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. Today, we will explore how markerless techniques can enhance our understanding of rodent behavior. First and foremost, we need to familiarize ourselves with the anatomy of the rodent forepaw, particularly the digits. As you can see in the image, the rodent forepaw, much like the human hand, features digits with joints such as the distal interphalangeal joint, proximal interphalangeal joint, and metacarpophalangeal joints.

A high-quality camera can capture this limb anatomy with clarity, allowing us to precisely place markers and label them within our algorithm. It's crucial to train the software to recognize these anatomical landmarks, identifying the tip of each digit, the various joints, and any other relevant points. Once trained, the software can automatically track the movements of these marked points in space, providing valuable data on the trajectory and kinematics of the forepaw during behavioral tasks.

Let's consider an example where we track the movement of a specific point on a digit. As the digit moves, the software detects its trajectory, capturing its path as it reaches for the pellet, grasps it, and retracts back into the setup box. This entire movement sequence is then fed into the training algorithm, enabling the software to learn and recognize the characteristic patterns associated with reaching, grasping, and retracting.

By analyzing the movements of the marked digits, we can further refine the algorithm and classify different outcomes of each step. For instance, we can label a trial as a "miss" if the rat fails to grasp the pellet, a "slip" if it fails to retract the pellet successfully, and a "drop" if the pellet is dropped before reaching the mouth. A "success" is recorded when the rat completes all steps, retracting the pellet into the box and consuming it.

This marker-based approach, where specific points on the digits are tracked, allows for a detailed analysis of movement patterns and outcomes. However, as we discussed in the previous session, markerless tracking offers an even more sophisticated and convenient alternative. By leveraging AI algorithms and training the software on anatomical landmarks and limb positions, we can achieve accurate tracking without the need for any physical markers on the rat.

So, we can see the extracted digit and hand trajectories. As previously mentioned, we selected a point on the distal interphalangeal joint. This software tracks the movement of that point, showing its path as it extends to reach the food pellet, grasps it, and then retracts back to its starting position. This visual representation gives us valuable insight into the trajectory taken by the extended digit. In a similar manner, if we were studying the movement of a flexed digit, such as during pronation and supination, we could track the trajectory of a marker placed on that digit, enabling us to observe those specific movements in isolation.

The beauty of this approach is that it eliminates the need for physical markers on the rodent's body. The software, once trained, can virtually track any point of interest on the forepaw, allowing for a detailed analysis of various aspects of movement. You can even define endpoints for trajectories. For instance, if the hand extends beyond a certain point, it could be classified as a "miss," indicating that the rat failed to reach the target accurately. Similarly, an incomplete retraction path due to stroke would be easily noticeable as a deviation from the trained pre-stroke data.

Markerless tracking streamlines analysis and provides automated, objective results. By simply observing the trajectories, you can quantify changes in posture and retraction patterns, obtaining more reliable and quantifiable data compared to subjective visual inspections. This objectivity enhances the validity of your findings and minimizes the risk of bias or misinterpretation. While visual analysis remains valuable, markerless tracking offers a significant advantage in terms of accuracy and efficiency.

Let's take a moment to watch a video that demonstrates the head-fixed cued multi-step prehension task. Observe how the disc moves after the cue, the rat grasps the pellet, brings it to its mouth, and then returns its forelimb to the resting position. The entire sequence unfolds within a remarkably short timeframe, often around 62 milliseconds. The video showcases the power of this setup, allowing for precise tracking of marked points on the forelimb, such as the distal interphalangeal joint, and the subsequent breakdown of movement into its constituent phases.

Each trial provides a wealth of information, and by conducting multiple trials and averaging the data, software like DeepLabCut can generate a comprehensive trajectory that represents the typical movement pattern for the task. This aggregated trajectory, visualized in the video, illustrates the consistency and precision of the rat's movements, further highlighting the benefits of markerless tracking and automated analysis.

In summary, markerless tracking techniques, coupled with sophisticated software like DeepLabCut, revolutionize the analysis of rodent behavior. They enable objective, automated tracking of movements, providing valuable insights into motor control and the impact of interventions. By eliminating the need for physical markers and automating the analysis process, these techniques offer greater accuracy, efficiency, and convenience, ultimately leading to a deeper understanding of the neural mechanisms underlying behavior.

As we observe the video, you'll notice that the darkest red line represents the most validated trajectory, which is the average path taken by the rat across all trials. While the blue lines depict individual trial trajectories, the red line highlights the most common path, providing a clear representation of the rat's typical movement pattern.

This averaged trajectory is invaluable when comparing pre- and post-stroke analyses. It allows for easy comparison against a normal distribution, encompassing all trials that fall within the curve. Additionally, it facilitates the calculation of standard deviation for behavioral analysis, enabling a more rigorous statistical evaluation of the data.

With this level of analysis, you can confidently determine whether the behavioral test has yielded the answers you seek. The experiment's success hinges on the ability to extract meaningful insights from the data. The objective and quantitative nature of the analysis ensures the validity and reliability of your findings.

Let's now shift our focus to another task, the lever press task, which serves as a valuable comparison. In the accompanying video, you can observe automated paw tracking in action. Instead of using markerless tracking, this setup utilizes a marker-based approach where the rat's right paw is tattooed with red ink.

The red channel is then thresholded through centroid analysis. As the rat presses the lever, the software analyzes the amplitude of movement associated with the red marker. This allows for tracking the trajectory of the rodent's forelimb and easily identifying any deviations from the expected pattern. The number of waves displayed corresponds to the number of trials conducted. This approach provides another perspective on the kinematics of the forelimb's movement during the task.

Comparing the lever press task to the Whishaw test, we can observe distinct differences in movement patterns. The lever press task primarily involves gross movements of the shoulder and elbow, with less emphasis on finer movements of the distal forelimb. It's also worth noting that the other forelimb might come into play during the task, further contributing to its gross nature.

While the Whishaw test excels at capturing fine motor control, it introduces noise due to chewing movements. This noise can be problematic when conducting electrophysiological analysis and recording neural signals, where minimal noise is desired. The lever press task, with its simpler movements, minimizes such noise interference, making it a suitable alternative when electrophysiological recordings are critical.

In conclusion, different behavioral tasks offer unique advantages and disadvantages. The choice of task depends on your specific research objectives and the type of data you aim to collect. Whether you prioritize fine motor control, noise reduction, or a combination of factors, careful consideration of the task's characteristics and available analysis techniques is crucial for achieving meaningful and reliable results.

Markerless tracking techniques, as demonstrated in the head-fixed cue multi-step prehension task, represent a significant advancement in behavioral analysis. They offer objectivity, automation, and the ability to synchronize movement data with neural recordings, providing a powerful tool for unraveling the complex relationship between brain activity and behavior.

In our next session, we will continue exploring various behavioral models and setups, delving into their applications and the insights they provide into rodent behavior and neural function.

In scenarios where noise might interfere with your signal acquisition, opting for a task that minimizes chewing noise, such as the lever press task, can be advantageous. However, it's important to acknowledge that trade-offs exist. You might need to compromise on certain aspects, such as the complexity of movements observed or the types of neural signals acquired. Careful consideration of these trade-offs is crucial when selecting the most appropriate task for your research question and hypothesis.

Before proceeding with stroke surgery, you'll train the rat on the chosen behavioral task. Once the stroke is induced, various behavioral analyses will help you assess the manifestation of the stroke in the subject.

Let's watch a video that clearly demonstrates the effects of a stroke. Observe how the rat's affected limb appears rigid and exhibits limited movement. Instead of purposeful reaching and grasping, the limb primarily engages in circumduction movements due to hemi-paralysis on the left side of its body. Only the right forelimb and hindlimb exhibit normal movement, resulting in this circular motion. If the forelimb is severely paralyzed, the rat won't be able to perform any of the previously discussed tasks. In such cases, the rat might not be suitable for these behavioral paradigms.

However, it's important to remember that stroke recovery is a dynamic process. Rats often exhibit some degree of spontaneous recovery, even without intervention. When selecting rats for translational neural engineering or interventional experiments involving neural stimulation, physiotherapy, or a combination thereof, it's crucial to choose subjects with a success rate of less than 60% on the behavioral task compared to their pre-stroke performance. This ensures that any observed improvements are genuinely attributable to your intervention, rather than natural recovery processes.

Choosing rats with a higher success rate (e.g., 80%) could lead to falsely positive results, suggesting that your intervention was effective when, in reality, the rat was simply experiencing natural recovery. It's essential to differentiate between the effects of your intervention and those of spontaneous recovery to draw accurate conclusions about the efficacy of your approach.

Natural stroke recovery can occur through various mechanisms, including brain plasticity, where new neural connections form over time, and revascularization, where new blood vessels develop to supply the affected area. These natural processes can contribute to functional improvements even in the absence of external interventions.

Therefore, when evaluating the recovery of a rat after a stroke, it's crucial to employ rigorous experimental design and appropriate controls to ensure that any observed improvements are genuinely due to your intervention and not simply a result of the brain's inherent capacity for repair and adaptation.

Therefore, it's imperative to keep the possibility of natural recovery in mind, as it can significantly confound your research, especially when investigating interventions aimed at improving the function of paralyzed limbs in stroke models. Failing to account for natural recovery could lead to misinterpreting the results and overestimating the effectiveness of your intervention.

With this crucial point emphasized, we conclude our discussion on the stroke model and its associated behavioral analysis. Remember, the principles and techniques we've covered can be applied and adapted to various other rodent models, such as epilepsy and spinal cord injury models. The stroke model serves as a foundational example, illustrating the importance of careful experimental design, objective behavioral analysis, and consideration of potential confounding factors like natural recovery.

By familiarizing yourselves with these advanced technologies and analysis techniques, you can significantly enhance the quality and reliability of your research. Failing to do so might result in missed opportunities to leverage cutting-edge tools and methodologies that could significantly benefit your investigations.

In our next session, we will shift our attention to the Parkinsonian model, another widely used model in neuroscience research. Beyond its therapeutic applications, the Parkinsonian model provides invaluable insights into the deep circuitry of the brain. It has been extensively studied in the context of deep brain stimulation strategies and other therapeutic interventions.

We've chosen the Parkinsonian model to offer a compelling comparison to the stroke model, highlighting the complexities involved and the valuable lessons that can be learned from its behavioral setups. By exploring these diverse models, you'll gain a broader understanding of the challenges and opportunities associated with rodent behavioral research and be better equipped to design and execute impactful experiments in your own work.

Thank you for your continued attention and engagement throughout this session. We look forward to delving into the fascinating world of the Parkinsonian model in our next session.