Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
Course Instructor: Dr. Shabari Girishan
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore
Week - 11
Lecture – 46

Greetings, everyone. In this session, we are going to delve into rodent behavioral models and experimental setups. We have already covered various neurosurgical and neuroanesthesia procedures. Now, it's crucial for all of you to grasp how to establish the experiments and replicate behavioral models that closely resemble actual disease processes in humans. This is the fundamental objective.

It's imperative to not only have the appropriate setup but also to thoroughly comprehend the disease process so that you can faithfully recreate it in the animal model.

Most diseases don't occur naturally in animal models. Therefore, you need to essentially construct the animal model from the ground up, often involving the injection of specific agents to induce manifestations that closely mirror the human disease process. This is vital not only concerning the animal but also to have the right experimental setup that will elicit the symptoms or manifestations in the animal, allowing you to analyze them and correlate them with your research question. In this regard, understanding the entire setup is of paramount importance.

Let's examine two significant models. Today, we will focus on stroke, and perhaps in the next session, we will address Parkinson's disease. I've selected these models primarily because they are quite prevalent and frequently utilized neural models. Another model that we won't be discussing today is epilepsy, which is also a valuable model to consider and commonly employed. I've chosen these two because their motor manifestations are relatively easier to elicit, and they necessitate a well-designed setup for conducting experiments and analyzing the animal's movements. Various apparatus need to be established to study and achieve the objectives of your specific project. Therefore, these models serve as excellent prototypes for any neural engineering project, whether you are investigating electrode implantation or exploring the functional aspects of the brain region under study.

Numerous applications are feasible, and they are also captivating. I've structured the models into sections where you'll need to understand what the stroke model entails, its clinical features, how to set up the test apparatus, the behaviors you'll be observing, and how to analyze them. We will proceed through each of these sections, concentrating solely on the stroke model for today's session.

First and foremost, as I mentioned, you truly need to grasp the nature of this disease process and what needs to be done. Essentially, a stroke results from ischemia. It's also referred to as a "brain attack," and I'm quite certain all of you are familiar with cardiac

arrest or heart attack as a well-known phenomenon. I presume most of you have also heard of stroke and its manifestations. However, what defines stroke as a clinical entity is either a blockage in a blood vessel or a rupture of a blood vessel, both of which lead to the loss of function in the affected brain region. This is crucial to understand.

There are two primary types of stroke, as I mentioned: ischemic stroke and hemorrhagic stroke. Ischemic stroke is caused by a blockage in a blood vessel. How does this blockage occur? Commonly, it might involve the normal carotid artery becoming diseased. You can observe the presence of a fibro-fatty blockage. This is a similar process to what happens in myocardial infarction or heart attack, also known as cardiac ischemia. Analogously, the blood vessel becomes obstructed with this fibro-fatty plaque along its wall, which can rupture and form a thrombus. This can completely occlude the blood vessel, resulting in the cessation of oxygen and nutrient supply to that part of the brain. Consequently, it will undergo cell death. The extent of this cell death depends on the segment of the vessel that is affected. For instance, if it's the very terminal end of the arterial supply, then only a small area is impacted. Conversely, if the blockage occurs further upstream, the entire hemisphere can be affected. The severity will vary depending on the precise location of the ischemia or blockage.

Sometimes, a clot can dislodge from the heart due to valvular heart disease or other reasons. This clot can then travel and obstruct the terminal vessels in the brain. This is referred to as an embolic stroke. There are numerous terminologies; you just need a general understanding of what these terms signify because we will be creating various models, and we are going to discuss a specific model in detail. Therefore, it's beneficial to have an understanding of the underlying pathophysiology and how the clinical manifestations arise so that you can accurately recreate them in the animal model. So, embolism is a term where the clot originates from a distant source, whereas ischemic stroke or thrombotic stroke involves the thrombus forming within the vessel itself, leading to an ischemic infarct. This is the essence of ischemic stroke.

It can be caused by a thrombus forming at the site or an embolism originating from a distant location. Now, let's consider hemorrhagic stroke, where the blood vessel ruptures. The most frequent cause of this is hypertension. When a blood vessel ruptures, the damage is catastrophic. As you can see, in ischemic stroke, there's a blockage. However, in hemorrhagic stroke, you'll have a substantial clot. Blood leaks into the brain tissue, causing damage wherever it seeps. Additionally, toxic metabolites released from this clot further impact the brain parenchyma, exacerbating the damage. This constitutes another form of stroke, known as hemorrhagic stroke.

That, in essence, is the pathophysiology of stroke. There are numerous intricacies to explore, but this provides a concise overview of what stroke entails, differentiating between ischemic and hemorrhagic stroke.

Let's now discuss the manifestations observed in patients suffering from a stroke. Firstly, it's crucial to recognize that stroke is a medical emergency. It typically presents abruptly, with a short onset time. If a stroke occurs in the morning, the patient usually arrives at the

hospital within one or two hours. This window of time is critical, as it determines how quickly clinical intervention can be initiated.

To emphasize the urgency of stroke manifestations and symptoms, the acronym BE FAST has been devised. "BE" stands for loss of balance, headache, or dizziness. "F" represents blurred vision. "A" signifies one side of the face drooping. "S" denotes arm or leg weakness. "T" stands for speech difficulty. And, of course, it's imperative to call for an ambulance immediately. This serves as both an awareness campaign and a reminder of the emergency nature of stroke. It's essential to recognize these symptoms as a stop sign to whatever the patient is doing and promptly transport them to a medical facility.

Why do these manifestations occur? As previously discussed in our neurophysiology session, the brain has distinct motor areas, speech areas, and visual areas. The specific manifestations depend on the part of the brain or blood vessel that is affected. If it's the middle cerebral artery, it generally results in speech and motor difficulties. Blockage of the posterior cerebral artery predominantly leads to visual loss. If the anterior cerebral artery (ACA) is affected, it primarily causes lower limb weakness. Thus, the manifestations vary based on the vascular distribution of the arterial tree. However, by and large, the predominant symptom will be weakness. In humans, this can also include speech difficulties, and the weakness can affect the face or limbs. Additionally, patients may experience decreased sensation in the limbs. These are the cardinal manifestations that aid in diagnosing a stroke.

Crucially, these are the manifestations that need to be reproduced in your animal model as well. There are numerous stroke models available in the literature. I recommend selecting a model that produces a substantial infarct in the brain and elicits these symptoms. This is vital for designing your stroke model and executing the experiment effectively.

The purpose of discussing these symptoms is to facilitate their replication in the animal model. Here's a pictorial representation of the manifestations mentioned earlier. Hemiplegia is a significant manifestation of stroke. When the blood supply to the sensorimotor area is compromised, it leads to facial drooping and limb weakness. The patient becomes unable to move their upper and lower limbs. This is how we diagnose a stroke and initiate intervention as early as possible.

Now, let's explore how to replicate these symptoms in a rat or mice model. First and foremost, it hinges on your research question. What are you aiming to investigate? Are you interested in the motor system itself, specifically the physiological aspects of the motor network? Perhaps you want to study how the premotor area, supplementary motor area, and primary motor area function. These are physiological considerations.

Alternatively, are you focusing on the anatomical basis of stroke recovery or stroke manifestations? Are you examining the disease itself, or are you interested in the rehabilitative aspects, studying how motor recovery occurs? Such objectives are crucial for designing your experimental subjects and setup.

For instance, if your study involves extensive motor movement analysis, it's vital to consider the species you'll be using. Will it be Long-Evans rats, Sprague-Dawley rats, or Wistar rats? There are many options. Which species is most suitable for your study?

Generally, Long-Evans rats are favored for cognitive studies and those involving significant motor movements. I'll elaborate on the species comparison later. Choosing the right species is paramount before even designing your study. Thoroughly review the literature, understand your objectives, and then determine how to select a species that will yield optimal performance in terms of behavior. Ultimately, you're aiming to analyze specific behaviors at the end of your experiment.

Therefore, the animal model you opt for should effectively elicit those clinical manifestations and behavioral targets, facilitating better analysis. This is of paramount importance.

Other parameters to consider include age, gender, and body weight. If you're examining specific parameters, ensure these factors are homogeneous across your groups. This prevents them from becoming confounding variables in your study, which is crucial in any research endeavor. List out the variables for your study and strive for group homogeneity. This homogeneity is vital, even in these specific animal models.

Furthermore, consider whether your chosen behavioral test apparatus aligns with your objectives regarding the muscle group, nervous tissue, or level of the nervous system you're studying. Are you focusing on the central nervous system, the peripheral nervous system, muscle movement, or something else entirely? This dictates the test apparatus you'll use. The key point is that the test apparatus should be congruent with your objectives.

Selecting the right apparatus, the appropriate species, and considering factors like age, gender, and body weight are all crucial parameters to finalize before settling on your study or experimental design.

Let's take the example of a lever press apparatus versus the Whishaw test and how to choose between them. The only way is to thoroughly review all available literature. This example serves to illustrate that selecting the wrong apparatus can hinder the elicitation of specific behavioral manifestations. While this is a somewhat general example and not an absolute rule (as there are distal fine movements involved in the lever press), I would generally favor the Whishaw test, where the rat needs to grasp a pellet in a pellet-reaching task. However, the Whishaw test has its drawbacks. The rat may engage in chewing, which can introduce noise if you're implanting electrodes and acquiring neural signals.

This highlights the importance of carefully considering every objective and weighing the advantages and disadvantages of each potential test. Select the test apparatus that best aligns with your objectives and yields the most valuable data.

In the lever press test, shoulder and elbow movements are more prominent than digit movements, making it suitable for studying proximal muscles or the rostral forelimb area. If you're interested in more distal functions requiring finer movements, I strongly recommend the Whishaw test.

Similar considerations apply to Parkinson's disease models. Are you examining the animal's gait or limb movements? The test apparatus should be tailored to your specific study objectives.

As I mentioned, there are various methods for inducing stroke. This image depicts the normal anatomy, highlighting the middle cerebral artery territory. This region is commonly targeted because it results in significant forelimb weakness and the classic hemiparesis we discussed earlier. This is due to the disruption of blood supply to the large bundle of motor fibers descending from the cortex.

If you aim to observe stroke manifestations, you would attempt to occlude this middle cerebral artery. There are multiple ways to achieve this and cause an infarct in the MCA territory. An infarct simply means the death of that particular brain area due to ischemia, which is reduced blood flow. Ischemia leads to an infarct. These are two essential terms to be familiar with when working with stroke models.

So, how do we induce stroke? Some methodologies involve intraluminal microinjection of thrombin to induce thrombosis, which, in simpler terms, means clot formation. You would trigger thrombosis in the vessel by directly injecting thrombin, a coagulant catalyst.

Alternatively, you could microinject endothelin-1, a potent vasoconstrictor that promotes platelet aggregation. These are indirect methods.

Another option is to use an intraluminal filament to cause an embolic stroke. To simulate an embolic stroke, you would perform an angio-intervention, passing a catheter from a proximal vessel to a distal one and leaving embolic material there.

However, the preferred and commonly used technique is electrocoagulation. I favor this technique because it's relatively straightforward and closely mimics the actual stroke process in humans.

Primarily, electrocoagulation allows for the coagulation of the most proximal portion of the middle cerebral artery. In contrast, intraluminal microinjection demands exceptional control to prevent vessel damage and bleeding. Moreover, it targets distal vessels, resulting in a smaller affected area. Endothelin-1, being an indirect method, may not always be effective and might necessitate supplementation with clip occlusion. On the other hand, directly inspecting the vessel, identifying the middle cerebral artery, and coagulating it using bipolar forceps offers a more direct approach to inducing stroke.

As covered in the neurosurgical session, this method necessitates a specific craniotomy to expose the vessel for coagulation. It's a fairly straightforward method for creating a stroke model. We will delve into the specifics of how to establish this model using electrocoagulation.

This particular technique involves occluding the right middle cerebral artery, followed by common carotid artery occlusion and temporary occlusion of the left common carotid artery. Briefly, rats possess both right and left carotid arteries in their necks. In the supine position (belly up), the large vessel in the neck branches into the anterior and middle cerebral arteries as it ascends towards the skull.

The middle cerebral artery occlusion model not only involves occluding the artery itself but also occluding the artery in the neck and temporarily occluding the opposite side. This is done to ensure the absence of collateral circulation, which is a frequent reason for stroke model failure.

Collateral circulation occurs when, for instance, only the middle cerebral artery is occluded. Blood flows through the common carotid artery to the anterior cerebral artery, which may have communicating branches to the middle cerebral artery. These branches can then supply blood to the area that was intended to be ischemic. The opposite vessel can also anastomose with the middle cerebral artery and provide blood flow.

To prevent collateral supply and ensure the manifestation of ischemia and infarct (the desired outcome), you occlude the opposite common carotid artery and permanently occlude the ipsilateral artery (the one on the same side as the intended stroke). This effectively mimics a right MCA stroke. This approach has been validated and even confirmed through preclinical MRI, demonstrating that the brain manifestations in this model are akin to those observed in human stroke, specifically causing selective middle cerebral artery ischemia and infarct. This is the rationale behind using this technique to create the stroke model.

I also prefer this method. As covered earlier, it involves exposing the neck, identifying the carotid artery, and permanently ligating it or occluding it with a clip on the opposite side. Then, the rat is turned into a lateral position, the middle cerebral artery is exposed, and coagulated. This was detailed in the neurosurgical sessions.

Once this is done, the occlusion is released after one hour. This constitutes the entire procedure for creating the stroke model.

Now, let's revisit species selection. If your study emphasizes movements and the cognitive aspect is crucial, then the preferred species is the Long-Evans rat. The literature demonstrates that Long-Evans rats consistently outperform Sprague-Dawley and Wistar rats, especially in motor movements and cognitive performance.

One reason is their pigmented eyes, which, compared to the albino eyes of albino rats, have better retinal pigmentation. This likely enhances their cognitive perception and

visual acuity compared to other rats. Additionally, although they are lab rats, the brains of Long-Evans rats appear to be evolutionarily superior in terms of performance compared to Sprague-Dawley rats.

How does this translate to performance differences? Observe the advanced movements; you'll notice that Long-Evans rats exhibit a greater range of motion compared to Sprague-Dawley rats. This is advantageous for video recording and kinematic analysis. You want the movements to be easily discernible in all frames captured during recording. If the movements are rapid and of small amplitude, as shown here, the amplitude or range of motion should be sufficient to be captured by both the naked eye and the video recording. Long-Evans rats are better suited for this purpose.

An example of such a movement is the advance step during pellet retrieval. We will discuss various steps that need to be observed and scored before evaluating the animal's performance. One such step is the advance. The subsequent step is the arpeggio movement, where the hand assumes a prone position to grasp the pellet.

Such movements in Long-Evans rats are more pronounced, with greater amplitude, compared to Sprague-Dawley rats, where the movement is briefer and retrieval is faster. Additionally, observe the arpeggio movement when grasping the pellet. Long-Evans rats utilize all their fingers, while Sprague-Dawley rats typically use only the index and middle fingers, resulting in a cruder grasp.

Therefore, the grasp is notably more refined in Long-Evans rats, and the drop rate is likely higher in Sprague-Dawley rats. Moreover, the duration of the movement is shorter in Long-Evans rats, indicating faster performance. The grasp is superior, and the time taken is significantly less than in other species.

The grasp-lift probability, or the likelihood of successfully lifting the pellet once grasped, is also very high in Long-Evans rats and lower in other species. Furthermore, the supination duration, the time it takes to supinate the paw and retrieve the pellet through the slit, is notably quicker in Long-Evans rats. This will become clearer when we examine the test apparatus.

Similarly, Long-Evans rats exhibit superior performance in various other movements compared to Sprague-Dawley rats. This underscores the importance of species selection.

This image illustrates the stroke surgery we discussed earlier, where one side is permanently occluded, and the opposite side is temporarily occluded to prevent collateral circulation from rescuing the targeted vessel.

Accurate identification of the middle cerebral artery is crucial, ensuring it's not confused with the inferior cerebral vein, as discussed in the neurosurgery session. A craniotomy is performed to expose the middle cerebral artery, which is then coagulated under direct vision using bipolar forceps or cautery. Coagulation ensures occlusion of the middle cerebral artery. Subsequently, the temporary occlusion is released after one hour.

This process guarantees that the targeted area experiences complete ischemia, resulting in cell death, or infarct. This is how the stroke model is surgically created.

In the next session, we will delve into the study plan. Thank you for your attentive listening.