Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Hello everyone, and welcome back to our session on Rodent Behavioral Models and Setups. In our last session, we explored various behavioral models, with a particular focus on the commonly used stroke model in neural engineering. We discussed the manifestations and clinical features of stroke and how to replicate those in rodent models. We also introduced the Whishaw test and its role in replicating stroke manifestations. Today, we will continue our discussion of the Whishaw test, exploring its significance and how it helps achieve research objectives through objective observation.

As mentioned previously, the Whishaw test involves a series of 10 steps where a rodent must retrieve a pellet through a slot in a box. The rat must use its forelimb to reach through the slit, grasp the pellet, and bring it to its mouth. The entire behavior of this single pellet retrieval is broken down into these 10 distinct steps, and video recording is essential for capturing them. The proper arrangement of cameras around the behavioral setup box, as explained earlier, is crucial for this process.

The importance of capturing these 10 individual steps lies in their ability to reveal subtle behavioral changes that may otherwise go unnoticed. For instance, you might anticipate that a stroke would lead to hemiparesis or hemiplegia, but if your stroke surgery is suboptimal and the rat can still retrieve the pellet, you might question whether there's any real difference in behavior before and after the stroke. This is where objective rating, such as the skill reaching rating scale, becomes crucial.

Each step should be meticulously recorded and compared with pre-stroke behavioral training videos to identify any subtle differences. This approach allows for a more objective evaluation of the behavioral test, rather than relying on mere visual inspection. Without these 10 distinct steps, you might simply observe that the rat can grab the pellet and feed itself, considering the behavior normal and potentially wasting a valuable research subject.

The skill reaching rating scale enables you to observe movements at the digit level, noting whether the digit moves to the midline, if it's semi-flexed, and whether the elbow reaches the midline. The camera angle is crucial for observing these subtle differences in limb and body posture. Additionally, you can assess whether pronation is complete or impaired. This point-to-point comparison is essential for confirming the presence of stroke manifestations and ensuring that the rat exhibits some weakness in performing the task.

Furthermore, if you're planning an intervention for a rat with a disability, these objective ratings become invaluable. They allow you to easily compare outcomes and assess improvements after subjecting the rat to various management strategies, such as medication or physiotherapy exercises. These objective measurements also prove crucial when incorporating translational neural engineering into the picture, as you can compare pre- and post-intervention recordings to gauge the effectiveness of your approach.

Now, let's delve into the specifics of these 10 steps, understanding their significance and how they contribute to a comprehensive behavioral assessment. While our focus is on the Whishaw test, the principles of objective rating and observation apply to any task, be it a lever press task, a head-fixed setup with a rotating disc for pellet retrieval, or any other experimental paradigm. The key takeaway is the necessity of objective measurements to validate your experimental setup and ensure the reliability of your behavioral data.

As we examine the 10 steps, it's important to note that the pictures provided illustrate the views from a front camera and a camera positioned below the setup. Depending on the specific task and research objectives, additional camera angles, such as a side view, may prove beneficial. The camera shot from below is particularly crucial, as it often provides a reference midline against which you can assess whether the rat's digits are approaching the midline during the preparatory phase of the grasping movement.

Essentially, we need to analyze the movements occurring at the shoulder, elbow, and wrist levels. The rat prepares itself by swinging its forepaw toward the midline, aligning it with the slit where the pellet is located. When observing Long-Evans rats, you might be surprised by the apparent lack of distinct steps, as they often retrieve the pellet with remarkable speed. However, digital recording and frame-by-frame analysis allow you to capture these subtle yet important steps, such as "digits to midline," and gain a deeper understanding of the behavioral nuances.

For accurate frame-by-frame analysis, it's imperative to use a camera with a high frame rate and fast shutter speed. This ensures that you can capture each movement with precision and identify any subtle impairments or improvements following interventions. The "digits to midline" step is just one example of the many critical movements that a detailed behavioral analysis can reveal, contributing to a more comprehensive understanding of motor function and recovery in rodent models.

The rat strategically reduces the girth of its hand, making it smaller to facilitate its passage through the narrow slit. This involves a subtle yet precise movement in the digits, characterized by semi-flexion. Comparing the semi-flexed digits to the fully extended ones in the image highlights this distinction. Each digit is semi-flexed, creating a more globular shape that can easily navigate the slit. This semi-flexion occurs after the digits are brought to the midline, demonstrating the sequential nature of these movements.

The third step involves the entire elbow moving towards the midline. Initially, only the digits approach the midline while the elbow remains in a mid-lateral position. As the movement progresses, the elbow also aligns with the midline. This exemplifies the remarkable way in which body posture dynamically adjusts to support the sequence of movements, a concept often referred to as ergonomics in humans. Efficient movement requires proper body and foot positioning, enabling the hand to execute finer, more precise movements.

In this scenario, the precise movement involves passing the hand through the slit, grasping the pellet, and retrieving it. To accomplish this, the rodent must strategically position its forepaw and maintain body balance. This careful coordination sets the stage for the subsequent steps. The movement of the elbow to the midline represents another crucial step in this process.

Next comes the "advance" phase. Here, the rat attempts to insert its forepaw into the slit while simultaneously raising its snout. This upward movement of the nostrils creates space for the forepaw to enter the slit, and the rat balances itself by placing its other forepaw on the ground. This coordinated action highlights the precise movement of the distal forelimb, emphasizing the richness of neural signals and information this task can provide about the distal forepaw compared to other available tasks.

Once the initial advance is made, the digits, which were previously semi-flexed in the third step, now extend. This extension allows the rat to further reach into the slit and prepare for the subsequent grasping motion. The transition from semi-flexion to extension demonstrates the adaptability and fine motor control exhibited by the rat during this task.

In this crucial step, the rat fully extends its digits, a subtle yet important difference that might be challenging to observe and record through mere visual inspection. This is where the camera and subsequent video analysis prove invaluable, allowing you to capture and scrutinize these minute changes. While automated analysis tools can aid in this process, a fundamental understanding of these movements, which involve anatomical changes in the rodent's forepaw to facilitate grasping, is essential for effective analysis using any software.

After extending its digits, the rat positions its hand on top of the pellet in a movement known as pronation. Pronation involves turning the hand palm down, while the opposite movement is called supination. Familiarization with the terms "prone" and "supine" is crucial here. For the rodent's body, prone refers to its natural position with the belly facing down, whereas supine indicates the belly facing upwards. Similarly, for the hand, the dorsum (back of the hand) is considered prone, while the palm is considered ventral. Therefore, when the dorsum of the hand faces upwards, it is referred to as supination.

Observing this pronation movement is vital, especially if the stroke or surgery is incomplete, the infarct is partial, or the rat is recovering well. In such cases, subtle differences might only manifest in these final movements. The pronation might be incomplete, the limb might remain semi-flexed, or each movement might take longer than observed before the stroke induction.

Pronation, or the downward positioning of the extended digits onto the pellet, sets the stage for the natural next step: grasping. The grasp movement, easily recognizable by the complete flexion of all digits, closely resembles the human grasping motion. While humans possess more individuated digit movements, such as opposition between the thumb and index finger, the rodent's grasp in this task offers the closest approximation for observation and analysis.

Following the grasp, the rat initiates a supination movement to retrieve the pellet into the box and consume it. This involves repositioning the forelimb, rotating it back from the pronated position. However, supination doesn't occur in a single, swift motion. If the full width of the hand were to enter the slit area during supination, it would likely get stuck.

Therefore, the rat employs a semi-supinated or semi-prone position to withdraw its forepaw, a movement referred to as "supination 1." Subsequently, as the pellet is pulled into the box and the rat prepares to eat, complete supination occurs, known as "supination 2." In supination 2, the hand is fully supinated, contrasting with the partial supination seen outside the box in supination 1.

Finally, the "release" step marks the culmination of the sequence, where the pellet is released into the rat's mouth before chewing and swallowing. These 10 individual steps provide a framework for detailed observation and analysis, enabling comparisons before and after stroke surgery or any interventional strategy. This approach allows you to comprehensively rate the behavior and assess the impact of your interventions.

Remember, the principles illustrated here extend beyond the Whishaw test. Any behavioral test can be broken down into a series of steps, whether it's 10 or 15, as long as there is consistency in their execution. This consistency is crucial, especially when conducting numerous trials (e.g., 30-40 pellet retrievals per day for 7 days), generating substantial video data for analysis. Before inducing a stroke, ensure that the chosen steps are consistently observed.

Even if you're not using Long-Evans rats or observing all 10 steps consistently, strive for at least 7-8 steps that are reliably reproduced each time the task is performed. Consistency throughout the experimental plan, from training to intervention and post-intervention analysis, is paramount. The chosen steps should be repeatable and comparable across all phases.

Now, let's explore automated apparatus. As mentioned earlier, manually conducting single pellet retrieval tasks and simultaneously managing camera recording and note-taking can be quite demanding, especially if you're working alone.

To address these challenges, automated apparatus can significantly streamline the experimental process. One such apparatus utilizes infrared detectors attached to the behavioral setup box and infrared LEDs positioned near the pellet tray. When the rat's movement is detected, a pellet dispenser automatically delivers a pellet into the tray. As the rat approaches the pellet, an IR LED outside the box, mounted above the tray, triggers the camera recording. This automated system replicates the essential sequences of the Whishaw test, freeing you to focus on observing and recording the rat's movements while ensuring that everything runs smoothly.

While automation simplifies the process, your supervision remains crucial to address any unforeseen issues. However, the apparatus handles most of the tasks, from pellet dispensing to camera activation, allowing for a more efficient and less labor-intensive experimental setup. Several automated apparatus options are available, so explore different setups to find one that suits your specific needs.

Now, let's transition to another task that represents a more advanced approach to behavioral analysis. In this setup, the rat's head is fixed, utilizing the cranial window we discussed in previous surgery sessions. Recall that a cranial window is created through craniotomy and the placement of a metal plate, enabling the use of two-photon microscopy to observe neuronal activity in real-time.

With its head fixed in a stereotactic frame, the rat engages in a head-fixed cue multi-step prehension task. Prehension, or grasping, is the core action, mirroring the single pellet retrieval task or Whishaw test. However, in this setup, the rat's head is immobilized, and a microscope visualizes neuronal activity during the task. You can observe fluorescent activity and even study different neuronal layers as they light up in response to the rat's movements. This technique, known as two-photon microscopy, capitalizes on calcium spikes that occur during neuronal activity, inducing fluorescence that is captured by the microscope.

A video camera records each movement, and an infrared camera provides automated kinematic analysis of the forepaw's movements. This combination of real-time neuronal imaging and kinematic analysis offers a powerful tool for understanding the neural underpinnings of motor control and the impact of interventions on both neuronal activity and behavior.

Let's take a closer look at how this setup operates. Each trial begins with an audio cue, prompting the disc to rotate and bring the pit containing the food pellet closer to the rat. The rat patiently rests its forepaw on a horizontal metal bar until the pellet is within reach. With its head fixed to the cranial window, the rat extends its forepaw, tracing a trajectory to grasp the pellet, much like in the Whishaw test. The colored blobs you observe represent kinematic analysis, performed by software called DeepLabCut.

This analysis involves tracking the rat's forepaw movements across multiple trials. In each trial, the rat's forepaw generally follows a similar path to reach and grasp the pellet. The software analyzes these trajectories and, after averaging across multiple trials, generates a representative path, showcasing the typical movement pattern.

This behavioral apparatus offers a significant advancement over the Whishaw test rating scale. It combines objective analysis with artificial intelligence, automating the tracking and analysis of movements. The software's algorithm, trained on the anatomical landmarks and positions of the limb, identifies and tracks the relevant markers without the need for any physical markers on the rat.

Let's delve deeper into the different levels of sophistication in behavioral analysis. The most basic level involves visual analysis, where you manually observe and compare pre- and post-stroke videos, noting any differences in the 10 steps. The next level involves using markers, such as colored dye or infrared reflective markers attached to the rat's forepaw. These markers are then tracked using cameras to analyze movement patterns. However, maintaining these markers can be challenging, as dye might fade, and infrared markers might detach during the rat's movements.

Markerless tracking represents the cutting edge of behavioral analysis. It utilizes AI algorithms to train software to recognize and track anatomical landmarks, eliminating the need for physical markers. Once trained, the software automatically analyzes the trajectory of the forepaw, as illustrated in the image. This approach offers greater accuracy, efficiency, and convenience compared to marker-based methods.

When choosing among these three levels of sophistication, the third, markerless tracking, stands out as the preferred option. Not only does it eliminate potential errors associated with physical markers, but it also offers greater objectivity and ease of analysis compared to manual visual analysis. If the opportunity arises to employ markerless tracking and utilize software like DeepLabCut, it presents a highly reliable and insightful approach.

The benefits of markerless tracking become even more apparent when considering the synchronization of neural signals and movement analysis. Neural signals operate on a millisecond timescale, whereas video analysis might capture events in milliseconds or even microseconds. This discrepancy underscores the importance of high frame rate video capture and fast camera shutter speeds to ensure accurate synchronization between neural activity and movement data.

To effectively leverage this high-resolution data, your analysis methods must match the efficiency of the camera. Automated software and AI algorithms play a crucial role in achieving this synchronization, enabling you to seamlessly correlate digit movements with neural spikes. Understanding which neural signals correspond to specific steps in the behavioral task is crucial for decoding and interpreting the captured neural data.

Synchronization is paramount. If the video and neural signals are not aligned, you risk misinterpreting the data. Analyzing movements on a different timescale than the neural activity could lead to overlooking crucial neural signals associated with specific actions.

In conclusion, strive to capture all movements and neural signals accurately and ensure their precise synchronization. This meticulous approach will pave the way for meaningful insights into the neural mechanisms underlying motor control and facilitate the development of effective interventions for neurological disorders.

In our next session, we will dive deeper into markerless techniques, exploring their principles and applications in greater detail. Thank you for your attention today.