort of behavioural setup like you know the lever press arm which delivers the water as in when the rat or mice presses the lever or pushes on to the lever or if it is the feeding test like the Vasar test where you have a slot and you keep the foot pellets. So, the entire behavioural setup should be able to accommodate the electronic apparatus that we just discussed all right.

So, that is about the microelectrode implantation in brief. Then the next few examples the

optogenetic stimulation as well as drug delivery cannula implantation. I am just trying to cover the major areas of research that are happening and optogenetic stimulation has come in a big way and is a very exciting field of studying the various areas of the brain using just the light. So, there is no direct electrical interference that is happening, but yes there are many pros and cons with the two different methodologies. So, you will have to choose it based on your objectives and aims. So, then what I am trying to cover is the simple surgical procedure that is required to achieve a good experimental outcome.

So, here are the steps basically which briefly tell you what the major steps involved in creating an optogenetic stimulation model. The first thing is that you need to sort of piece together the genetic construct which is going to give us the fluoro sensitive you know outcome of opening up an ion channel. So, to get the fluorescence expressed in that particular target you need to sort of include this into the rat's brain. So, that particular gene is incorporated and it starts expressing the channel which will be fluorescent whenever the action potential you know is propagated. That is the idea in brief. So, to get that area to encode this particular channel adoption is one example wherein the gene expresses the fluorescence whenever the option channel opens up.

So, that particular viral I mean genetic construct needs to be inserted into the virus. Once that is done then the entire viral vector is instilled into the same target that we just discussed in the first few slides wherein the Hamilton syringe has the viral vector which will be implanted first all right. So, once that is done then the optrode is a fiber optic cable plus an electrode all right. So, either you can use just the fibre optic cable or you can combine it with an electrode which can gather the electrical signals not just by stimulating and then you see only the response you can also record it from the electrode and that is called optrode. So, then it all goes to the same cannula that we have just been discussing all you have to make is a burr hole and then you can implant the cannula which will hold the optrode or the fibre optic cable which will pass through the cannula which you have just implanted by making a burr hole.

So, once you do that a laser light of a specific wavelength opens the ion channel in the neuron and it flows. So, that gives the action potential which will be picked up by that particular electrode. So, that is the whole idea of having an optogenetic stimulation where you make the target the behaviour of the target based on the stimulation. So, in a way you are trying to control the behaviour of that particular target you can open and close the channel when we need it and we can study the spontaneous activity as well when the rat behaves in different ways. So, this is how we do it wherein you keep the target, select the target and leave the optic fibre right on top of it.

And here is an example of a nucleus accumbens target wherein the optical fiber is right

on top of it and partly into it and you have a guide cannula through which the optic fibre that you are seeing here will be passed to stimulated all right. So, this is a cranial window that we have performed and if you all recollect a cranial window is performed and there is a PDMS sheet you can have or you can have an artificial CSF or you can have a permanent cranial frame which will hold the guide cannula or for that matter, you can fix it permanently using dental cement and leave the cannula in C2 which can be closed or open as and when you want to do the experiment using the optic fiber cable which will pass through the cannula and stimulate that particular target all right. So, another important additional apparatus that can be used along a stereotactic frame is this rigid cranial fixation. If you want to do an in vivo acute study wherein the rat is habituated for this sort of a cranial construct or the cranial setup and as the rat behaves you can study the brain signals using this optogenetic stimulation whether that performs various tasks like for example, a foot pellet grabbing task or lever press task or even for that matter it runs over a big ball for the navigational electrophysiological studies. So, there is a whole lot of apparatus wherein the head is rigidly fixed onto a stereotactic apparatus using such a cranial stabilization device and then you gather the signal either by using optogenetic stimulation or even for that matter microelectrode implantation.

So, this is one such device implantation where a light emitting device implantation for wireless optogenetics. So, all you saw was the wired cable wired optogenetic stimulation with a cable running out of the head, this is one such device where the light emitting happens and it works on a wireless tech, but the point of highlight is the adapter usage as I said for a flat tool that you are going to for the flat implant that you are going to use it you can use this sort of an adapter as suppose to the adapters that you just saw for microelectrode and drug delivery using Hamilton syringe. So, this particular adapter again comes with the stereotactic apparatus where a flat implant can be held which in this case is a light-emitting device. As you can see it is nicely shaven and the skull is exposed, your Bragman lambda is available. You get the coordinates and you lower your drill bit, this is a different type of drill bit which is called a twist drill where the length of the drill bit is pretty long. I generally would not recommend this particular drill bit because you need to have really good control to make sure that this does not go beyond the thickness of the skull all right.

So, again the basic principle of making a burr hole and then bridging the dura and here once the burr holes are made the first thing as I said is to implant the screws to aid in rigid fixation. Once two screws are implanted and screwed in as I said, leave enough space for the skull and the top of the screws to hold the dental acrylic and then as per the target entry point, you go in with the implant final implant. In this case, it is a light-emitting device which has been implanted into the burr hole in the middle and once that is done you start adding the dental acrylic here. So, the dental acrylic goes around the

implant and then occupies the entire area of the screws and you can even further cover the entire implant with the acrylic leaving out the light-emitting wireless electrode outside the implant for electrical communication and that is the picture of this particular adapter holding the device.

So, that is about the various devices and implantation. Then briefly I would cover the drug delivery cannula implantation because this is another very important armamentarium that one needs to be familiar with if you are trying to choose a particular study and if you are wondering which is a good study which is a good methodology that you need to follow. Drug delivery is another important methodology that you should be familiar with which can be used for various neurophysiological experiments. So, what has been shown here again is that once the burr hole is made, in this case I think a large cranial window has been made. It is not a small burr hole; a large cranial window has been made and then you put the dental cement screwed all right. So, a partial thickness window has been made where a part of the bone is still there into which the screws go in and then you fill it with the dental cement around the cannula that has been implanted right on top of the target. So, the injection cannula will go completely throughout the length of the trajectory whereas, the drive tube has to stop around 1 or 2 millimetres above it all right.

It is only the injection catheter and cannula that goes right up to the target, the guide tube has to stop it 1 or 2 millimetres above it. A guide tube is something that has been implanted using the stereotactic coordinate. Once the stereotactic apparatus is out you can use this particular cannula anytime without the use of the stereotactic setup by using the drive tube which is going to maintain the coordinates that you have used this drive tube has been fixed rigidly to the skull using all these dental cement and the screws. This is the idea even for the opportunity cannula we just discussed and even for that matter a microelectrode implantation. A good rigid fixation once the major tube goes in and this guide tube can be used even for microelectrodes which will maintain the trajectory and implanted after the acute study after the rat is recovered and this particular guide tube will maintain the trajectory and through that you will pass the drug solution.

If it is a 6-order this is the microinjection syringe pump I was discussing. For each drug, there is a set microlitre per minute drug delivery that needs to be set up on this and then it automatically injects that much amount of the drug through this injection cannula into the target which is very very important to prevent damage due to the higher pressure which comes when you try to inject manually without using this syringe pump. So, this has to be pre-filled and make sure this air bubble is in place which will let us know where this drug solution stops and where the distilled water begins which will distilled water is used as a driving force and the air bubble gives us the idea as to how much the drug has been

passed and when the drug has reached its target all right. So, here I will show one of the videos taken from the JOWI itself, a wonderful video that will tell you how to perform the survivable stereotactic surgery. So, I have started the video from the point of exposure of the skull and you can see that the bregma and lambda points are marked if you do not have the digital display of the stereotactic coordinates as I showed in the earlier session you need to manually check the coordinate for the bregma note it down, choose your target and then accordingly you transfer it onto the coordinates based on your bregma either you add and or you subtract if it is posterior add if it is anterior. Similarly, the lateral coordinates and the ventral coordinates of all three coordinates are planned and you will mark the area where the drug delivery canal is going to be implanted all right.

So, once you have marked the portal of entry then you use the drill gently and make sure the skull is penetrated without damaging the underlying dura. Here you can see how the burr hole is made with the perpendicular movement and you can see the drug delivery cannula being implanted. So, once you make sure that the dura is not punctured, what you saw now is basically how they puncture the dura and see either a drop of blood or CSF which comes out and those four holes we just discussed are for the screws to give us the good rigid fixation. So, once those extra burr holes are made the screws are implanted as shown here all right. So, then you fix it with the artificial CSF, dip it in artificial CSF and then mount it onto the adapter and that is the adapter for drug delivery cannula implantation or for that matter any cannulas that need to be held perpendicularly according to the coordinates.

Once the cannula is in place in the target one needs to mix the dental acrylic cement and make sure it forms a nice paste using the spatula nicely spread it out around the screws and your cannula. You can have these sorts of constructs to make sure that it does not overflow beyond you know the edges of the skin and it also prevents overflowing onto the eyes and other areas that are unwanted and gives a nice good shape for your implant which will make sure that it is implanted rigidly on to the skull. So, it forms a sort of crown all around the implant in this case it is a drug delivery cannula. Once that is done you dismounted and dismantled it from the adapter and then allowed the rat to recover and you can even add tissue glue.

So, that is about the drug delivery cannula implantation. So, that completes our stereotactic surgeries. So, as I said it is very important that you all go and practice if possible on a cadaver using all these steps that I just described. There are n number of materials also available on the net to know more about the topics which I just described and these are the various methodologies which are very useful to adopt for your neurophysiological experiments. It is sort of like we are trying to lay a foundation here on top of which you need to improve your technique on these basic general neurosurgical

principles that we just described. Briefly, I will just touch upon the wound closure and healing which in itself is a big session, but then again what has been shown here on the left-hand side is a practice suturing set that is available on any online shopping website where you can procure and practice suturing is a very important surgical step, but these days there are n number of surgical staples that are available which are easy to use expensive than the regular sutures.

But then it is important to know the sutures themselves because even in deeper areas where we learn carotid ligation techniques, many other deeper areas might require suturing. And when you are dealing with GI surgeries or thoracic surgeries which is an additional surgical technique you need to adopt, then you need to know how to do simple sutures at least. There are various types of sutures like mattress sutures, continuous running, or continuous interrupted sutures, but at least there are simple sutures that you need to understand. So, you can do the suturing in either two layers or one layer. Before the rat model generally, one layer should suffice because the scalp layer is just one layer and beneath which you have muscle only for the temporal craniotomy if the temporal muscle usually it is excised.

So, you do not have to suture it back as we saw it, but if it is the abdomen or if it is a spine then you will have to close in two layers where muscular layers from one layer and then on top of that skin can come in all right. So, these are the examples of simple sutures. If there are sort of bleeders within the muscle you can take two or three rows, three throws using the needle holder as you can see here make a loop using the needle holder and then you pull the tip inside it. So, that one throw goes on top of it and similarly you repeat the same step of putting another two or three throws and then make a final knot all right. So, this again requires practice to master wound closure and generally, if you are trying to use post-operative antibiotics and good care post-operative care the wound healing is pretty good.

So far I have not seen any gross infection of the wound or gross wound gaping after the surgeries. So, the scalp layer is very rich in blood supply. So, the healing is pretty good. So, you can handle it with simple sutures. If it is a really big hassle surgical staples can be used which can clip the two edges and by delivering a clip.

So, that is the end of today's session. So, to summarize I would say we have gone through the major stereotactic procedures that form the workhorse of any neurosurgical approaches for neural experiments or various neural engineering techniques. So, it is very important whether you are going to use a cranial window or you are going to do a craniotomy is a different matter. But if you master one technique that is drilling then I guess most of the procedures can be performed with ease. So, start with simple burr holes

and then start you know from making cranial windows on cadavers and then start learning how to suture and put a knot and or how to close the wound using surgical staples, how do you use the dental acrylic, what is the right amount of time that you are going to wait for it and what is the sort of viscosity that is required all these will have learning curve. So, either you ask for a higher number of rats to make sure that you get over that learning curve or use the rat's dead body or the carcass to preserve it in formalin and practice all these steps.

So, but then stereotactic neurosurgical procedures are very basic procedures that form the foundation for any neural experiment. So, that is the end of the session today. In the next session, we will try to cover the spinal anatomy, and spinal procedures because most of the cranial approaches might require an additional spinal arm. For example, if there is a stroke model you would want to sort of implant an electrode in the epidural space of the spinal canal if there is a translational approach that you are planning to do it or for that matter even how to expose the peripheral nerves wherein you can put an electrode around that nerve and then gather signal as the rat performs various studies. So, in the next few sessions we will cover the spinal surgeries and peripheral nerve surgeries and preceding that session there will be spinal anatomy and peripheral nerve anatomy as well to understand better all the surgical approaches and the structure that will be exposed and operated upon, alright. So, see you in the next session. Thank you.