Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Welcome back to the module on Rodent Neurosurgery. In the last session, we comprehensively covered the process of euthanasia, and now, we will move on to the subsequent and equally critical step: organ harvesting. Understanding how to extract the brain and spinal cord while preserving their structural integrity is paramount.

This session holds significance for two primary reasons. Firstly, it is imperative to grasp the technical aspects of the procedure itself, understanding the precise steps involved in organ retrieval. Secondly, it is equally crucial to appreciate the importance of performing this procedure with utmost care and delicacy. While there are numerous ways to extract organs from a deceased animal, the ideal technique is one that minimizes damage to the harvested organ. This is particularly important for organs like the brain and spinal cord, especially when implantation of sensors or drug delivery devices has been carried out. Post-experiment histopathological slicing often necessitates confirming the precise location of any piercing or intervention, verifying the accuracy of targeting, and assessing any potential histopathological changes.

To preserve the intricate architecture of the brain, spinal cord, or any other organ for that matter, it is essential to remove it from the body cavity with meticulous care, inflicting minimal damage. The techniques we will discuss in this session, when executed as described, not only yield a clean and well-preserved brain and spinal cord but are also remarkably efficient. In a relatively short time, these methods enable the successful retrieval of the desired organs. Typically, researchers often harvest the brain alongside other organs. However, given the time constraints and the specific scope of this module, we will focus exclusively on the brain and spinal cord. I encourage you to explore the literature for techniques related to the retrieval of other organs, which are generally less complex compared to the brain and spinal cord.

The outline for today's discussion encompasses two primary aspects. We will begin by addressing how to harvest the brain and the spinal cord individually. Subsequently, we will delve into the slightly modified technique required when your experiment necessitates the simultaneous retrieval of both the brain and spinal cord. Understanding these distinct approaches is crucial for adapting the procedure to your specific research needs. Let's commence by exploring the individual organ harvesting techniques for the brain and spinal cord, laying a solid foundation for the subsequent discussion on simultaneous retrieval.

Now, let's turn our attention to the process of mouse brain harvesting. I've chosen the mouse as an example because it's a commonly used small animal in research. If your

experiment involves rats, the primary differences lie in the skull's characteristics and the overall larger size of the animal. Specifically, the rat skull tends to be thicker, which can pose a slight challenge during harvesting compared to the mouse skull.

As you can see in the image, the rat has been completely skinned, revealing the skull with its eyes and ears intact. I want to draw your attention to the relative transparency of the mouse skull bone. This characteristic makes it comparatively easier to harvest the brain from a mouse than from a rat. In the case of rats, the use of bone rongeurs might be necessary, or you may need to employ slightly sturdier instruments. However, the fundamental steps remain similar. In the mouse, you can even observe the brain's surface capillaries through the skull, highlighting its thinness.

With the right technique, mastering brain harvesting becomes relatively straightforward. However, it is absolutely crucial to follow and comprehend each step of the procedure. As I've mentioned in previous sessions, there is undeniably a learning curve involved, but once you've mastered the technique, it becomes a remarkably smooth and efficient process.

Now, let's review the essential instruments required for this procedure. I've listed only the most critical ones here; other general surgical instruments, which we may have discussed in detail previously, are also necessary. Straight scissors are indispensable tools for harvesting surgeries, used for cutting and dividing the skull to expose the brain. Toothed forceps are essential for handling the cut sections of the skull. Finally, a lab sampling spoon proves invaluable for detaching the brain from the base of the skull and carefully scooping it out of the cranial cavity.

Before we proceed to the surgical steps, I strongly recommend that you refresh your understanding of skull anatomy. While I won't be revisiting the anatomy in detail here, as we have covered it extensively in past sessions, it's crucial to recall the names and locations of the skull bones we'll be handling today. Additionally, understanding the attachments near the base of the skull is vital, as these structures need to be carefully separated before extracting the brain.

Now, let's examine the surgical steps one by one. Following that, I'll present a demonstrative video that illustrates how these steps seamlessly come together in the brain harvesting process. As I mentioned earlier, the first step involves completely stripping off the skin, starting from the upper neck region.

From the upper neck region, the skin is meticulously stripped away, extending towards the nose until the eyes are fully exposed. It is crucial to stabilize the nasal area of the skull using forceps, as this provides a secure point of fixation for subsequent steps. While some techniques involve decapitating the head before proceeding, I recommend keeping the head attached to the body as this facilitates handling and provides additional tissue for anchoring and stabilizing the skull during the brain harvesting process.

Therefore, it is advisable to keep the animal largely intact, preserving the skin, muscles, and other tissues that can serve as tethering points for manipulating the skull and extracting the brain. Once the skin has been stripped and the subcutaneous tissue exposed, revealing the entire skull, it is stabilized using toothed forceps, gripping the nasal bone firmly.

The next critical step involves separating the foramen magnum from the atlas, which is the first cervical vertebra (C1). As you may recall from our discussions on skeletal anatomy, the cervical vertebrae comprise the atlas, axis, and so on. Achieving craniovertebral disjunction can be challenging without resorting to decapitation, as we are working directly with the skull bone and vertebral body without severing the overlying skin and tissues. Thus, this procedure doesn't strictly adhere to the conventional definition of decapitation. Instead, we are meticulously stripping the skin, removing soft tissue, and exposing the skull bone. After cutting through the muscle, we perform a craniovertebral disjunction, effectively separating the cranial bone from the vertebral skeletal system.

This separation is essential for gaining access to the occipital region of the skull. If you recall the skull's anatomy, it consists of the frontal bone, parietal bone, interparietal bone, and finally, the occipital bone, which articulates with the atlas. This region represents the continuation of the brain into the spinal cord. Therefore, if your experiment doesn't require harvesting the spinal cord, you can proceed with craniovertebral separation. This separation is crucial because it provides access to the cranial cavity through the foramen magnum, a large opening at the base of the skull where the brain transitions into the spinal cord. This access point will be pivotal for the subsequent steps in the brain harvesting process.

Once the separation of the skull from the vertebral body is accomplished, the next step involves carefully inserting the tip of the straight scissors into the newly created opening at the foramen magnum. The scissors should be held vertically and gently slid along the sagittal suture. Remember the skull anatomy: we have the coronal suture, the sagittal suture, the bregma (the intersection of the coronal and sagittal sutures), and the lambda (the intersection of the sagittal and lambdoid sutures).

After the craniovertebral disjunction, this is where you introduce the scissors. The sagittal suture forms the bregma, and you will be carefully cutting along this suture line. It's generally easier to separate the skull bone along existing suture lines rather than attempting to cut through intact bone, which can not only damage the skull but also tear the delicate brain surface, defeating the purpose of the procedure. Our goal is to maintain the brain's jelly-like structure intact while removing the tough skull bones that encase it.

Once you've passed the scissors vertically along the sagittal suture, the next step is to flatten the scissors. By flattening and opening the scissors, the parietal bones will separate, much like opening a book. This step is crucial: you go in vertically and then make a 90-degree turn so that the flat surface of the scissors rests against the brain. Then, you gently open the scissors. This maneuver minimizes damage to the brain because the

flat surface of the scissors provides a smooth interface. The opening force not only separates the sagittal suture but also causes separation along the lateral sutures.

Remember the side profile of the skull: there's the sagittal suture on top, and on the lateral side, there's the temporoparietal suture. The lateral sutures also need to break for the bone to be completely removed, revealing the majority of the brain.

At this point, the only remaining bone is the frontal bone, which covers the anterior part of the frontal lobe and the olfactory bulbs. After separating the parietal and occipital bones, you'll continue with a similar step, exercising extreme caution to avoid piercing the delicate brain tissue underneath. You now have more leverage and can use the lower prong of the scissors to gently lift upward and slide the scissors over the surface of the sagittal vein. This area contains the sagittal vein but no brain tissue, making it a relatively safe path for the scissors.

In the event that your study permits some degree of injury to the vein, you can utilize it as a support for the scissors while cutting the remaining portion of the sagittal suture. Essentially, you are replicating the initial steps for the remaining section of the skull. After sliding and cutting the central part of the sagittal suture, you rotate the scissors again, ensuring the flat surface lies gently against the brain. This action will cause the remaining part of the skull to open up, fully exposing the intact brain, which presents a smooth and glistening surface known as the pia mater. Beneath this, you'll find the arachnoid mater, and deeper still, the dura mater. These layers protect the cortical gray matter of the brain.

It's crucial to preserve at least the pia mater and arachnoid mater during the harvesting process. While maintaining the dura mater is ideal, it's not mandatory, as it often adheres to the underside of the skull and gets ripped off during skull removal. As you can observe, there are no visible blood vessels on the exposed brain surface. This is because the dura mater, along with the venous sinuses it houses, is typically removed with the skull.

Throughout the surgical steps we've discussed, maintaining a secure grip on the nasal bone with the forceps is paramount. Any movement during skull removal could inadvertently damage the underlying brain.

Once the skull has been adequately opened, a drug sampling spatula is introduced. It is carefully inserted in front of the olfactory bulb, and a gentle tug is applied posteriorly, or backward. To visualize this, imagine the brain in side profile, attached to the optic nerve, with numerous blood vessels coursing over it from the Circle of Willis, the arterial network at the base of the brain. The spatula is inserted from the top, and a backward tug is exerted on the skull.

This action effectively tears the olfactory nerve, the optic nerve, and the blood vessels at the base of the skull, causing the brain to shift forward. Once this movement is achieved, you'll know that the brain is ready to be lifted from the cranial cavity. Omitting this step

can lead to lacerations on the underside of the brain, as the tethering elements of the optic nerve and blood vessels would pull on the underlying structures, potentially damaging the lower portion of the brain.

The overarching goal of these meticulous steps is to ensure the brain's preservation to the greatest extent possible. This meticulous preservation enables clear slicing and in situ studies of various brain structures, maintaining their natural arrangement and relationships. It is vital to retain the brain's structural integrity, especially when considering procedures such as implantations. Post-experiment analyses often necessitate histopathological examinations to confirm the precise placement of implants and to assess any potential tissue reactions or alterations.

Once the brain is freed from its attachments, the same spatula is gently passed under its surface to detach any remaining tethering elements at the base of the skull. At this point, the brain can be carefully lifted and placed on a petri dish, completing the extraction process. When performed correctly, the entire surgical procedure, from start to finish, should take no more than 10 to 15 minutes. While harvesting a rat brain might require slightly more time due to its larger size and thicker skull, employing a heavier pair of scissors or utilizing techniques like drilling can still facilitate successful extraction.

Now, let's transition to a surgical video that demonstrates the steps we've just discussed. In the video, you'll observe the initial fixation of the skull, followed by the careful cutting of the skull along the midline using scissors. The scissors are then flattened, and the separation occurs with the flat surface gently resting against the brain's surface. This ensures complete separation of the sagittal suture, bringing the entire brain into view. You'll also notice a break along the lateral temporoparietal sutures, as mentioned earlier, leading to the complete opening of the skull. The same steps are repeated for the remaining portion of the bone: a vertical slit is made, the scissors are turned, and the bone is spread apart. Next, the spoon is introduced, and a gentle backward tug is applied, severing the remaining attachments. Finally, the spatula is passed under the brain to lift it out of the cranial cavity.

This video encapsulates the entire surgical process for harvesting a mouse brain. It is evident that with practice and adherence to these principles and techniques, the procedure becomes relatively straightforward.

Now, let's shift our focus to the spinal cord. If your research involves spinal cord studies, such as investigating spinal cord contusion models or implanting devices like drug delivery cannulas or microelectrodes, it is imperative to harvest the spinal cord intact to enable subsequent histopathological analysis. The surface of the spinal cord must remain undamaged, as most histopathological assessments involve examining both the surface gray matter and the deeper white matter.

Given the thin and delicate nature of the spinal cord in small animals, extracting it without causing damage can be quite challenging. It is crucial to avoid opening the entire

skeletal system and drilling into each cervical vertebra. Such an approach would almost certainly destroy the delicate spinal cord housed within the rigid vertebral column.

I strongly recommend adhering to the techniques we are discussing today, as they will ensure a smooth and efficient process, ultimately yielding an intact spinal cord suitable for histopathological analysis.

There are two primary approaches to spinal cord harvesting. In the first part, I will elucidate the surgical steps involved in removing the spinal cord independently, after the skull has been extracted. The image depicts the entire vertebral column, extending from the first cervical vertebra (C1) to the lumbosacral junction. Recall from our previous discussions that the spinal cord typically terminates around the L3 to L4 level, where it tapers and ends. The skeletal column, however, continues as the tailbone.

The initial cuts are made at the C1 level and the lumbosacral level, encompassing the entire length of the spinal cord. Subsequently, another separation is made along the lateral edges, severing the rib cage at the thoracic junction. The skin and muscle are then completely removed, leaving behind the spinal column along with the paraspinal musculature. This preliminary preparation is necessary before proceeding with the actual spinal cord harvesting.

I won't delve into the specifics of removing the surrounding tissue, as it primarily involves dismembering the skeletal structure after making the two initial cuts. The aim is to isolate the spinal column with its paraspinal muscles, creating a distinct block with two openings: one at the cervical vertebra (C1 or atlas) and the other at the sacral region, which was originally connected to the rest of the body (the hip bone and hind paw). The former opening is near the forepaw and head, while the latter is near the hindquarters.

The subsequent step involves inserting a syringe cannula into the lumbar segment's opening, or foramina. It's crucial to remember that the spinal cord isn't merely a thin, hollow tube; it has attachments in the form of cervical and lumbar roots. As we discussed in the previous session, these roots emerge from the spinal cord and combine to form peripheral nerves, connecting the central nervous system to the rest of the body.

Dissecting each nerve root and cord segment individually through microsurgery would be excessively time-consuming and impractical in small animals. Fortunately, the small size and slender nature of the spinal cord in these animals offer an advantage. By applying hydrostatic pressure using saline injected through the lumbar foramina into the spinal canal, we can leverage the fluid's shear force to sever the nerve roots within the canal. This forceful injection will cause the spinal cord to be extruded from the cervical foramina of the vertebra.

Therefore, in the initial phase of the surgery, you have the isolated spinal column block with its two openings and surrounding muscles. Saline is then forcefully injected into the spinal canal using a syringe. The resulting pressure within the canal effectively severs the nerve roots, causing them to detach from the spinal cord. While this may sound alarming,

it fortunately doesn't inflict significant damage. The roots are delicate and their severance doesn't compromise the overall architecture of the spinal cord. The final outcome is the extrusion of the spinal cord from the spinal canal, facilitating its collection.

This technique, while seemingly unconventional, offers an efficient and relatively atraumatic way to harvest the spinal cord in small animals, preserving its structural integrity for further analysis.

The overarching objective of these techniques is to ensure the optimal preservation of the brain, facilitating clear slicing and enabling in-depth study of its intricate structures in their natural context. This is particularly vital when implants are involved, as post-experiment histopathological analysis often necessitates precise visualization of the implant's placement and any potential tissue responses.

Once the brain is fully detached, the same spatula is gently inserted beneath it to release any remaining connections to the base of the skull. The brain can then be carefully lifted and transferred to a petri dish, marking the completion of the extraction process. When executed proficiently, this entire surgical procedure, from incision to extraction, should be achievable within 10 to 15 minutes. While harvesting a rat brain might necessitate a bit more time due to its larger size and denser skull, employing slightly heavier scissors or techniques such as drilling can still yield successful results.

Now, let's transition to a surgical video that vividly demonstrates the steps we have just elucidated. In this video, you'll observe the initial fixation of the skull, followed by the precise cutting of the skull along the midline using scissors. Subsequently, the scissors are flattened, and the separation is executed with the flat surface delicately placed against the brain's surface. This ensures a clean separation along the sagittal suture, revealing the entire brain. As previously mentioned, you'll also witness a separation along the lateral temporoparietal sutures, resulting in the complete opening of the skull. The same steps are then replicated for the remaining portion of the bone: a vertical slit is created, the scissors are rotated, and the bone is gently spread apart. Next, the spoon is introduced, and a gentle backward tug is applied to sever any lingering attachments. Finally, the spatula is carefully inserted beneath the brain to lift it out of the cranial cavity.

This video encapsulates the entire surgical process for harvesting a mouse brain. It becomes evident that with practice and adherence to these principles and techniques, the procedure is quite manageable.

Let's now shift our focus to scenarios where an intact brain and spinal cord are required for histopathological analysis. These instances are particularly relevant in circuit studies where researchers aim to investigate tracts originating in the brain and extending into the spinal cord. For example, when studying motor cortex pathways that descend from the motor cortex into the spinal cord, maintaining the continuity of the central nervous system is essential for comprehensive histopathological analysis. Disrupting the spinal cord would lead to the loss of crucial sections necessary for understanding the complete pathway.

So, how does the procedure differ when harvesting both the brain and spinal cord intact? Essentially, we combine the surgical steps for individual brain and spinal cord harvesting, with certain modifications to ensure the preservation of their connection.

The first step remains the same: exposing the skull. However, it is crucial to also expose the upper part of the cervical vertebrae. Once this is achieved, the animal's neck is gently bent to bring the cervical vertebrae into a more superficial position, facilitating the exposure of the foramen magnum and the C1 vertebra. All muscles supporting the craniovertebral junction are then meticulously separated, exposing the underlying bone.

Next, a craniovertebral disjunction is performed, effectively separating the skull from the vertebral column. This exposes the upper part of the spinal cord (the cervical segment) and the lowermost part of the brain (the cerebellum).

Following this, the area around the foramen magnum is further dissected to fully reveal the neural structures at the craniovertebral junction, or more precisely, the cervicomedullary junction. While the terminology may seem complex, it simply refers to the point where the lowermost part of the skull meets the uppermost part of the vertebral column. You can identify this junction by palpating the skull's surface; where the skull ends (the occipital bone) and the ring-like vertebral bodies begin marks the junction.

At this junction, careful dissection is performed to remove the skull bones overlying the neural structures. In rats, this might necessitate drilling, but in mice, it can often be achieved with scissors. The goal is to expose the white, glistening neural tissue.

Once this is accomplished, the complete skeletal separation is performed. With the separation complete, lifting the skull bone becomes relatively straightforward. From this point onward, the steps for brain harvesting proceed as previously described.

Once you have successfully separated all the skull structures in the neck region from the vertebral body, a noticeable gap will be created by the positioning of the neck, which you have previously bent. You can then carefully lift the dorsal aspect of the skull bone. From this point onwards, the process for brain harvesting that we discussed earlier is essentially repeated. However, it is crucial to remember that in this combined brain and spinal cord harvest, we are not dismembering the head from the body. We are merely removing the top portion of the skull and the vertebrae to expose the brain and spinal cord from above.

Let me reiterate this point for clarity, using a sagittal section as an example. Imagine the vertebral bodies and the skull; we are only removing the dorsal part of the skull and the vertebrae, just enough to reveal the brain and spinal cord from a superior view. We are not severing the head completely.

Once the dorsal aspect of the skeletal structure is removed, you gently insert the instrument and lift it upwards. This creates sufficient space between the brain surface and the skull bone to allow for the passage of the scissors.

From here, the surgical steps for brain harvesting are replicated. The scissors are passed vertically, the sagittal suture is separated, the scissors are turned 90 degrees and flattened, and then opened to expose the entire brain, except for the anterior part. The same steps are then continued for the remaining portion of the skull: vertical insertion, rotation, and opening of the scissors. However, in this combined procedure, you will not be using the spatula to lift the brain out at this stage. You need to wait until the spinal cord harvesting is complete before proceeding with that step.

Therefore, in this particular brain and spinal cord harvest, the goal is to open up the skull and expose the brain completely, keeping it in place until the spinal cord extraction is finished. After exposing the brain, you'll shift your attention to the lumbosacral junction near the hind limb. The skin is opened and retracted in this area, revealing the spinal column at the center, surrounded by musculature. This is referred to as lumbosacral exposure.

Next, the paraspinal muscles surrounding the vertebral column are carefully cut to expose the skeletal system. The glistening structure you observe at this point is the lumbosacral junction, with the spinous processes projecting upwards. The junction is then separated, and the spinal column is gently bent to bring the neural structures and the spinal canal, housing the spinal cord, into view. Essentially, you are opening up the spinal column at both ends, creating access points for the saline injection.

From this point forward, the steps mirror those of the spinal cord harvesting procedure we discussed earlier. Once the lumbosacral junction is cut and the canal is exposed, the cannula is inserted into the foramen, and saline is injected under high pressure. You'll notice the saline entering the spinal canal and the entire spinal cord being extruded from the cervical foramen, which you exposed earlier. This results in an intact brain and spinal cord, ready for harvesting.

Finally, you'll repeat the step from brain harvesting, where the spatula is passed in front of the olfactory tubercle and a gentle backward tug is applied to sever any remaining attachments at the base of the skull. You can then carefully extract the intact brain and spinal cord from the body cavity.

In summary, we have now explored how to harvest the brain and spinal cord separately, as well as how to harvest them together as an intact unit, preserving their structural integrity throughout the process. This is critical for various research applications, particularly those involving circuit studies or investigations requiring the examination of neural pathways that traverse both the brain and spinal cord.

While this session focused on the brain and spinal cord, other organs such as the heart and lungs might need to be harvested for chemical analysis or systemic toxicity studies. Additionally, blood collection might be necessary. I encourage you to consult relevant literature for the procedures involved in harvesting these other organs, as they fall outside the primary scope of this rodent neurosurgery session.

In our next session, we may also touch upon histopathological analysis techniques. Subsequently, we will explore some of the behavioral setups commonly employed in neuroscience research, specifically focusing on stroke and Parkinsonian models, two widely used and significant models in the study of the nervous system.

Thank you all for your attentive participation.