Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Lecture – 47

In this session, we're focusing on a crucial aspect of your research: the study plan. This meticulous outline is vital for a successful experiment, guiding you through the entire process from preparation to data analysis. Before you even begin, it's essential to have a clear plan, allocating time for training, determining the necessary number of sessions, and establishing a timeline for pre-stroke testing.

In the case of Parkinsonian models, pre-surgical tests act as crucial control data, offering a baseline to compare with post-stroke performance. If no other control groups exist, these pre-surgical tests serve as the primary point of reference.

Once the lesion is induced, a recovery period is imperative. This allows for the brain to heal from swelling, any wounds to close, and consciousness to be fully restored. Only when the animal has fully recuperated should testing resume.

Similarly, if electrode implantation is part of the procedure, additional recovery time is necessary before signal acquisition begins. Drug delivery also requires a recovery phase before testing can continue. This comprehensive study plan, along with the specific performance metrics you'll be monitoring, should be firmly established before the experiment commences.

Let's briefly touch on the Whishaw test apparatus, particularly relevant when studying the motor cortex and finer forelimb movements in stroke models. Recovery in proximal muscles tends to be relatively good, while distal muscles often experience more lasting impairment. This pattern is observed in both humans and rodents, though rodents exhibit some proximal recovery due to the redundancy within their sensory nervous systems.

If your focus is on the caudal forelimb area, particularly digit or fore palm movement, and you're less interested in shoulder and elbow movement, the Whishaw apparatus is an excellent choice. So, how do you integrate this apparatus into your study plan?

The Whishaw apparatus features a slit, with overall dimensions of approximately 36 centimeters in height. The specific measurements and configuration will depend on your research goals and the size of the animals you're working with. The apparatus provides a controlled environment to assess fine motor skills and coordination in rodents, offering valuable insights into the effects of stroke and the efficacy of potential treatments.

In front of the slit, there's a shelf positioned approximately 4 centimeters from the anterior wall or front of the chamber. This shelf has a plate or platform with two wells where food pellets are placed, and this is the target for the rat. The rat must extend its forelimb through the slit, reach the well, grasp a pellet, retract its limb, and then consume the pellet. This sequence of actions is the core of the task, and despite the apparatus's simplicity, it allows for the observation and recording of complex movements once the rat is adequately trained.

While this setup is the foundation, you have the flexibility to modify the test apparatus to suit your specific research needs. For instance, if electrical signal acquisition is part of your study, the entire apparatus can be enclosed within a Faraday cage to shield it from electrical noise in the surrounding environment. We have previously covered this concept.

Additionally, you need to plan for your video recording setup. Ideally, cameras should be positioned above, below, in front, and to the side of the apparatus to capture the forelimb movements from all angles. This allows for kinematic analysis using various available software tools to objectively study and quantify these movements.

The goal is to reduce subjective variation that can arise from purely visual inspection. When electrophysiological studies are incorporated, the ability to correlate neural signals with specific movements becomes even more critical. In such cases, video recording and kinematic analysis play a crucial role, especially when attempting to decode the neural signals responsible for particular movements.

Therefore, based on your research objectives, you can add numerous accessories to this apparatus to tailor it to your needs. Moreover, the apparatus itself can be modified in various ways to accommodate different experimental designs.

The provided example demonstrates how a rat performs the single pellet retrieval task. This specific task involves retrieving a single food pellet and is the behavior the rat will exhibit once properly trained. We will delve into the training process in more detail in upcoming sessions and slides. The additional examples illustrate how the rat inserts its paw and grasps the pellet, providing further visual context for the task.

If you observe closely, the rat's grasp does resemble that of a human hand. This is one of the beauties of this apparatus—it allows us to study potential parallels between human and rodent brain function by replicating human-like movements in rodents. You can see how the hand is brought in, and the Long-Evans rat, with its distinctive digits and long phalangeal joints, offers additional advantages for this type of research.

In essence, the core of the test involves the rat bringing its forelimb through the slit, grasping and retrieving the pellet, then taking it back inside to consume. This is the overarching sequence of events we're interested in.

Naturally, your camera's focus will be on this particular part of the apparatus. The image provided is a snapshot from the front camera, but you'll also require a side camera at the very least to conduct a thorough movement analysis. If you have the resources, adding cameras below and above can provide even more comprehensive data for further examination.

Now, let's address a critical aspect of working with small animals: training. Unlike humans, you can't give verbal instructions to rodents. Therefore, training becomes paramount, especially when using animal models to replicate disease processes and study behavior. Training demands significant time and effort, but with experience, the process becomes more efficient. However, careful consideration must be given to the ethical implications of food deprivation, a common training technique.

Before embarking on any experiment involving food deprivation, it's imperative to consult the guidelines set forth by your institution's animal ethical committee. An underfed rat may not perform the experiment at all, while a well-fed rat may lack the motivation to even attempt the task. The key is to strike a balance—ensuring the rat remains healthy and active without compromising its performance due to hunger or fatigue.

In the following slides, we'll explore how to train rats or mice for this particular experiment. Food deprivation is the first and most crucial step, but it must always be conducted within ethical boundaries. This is vital to ensure the well-being of the animals and the validity of your research findings.

In general, it's recommended to initiate food deprivation approximately three days before starting the training process. This deprivation can then continue throughout the training period. However, it is crucial to weigh each mouse and establish a baseline body weight before food restriction begins. This baseline is essential for monitoring the animal's weight loss, as it should not exceed 10 percent of its body weight per day. Failure to adhere to this guideline could result in the animal underperforming due to malnutrition.

Traditionally, three days prior to pre-training, the animal is transitioned to a standard lab diet. While there are numerous options available, if your study involves single pellet retrieval, the ideal diet is precision food pellets. These pellets are readily available in the West, but in India, their procurement involves navigating licensing issues and importing from specific food suppliers. These round pellets are designed to fit comfortably within the animal's forepaw grasp, facilitating smooth learning and consistent behavior.

In contrast, using small pieces of regular food chunks, which vary in shape and size, can lead to challenges. The rat may take longer to learn the task, dislike the food, or even refuse to eat, potentially leading to starvation. Even if the rat does attempt the task while hungry, it might become hyperactive, increasing drop and failure rates.

To ensure optimal performance and minimize these issues, precision food pellets are the recommended choice. The suggested feeding regimen involves providing 0.05 grams of food per gram of body weight per day, given for at least 12 hours, followed by regular feed. Water should be available ad libitum.

For a typical rat weighing 200 grams, you would provide 10 grams of food. Once the rat is on this feeding schedule, it's vital to monitor its body weight closely to ensure it doesn't lose more than 10 percent per day.

Furthermore, before subjecting the animal to the actual test, it's crucial to allow it to acclimate to the testing environment by placing it in the training box without the food pellet. This familiarization process is essential to prevent the rat from becoming overly anxious or exploring excessively during testing, which could lead to wasted time and further weight loss.

By taking these careful preparatory steps, you can optimize the animal's performance and ensure the ethical and scientific integrity of your research.

Before the actual Whishaw test, which employs an apparatus with a single slit, there's a preparatory phase involving a "training box." This box has multiple slits and a platform outside with ample food. This allows the rat to become acquainted with retrieving food through slits, familiarizing it with the core action required in the actual test. If a training box isn't available, you can directly train the rat in the test apparatus itself, though this may take longer. In such cases, conduct food deprivation sessions in the test apparatus for 10 to 15 minutes daily for three days. This serves as a pre-training phase, where the rat is left in the training box with around 20 pellets on the platform, encouraging it to reach through the slits to grab them. Following this pre-training along with food deprivation, the rat should be ready for the test apparatus.

The next stage, spanning 3 to 5 days, involves "shaping," which is the process of determining the rat's dominant forelimb. Similar to humans, animals exhibit a preference for using one limb over the other. It's crucial to identify this dominance, as inducing a stroke in a right-handed animal would require targeting the left hemisphere to paralyze the right forelimb, facilitating subsequent studies.

To determine dominance, position the single slit side of the training chamber downwards and gently press the food tray against the front wall to make the food easily accessible. This essentially encourages direct feeding, allowing you to observe which hand the animal uses to grab the readily available pellets. Aim for 20 reaching attempts within 20 minutes. The limb used for more than 70 percent of these reaches is considered the dominant limb.

Following shaping, the actual training commences, typically lasting for seven days. During this phase, the individual seeds or food pellets are placed in the two food wells on the platform in front of the slit. The rat must now reach through the slit, grasp a pellet, retract its limb, and consume it, replicating the core actions of the test.

You'll be placing the food pellet in the well, and then carefully observing which paw the mouse prefers to use. If it's a right-handed mouse, it will naturally use its right paw. This observation of paw preference is crucial.

Once the mouse begins its attempts, you need to categorize each one into three distinct outcomes: success, drop, or failure. A successful attempt is when the mouse uses its preferred paw to grasp the pellet, retrieve it, and bring it to its mouth for consumption. The entire sequence must be completed for it to be considered a success.

If the mouse uses its paw to grasp the pellet but drops it before reaching its mouth, it's classified as a drop. If the mouse misses the pellet entirely or knocks it off the holding plate, it's recorded as a failure.

You'll need to conduct multiple trials, providing the mouse with opportunities for at least 30 attempts or 20 minutes, whichever comes first, each day. This allows you to observe and record its performance. After each training session, return the mouse to its home cage and provide its regular daily food allotment.

In the next session, we'll delve deeper into the intricacies of the Whishaw test. However, to summarize, you essentially assign a score based on the success rate. A normal, unimpaired performance would receive a score of 0. The goal is to assess any impairment in reaching movements. Once the training is complete and the success rate is satisfactory, you'll then rate the specific movements of the rat's forelimb as it grabs and consumes the pellet. This involves breaking down the action into 10 distinct steps and scoring each one.

A mildly impaired movement might receive a score of 0.5, while a completely impaired movement would be scored as 1. Typically, a hemiplegic rat post-stroke would have a score close to 10, or at least within the range of 8 to 10. This scoring system provides an objective measure of the rat's impairment and its subsequent recovery progress, which can occur due to plasticity and collateral blood supply.

With that, we'll conclude today's session. In the next session, we'll comprehensively cover the Whishaw test scores and how to evaluate each movement. We'll also explore potential improvements and automation possibilities. Manually assessing every single step can be laborious and time-consuming, so we'll discuss advancements like automated pellet delivery, movement recording, and AI-powered kinematic analysis. Following that, we'll shift our focus to Parkinsonian models.

Thank you for your attentive participation.