Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Hello everyone. So, after we covered all the relevant details in spinal neuroanatomy and spinal cord anatomy, today we can move on to the actual surgical steps involved in spinal cord exposure. Today, I will walk you through the major setup required to perform spinal surgeries because it is very important as it is quite different from the cranial surgery setup. More or less, at least for cervical vertebrae exposure, you can use the stereotactic setup that is useful for brain surgery, but for the thoracic vertebra and lumbar vertebrae, especially if it involves spinal cord injury models, the setup is quite different, and the exposure and handling of the spinal cord and vertebrae differ from cranial neurosurgical approaches. So, with that, this is the outline we plan to have. I will walk you through the instrument stabilization system and the retractors because they form a very important aspect of the entire spinal surgery.

As I said earlier, we are dealing with a very focal part of the spinal cord, and we are trying to expose a small segment of it to create injury, inject viral vectors, administer drugs, or even drain the CSF and perform microdialysis for whatever spinal neurosurgical procedure you are planning. The stabilization system and retractors are very important to ensure the spinal cord is not injured during the surgery, similar to cranial surgery. So, whenever dealing with the neural system, stabilization systems and retractors are very, very important, unlike other areas of the rodent body. Once we deal with the stabilization system and retractors, we will go through the different sections of the spinal skeletal system, including cervical vertebrae and thoracic vertebrae exposure.

As I said, it is different, and then we will look at the creation of spinal windows, similar to the cranial windows we discussed, and briefly discuss the spinal cord injury model, which is one of the most common models studied. Then, briefly, I will take you through microelectrode implantation as well, and those steps are pretty common for other strategies with slight improvisation. So, the first half of the session will cover the setup itself. The first part is spinal surgery instruments. If you look at the instruments involved in spinal surgery, they are slightly larger, and you have more bone instruments than in cranial surgeries. Here, from top to bottom, we have listed a steel clip, a plier, and a mosquito clamp, but if you want to go according to the exposure, then the first thing that would come is the knife—there is the handle with the blade—and then comes the dissecting scissors, followed by toothed forceps and non-toothed forceps, and then the bone rongeurs.

Once the dissection is made in the muscle, you will be performing the laminectomy using these bone rongeurs. You can see how heavy this instrument is, but the tip of the instrument is quite fine, rigid, and sharp. However, if you are not using the drill—even though drills can be used to perform the laminectomy, it is much safer to use the drill, the only thing is there is a learning curve involved in drilling for spinal surgeries, which is quite different from cranial surgery drills. So, if you are well-versed and good at using drills, that is the best instrument for laminectomy.

If, however, laminectomy—I mean if drilling is difficult, or if you do not have the drill setup—then only would I recommend using these rongeurs to remove the lamina or the spinous process. The spinous process is fine because you still have the lamina intact, covering the spinal cord. When you use this instrument the entire spinal system will move along with your force to remove the bone. That is exactly why we need a stabilization setup, where the spine is fixed at some point so that there is counter traction on the rodent body when you remove a segment of the spinal canal. Then, of course, instruments like micro scissors and forceps will be used, and once the surgery is over, you will use stainless steel clips to close the wound, which are similar.

So, the most important part is the spinal stabilization system. Here, I am using the stabilization system, not the stereotactic system. You do not need a stereotactic system if you know which level you are targeting, and you are making a pretty large incision, exposing the whole length of the thoracic or lumbar vertebra. You do not need a stereotactic system. It is not that commonly used; the stereotactic atlases are not that commonly used, and for a simple reason, the cord varies a lot, and those coordinates are going to be arbitrary because of the shift and the movement—the maintenance of the stereotactic position is very, very difficult compared to cranial surgeries. Of course, the atlases are available even for stereotactic surgeries of the spinal cord, but they are not used as frequently as in cranial surgery because of the similarities between the cord segments. Even if you are one segment higher or lower, you can still manage unless your experimental outcome is that rigid and you need specific segments involved.

Only then does accuracy matter that much in spinal cord surgeries. So, what you are seeing here is the stabilization system. Clamps and retractors are more important—these are self-retaining retractors. Once the skin incision is made, imagine the rat lying down in the prone position. So, once you make the incision and then expose it, the skin will be held initially; later, when you go in-depth, mainly to hold the muscle apart.

So, that is the importance of having these clamps. Not only that, but you can also bring them closer and have a rigid fixation of the spinous process. I hope you all remember the spinal canal anatomy that we discussed. The spinous process of the vertebra will be fixed at two points, and in the centre, you can perform the laminectomy. When it is fixed at two rigid points, you can remove the bone, expose the spinal cord, and even use the impactors. That is one way of fixing it.

On the right side, you can see that there are two rigid fixators on both sides, which are brought to the midline and then fixed onto the lateral edge of the spinal canal, or for that matter, closer to the transverse process. If you remember the anatomy, this is the spinous process, and that is the transverse process. There will be the spinal canal and then the body. So, that is how it lies. This will go under the transverse process, specifically if possible, after dissecting the muscle. You can bring it closer, expose the transverse process, and get these rigid fixating bars underneath the transverse process so that the spinal cord or spinal column does not move during the laminectomy steps when you will be using the heavy rongeurs to remove the lamina. Even while using the drills, you do not want the spinal canal, which is otherwise mobile, to move around.

This sort of stabilization system is very important before we proceed to the next step. Here is a comparison of various levels and techniques of using the fixators and clamps. What you see here is the movement of the spinal column when you use the impactor from the top. This is the classical spinal cord injury model, where you want to induce injury by contusing the cord. It is called a contusion, where something hits the cord. In this case, it is an impactor, which is a measured one that will be shown in the next few slides. However, what I want you all to focus on is these clamps, the area of fixation.

The closer the fixation is to the area of surgery, the less movement there will be. As you can see, when the clamp is applied to the top and bottom, the movement is much more than when it is next to the laminectomy level. However, if you see, the movement is absolutely nil when the fixating bar goes underneath the transverse process. This is the spinous process. This is why we always tend to use these fixations under the transverse process unless it is very difficult to expose this particular transverse process. Only then will you resort to using the clamp on the spinous process above and below the level of laminectomy. Here is where the laminectomy will be performed.

I hope you all remember what a laminectomy is. The lamina is the covering of the posterior arch of the vertebra. This is the spinal canal, and that is the body of the vertebra. That is the transverse process, and that is the spinous process. We need to remove the lamina to expose the spinal canal within which the spinal cord sits in the horseshoe-shaped frame, matter, and white matter. So, that is how the fixations are recommended. This is the preferred way of fixating the spinal column when you are using the injury model or, for that matter, any laminectomy procedure. This is the best way of fixation. The setup used to achieve that sort of fixation under the transverse process is this.

You can see this is the trough of the U-shaped rat container, which lies down. Once you expose the spinal column, you will expose the spinous process first after making the skin incision, then remove the muscles on the sides, and then you will have the transverse process there. So, get these bars under the transverse process, and that gives a rigid fixation. You can see there are good serrated edges to grasp the lateral vertebral processes or transverse processes. That is very important, and here is the picture where the surgery is in progress with this container holding the rat. The fixators have gone in under the transverse process after the exposure, and then you have this vibrating assembly wherein there is an impactor that can go and cause the cord injury. Or, you can have any sort of instrument. For example, here they have used the blade and blade holder, wherein you can make an incision in the cord. There are various models and methodologies available to create spinal cord injury models. In this case, they are using the blade to cut the cord and induce it, but the natural injuries are all caused by using the impactors, which reflect the natural traumatic cord injuries in humans.

There are pros and cons for various injury models. Another way is to grasp the spinous process using such a stabilization system. There is another modified stabilizer system that concentrates again on the spinous process. Suppose you need a larger area of exposure and want to perform the laminectomy because, when you use the transverse process, which is coming from the side, it may hinder the other surgical steps. This is one way where you can modify and hold the spinous process, but there are chances that it can slip downward no matter how tight the grip is when vertical force is involved. If no vertical force is involved, and it is just for the laminectomy, this is very convenient to use because all you have to do is expose the spinous process above and below the level of interest and then bring in the stabilization system, which grasps the spinous process. This is good enough for small injections, microdialysis, or something where the spinal cord is handled more than the injury models.

The impact of injury models is one thing where the spinal column is going to move more. There, you need a much more rigid fixation, and that is where you use those serrated bars to fix it under the transverse process. This is another stabilization system shown here, and the beauty of this stabilization system is that there are retractors involved, which will help immensely with the exposure. This is one of the cervicothoracic cord exposures where the oral point, which is below the auricle, has been used as the fixation point, something similar to cranial stabilization. What you see here are retractors that can be modified as per the need, where the skin and muscles are held out of the surgical area. That retraction is an amazing step that clears the exposure of the spinal cord and ensures that the tissue does not interfere with any of the surgical steps. Unwanted tissue will not hinder the progress of the surgery.

So, that is another good spinal stabilization system. This is what I was talking to you all about when you are trying to use the impactor or an impounder. It is better to have a very good stabilization system. This is another manual impounder or impactor where you can use the metal rod to create a spinal cord injury. These forceps are used to grasp the spinous process again above and below. This is another spinal stabilization system commonly used, and the other one I showed where the transverse process is grasped is an improvement on this basic system.

But here, the impounder is sort of blended into the entire stabilization system. It is like one system wherein there is a fixation that can be moved freely in different directions and axes. So, irrespective of the size of the rodent that you are handling, be it a mouse or a rat, this particular system can be used to stabilize the spinous process. This is the automated impactor system. There is a similar stabilization system where the spinous process will be held by this, and the laminectomy has been performed, exposing the spinal cord.

I would say that is a rat, and that is how this is going to come and hit the cord and create the spinal cord injury model. So, these are the automated impactors similarly used here, where the exact amount of force can be calibrated and then adjusted, and only that specific force will be delivered to create a controlled contusion of the rat's spinal cord. Unlike the mechanical impactor, where the accuracy is a little lower and the injury may be more than what is expected. The next remaining part of the presentation is about the cervical vertebrae exposure. Again, for spinal surgery, the positioning is entirely different.

You need to make sure the area of importance or the area that you need to expose should be at a higher level. If you all remember the discussion, there is cervical lordosis. The curve of the spine is this way. That is the head of the rat, and that is the skeletal system, and then there is thoracic kyphosis, and then there is again a slight downward dip, and then the tail comes. That is how the natural curve of the spine system is. If you are doing surgery here, then you have to make sure that the cervical vertebra is on top; otherwise, what will happen is that crowding of vertebrae occurs, and all the spinous processes will be together. It is very, very difficult to expose and do the laminectomy for the simple reason that you can consider this as multiple rings crowded like that. That is because of the natural process of, I mean, the natural curve of the cervical spine. You want this to be outwardly arranged so that the spinal canal, which is in the centre, is easier to expose after removing the lamina of the exposed vertebra.

To do that, you need to give a wedge or a pillow, as shown on the right-hand side, where the pillow gets the curve and makes it kyphotic that way. So, from this to this, the change is brought about by the wedge, where the spinous process opens up, and the spinal canal is easier to view. Not only that, you can even pass your instrument, rongeur, or drill easily and remove the spinous process and lamina and expose the spinal cord. For example, if you want to use a drill here or use the bone rongeur, you do not have an edge of the bone to handle, and that is the whole importance of this positioning. Whereas in the thoracic vertebra, this problem does not arise because naturally, it is kyphotic; however, you need some rigid support under the rat and also make sure that it does not compress too much.

For example, the thorax is coming in contact with this pillow or wedge; it should not be overdone so that there is compression of the chest wall, and it hinders the breathing of the rat. For the same reason, you are not supposed to keep your hand or any sort of instrument on the body of the rat during the surgery. That is very, very important because that would compromise the chest wall expansion and would lead to oxygenation deficiency, and that is again the reason why you need to monitor the vitals. This will be dealt with in detail in the anaesthesia section, where we will discuss all the vital monitoring steps and vital monitoring apparatuses. So, that is briefly about the positioning required for cervical vertebrae exposure. Once the positioning is done, you are good to go for the exposure of the vertebrae.

The incision technique is the same; you will be indenting or piercing it with the tip of the surgical knife and then using the belly and making a slit. That technique is the same. When you do that, keep your index finger and thumb on both sides and then spread them apart as you make the incision. So, you know how far deep you have gone with the incision. Once you do that, at the cervical level, this is the muscle that comes into view, is the trapezius muscle, which controls neck movement, and then there is some adipose tissue and subcutaneous fat that comes into view. These are the self-retaining retractors, which are different in different stabilization systems. One of the commonly used retractors is something like a catspaw retractor.

Those retractors will be used on the edges, and then the exposure is maintained until the exposure of the muscle. Once that is exposed, the first bony prominence that is seen is always the T2 spinous process. In humans, it is always the C7 vertebra; in rats, it is the T2 spinous process. That is how you know at what level you are before you start to expose a different lamina or different vertebrae. Once you see this spinous process, you are confident enough to proceed with the exposure of the remaining vertebrae. If you do not see it, if you stop your incision there and expose it, and you only see muscle without T2, then you need to extend your incision until you see the T2 spinous process. That is your whole idea, where T2 can be used as a very good reference landmark.

So, once the T2 is seen, we can proceed and make an incision or split the muscle. If you go in the midline, you will get a beautiful avascular plane. Avascular refers to the absence of a major blood supply, wherein you have only loose areolar tissue. These are some of the tissue terminologies where you have tissue without major prominent blood vessels, which you will encounter. If you go through the muscle, that leads to a lot of blood loss, and for a small animal as low as, for example, 20-gram or 30-gram animals (pups), that sort of blood loss is enough to cause hypovolemic shock. We will discuss this further when we cover the anaesthesia part. So, it is important that the blood loss is kept to a minimum.

If you are in the strict midline, you will ensure that it goes through the avascular plane, and then you can expose the vertebra as shown here. So, that was the midline. From the T2, you can count upwards: T1, 7, 6, 5, 4. If you want to create a spinal cord injury model at the C5 level, you need to remove this lamina, either by using a drill or, as you learned in the previous sessions, by using the bone rongeur. As you can see, these bones are really slender and very soft compared to the cranial bones. Of course, the hardness of the bone depends on the age of the rat, and if it is a mouse, it is going to be soft, where you can sometimes cut it with scissors, or you can use the bone rongeur to make a nick on both edges and lift the vertebra.

This allows the spinal cord to come into view. This is how the surgery progresses. Once you expose the vertebra, this is a zoomed-in view of the area. This area has been zoomed in where all the surgeries are best performed under a microscope. I am not changing this, as I am presuming that you know we will be using a microscope for all surgeries. The simple reason is that these vertebrae are pretty small to appreciate without the microscope. Especially when you do the bone work, if you are not careful enough, you will injure the spinal cord before the experiment even begins. So, every single step must be performed using the microscope, which again requires a lot of hand-eye coordination exercises.

Once you are good at using the microscope, these steps become easy. So, what I was saying is that this area is zoomed in to expose the lamina at the C4, C5, and C6 levels. Somewhere there was the T2, so the orientation is this way: T2, T1, C7, C6, C5, and C4. That is the spinous process. That is how the rat is lying—rostral to caudal. This is called a coronal orientation. This is the dorsum, and we are moving downward as we approach. The skin has been retracted, the muscle has been retracted, and the spinous process has been exposed, and this is the lamina that we have exposed. Using a drill or the bone rongeurs, you open the lamina in that area.

You start the drill and continue as you did for the craniotomy, or use the bone rongeur to make a sharp cut here. Medially, this is lateral, and this is medial—closer to the midline is medial, and away from the midline is lateral. When you do that, you will see the spinal cord. This is the blood supply you learned about in the previous anatomy session because those are the roots. This is the midline. As you remember, the blood supply on the dorsum (the top surface of the spinal cord) comes from the root, then climbs up onto the surface and supplies the dorsal surface of the spinal cord. Once the dura is exposed, you can remove it if your experiment involves some injections, microdialysis, or any sort of experiment that requires exposure to the cord. In this case, because it was a spinal cord injury model, you can remove the dura to appreciate the contusional changes that are going to happen, or even maintain the dura and then bring your impactor after this step to cause this contusion.

If you compare the post-laminectomy and post-spinal cord injury pictures, you can see the dark discolouration here. That is a sort of ecchymosis or contusional haemorrhage, to use a medical term, where there is a contusion caused by the impactor, which comes and contuses the spinal cord. So, that is how the spinal cord injury model is created. That concludes today's session. We have considered the entire spinal surgical process in which we exposed the cervical vertebra and, as an example, created a spinal cord injury model at the level of C5.

That covers the entire stabilization system and the cervical vertebra exposure. In the next session, we will deal with thoracic vertebra exposure and, of course, microelectrode implantation, how it is done, and those particular steps that are a little different from these surgeries. With that, we will complete the spinal surgery. In the next session, we will take up those other sections and discuss them in detail. Thank you.