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Title: Automated Gait Analysis to Assess Functional Recovery in Rodents with Peripheral Nerve or Spinal Cord Contusion Injury

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen captures provided, do not film

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **18**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **David Hercher**: Automated gait analysis is a widely used method for evaluating functional recovery in rodent models of peripheral nerve injury, repair, and regeneration **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **David Hercher**: The method can be used to study changes in gait after peripheral nerve injury, relating both to motor and sensory nerve function **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **David Hercher**: Researchers new to the method might experience difficulties in acquiring high quality data. Typical hurdles are adequate animal training and accurate hardware and software calibration **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Animal Protocol Review Board of the City Government of Vienna.

Protocol

2. Automated Gait Analysis Training

2.1. During training sessions and on the test day, turn off all of the light sources in the behavioral test room **[1-TXT]** and face the computer screen for data acquisition away from the camera to prevent its light from interfering with the camera **[2]**.

2.1.1. WIDE: Talent turning off lights **TEXT: Perform all testing procedures in dark and absence of disturbing noise**

2.1.2. Talent adjusting computer screen angle

2.2. Confirm that the device is installed in a stable position in a way that prevents any form of vibration **[1]** and bring the rats into the behavioral test room in their own home cage for at least 30 minutes before the test **[2]**.

2.2.1. Talent checking device position/vibration
2.2.2. Talent bringing cage into room/placing onto bench

2.3. On the first day of training, gently grasp an animal under its trunk **[1]** and carefully place it in the walkway entrance **[2]**.

2.3.1. Rat being grasped *Videographer: Important step*
2.3.2. Talent carrying/placing rat *Videographer: Important step*

2.4. Let the rat explore the opening of the corridor without any interference **[1]** and wait until the animal voluntarily crosses the walkway to reach its home cage without any external stimulus or motivation **[2]**.

2.4.1. Rat exploring opening *Videographer: Important/difficult step*
2.4.2. Rat crossing walkway to home cage *Videographer: Important/difficult step*

2.5. On the second day of training, allow the animals to become accustomed to entering the walkway and returning to their home cage without hesitation **[1]**.

2.5.1. Talent placing rat/rat crossing to cage without hesitation

2.6. On the third day of training, confirm that the animals have learned to cross the walkway at a uniform speed without hesitation, sniffing, or other explorative

movements **[1]**.

2.6.1. Rat crossing walkway at uniform speed without distraction

2.7. On the fourth and fifth, repeat the training to reinforce the testing procedure **[1]**.

2.7.1. Talent placing rat into walkway entrance

NOTE: Take 1 and 2 of 2.7.1 show also partial explorative behavior→ could be used for 2.4.1

3. Automated Gait Analysis

3.1. On the day of the experiment, use commercial glass cleaner and a squeegee to clean the top and bottom of the walkway **[1-TXT]**, taking care to remove all of the fluid from the ends of the walkway **[2]**.

3.1.1. WIDE: Talent spraying/wiping walkway *Videographer: Important step* **TEXT: Repeat cleaning as necessary throughout experiment**

3.1.2. Fluid being removed *Videographer: Important step*

3.2. Place both the lightest **[1]** and heaviest rats onto the walkway **[2]** and select a registered camera from the **Setup** tab **[3]** to allow the **Camera Gain**, **Red Ceiling Light**, **Green Walkway Light**, and **Green Intensity Threshold** to be adjusted to ensure an optimal paw print detection for all of the animals in the analysis **[4]**.

3.2.1. Talent placing lightest rat onto walkway *Videographer: Important/difficult step*

3.2.2. Talent placing heaviest rat onto walkway *Videographer: Important/difficult step*

3.2.3. Talent at computer, selecting camera, with monitor visible in frame

3.2.4. SCREEN: screenshot_1: 00:02-00:22 *Video Editor: please speed up*

3.3. Next, click **Open Acquisition** and acquire a snapshot of the empty, cleaned walkway to be used as a reference throughout the data acquisition procedure. The status will change from **Waiting for Snapshot** to **Ready for Acquisition [1]**.

3.3.1. SCREEN: screenshot_1: 00:22-00:26 *Video Editor: please emphasize status changing*

3.4. Click **Start Acquisition**. The status will change from **Ready for Acquisition** to **Waiting for Run to Start [1]**.

3.4.1. SCREEN: screenshot_1: 00:26-00:29 *Video Editor: please emphasize status changing as appropriate*

3.5. When the camera is ready, place a rat onto the walkway **[1]** and follow the animal's movement on the computer screen. Note the status change from **Waiting for Run to Start** to **Recording Run [2-TXT]**.

3.5.1. Talent placing rat onto runway
3.5.2. SCREEN: screenshot_4: 00:13-00:17 *Video Editor: please emphasize status changing as* **TEXT: Repeat for each rat**

4. Data Classification and Statistical Analysis

4.1. At the end of the analysis, click **Classify** in the **Experimental Explorer** tab of the trial to be classified **[1]** and play the acquired run at normal speed to determine whether the data conforms to the classification requirements **[2]**.

4.1.1. WIDE: Talent clicking Classify, with monitor visible in frame
4.1.2. SCREEN: screenshot_2: 00:06-00:11 Run being played at normal speed

4.2. Click **Auto Classify** for automatic classification of the paw prints by the software **[1]**.

4.2.1. SCREEN: screenshot_2: 00:11-00:16 Auto Classify being clicked

4.3. For correct calculation of the Normal Step Sequence Patterns, make sure that the classifying algorithm is not confused by non-visible paw prints, leading to flawed NSSP (N-S-S-P) **[1]**, and that only paw prints that are detectable while the contralateral paw is also visible for NSSP calculations are included **[2]**.

4.3.1. SCREEN: screenshot_5: 00:00-00:59 *Video Editor: please speed up*
4.3.2. SCREEN: screenshot_5: 01:00-01:18 *Video Editor: please speed up*

4.4. For statistical analysis of the data, click **View Run Statistics** to obtain a comprehensive overview of the run statistics **[1]**.

4.4.1. SCREEN: screenshot_3: 00:02-00:06

4.5. Then select **File** and **Export** to export the run or trial statistics into a spreadsheet software **[1]**.

4.5.1. SCREEN: screenshot_3: 00:06-00:15

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.3., 2.4., 3.1., 3.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
2.4., 3.2.

Results

5. Results: Representative Gait and Functional Recovery Analyses After Peripheral Nerve or Spinal Cord Contusion Injury

5.1. Following sciatic nerve injury **[1]**, rats use the heel of the paw for weight support only and the limb is moved in a sweeping circumductory movement **[2]**.

5.1.1. LAB MEDIA: Figure 3
5.1.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize Figures 3B-3E RH images*

5.2. Therefore, locomotor changes assessed via Automated Gait Analysis become apparent **[1]** by a significantly reduced Print Area **[2]** and significantly increased Swing Time **[3]**.

5.2.1. LAB MEDIA: Figure 4
5.2.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize Print Area data line*
5.2.3. LAB MEDIA: Figure 4 *Video Editor: please emphasize Swing Time data line*

5.3. Femoral nerve resection results in denervation of the quadriceps muscle of the thigh **[1]**, impairing knee extension and inducing hyperflexion of the ankle joint with consecutive lifting of the corresponding heel **[2]**.

5.3.1. LAB MEDIA: Figure 6
5.3.2. LAB MEDIA: Figure 6 *Video Editor: please emphasize Figure 6B RH image*

5.4. Starting from post-operative week 4 **[1]**, reinnervation of the quadriceps by the regenerating femoral nerve leads to reversal of these changes **[2]**.

5.4.1. LAB MEDIA: Figure 7A *Video Editor: please emphasize week 4 data point*
5.4.2. LAB MEDIA: Figure 7A *Video Editor: please emphasize data line from week 4 to end of graph*

5.5. As the quadriceps muscle of the thigh also plays a role in the swing phase of the respective paw **[1]**, the Swing Time is greatly prolonged in rats with femoral nerve injury **[2]**.

5.5.1. LAB MEDIA: Figure 7
5.5.2. LAB MEDIA: Figure 7 *Video Editor: please emphasize data line from week 4 to end of graph in Figure 7B*

5.6. Gait analysis reveals markedly altered paw prints after thoracic spinal cord contusion

injury **[1]**, with a notable decrement in the Print Area and a marked internal rotation of the hind paws at post-operative week 2 **[2]**.

5.6.1. LAB MEDIA: Figure 8
5.6.2. LAB MEDIA: Figure 8 *Video Editor: please emphasize Figure 8B RH image*

5.7. Spinal cord contusion at the thoracic 11-level also results **[1]** in a decrease of the Print Area Ratio **[2]** and increment of the Swing Time Ratio **[3]**.

5.7.1. LAB MEDIA: Figures 9A and 9B
5.7.2. LAB MEDIA: Figures 9A and 9B *Video Editor: please emphasize Figure 9A data line*
5.7.3. LAB MEDIA: Figures 9A and 9B *Video Editor: please emphasize Figure 9B data line*

5.8. The coordination-related Regularity Index also decreases at post-operative week 2 **[1]**, while the Base of Support of the hind paws shows a marked increase **[2]**.

5.8.1. LAB MEDIA: Figures 9C and 9D *Video Editor: please emphasize week 2 data point in Figure 9C*
5.8.2. LAB MEDIA: Figures 9C and 9D *Video Editor: please add/emphasize asterisks in Figure 9D*

Conclusion

6. Conclusion Interview Statements

6.1. **Viola Oberhauser**: When using Automated Gait Analysis to evaluate functional recovery in rodents, be sure to carefully train the animals and to be meticulous when making any software or hardware adjustments **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.4., 3.2.)

6.2. **David Hercher**: By using Automated Gait Analysis, injury and regeneration of the central and peripheral nervous system can be evaluated in various rodent models, including yet unstudied forelimb nerve models, such as the median nerve **[1]**.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera