Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Hello everyone. So, in today's session, we will continue the spinal microneurosurgery that we discussed in the last session. So, in the last session, we went through all the spinal stabilization setups and the instruments that are required for the exposure of the vertebra and the spinal cord. We also covered the major portion of cervical vertebrae exposure how to do the laminectomy and how to expose the cord and the dorsal root ganglion. So, in today's session, we will go through the remaining aspects of thoracic vertebrae exposure and thoracolumbar vertebrae exposure after which briefly we will deal with the implantation strategies that are commonly used. So, having said that the basic steps remain the same you know the layers of exposure and the sequence of exposure almost remain the same.

Some nuances keep adding as per the strategy and these are the same nuances I keep emphasizing that you will sort of adopt to improvise your methodology based on the research question and the research objective that you are planning to achieve. So, with that introduction let us look at what we are trying to do today. So, cervical vertebrae exposure we completed we could not finish the thoracic vertebrae exposure that we will be covering today. And then the spinal cord injury model which is the most common model that is used so you all should have some brief idea of all the research that is commonly employed with the spinal cord.

Then bit about microelectrode implantation and the strategies used. Then as I covered in the brain surgery a little bit about the spinal windows alright. So, let us look at the thoracic vertebrae exposure. So, on the left-hand side, this is before you bring in the stabilization system you can make the skin incision and then try to expose the spinal column that comes right in the middle. So, this picture also tells you what sort of layers that would come.

The draping and I mean the painting and cleaning and all that has been done somehow this picture does not include the draping, but draping is always a good practice to include. Then on the right-hand side, you can see that there is a T-10 level exposure that has been done but before that, they have tried to dissect the muscle and then try to get the exposure of the spinal column as per se. So, once the spinal column comes into view then you can bring in this spinal stabilization setup which we discussed last time. This is more of a

mechanical stabilization with the mechanical impounder which has around a 10-gram weight which is a metal rod with a blunt tip to induce the spinal cord injury. And these are the fixation forceps which as I said earlier will be used to hold the spinous process and then you turn in this you know these screws and so, tighten them.

So, that it grips the spinous process. So, again as I said earlier you need to sort of figure out what sort of impact is going to happen and how much of movement is going to happen with the actual surgical setup and then obviously the target and the objective that you are planning to employ. And if it is something where if it is a surgery that does not allow any sort of movement to happen for example, you are handling the implantation of the spinal cord then this setup might not suffice because when a vertical movement and the vertical force is acting on the spinal cord concerning any of the surgical steps then it is better to fix the transverse process which gives us the better rigid fixation. As I explained earlier when we compared various technique of various techniques of spinal cord stabilization during the surgery, the retractors or the stabilization system going under the transverse process gives the best rigid fixation. But for the impact, if it is on the thoracic cord where there is a hump naturally occurring and the spinal column is naturally convex on the top unlike the cervical section there is a natural hump that is visible and palpable.

So, this sort of fixation probably would suffice most of the time. And then so, once you bring in the stabilization system where the upper spinous process, and lower spinous process both have been fixed and then what you can see here is another retraction system. This is the retraction system wherein when you twist and they keep tightening it these prongs that are seen here would sort of retract the skin and muscle tissue away from the midline you know. So, it keeps pulling it out. So, that is the retractor called self you know retraction.

So, SR or automated self retractor is a very useful self-retaining retractor wherein you screw in and it retains the tissue retains and you do not have to spend too much time in taking a stitch and putting a fixation. So, such self-retaining retractors are very useful at this particular point. And then you bring in the impactor which will give a dorsal force right onto the spinal cord once the vertebra is exposed all right. So, that is how the impaction is done. You can see this is a zoomed-in view wherein you fixate the spinal process using those rigid fixators which are forceps holding the spinous process on both sides.

Then there are the retractor blades where it is holding the skin and muscle tissue which is self-retaining. And then the tip of the impounder is closer to the cord after you expose the cord. On the right-hand side, you can see the zoomed-in view where the spinal cord is

exposed and the dura is seen. This is after the impaction by the impounder there where the tip has moved already and caused an impact and contusion is seen. This bluish discoloration is the contusion which is a terminology used generally, a contusion of the cord.

So, which is very close to what happens when somebody meets with a road traffic accident this is what happens. So, we are trying to simulate using this artificial impaction or using this impounder and creating a contusion in the spinal cord. Then the various subjects of what changes happen in the spinal cord, what sort of cellular changes are happening, what sort of biochemical markers can be studied, how this degeneration happens following the trauma to the spinal cord all those can be studied after this basic step has been achieved. So, once the spinous process is exposed just similar to the cervical vertebrae exposure that we discussed last time, you process you proceed with laminectomy which is again covered and then the dura and the spinal cord are exposed bringing in the impounder and causing the impaction. That is the end of the thoracic spinal cord impaction a model where the entire surgical step has been shown.

So, the other method is that this again can simulate the natural injury that you know happens in many of the road traffic accident victims where in you the direct bony injury. So, on the right-hand side, you can see especially here the right half of the lamina all right. So, just to orient yourself this is the lamina that is the spinous process. If you all recollect from the previous anatomy class that will be the rib and you can see the artificial impaction that is been done which is pretty similar to what happens naturally in an accident where there is a fracture of the spinous process and the lamina especially the right half of the lamina. So, if you take an axial cut this is an imaging this is a CT imaging which is not the luxury that most of us can have it is a preclinical imaging it is called preclinical imaging.

Clinical imaging is what we use in patients MRI CT any sort of imaging whenever an animal model employs an imaging process is called preclinical imaging. So, this is a preclinical imaging of the rodent where the CT scan has been employed. This is the 3D remodelling after you acquire the CT scan this entire thing is 3D modelling. This is the actual bone window of the CT scan wherein the impact has been created artificially directly onto the spinal column there before you expose the muscle that is another model that we can use wherein you do not have to do laminectomy you open up the spine all that need not be done. Suppose the spinal column after you make an incision with skin and subcutaneous tissue and then these stabilizers are used wherein the spinal column is stabilized by you know by a medial force.

There is a fixator that will tighten these screws here and then make sure that the medial

force has been applied and which should be equal on both sides. So, that it does not induce any sort of curvature in the spine dead neutral positioning is maintained before you bring in the impounder which causes the fracture and then presses the cord which is seen here on the right-hand side. Suppose if there is a cord this is how the cord will be all right. So, when the impaction happens there is a fracture. So, what you are seeing here is the fracture and the fracture fragment will cause again the impact on the spinal cord.

So, both the bony injury as well as the cord injury can happen. So, this is a compound injury model wherein there is a fracture of the bone bony canal as well as the spinal cord all right. So, this is another spinal cord injury model with similar methods of surgical exposure or thoracic vertebrae exposure all right. So, now comes the thoracolumbar vertebra wherein so, this is the cervical vertebra till there is the around thoracic vertebra from here it becomes the thoracolumbar and then the sacrum and the coccygeal and the tail bone is how the spinal column that we have seen so far. This is something similar to the thoracic vertebrae exposure.

So, just to orient yourself, this is the skin after you clip the hair or shave the hair off. Then let us look at how the micro LED implantation happens. So, this is another important methodology that is commonly employed now, wherein you inject the virus adenovirus and then which is tagged to a fluorescent dye and then make the nervous tissue express those fluorescent proteins which with the wavelength of the laser light the responses are seen all right. So, it stimulates something like an optogenetic stimulation. So, this is the employment of optogenetic stimulation in the spinal model.

So, this is the LED implantation to bring about such a methodology. So, the process is going to be the same for any implant for that matter, but then all you need to decide is what implant you are going to use and which layer are you going to use. So, as I said earlier in the brain surgery you need to sort of figure out which layer you are going to expose where the implant is going to be fixed and how are you going to maintain that implant for a chronic study all right? If it is an acute study then you do not have to worry about closure, a fixation and then survival of the ride all that does not matter if it is just an acute study. But if it is a chronic implant study where you need to acquire the data as the animal behaves in various behavioral setups and experiments then this surgical setup matters a lot you know the entire survival of your equipment as well as the rodent depends on these surgical steps.

So, that is why that is the reason why I keep saying that you need to plan it you know every minute and till from the opening to closure and simulation of all your surgical steps are important and it is better if you can have a dry run using a cadaver you know. So, go through all the steps to see if the implant fits in because there are a lot of improvisation

will be required. So, you do not have to waste your allotted animal subjects just to cover up your learning curve. So, a cadaver is the best way to circumvent that. So, these basic surgical steps will keep getting repeated but try to focus on the step which is a little different based on the experimental methodology that I am trying to explain all right.

So, on the left-hand side, you make the skin incision to expose the muscular plane and the spinal column. On the right-hand side, this sort of exposure we have already discussed, where once the skin and subperiosteal tissue are open and then retracted laterally. Here is when you can use the self-retaining retractors, as I said earlier. Those are things with the screw at the top, whereas you twist it, this will keep opening up—that is all it is, which is called self-retaining retractors. All right. So, once you bring in that, it keeps maintaining the layers that you are exposing; that is the whole point. When you really do not have to waste time by taking stitches in every layer and then putting it down to the side, which not only increases the surgical time but also increases the anaesthesia time. Of course, your surgical field is also going to be bigger and bigger as you go deeper.

So, once you do that, then you expose the spinal process. What has been exposed here is the spinous process. So, these steps we have already gone through in the surgical step that we were discussing for laminectomy. So, the spinous process has been exposed. Once the spinous process and the vertebra have been exposed by dissecting the muscular tissue all around, then you are ready to make a laminectomy. As I said, a partial laminectomy has been done. What you are seeing in the midline is your dorsal spinal vein, not really an artery; the dorsal spinal vein. Then you can see the dura covering the spinal cord.

So, once that step is achieved, then the next few steps depend on what sort of implant you are planning to use. Are you planning to inject it into the spinal cord? Then you have to sort of expose the actual cord by incising the dura and exposing the spinal cord. Or if you are happy enough to put your implant outside the dura, then it is called an epidural. These are the terminologies usually used in various implants that you are going to do wherein there is a dura mater. This is a sagittal section; which is the dural covering. If your implant is going to go outside, then it is called an extradural; if it is going to go under the dura but over the spinal cord, then it is called a subdural.

So, you have an extradural implant, you have a subdural implant; something inside the dural sac is intra-dural, in which case you need to decide where you are going to leave it on the spinal cord, and then it is called extra-axial. If you are going to implant within the substance of the spinal cord, it is called intra-axial. These are the various terminologies that are surgical and very important to document and communicate. Of course, you will learn this many, you know, you are supposed to go into the details of these surgical steps which are available in the literature. But to cover briefly, this is how it is done. So, once

the dura is exposed, all you need to do is slide in the implant under the spinous process because there is a potential space between the spinous process and the dura, which is called the epidural space. Epi or extra can be interchanged. So, there is an extra dural space that will have fat, and this fat will have veins which are called venous plexus.

No tissues in the body are completely devoid of vessels, understood? So, everywhere, every step you need to be conscious about what sort of vascular injury you are going to do to the rat and then be aware of those planes and those structures that you are going to handle. Then the surgical morbidity comes down drastically. So, as I said, this is a dural sac; that is the spinal cord here. You are going to leave the implant outside the dura, which is extradural. So, you do not have to do a complete laminectomy or make a wide exposure of the spinal sac. You can just make a smaller opening and then pass a dissector, an instrument called a dissector, you pass under the bony space make some space, and then slide such implants underneath it.

So, that would suffice a lot. Here, in case this is the LED; this was the LED. So, it was supposed to be implanted intramurally. Extra durable, this is a coiled copper wire that works with induction, which is a sort of auto-generative model implant. I am not going into the details of this implant, but it has its characteristics. This is a battery-less implant. It does not have a battery; it works with an induction principle. But this tip of this thing has an LED, you know, light-emitting diode, which has to go under the dura and over the spinal cord.

So, they make a small nick, and when you do a small nick, there is an egress of CSF. That is how you know for sure that you have cut the dura or zoom in with your microscope and see the cut of the dura. But it is important to let the CSF out. So, that the spinal cord and the spinal column become relaxed and then you slide in the implant so that the friction does not come into the picture where the electrode grazes over the spinal cord and causes injury by itself. So, that is how the final implant is going to look like. So, if you remember, you have been exposed and these are the muscles.

This is the bony edge where you have removed the lamina, and from the previous slide, this is the upper part of the coil. The tip that is a light-emitting diode has actually gone inside the dura, and it is lying like that, and then you put the skin on top of it. The major advantage of the rodent model is that you have a lot of loose skin to house all these sorts of implants. All right, that is the big benefit. But of course, your wound should be smaller, and then it should be friendly enough to these biological tissues and planes. And what has been shown here are the surgical clips. Nowadays, things are smooth and easy and you do not have to sit and suture. It is always good to practice suturing and wound closure, but clips will do the same job much more easily.

Another one is Derma Bond, where you apply the gel and then stick the plasters across. Again, that also will work. But then all you have to make sure is that if this is the cut edge of the skin, you have to make sure that there is a good approximation of the raw edges. If the wound overlaps with the skin edges, then that sort of wound gives and there will be a lot of healing issues. Then it gets infected with bacteria, and your implant will fail. All right. So, it is very important whether you use sutures or clips or Derma Bond or whatever material, you need to make sure this approximation of wound edges is perfect.

Then the healing will take place. All right. So, this is a similar experimental methodology, but what you are seeing here is multi-level micro LED implantation. The reason is not to emphasize the LED part of it but to know how smooth the epidural spaces are and to what extent you really can slide these implants. And if you have a chance to do imaging, you know, an X-ray or, you know, under fluorescence, where we call usually the CM fluorescence, where there is a transmitter and then a receiver connected with an arc. We have that; then it is amazing. But even if you do not, you really can use tactile feedback and see where the implant can be slid across the entire length of the epidural space. For example, if this is a T13, they have slid the implant almost up to, you know, T13, T12, T11, T10. Until T10, they have slid the electrode. But the good thing is that you can sort of have an additional exposure here at T10 and use the loop to pull it across.

So, all these sorts of surgical steps are possible if you are in the right plane. That is very, very important. For example, if you are in the epidural space, you will get this much freedom, whereas, if you are inside the dura, then you have to be very careful to avoid injury to these rootlets. Then there are veins coming in and arteries coming in. So, you need to make sure that you are in the right plane. One way to look at it is that if no CSF is coming out, if there is no CSF egress, which is inside the dura, then you are safe. You need to make sure this dura is intact. At any cost, there should not be any injury to the dura and CSF leak should never happen. Only then can you make sure that there is no injury to the cord as well.

If by chance during a laminectomy the dura opens, then you will need to increase the bony exposure wide enough to see the normal dura, and then slide the implant on top of it. These are some of the technical nuances that you will eventually learn during the process, but just to complete the picture, I am trying to show this particular aspect. The next aspect is the spinal window. We did learn about the cranial window; the process is almost similar to the cranial windows, only the bone fixation is going to be different compared to the cranial windows. The most important aspect is that if you are using such rigid mechanical fixators rather than just the disc, there are many modifications available to maintain the spinal window, one of which is this metal rigid fixation.

It depends on how long you are going to maintain the spinal window and what accessories you are going to use along with this spinal window. That is what is going to decide whether you need such mechanical rigid fixators or if it is enough to keep a small loop and then put glue all around so that it maintains the skin away from exposure to skin and muscles. All that is done here is after the skin incision is made, the muscle is retracted, and the laminectomy is done. Then that window is maintained by this. Here, you have to remove the entire layer. The skin can be retracted, but the muscle plane has to be removed and then the bone laminectomy has to be done. Then your dura or the spinal cord itself can be used.

Preferably, the dura will be maintained most of the time unless it involves some spinal cord injections. Once that is done, these fixators have to be screwed into the lateral bony edges, which are mostly the facet joints. Either you use the facet or the transverse process. If you remember the bony anatomy, these are the bony structures wherein you can screw in these fixators over which the metal window will be fixed. That is the rat where the spinal window has been fixed. Most of the time, these kinds of windows are used for two-photon microscopy.

We have already discussed this. In a live rat, it is amazing to see the brain in action. This is one way where you can look at the live brain in action when you use two-photon microscopy, where the rat is alive, most probably with injectable anaesthetics. The same thing can be done with inhalational anaesthetics as well, which we will discuss in the anaesthesia section. This is another way of fixating the spinal window. As I said, there are retractors used, then the dura has been opened, and then artificial dura is used, mainly to be thinner and transparent enough to allow microscopy. This is custom-made; you can even 3D print such windows to customize them for your experiments. This is just another way of maintaining the spinal window.

This is how two-photon microscopy generally happens. You expose the spinal canal, maintain those tissues all around which are opened, and then maintain it with either retractors and glue or metal frames, as discussed. Then bring the whole rat under the two-photon microscope. The principle is that femtosecond lasers are used to send laser impulses within a very short time, wherein specific layers are focused, and then you can see the nerve cells illuminated with various fluorescent tag markers. That is the general principle of two-photon microscopy, and this is how the entire surgical setup would look in spinal two-photon microscopy. There are many exciting spinal column and spinal cord research projects happening, and it all depends on what questions you are trying to address.

Another important experiment is spinal cord injections. Whether it be stem cell or adenovirus injections, these are used to label any part of the cord segment. The surgical

principle remains the same. You either inject it blindly into the spinal cord, which is not advisable, or perform a very superficial exposure where the skin and muscles are retracted, the spinal column is stabilized using these fixators, and then partly the muscle is opened up to find what is known as the interlaminar space. Here, they have shown a cross mark indicating the interlaminar space. You can avoid doing the laminectomy, which is time-consuming and can injure the dura.

For implants where you want to access the epidural space and need to create a spinal window, extensive dissection and laminectomy are required. But if you can avoid laminectomy, that is ideal. For injections and microdialysis to collect the CSF, you can use the interlaminar space shaded in blue on the right side. This space can be accessed using a microscope to visualize the interlaminar space. The depth is only around 250 micrometres. The coordinates used are 500 micrometres from the midline and 250 micrometres depth to reach the target.

They have used CT imaging to inject without doing a laminectomy. This is for intraspinal cord injections where preclinical imaging is used to get an idea of the size of the interlaminar spaces. There is a significant learning curve where you may need to perform trial and error to access the space, feel the loss of resistance, and walk down or up the lamina. These steps involve feeling the lamina and space where there is no resistance, entering, and aspirating. When you see CSF coming into your syringe, you know that you are inside the dura. If you remember the steps discussed last time in a spinal cord, let me draw it neatly so you can understand. For example, if this is the spinal column, you will have a spinous process like that.

So, you try to pass the needle like that and you will hit the lamina first, which is the bone, and then you walk along the lamina until you obtain the free space in between the lamina, which is shaded in blue. You will feel the loss of resistance and then you poke in. When you feel that second loss of resistance, you aspirate and see if you can aspirate cerebrospinal fluid, which is present between the dura and the spinal cord. This is the spinal cord, and I think I will have another slide where, yes, exactly. If you are not doing the injection and want to aspirate, this is one way of collecting the CSF. This is exactly what I was trying to explain: that is the dura, and this is the spinal brain substance—in this case, it is cisterna magna.

Cisterna magna is the dilated CSF space. This blue area is entirely CSF. Wherever the CSF space is dilated, it is called a cistern, and these cisterns around the brain have names such as cisterna magna, cisterna ambiens, sclerulis, and many others. One such space that is larger and easy to access is the cisterna magna. "Magna" obviously means large cistern, which is available in the dorsal aspect of the rat, between the cerebellum and the spinal cord, at the junction known as the cervical medullary junction.

When you go through the spinous process, as I was saying, you will feel the second loss of resistance in your aspiration of CSF. Then you can either inject into the CSF space, which is called an intrathecal injection, or subarachnoid space. If you remember the covering of the central nervous system, there is the dura mater—this will give you a brief recap—arachnoid mater, and then the pia mater. This is the second layer which contains CSF in its space, and this is the pia mater, which adheres tightly to the actual neural substance. When your needle passes through the dura and hits the arachnoid, you are in the fluid, and this space is very narrow and thin.

You need to be careful to feel the loss of resistance, which is entirely tactile feedback, and then you aspirate and collect the CSF as seen in this. CSF collection or microdialysis is another minimally invasive way of examining the central nervous system. If your study involves the collection of these biological fluids, this is one way of doing it. You need to know the anatomy inside out if you are trying to access these kinds of spaces. If it is the spinal cord and spinal canal, it is called a lumbar puncture. It is a very important diagnostic tool in patients with various infectious diseases, like meningitis, or, for example, even if there is a malignancy involving cancer patients. We need to collect the CSF for studies, which will have biochemical markers as well as cellular markers for the pathology you are dealing with.

This is one way of collecting the CSF, which is very important to understand. So, with that, we will conclude today's session. Most of the basic procedures have been covered. As I keep reiterating, the same fact applies: according to your objectives and research question, you need to tailor these basic surgical steps, improvise them, and then adopt them for your research methodology. Hopefully, these methodologies should be enough to start, and with the various literature on surgery and anatomy, you need to combine them and make the best use of them.

Thank you all. In the next session, we will be dealing with the anaesthesia part, and then there will be some animal handling sessions. Most of the surgical sessions are covered in the earlier lectures. Thank you all.