Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
Course Instructor: Dr. Shabari Girishan
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore
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Hello everyone, following the previous lectures on rodent neuroanatomy and stereotactic principles, it's now the right time to delve into rodent neurosurgery. Today's lecture will focus on two main types of surgeries: craniotomy and stereotactic implantation surgery. These surgeries vary significantly, so I'll start by briefly introducing them and then move on to discuss the essential surgical setup required for rodent neurosurgery.

The outline for today's session includes covering topics such as head positioning, fixation, the complete surgical setup needed for the procedures, and a bit about prepping for surgery. In subsequent sessions, we'll delve deeper into surgical basics and examine a few examples of implantation surgery. Now, let's begin by understanding the fundamental aspects of rodent neurosurgery and the critical equipment and procedures involved in these surgeries.

The setup we employ in neurosurgery is highly sophisticated, ensuring that we address patients' issues with minimal risk to their lives and minimal postoperative deficits. This means they retain the same level of functionality they had before undergoing surgery. To achieve this, we must maintain a high level of aseptic precautions and expertise. Additionally, we rely on various technologies such as microscopes and electrical and electronic equipment. These advancements are made possible by ongoing research, especially in animal models, which helps us refine our techniques for human applications.

One prominent feature of our setup is the operative microscope, which serves as a cornerstone for many neurosurgical procedures. Alongside this, we utilize bi-polar instruments, suction apparatus, and even robotic gadgets like the necessity arm, showcasing the remarkable progress in human neurosurgery. Another significant advancement not depicted here is image-guided neurosurgery, which adds another layer of precision and efficacy to our procedures, representing the beauty and complexity of modern neurosurgery.

Despite the advancements in human neurosurgery, there is still significant progress to be made when comparing it to rodent micro neurosurgery. One area where we haven't fully embraced the technology yet is image-guided surgery, although some centers do utilize it to some extent. However, the predominant method still relies on stereotactic coordinates

for guidance. Now, let's delve into the two primary types of rodent neurosurgery. Today, I'll introduce the term "craniotomy."

The term "craniotomy" breaks down into "cranium," referring to the skull, and "tome," meaning an opening. Similar terms include "laprotomy" for opening the abdomen and "thoracotomy" for opening the thorax. In essence, "craniotomy" involves creating an opening in the skull to access various parts of the brain once the skull is opened. This procedure allows us to approach specific brain regions with precision and care.

Unlike laparotomy, where a single midline incision provides access to the entire abdomen, skull exposure in craniotomy is specific to the area of interest. For instance, in our Parkinsonian model experiment, we conducted an extensive craniotomy, although we plan to transition to smaller craniotomies in the future. It's crucial to note that not all surgeries are as extensive, and focal craniotomy is preferred to minimize morbidity and mortality rates in rodents, a principle that also applies in human surgeries. Prior to surgery, meticulous planning is essential, ensuring that every step is finalized before making an incision in the patient's head. Similarly, in rodent surgeries, to reduce the number of animals used, I recommend utilizing rodent carcasses as cadavers for practice and training purposes. This approach aligns with ethical considerations and promotes efficient use of resources in neurosurgical research.

Once the surgery is completed and you are sacrificing the animal, it's crucial to ensure that you have gained satisfactory surgical experience and have climbed the surgical learning curve before embarking on another experiment. Proficiency in tissue handling is fundamental in surgical procedures and develops through repeated exposure to various surgical techniques. During your experiments, when using most of the allotted rats for specific studies, it's essential to prioritize fulfilling the experimental requirements and procedures rather than using those numbers solely to achieve your surgical learning curve. As you are aware, animal ethical committees are stringent nowadays, and it's also humane to develop the surgical curve using cadavers rather than utilizing actual experimental subjects. With that said, craniotomy is a procedure that involves a significant learning curve compared to stereotactic fistril craniostomies, where a small burr hole is made. This distinction underscores the importance of training and experience, especially in complex surgeries like craniotomy, to ensure successful outcomes and minimize risks to experimental subjects.

Craniotomy indeed demands significant time, exposure, and practice to master. In detail, I will discuss the various steps involved in performing a successful craniotomy, where preserving the delicate pink jelly-like structure of the brain is paramount. Any damage incurred during the craniotomy, especially if your target is the cortical surface, can compromise the structure even before the experiment begins. This course aims to equip you with a thorough understanding of all the steps and principles involved, culminating in

hands-on experience with rodent cadavers. The core objective of this course is to ensure that participants are well-versed in the procedures, comprehend the underlying principles, and gain practical experience. This approach is crucial to minimize risks and ensure successful outcomes in experimental neurosurgery, particularly when dealing with surface cortical targets. The ultimate goal is to conduct surgeries with precision and care, preserving the integrity of brain structures throughout the procedure.

Today, I'll provide a brief overview of the setup required for performing a craniotomy, as well as discuss another approach to rodent neurosurgery, which is stereotactic microelectrode implantation. Here, we have two types of studies: acute and chronic. An acute study involves procedures conducted on an anesthetized rat, such as implanting an electrode, performing lesioning procedures, or instilling drugs into a specific target. On the other hand, a chronic study involves implanting or creating a lesion, allowing the rat to recover from anesthesia, and then collecting data using the implanted electrode after the surgery.

It's crucial to master both procedures because they are integral to various behavioral experiments. For instance, in our current study focusing on Parkinsonian behavior in rats, we record brain signals while the rat exhibits certain behaviors. This requires fabricating various sensors, which is another aspect of this course. Once the sensors are ready, they are implanted using the techniques covered in this course. Subsequently, we must ensure proper maintenance of these electrodes to obtain valid data, free from excessive electrical noise or interference. The significance of each step in surgical principles cannot be overstated. Not only should the surgery be successfully performed, but the rat should also be alive and healthy enough post-surgery to provide accurate neural signals. This underscores the importance of meticulous attention to detail throughout the surgical process to achieve favorable outcomes.

So, with that brief introduction to the two different kinds of surgeries, let's delve into the entire setup required. It's important to note that rodent neurosurgery entails a significant investment, and I strongly advise against compromising on any of the aspects we'll discuss today. These are the bare minimum requirements essential for conducting effective neural engineering work and meaningful neural experiments. Any compromise on these setups can increase morbidity and mortality rates, and even if the rat survives, the quality of the signals obtained may be poor, rendering the entire experiment less valuable. Now, let's discuss the basic surgical instruments used for craniotomies and microsurgical electrode implantation in stereotactic procedures. Firstly, scalpels and scissors are standard tools. However, I'd like to draw your attention to the Dumont forceps, which are extremely valuable, especially when working under a microscope or handling delicate structures like the dura or small electrodes for implantation or surface

sensors placement. These forceps offer precision and control, making them indispensable in neurosurgical procedures.

The Dumont micro forceps are invaluable in this scenario, and the cautery tool is very vital. I strongly suggest opting for a bipolar cautery if possible, or at least having a battery-operated monopolar diathermy device. However, it's important to note that using monopolar cautery in brain surgery carries a risk because you may not know the extent of the lesion that can occur when it's applied directly to the brain tissue. On the other hand, bipolar forceps ensure that the energy transfer is strictly between the tips of the forceps, significantly increasing safety levels. You might be wondering where we would use these bipolar forceps and bipolar coagulation. One particular application is in stroke models to study the effects of strokes and various neural sensors required for stroke rehabilitation or other related studies. Bipolar forceps are essential not only in stroke models but also in cases of dural bleeds or cortical bleeds during forceps implantation, making them invaluable tools in neurosurgery.

So, hemostasis refers to stopping bleeding during surgical steps. Until the dura is reached, monopolar cautery can be used effectively. However, once inside the cranium and inside the dura, bipolar forceps are always preferred for hemostasis. Apart from that, various retractors are available to hold back the skin flaps, which we will discuss in due course. If retractors are not affordable, silk sutures can be used and held in place with artery forceps or clamps. Artery clamps are useful for securing these ties. Additionally, bone rongeurs are used in craniotomy. After creating a small hole, rongeurs can enlarge the hole, or they can thin out the skull for a larger craniotomy and lift it. These rongeurs are particularly useful below the temporal line for creating stroke models, which we will cover later on.

Another invaluable instrument is the drill. There are two types: a dental drill where the drill bit is perpendicular to the handle, and a coaxial drill where the entire body is in line with the drill bit. The coaxial drill can be mounted onto a stereotactic apparatus for fine manipulation. These instruments are essential for basic rodent neurosurgical procedures. Before delving into surgery, it's crucial to discuss the neuroanesthetic setup, although it deserves its own session.

There are two types of anesthesia: injection anesthesia, where drugs like ketamine and xylazine are given intraperitoneally, and inhalation anesthesia. In rodent neurosurgery, inhalation anesthesia is preferred as it ensures smooth anesthesia without the need for repeated injections. Isoflurane is commonly used and delivered in gaseous form through tubing mounted on the stereotactic setup. This setup allows continuous delivery of anesthesia without interruptions during surgery.

Briefly, the neuroanesthetic setup has been discussed, and a comprehensive session will cover neuroanesthesia separately. Another crucial aspect is the stereotactic setup, which is essential for rodent neurosurgery due to the absence of image guidance. While image-guided surgeries rely on preoperative images to guide procedures, we currently depend on the stereotactic setup as our primary tool. Mastery of this setup is vital, and we must utilize it to its fullest potential until we transition to more advanced image-guided techniques in the future.

The principles of the stereotactic setup have already been covered in previous discussions. Today, we will directly delve into the arrangements, head fixation, and how to perform surgery using the stereotactic setup. However, as a brief recap, the stereotactic setup involves rigid head fixation, which I will explain in the upcoming slides. Once the head is securely fixed, I prefer using stereotactic coordinates for both craniotomy and stereotactic micro-implantation. This preference is based on the fact that these coordinates provide a precise target location, which can be transferred to the stereotactic setup to guide the craniotomy area effectively.

It is always preferable to use the stereotactic setup before the craniotomy itself because it helps optimize head fixation, instrument handling, placement of retractors, and positioning of the drill. Finalizing these details before the actual procedure starts ensures a smoother surgery. Mastering the principles of the stereotactic setup, including how to fix the head and calculate coordinates for deep-seated targets, is crucial for effective rodent neurosurgery. Another critical aspect of rodent neurosurgery, as well as any neurosurgical procedure, is the operative microscope. This equipment is essential due to its excellent magnification and lighting capabilities. It is not limited to neurosurgery and can be used for various procedures. However, using the operative microscope requires a significant learning curve. One must have good hand-eye coordination because you view the surgical site through the eyepiece of the microscope while performing precise movements with your hands. This adds complexity as you navigate a three-dimensional space during surgery.

That is something very important to note. It's always a good idea to use rodent cadavers to master the operative microscope, learning how to make incisions, use drills, and handle various micro instruments under its magnification. Once you're comfortable with these techniques, procedures like implanting cortical sensors or any surgery involving the microscope become much easier. Understanding the various controls of the microscope and practicing sterile techniques are crucial before starting any procedure.

Sterility is a key aspect that I'll emphasize throughout today's presentation. It involves preventing the introduction of harmful microorganisms during the procedure, ensuring a safe and successful experimental outcome. This focus on sterility, safety, and the overall

success of your experiments is particularly crucial in chronic studies, where maintaining sterility becomes even more critical.

If you're implanting a sensor or planning to leave a drug delivery cannula for a chronic study, it becomes even more crucial to follow all possible aseptic precautions. One such precaution is cleaning the microscope with alcohol or using an operative drape to avoid frequent cleaning, although the latter can be expensive. Typically, the microscope is cleaned right before surgery. Another method of sterilizing the microscope is using formalin-soaked cotton left under the microscope in a closed environment to sterilize it before the procedure. It's vital to set up and sterilize the microscope properly before starting the procedure to ensure aseptic conditions.

To minimize handling of the microscope, it's best to bring it in only after fixing the head, arranging the stereotactic coordinates, and setting up the stereotactic apparatus. Detailed steps requiring the microscope will be covered later. For those unfamiliar with the microscope, it consists of the eyepiece through which the operating surgeon views, and there's an adjustment for focus with different settings like 2x, 5x, and 10x magnification.

It's crucial to understand that movements are magnified under high zoom, so it's preferable to keep the focus away from the field to get a bigger picture and avoid potential damage when instruments approach closely with high zoom. Another important aspect is having a camera setup to record surgeries for later review or educational purposes. Monitoring the surgery through a screen can also be helpful for others involved in the procedure.

There are also arm controls on the side of the microscope that can be rotated in different axes, which is something you need to practice before the procedure. A decent microscope in Indian rupees would cost around 3 to 4 lakhs, but starting with any dissecting microscope is also fine as long as the base of the microscope is away from your operative area to ensure smooth surgery and workflow. Another important aspect, especially if you're interested in neural engineering, is understanding the different surgeries involved, such as creating lesions and studying behavioral aspects to comprehend various brain functions.

Another area to explore is the study of the effects of various drugs by administering them into different targets within the brain. The third aspect involves electrophysiological experiments, where a microelectrode is implanted to collect data while the rat behaves in different setups. If your experiments include electrophysiological recordings, it's crucial to have a reliable data acquisition system, which you can obtain from various commercial setups. However, if your experiments require customization, you may need to build the system from scratch with the assistance of electronic engineers. The electronic setup

plays a vital role, and it's important to ensure that the neural signals you gather are electrically isolated from other components in your operative arena.

So, that's the Faraday cage, which isolates electrical noise from the external aspects of the behavioral setup. In this setup, water is delivered here, and the only electrical components are your preamplifier and camera. Everything else is isolated using the Faraday cage. It's crucial to understand the different aspects of the data acquisition system, and we will have a dedicated session for that in due course. When you assemble all the components together, this is how the entire rodent neurosurgical setup would look.

The operative microscope is positioned in a corner on its base. Once your stereotactic apparatus is ready, you can swing the operative microscope arm into the field to begin the experiment. Your electrical apparatus can be kept nearby or isolated to the opposite side. Additionally, you'll need the vital oxygen cylinder and anesthetic setup. On the other side, you can set up the behavioral setup as needed.

This setup is from the Central Animal Facility at IISc, where we have established the rodent neurosurgical setup. Maintaining a smooth neurosurgical workflow is crucial to ensuring asepsis, sterility, and the success of the surgery, ultimately determining the outcome. With that introduction, let's delve into the actual neurosurgical steps required. Today, I will cover only up to skull exposure, while the remaining steps will be discussed in the next session.

First and foremost in neurosurgery is head positioning and fixation. Some of this has already been discussed in the stereotactic principles. I won't repeat everything, but it's crucial to understand how to use the ear bars and nose bars. These two components are vital to ensure that the head remains immobile during surgery. Any movement of the head during drilling or final implantation can lead to disastrous consequences. If the head shifts, the entire experiment may need to be abandoned because the target is altered in stereotactic procedures, or brain damage can occur during craniotomies.

As mentioned earlier, you should identify the ear bone spur by lifting the rodent's auricle, and then the ear bar is inserted and secured just behind the ear bone spur. Similarly, on the opposite side, the ear bar is inserted into the external auditory canal of the rat. Once this is done, the next crucial step is to position the nose bar and the incisor bar correctly. The rodent's incisor is placed on the incisor bar, which is a long bar designed for this purpose, while the nose bar is positioned on the dorsal aspect of the nasal cavity. It's crucial to ensure that the nostril of the rodent is not too tightly secured, as this could compromise air entry. Although the bony framework provides some protection, over-tightening can still hinder oxygenation, making optimal adjustment using the screws and bolts provided essential.

The image illustrates the rigid fixation achieved using the ear bar and nose bar, emphasizing the importance of aligning the ear bar with the nose bar and ensuring optimal tension, which can be adjusted using the screws and bolts shown. These components play a crucial role in centering the head properly in the surgical field.

Centering the head is crucial to ensure that the nose bar and incisor bar align with the midline of the rodent's body, perpendicular to the ear bar. This alignment provides a flat skull orientation, enhancing surgical precision. Additionally, it prevents the head from slipping during the procedure and facilitates easy coordination with the stereotactic setup and coordinates. Ensuring proper centering is fundamental for efficient stereotactic arrangements and accurate execution of stereotactic experiments. After making the necessary adjustments and achieving centering, the surgical field will appear as shown in the image. At this point, the stereotactic arm can be swung into position, allowing for the attachment of adapters and various stereotactic instruments to conduct precise stereotactic experiments. This setup enables researchers to carry out procedures with accuracy and reliability, ensuring successful outcomes in neurosurgical experiments.

Preparing the surgical field is crucial to ensure aseptic conditions and prevent sepsis during neurosurgery. While there are debates in human neurosurgery about shaving the head, in rodent neurosurgery, I prefer shaving the hair for two primary reasons. First, hair can harbor various organisms, and rodents' environments are not as sterile as human environments. Second, leaving hair behind increases the risk of contamination entering the surgical area and affecting the entire procedure.

Shaving the hair makes it easier to handle the scalp and facilitates the application of antiseptic solutions. After shaving, the next step is to paint the area using various agents. Typically, we use a 10 percent Povidone iodine solution applied with a swap stick. This disinfects the area where the incision will be made, ensuring a sterile field for the surgery. Povidone iodine is preferred due to its effectiveness against spores, providing an advantage over other antiseptic solutions used for painting and scrubbing. However, chlorhexidine solution is also a viable alternative for painting the area. Ensuring the surgical site is thoroughly disinfected before the incision is crucial for reducing the risk of infection and maintaining sterility throughout the procedure. The choice between Povidone iodine and chlorhexidine depends on factors such as efficacy, availability, and surgeon preference. Both solutions are effective in creating a sterile field and minimizing the chances of contamination during neurosurgical interventions.

After painting the surgical area, it's crucial to protect the eyes using two different methods depending on the type of craniotomy being performed. For procedures like stroke models where the craniotomy is between the ear and oracle, we shave the area and stitch the eyelids together to ensure absolute protection since the operative area is very close. However, if the craniotomy is on top of the skull, covering the eyes with eye

ointment and a moist cotton swab is sufficient. This precaution is vital to prevent injuries to the cornea or eyes, especially if the experiment involves behavioral aspects, as it would be challenging to continue the experiment if such injuries occur. Once the painting and eye protection are done, the next crucial step is draping. Draping is essential as it enlarges the sterile area, as depicted in the image. This expanded sterile field is crucial for maintaining aseptic conditions during the surgery, reducing the risk of infections, and ensuring the success of the procedure.

It's crucial to paint the area before making the incision to ensure sterility, but I recommend using drapes around the operative area as well. Additional sterile drapes can cover the entire operative field, allowing freedom of movement during surgery. It's essential to prevent contact between sterile surgical equipment like needle holders and sterile threads with unsterile apparatus. While cleaning these tools is necessary, it's not sufficient; ideally, all equipment should be autoclaved, but this isn't always practical for complex apparatus like stereotactic setups or operative microscopes. Therefore, draping these areas close to the surgical field that will come into contact with surgical equipment is critical. Draping is particularly significant in rodent neurosurgery, especially when leaving indwelling catheters or electrodes that can harbor organisms and lead to infections, abscess formation, or pus formation post-surgery. Such complications can result in the failure of the entire experiment, necessitating the discard of the rodent and wasting the extensive time spent on the experiment. Therefore, maintaining sterility during surgery is crucial, and proper draping is one way to achieve this.

Another crucial aspect I want to address in this session is the incision. Knowing where to start and end the incision is vital to avoid excessive tissue handling, unnecessary wide exposure, and to understand the surface landmarks of the area of interest. It's important to have a clear idea of the extent of the brain and the area you'll be working with when making the incision. This slide highlights that before opening the skull, you must determine where to begin and end the incision.

Roughly speaking, the mid-pupillary line, which is the middle of the eye to the posterior end of the oracle (the back end of the ear), serves as a suitable landmark for the extent of the incision. Understanding the orientation of anterior (front) and posterior (back) in relation to these landmarks is crucial. The incision should extend from behind the eye, where the olfactory tubercle of the brain begins, to the posterior aspect of the occipital bone, which roughly aligns with the interaural line or the line passing through the oracle. While you'll only fully see these landmarks after removing the entire skull, having a rough landmark based on anatomical exposure is beneficial for starting the incision correctly. Identifying these landmarks ensures that your incision is neither too anterior nor too posterior, saving time and avoiding the need to extend the incision after skull exposure. The final coordinate determination point comes after exposing bregma, which

is several layers deep beneath the skin. This strategic approach helps streamline the surgical procedure and ensures precision in accessing the target area within the brain.

If you cannot visualize bregma or lambda, it will require additional time to extend the incision and clear away tissue from the skull. This underscores the importance of being aware of these landmarks before starting the surgery. Once these landmarks are determined, you are ready to begin the procedure. Knowing how to grip the scalpel and initiate the surgery is crucial. Practicing on cadavers can help ensure clean cuts instead of jagged edges from hesitant cuts that can hinder wound healing.

The scalpel blade, whether a number 21 or 10, should be positioned with the tip penetrating the tissue substance and the belly of the knife lowered down. This technique follows general surgical principles critical for neurosurgical procedures. The advantage of working with the skull is that it provides a barrier between the brain and scalp, allowing for better depth control. You can poke directly onto the skull with the tip and then extend the cut posteriorly using the belly until reaching the back end of the oracle, as demonstrated in the illustration. This methodical approach ensures precision and reduces the risk of unintended tissue damage during the incision.

Once the initial incision is made and considering the various layers of the rodent scalp, there are loose tissue areas and pericranial layers that need to be carefully moved aside to expose the skull, as depicted in this slide. Achieving this skull exposure requires patience and careful handling. Depending on the procedure, such as stereotactic implantation of electrodes or placement of stereotactic burr holes, the level of exposure may vary. For instance, minimal exposure is needed for stereotactic implantation, whereas a craniotomy requires a larger area to be exposed.

After making the incision, you can use your thumb and index finger of the other hand to spread the incision and keep the scalp away from the area of interest. If you are right-handed, you would typically make the incision with your right hand while using your left hand to spread the incision apart using the thumb and index finger. This technique ensures proper exposure while minimizing tissue trauma and interference with the surgical site.

To ensure deeper tissue visibility, it's crucial to move aside the thin layer of pericranium using a swab stick. This step is vital for achieving a good exposure of the skull. Drilling without properly moving the pericranium can lead to slipping, which can be damaging, especially if you've already exposed the brain and need to extend the area. By completely exposing the skull and moving away the pericranium, you ensure that all anatomical landmarks such as bregma, lambda, coronal sutures, and the sagittal suture come into clear view.

The skin can be held in place using hemostats, bulldog clamps, or by placing a stitch and using artery forceps. Personally, I prefer using a stitch because it keeps the bulky instruments away from the surgical field. You can use 4-stage sutures with an artery forceps at the end and secure them to the operative table, ensuring that these instruments don't interfere with the actual craniotomy or stereotactic implantation procedure. This completes the process of exposing the skull for surgery.

In conclusion, it is crucial to become familiar with the various setups required for neurosurgical procedures, procure the appropriate equipment, and master the use of these instruments to minimize mobility and mortality risks. Strict adherence to aseptic practices throughout the procedure is also paramount and significantly more vital compared to general surgical procedures. With that, we conclude today's session. In the next session, we will delve into the actual neurosurgical steps involved in exposing the brain during craniotomy. If time permits, we will also cover stereotactic implantation techniques for various electrodes and catheters. Thank you.