Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Week - 05
Lecture - 19

Hello everyone, welcome back to the course on Rodent Neurosurgery. Last session, we went through all the SFT precautions that are needed and how to drape and prepare the rat's head. Today, we will go through the actual craniotomy steps and briefly discuss how the cranial window differs from the craniotomy per se. Today's session is mostly on craniotomy. In the next session, we will discuss stereotactic burr hole preparation and twist drill craniostomy. It is essential that you understand the general principles behind it, and every experiment will be customized to achieve the particular objective or aim of your study.

I am trying to cover all the general surgical principles involved in craniotomy, which will be useful for you to switch, improvise, or expand the usage of these surgical steps. The aim is for you to handle both craniotomy and stereotactic techniques and customize them as per your needs. Today's session focuses entirely on craniotomy and cranial window surgeries.

I will briefly touch upon the exposure of the skull for continuity from the previous session, and then discuss planning, mostly focusing on drilling techniques. This step is crucial in rodent neurosurgery, common to both craniotomy and stereotactic techniques. Mastering the usage of the drill is significant, as around 60 to 70 percent of the work is done if you are proficient with it. There is a learning curve in using the drill, and I suggest practicing on cadaver dissections before using live subjects or experimental rats.

I will also cover special scenarios where the steps differ, such as temporal craniotomy, with different anatomy and positioning than usual craniotomies. Additionally, I'll touch on cranial and spinal windows, which are part of the craniotomy but with a different application than regular craniotomies. This brief introduction to cranial and spinal window techniques will help you understand their differences from stereotactic planning.

In craniotomy planning, you aim for a wide exposure of the skull. You can opt for a limited exposure, but it doesn't add much if you limit the length of the incision. So, I guess the length of the incision, whether it is for a stereotactic procedure or for a craniotomy, is more or less the same, and those craniotomy, I mean, the incision limits have already been discussed. What I want to highlight here is the draping part of it. What has been shown here is the opposite, which is a transparent adhesive layer that you can actually stick onto the entire rat's body, and this is going to be sterile. So, this sterile sheet

on top of which you can start using the sterile drapes as and when required. The idea behind having this impervious waterproof layer is that when we talk about asepsis and use cloth material directly over the rat, and you will be using irrigation during the surgery.

When that happens, and the cloth becomes wet, then the entire field is considered unsterile, alright? So, pathogenic organisms can easily get into the field. So, it is always good to have this waterproof, impervious layer, preferably, usually it is offsite, or sometimes we can even use the Ioban. Ioban is, again, a plastic transparent sheet with an adhesive layer in which the betadine or the povidone iodine antiseptic agent has been impregnated. So, that is best, and that is what we use in human neurosurgeries.

But then, I guess offsite will do the job, and on top of this, if you are draping for a small animal, this should do, and that is the classical positioning for the craniotomy, and this head fixation, how do we fix the ear bar, and how to fix the nose bar, what are the nuances in which the coordinates and targets can change, all that has been discussed in the previous session. So, just to briefly recap what is been shown here is the exposure of the skull. One can use this swab stick to push away the pericranium. As I said earlier, the pericranium is a very thin, soft tissue layer on top of the skull, and it is very important that you try to avoid using a drill on this slippery, pericranial tissue because it generally slips. So, that is one important thing, and moreover, you need to expose the surface landmarks of Bregma, Lambda, and coronal suture, sagittal suture.

So, it is always good to expose the entire skull before you actually begin the process of craniotomy. That was a brief recap on the exposure of the skull, and when it comes to planning, it is very, very important to sort of know which is the area that you would want to expose. And I have emphasized earlier that it is very important that you do not really try to open up as much skull as possible for the simple reason that you do not want injury to the areas of the brain which are not needed to be exposed or targeted. So, it is always safer to know beforehand which part of the brain you are going to expose, and the remaining part of the skull area is definitely useful for anchoring. That is one reason why we expose the entire skull in rodent neurosurgery, unlike in human neurosurgery.

In rodent neurosurgery, you need to expose the entire skull, though the craniotomy is only here because the remaining part of the area is going to be the anchoring screws, and if the electrophysiological experiment you will be using a lot of ground and reference electrodes. So, whereas, various uses of these exposures, which we will learn in the subsequent slide. What has been shown here on the right-hand side, you can see, and if you all remember from the previous session, this is the Allen's mouse brain atlas 3D atlas where the M2 area has been highlighted. M2 is the secondary motor area, which is an associative motor area wherein it takes inputs from the sensory areas and other cerebellar and other motor network areas and then feeds it into the M1 where the final processing

happens and then sends the impulses down the spinal cord. So, one of the studies that we are doing on Parkinsonism or epilepsy models and various other models, whichever model that you are choosing, you need to know which is the largest area that needs to be exposed in the craniotomy.

For example, if your area is on, I mean, if your study is on the barrel cortex in the somatosensory area, that is the area that you need to target. So, you need to know the extent of the area that needs to be exposed beforehand. For example, in this study, we are sensor the cortical surface array needs to be implanted in this M2 area. So, for that, we needed to expose that 8-millimeter area is our minimum exposure that is required. Over and above if I remove the skull then there would not be space for me to put the ground and reference electrodes, there would not be enough space for me to use the anchoring screws and stuff.

So, that is the limited exposure, and you need to know whether you really need to expose both sides of the skull that is very important to know beforehand because the midline sagittal suture that is the sagittal suture this is the lambdoid suture and that is the coronal suture, alright, and that is bregma and that is lambda. So, usually we do not expose the midline for a simple reason that there is a large sagittal sinus venous sinus that comes in and if it bleeds, it that is really detrimental to the survival of the rat. They will go in for a complication on as cortical venous thrombosis or sagittal sinus thrombosis or even injury can kill the rat instantaneously on the on your OT table. So, try and avoid this midline exposure as much as possible. I am not saying it is impossible it is possible to expose and we have exposed many times, but then it is prone to get injured because that part of the dura is adherent to the skull more than the rest of the area, alright.

So, you need to know are you going to do only a right-side craniotomy or the left-side craniotomy that is partly decided by the dominance of the rodent. So, when we talk about behavioral experiments we talk about something about shaping. So, shaping is basically to determine which is the dominant hand for the rat or mice that you are going to use. For example, if it grabs a foot pellet using its right hand then it is right dominant and there is a crisscross wiring from the brain to the spinal cord. So, the left hemisphere controls the right half of the body, the right hemisphere controls the left half of the body which this has been covered in the neuroanatomy session as well.

So, during your training session of the behavioral task, you will come to know which is the hand that you are going to use it, and then based on that, you do either left side craniotomy or the right side craniotomy or if your study requires a bilateral arrangement like in this case where we had to expose both sides of the skull we had planned to do the craniotomy which is called bifrontal craniotomy. So, frontal bone anterior to the coronal suture on both sides was planned to remove. So, that sort of idea and the exposure and the craniotomy plan have to be ready before you begin the procedure. So, and there may be

instances where you may be using both surface electrodes and the depth electrodes. So, when simultaneously you are using then both types of electrodes then it is very important that you have a very wide exposure of the skull because the number of ground and reference electrodes are going to go up and then of course, the area that is required for anchoring screws are also less.

So, it is very important that you pre-plan it in the sense as to where what is the entry point of your depth electrode. For example, on the right-hand side, you can see that these two are the depth electrodes entering the deeper subthalamic nucleus in the basal ganglia, and that is the surface electrode. So, if your study involves electrophysiology, then you need to keep in mind that there will be a lot of areas which will be used for the head stage, there will be areas for PCBs, and then there will be areas for ground and reference electrodes as I said earlier, and you need to plan the entire electrodes with the rigid fixation that is very, very important. So, that is the reason that you need to plan accordingly if you are using the depth penetrating electrodes; sometimes, you may be using the drug delivery cannula along with the cranial sensor. So, plan it accordingly.

These kinds of planning and the objectives need to be ready before you start the craniotomy procedure. Once the craniotomy is done, it is very, very difficult to undo the steps and go back and change your strategy, and that rat subject will be of waste, you know. So, we need to be really careful before you begin the procedure. So, having said that, do we really need to use the coordinates and I would suggest it is very important whether it is a microsurgical procedure like craniotomy or it is a stereotactic procedure like placing a burr hole; both instances I would definitely recommend using the stereotactic coordinates. For a simple reason, the area that is exposed needs to be in the center of your field; it is not enough that you do a craniotomy and the cortical surface target is one corner of the field, then the edge of the bone is going to hinder the various attachments that are going to come later.

For example, let us say if you want to insert a drug delivery cannula, alright, and you can see here let me use the pointer here. So, as I said earlier, this is the craniotomy area that you have chosen based on stereotactic coordinate then you can make sure that the drug delivery cannula is right in the center or if you are using an electrode, the surface electrode is right in the center. So, centering of your experimental tool, the ultimate tool that you are going to use, be it an electrode, be it a delivery cannula, be it an optogenetic stimulator electrode fiber optic cannula, n number of peripherals that you are going to use, you need to plan it in the center of your craniotomy, and that center is based on the coordinate. So, if we do not use the craniotomy coordinates and you generally do that sort of a craniotomy which goes way beyond and if you are, you know the end peripheral device that you are going to use if it comes in the edge of the craniotomy that is going to be a big hassle for fixations to come in and it is sort of damaging as well where the bleeding if it is from the edge of under the bone is very difficult to be arrested. So, I

always recommend that you plan the craniotomy such that the electrode or cannula or whatever end effector that you are planning to use will come in the center of the craniotomy field.

So, that is the reason why one would generally use the stereotactic coordinates, and once you determine the bregma and you know the coordinates, accordingly you develop the final target coordinate onto the skull. So, these stereotactic principles and planning the coordinate have all been covered in the previous session. Once you decide where to open up the skull, then the next very important step is the drilling. You need to remember that the skull is a thick structure, and underneath it, there is a jelly-like structure of the brain, and in between the skull and the brain, you all will know, you all remember from the previous session, there are n number of cortical veins and arteries. These are very thin, delicate blood vessels which are easy to get damaged, and before you even begin your experiment, either the rat will die from the direct injury or it is going to develop brain swelling, and then later in the recovery time, we might lose the rat.

So, unless until you master the usage of the drill on the skull of the rat, you are going to face a lot of problems with the experiments. So, I would say it is very, very important to practice on the cadaver and then take it forward. You need also to decide which type of drill you are going to use. On the left-hand side is the dental drill which is popular because of its smaller size, smaller handpiece, easy to handle, but then there is a disadvantage, it is difficult to mount it on the stereotactic adapter as seen on the right side. On the right, you can see that this coaxial system of drill has been mounted on the stereotactic apparatus, but then it is not that practical.

For example, you will not be able to see the skull which is in front of the adapter, but nevertheless for a beginner, when you need a little more training, it is always safer to use this sort of adapter and you know where you can use a micromanipulator which is in the stereotactic system, which can drive axially up and down without any extra movements, but then the biggest disadvantage is that you will not get haptic feedback. That is when the skull is through and it is entering, there is a sense of giving way. So, that sort of tactile feedback is very important to stop drilling. So, with practice, you will be able to do it with this as well, and then to use this particular system, you need to make sure that the head is fixed rigidly without any dorsal-ventral, that is up and down movement of the skull because if that is going to happen, then there is a sudden plunge when the bone is drilled and when the extradural space is reached. So, it is very important that either you use these adapters or use a freehand technique to drill. It is very important to know when the depth is reached or when the dura is reached. It is very important that you do not puncture the dura. Once the dura dural puncture is done with the drill, then it is understood that the brain is injured under the area of the dural puncture, all right.

So, these are two types of drills. But then, how do you decide the size? Generally, the diameter is pretty much standard; it is between 0.6 to 0.8 millimeter diameter. So, it is a micro drill, and these are the examples of how you make burr holes before any sort of surgery. It is very important to practice this particular step because these are important both for craniotomy as well as for stereotactic surgeries.

These are the holes for the anchoring screws, and if you all wonder why these screws are used, these screws are used to anchor the equipment or the interface device that is going to come on top of the skull of the rat. Be it a drug delivery cannula, so that will go through this bigger burr hole, or for that matter, even if it is a depth electrode that you are trying to pass into the brain substance. Once it goes in, you want it to stay within that area, and it is not enough to just put dental cement just around it because that is going to, the rat is going to pluck it out. So, in order to get a rigid fixation of any implant that you are going to do, we generally use these anchoring screws, and it is very important that you match the size of the screws with the drill bit. So, here I have taken an example of a 1-millimeter diameter screw, which is around 2 mm bigger than the drill bit per se, and that is important for it to have a good purchase on the skull.

When you screw it into the skull substance, once the hole is smaller, the extra 2-millimeter diameter of the screw will make sure that the fit is good. Now, that is called the purchase; it has threads on it. So, that will have a very good grip onto the skull, and it is very important to maintain a gap between the surface of the skull and those screws when you finally screw it in so that the dental cement occupies those spaces and the fixation is rigid. So, this will form one unit along with the implant that you are planning to put in. The idea of this anyway will be dealt with in detail in the next session where stereotactic surgeries will be discussed. The idea of this slide at this point of time is that when you choose a drill bit diameter and when you choose a screw, you need to make sure this drill bit size is a little smaller than the screw for a good purchase.

So, there will be a little bit of trial and error when you do not get an exact size which we all will go through, but then it is very important to make sure that once you put in the screws, there has to be a good purchase when you tighten it, okay, that is very important. Generally, this depth is based on the thickness of the skull, alright. So, it should not be too long that it will puncture the dura and injure the brain, neither too short that it will inadequately cover the thickness of the skull. So, the optimal size that we have come across is for the vista rat that we were doing, and we came across this particular size, and with this, you can always decide based on your experiment and other requirements. So, once you know about the drilling, the drill part, and the setup that is required for drilling, now you can, you are good to go for drilling.

So, that is the skull which is exposed completely. So, that is bregma. So, this is the parietal bone. So, here, they are doing a parietal craniotomy, and this can be used as a

cranial window as well. So, one needs to, there are different ways as to how the craniotomy can be performed, but the standard way of doing it is that it's better to go ahead with making a groove along the line of the craniotomy

If this is your requirement for craniotomy, you do not make a burr hole straight and then take a craniotomy and then do it. So, that is the standard procedure in humans wherein a burr hole is made and then there is a cutter which goes with a footplate that goes all around and cuts it. Because the skull is so thin, it is sensible to thin it out further with the drill and then separate it from the surrounding skull. So, as you can see here, this is the burr, all right, the drill burr that the tip of the drill bit is called burr and the edges, the sharp edges on it are called flutes, all right. One needs to know which way when it's rotating clockwise you need to use the drill, you know in the reverse manner.

So, that it does not slip forward and then injure the neighboring structure, that is very, very important to understand. And either you can go straight perpendicular down onto the skull or the preferred technique would be to use it in a slightly slant position where you can see the tip of the drill bit and how it comes in contact with the skull. So, with that in a circular manner, you generally thin it out and then look for the separation from the edge of the bone then only we are going to lift the skull bone away from the dura that is very, very important. So, with the video I can show you all how this particular step is performed, all right.

So, here is an example. So, this is a mouse's head that has been fixed rigidly, and you can see, but one step is that the eye closure has not been done, this is one of the online versions of the video which we had taken. The eye has to be covered completely so that it is not you know kept open like this because prone to injury and this is probably one of the acute studies where they are going to implant the drug delivery cannula. So, once so, instead of making an incision, there is always an option of you know excising the skin flap especially if it is an acute study and you really do not worry about closing it back the easier way is to excise it because if you make an incision you need to make sure that there are retractors to hold the skin flap back or you need to even take sutures to keep it separate. And what they are doing at this particular step is to remove the pericranium is very, very important and you roughen the skull.

So, that you know the skull is rough enough for to hold the drill bit in place and what has been what you are seeing here now is the groove making, you know using the drill bit. This is the dental drill where you can have a slant edge onto the top of the skull rather than having perpendicular and once the groove is made you check whether you can see that it is getting depressed that means the skull is separated, that part of the skull is separated from the remaining part of the skull and what is placed here is the artificial shear and then try to gently lift up. You can see that the edge of the bone that is separated and then with the Dumont micro forceps, you can lift up the skull. I mean in mice, in a

young mouse, the skull is going to be pretty thin. So, because of that you may be able to lift it up that way, but then it may not be that thin in the case of the wister rat or older rats wherein you need to sort of deepen this groove and make sure that it is completely separate from the edge of the bone and that is made sure by giving a firm pressure on the center of the craniotomy bone and see that if it is separate from the remaining part of the skull.

Once it is confirmed, then only are you going to lift it up, and it is very important in that process where you elevate the bone table not to injure the dura, all right. There are many instruments; a flat dissector is usually used to keep the dura intact before you lift up the skull. If the dura tears along with the skull, usually, you can see here there is this vessel there which can get injured, and if it starts bleeding, that part of the area which you actually wanted to study will get destroyed. It is called an ischemic infarct or stroke that can happen because of the iatrogenic that is something introduced by the physician or, for that matter, a person who is conducting the experiment. So, that would affect the entire experiment and the outcome.

So, it is very important that you maintain the dura, which is a thick tough layer, but then bound to get injured if you are not careful enough to protect it in the step of drilling. So, this is a very critical step, all right. So, once the craniotomy is done. So, next important a different technique that one needs to know is the creation of a stroke model, which is a little different from the usual craniotomy that you just witnessed. So, a surface craniotomy is slightly easier to handle because it is right on top, and the coordinates are easier, the position is standard, everything is standard.

Whereas, if you are trying to do anything below the temporal ridge of the rat, then the position is lateral, all right. So, all the position that you use for parietal craniotomy or frontal craniotomy, the rat is going to lie on its belly, and it is a pretty standard position; it is easier to handle. The minute you want to handle these kinds of arteries or if you want to expose the temporal lobe or the lateral surface of the brain, then the rat has to be placed in a lateral position. Here what you all can see is the middle cerebral artery occlusion model. There are various stroke models; one of the stroke models is that you inject an exotic drug into the area that you want to create an infarct.

An infarct, as I said earlier, is basically the part of the brain which is necrosed or dead in simple plain language; part of the brain will be dead because of the lack of blood supply which carries oxygen and glucose. So, that is ischemia, and that is an infarct. Ischemia is basically a loss of blood supply, infarct is the result of ischemia wherein part of the brain is dead, all right. So, what is been shown here is the middle cerebral artery. If you all remember from the neuroanatomy session, the middle cerebral artery comes from the base of the brain.

This is the top of the brain, all right, and these are the ventricles and the vasculature which goes from below upwards, and that is the middle cerebral artery, and it ramifies into different branches and then supplies all the cortical brain tissue. So, if you want to create a stroke, then it's best to coagulate using the bipolar quatric, not the monopolar as I said earlier; this has been discussed again. So, only the current will be between the two tips of the bipolar forceps. Whereas, if it is monopolar, there's usually a sink of an electrical field around it and it will injure or affect the neighboring vessel as well. That is why it is preferred always to use the bipolar forceps to coagulate.

So, how do we expose this? It is pretty tricky because at the base of the skull, when you expose that, you are very close to the area of the eye and the ear. So, there are certain landmarks that we need to follow, and what is very close to this artery is the inferior cerebral vein. So, let us see how we can do this part, use this particular model, and try to cover this model mainly because the craniotomy that we just discussed is not going to be useful for this particular model, and it is pretty different. So, as I said, the rat has to lie in the lateral position, all right, and the area that we are going to use is between the auricle and the eye. So, the rat is lying on its lateral side with the side up; for example, this is the left side of the rat because the rat is right-dominant, and you want to induce the infarct and create a stroke model in the left hemisphere, all right.

So, what is known as temporal craniotomy? This temporal craniotomy is pretty different from the parietal or frontal craniotomy we just discussed. So, the incision for the temporal craniotomy is the midpoint between the eye and the auricle, all right. So, that is the area that you are going to make an incision. So, we need to remove the hairs and prepare the area and drape that particular area as we just discussed in the previous session. So, that is the area of the bone from which the flattened surface of the skull, which is the top of the skull, is medial, and below that is the temporal bone.

So, there is a thick ridge on the edge of the top surface of the skull, which is called linear temporalis, all right. So, the entire craniotomy is going to be below the linear temporalis, and you can feel another bony ridge called the zygomatic arch, which is closer to the maxilla or the upper jaw of the rat. So, between these two bones is where we are going to create a cranial window or make an opening. So, you make a vertical incision like that. So, the rat has been draped, you can see the plastic thin sheet over the auricle, and the eye has been stitched here.

As I said, as your operative field is closer to the eye, it is always wiser either to stitch it or at least protect it with the eye ointment and keep a wet moist cotton on top of it so that it does not injure the cornea, which is pretty sensitive. So, in this scenario, we have stitched the eyelids so that the operative procedure does not interfere with the eye. So, once the incision is made, you will expose what is known as the temporalis muscle.

So, that is the biggest difference between top of the skull versus temporal craniotomy, that is, parietal craniotomy versus temporal craniotomy, that you come across a very thick muscle soon after the skin incision. So, we need to split that muscle to expose the temporal bone, all right. Here, the muscle has been split, and you will come across, in depth, what is known as the coronoid process, okay. That is the zygomatic arch, and that is the linear temporalis, medial to that or closer to the midline away from the linear temporalis is the flat surface of the skull. So, that is your upper limit, linear temporalis upper limit, and zygomatic arch is the lower limit.

Once you split the muscle, now what you see is your craniectomy edges. The word craniectomy is used instead of craniotomy because you are not going to replace the skull flap here, whereas, in parietal craniotomy, once you make a window, for example, you are cutting a surface electrode, you can replace the bone flap, then the word craniotomy will be used; if you are removing the bone permanently, then the word ectomy, ectomy is cutting off. So, craniectomy terminology will be used. Anyway, in any terms, you are basically trying to expose the area of the brain below that particular area, all right. So, posteriorly, that is backward, that is there is an oracle in front, you have the eye lower down, the zygomatic arch, and upper limit is linear temporalis.

You need to drill in this particular area. Once the drill is done and the bone is removed in a similar fashion that we discussed earlier, then you will expose the dura there. Once you expose the dura, if you remember the first picture I had shown, the middle cerebral artery comes from the base. So, as you know that this is the lower surface, lower part of the animal that is towards the belly, this is towards the head, all right. It comes from the base of the skull, that is the middle cerebral artery, and there is another landmark which is a little difficult to expose and if you have that, that is pretty good, olfactory tract, that is the pathway carrying the olfaction. So, that is the surface landmark once you expose this area to identify the middle cerebral artery nonetheless, it is a very prominent vessel difficult to miss it if you are following other landmarks correctly.

Once the middle cerebral artery is exposed, you need to differentiate it from the vein that I just discussed, the inferior cerebral vein that should not be injured or by mistake occluded, thinking that is an MCA. The ICV or the vein is perpendicular to the middle cerebral artery which comes from the base of the skull. So, once you recognize this skull, now you are good to expose the actual artery and the brain by removing the dura. If you remember from the previous slide, you can see a thin hazy layer on it that has been removed here, dura has been opened and the vessels are exposed.

So, that is a vein, that is the artery. In humans, it is pretty easy to differentiate between vein and artery, but then in rodents though it is a very small animal, it is difficult to differentiate between by the color of it, all right. So, that is the MCA vessel, and that has been coagulated using the bipolar forceps, and it turns black to brown. So, that is the

classical MCA occlusion using bipolar coagulation once the middle sublateral is exposed by doing a temporal craniotomy. This is a very important difference with the usual craniotomy, and I am sure this craniotomy can be used for many other approaches to the various brain structures, all right.

So, this is a temporal craniotomy. Once that is done, you need to once the coagulation is done, you will be transacting it again to make sure that there is a complete division of the middle cerebral artery, alright, and then the surgical wound is closed either by stitches or by staples, and then the eye suture is opened, and you will see that and that is intact, alright. Another major additional surgical procedure that accompanies the creation of a stroke model is the ligation of the carotid and middle cerebral artery. It is always combined to achieve a good stroke model. So, what I am trying to say is that it is not enough just to expose the middle cerebral artery and occlude it with bipolar forceps. What we are showing here is the neck dissection. You make a midline incision from the lower jaw to the sternum.

So, this is in the supine position, alright. So, ideally, this is done first before we actually do the craniotomy. That is the supine position where you can see the skin has been opened up widely and then put a stage sutures to keep the structures in view. This also has a significant learning curve which can be mastered by doing a carotid section. What we are doing is after exposing the cervical glands which come superficially, you go deeper right next to the trachea. What we are seeing in the middle is the windpipe next to that is the carotid artery. One side it is occluded and one side it is ligated permanently. So, one side there is a temporary occlusion wherein you take a loop of the silk suture and then tie it down, but do not clamp it completely and then close it for the later half of the surgery and then close the wound. You will be revisiting this once the middle cerebral artery is occluded. This one side of the artery is required to make sure that the general blood supply of the brain is maintained. It is impossible that you take off both the carotid because that will cause bilateral infarcts which are detrimental to the survival of the rat.

So, one side you permanently occlude it, and the other side you temporarily occlude it for around 40 to 45 minutes, by which time you do the middle cerebral artery occlusion by doing a temporal craniotomy. So, this combined carotid and middle cerebral artery occlusion is very important to do so that the collateral supply, let us say if you have done the occlusion only for the middle cerebral artery, then the other side of the carotid artery sort of comes and compensates for the loss due to the middle cerebral artery occlusion. So, that is the reason why you also do an ipsilateral carotid artery occlusion along with the middle cerebral artery. This is one extra surgical procedure that you need to practice if you are considering the stroke model which is widely used rat model for various experiments.

Briefly, I will cover the cranial and spinal windows because anyway we are discussing the craniotomy. Here, the only extra few steps that are needed are to fix the cranial window apparatus or the setup to use it for your various other experiments.

So, generally, this is used for two-photon microscopy, which is coming in a big way now. Then, many experiments are used using this particular technique of optogenetic stimulation or, for that matter, two-photon microscopy where you can watch the live brain in action. That is the beauty of this two-photon microscopy where you can actually see the neurons in action, where the fluorescence lights up with the action potential. So, in order to see that, you will use what is known as two-photon microscopy, and for the microscopy to see through the different layers, you need to have the brain being exposed and maintained, alright.

So, here, there are various examples shown. One is the cerebral cortex window. It only depends on the various stereotactic coordinates and the location of the area of interest for study. If you want to study the cerebellum, you are creating a cerebellum window by making an opening in the occipital bone, and this is the frontal cerebral window, and that is the spinal cord chamber. If you want to study the spinal cord, it is a similar technique, but then different in terms of the removal of the laminar plate which we will be discussing in the next few sessions, and then you have the olfactory bulb window. So, there are various windows to peer through the central nervous system in action. It is very important to know that these are also available as an option if you are trying to do rodent experiments.

These are the various steps wherein the cranial windows are created. So, as you can see, once the craniotomy is performed, it is filled with artificial CSF. It is very important that the CSF is not leaked out because you are planning to use the cover glass on top of it. You cannot have the cover glass directly on top of the brain because it needs a media to see through, and then you need to fix it to the edge of the skull using dental cement or bone cement. So, those are the extra few steps needed to create a cranial window, and what you can do is have a sort of protective plug on top of it where you can thread it, remove it, and you can reuse it, or you can have a PDMS layer through which another sampling of the brain can be done or neurophysiological recordings can be done. So, there are various options for cranial windows: either you can have a cover glass with the artificial CSF, or you can have a protective plug through which the microscope can be used, or you can have a PDMS covering through which the brain can be sampled again and again, either to deliver the drug or to record the neural signals.

The idea is that the brain is exposed, and the rat can be allowed to recover from this stage onwards, and the brain is exposed all the time, either you want to use the microscope or you want to deliver certain drugs; the brain is available, alright. So, it is very important that the superior sagittal sinus is left intact whenever you create the

windows here, and that is pretty important. So, that is the superior sagittal sinus that I was talking about. So, this particular sinus, if it is injured during the cranial window creation, that is very detrimental to the life of the rat. So, these are some of the live examples; you can see that once the window is created, the microscope is brought into action to see various activities. And this is two-photon microscopy where the fluorescent dye has been used, and along the z-axis, it runs, and then different layers of the rodent brain can be visualized, and then there is a readout of the calcium signaling that can happen, and you can even see the fluorescent activity in the areas of the brain which are active.

So, the rat is fixed, and there is a cranial stabilization system where the microscope can be used as the rat is performing various activities; even that option is available. So, that is the advantage. And as I said, if you have a PDMS layer, you can even send a pipette through it and deliver the vector viral agents or deliver any sort of drugs, toxic agents, or you can even fix up a cranial window setup which can be used at a later stage once a rat recovers. So, these are the various modifications that can be used, and this is just to cover the topic which you can also create various spinal windows similarly, but one needs to design this particular plate that needs to be fixed on the bony structure. You can see the screws there, and then you will pour the dental cement on the side. So, this sort of metal frame is required to keep the cranial windows as well as spinal windows if your study requires the repeated sampling or repeated delivery of the drug or anything that requires a chronic extension of the study.

With that, we will cover today's session. So, to summarize, we have covered the craniotomy, the basic steps involved in the craniotomy, and as I said, it is very difficult to cover all the practical steps that are involved. I have tried to demonstrate and explain the steps and principles that are required, but whatever is said and done, unless you all practice these particular steps on the rodent cadavers, it is difficult to master those steps. So, please go through this part of the session again and again and understand all the principles, read up a little bit more to understand the various modifications and nuances of creating a cranial or spinal window, and then master the various craniotomy steps as well. As I said earlier, you will have to modify all these steps as per the requirement, but the basic step of drilling, using the anchoring screws, using the dental cement, all these are going to be more or less similar, alright.

In the next session, we will try to cover all the stereotactic implantation steps. Now, implantation of drug delivery cannula, implantation of electrodes, but all of those procedures will have one common procedure which needs to be followed, and then it is only the implant that is going to be different, and the location is going to be different, but the general principle behind all these procedures is going to be more or less the same. So, let us try to cover the stereotactic implantation steps in the next session. Thank you.