2.3. Cardiovascular treadmill endurance assay in conscious mice

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A. OBJECTIVE

To evaluate cardiac exercise endurance in intact conscious mice.

B. CAUTIONS

- For all animal experiments, make sure to get approval from the Institute's Animal Care and Use Committee and follow NIH guidelines.
- Prior to the experimental testing of cardiovascular treadmill endurance on day 6, mice must be subjected to 5 days of increasing treadmill exposure to train them for treadmill running.
- The ideal room temperature for treadmill running is ~ 70ºF. If higher than 80ºF, mouse performance will be affected. A mild electric shock is used as a negative stimulus to discourage the mouse from standing/sitting at the end of the belt rather than running on the track. If the electric shock is too strong, it may damage the mouse. Since toe clipping may affect the gait, we recommend not use mice that have undergone toe clipping.
- The strain, age and sex influence treadmill performance.
- Each training session takes about 30 to 40 min. We recommend perform training once per day. It is important to always perform the training at the same time of the day (for example, always at 9 am in the morning). This should also be the time of the test. The mouse should be continuously monitored throughout the entire session. When speed reaches 10 m/min, some mice may stop running. This usually does not mean these mice are exhausted. Coax the mice to run by gently brush the tail. Occasionally, a mouse may run towards the direction of the electric shocker. If this happens, stop the treadmill, manually place the mouse to the right direction and re-start. It may take several rounds for some mice to learn. At the end of each session, check the mouse for feet or toe damage. Exclude the mice that are either injured or reluctant to run. Up to 20% mice may fail to acclimate.
- Caution should be taken when using the time spent on the electric shocker as an indicator for exhaustion. Individual animal may display different running styles. Mice that do not run willingly or do not show a consistent running style should be excluded from the study.
- To demonstrate therapeutic efficacy, one should always include age and gender-matched untreated controls and normal mouse controls. We recommend start with at least 7 mice for each group.
- It is important to point out that the treadmill is set at 7º uphill for cardiovascular treadmill endurance assay. It is different from the downhill setting which is often used to test skeletal muscle function.

C. MATERIALS

- Exer 3/6 treadmill system (Columbus Instruments). The system includes a treadmill with an electrical stimulus and a control unit. The control unit regulates the treadmill speed (3 to 100 meter per minute, m/min) and the intensity of the electrical shock. The system allows simultaneous analysis up to 6 animals in separate compartments.
- 9-inch cooling fan (Lasko, West Chester, PA, USA).
- Jiffy jack (Cole Parmer, Vernon Hills, Illinois, USA).
- Timer.

D. METHODS

1. Prior to the study, Check to make sure there is no visible injury in the limbs and toes. Record room temperature. Set up the electric shocker at the intensity of 7 and the repetition rate of 9. Adjust the inclination of the treadmill platform with the Jiffy jack. Turn on the fan to the low setting and have the air blows in the same direction as the mouse is running
2. On day 1, place mouse on unmoving flat treadmill for 2 minutes. Place mouse on unmoving 7˚ inclined treadmill for 5 minutes. Next, make a positive 7˚ inclined treadmill by setting the crank at zero and placing the incline rod into the hole on the distal end of the treadmill. Then, insert the pin into the first hole on the rod with the treadmill raised above the hole and lower the treadmill onto the pin. Run mouse at 5 m/min on 7˚ inclined treadmill for 15 minutes. Run mouse at 10 m/min for 5 minutes. A ruler or other flat instrument may be used to help mouse stay in the proper orientation on the running lanes and help mouse off the shocking grid.
3. On day 2, place mouse on unmoving flat treadmill for 2 minutes. Run mouse on 7˚ treadmill at 5 m/min for 5 minutes. Run mouse on 7˚ treadmill at 10 m/min for 15 minutes. Run mouse on 7˚ treadmill at 12 m/min for 5 minutes.
4. On day 3, place mouse on unmoving flat treadmill for 2 minutes. Run mouse on 7˚ treadmill at 5 m/min for 5 minutes. Run mouse on 7˚ treadmill at 10 m/min for 15 minutes. Run mouse on 7˚ treadmill at 12 m/min for 10 minutes.
5. On day 4, place mouse on unmoving flat treadmill for 2 minutes. Run mouse on 7˚ treadmill at 5 m/min for 5 minutes. Run mouse on 7˚ treadmill at 10 m/min for 20 minutes. Run mouse on 7˚ treadmill at 12 m/min for 5 minutes. Run mouse on 7˚ treadmill at 15 m/min for 5 minutes.
6. On day 5, place mouse on unmoving flat treadmill for 2 minutes. Run mouse on 7˚ treadmill at 5 m/min for 5 minutes. Run mouse on 7˚ treadmill at 10 m/min for 20 minutes. Run mouse on 7˚ treadmill at 12 m/min for 5 minutes. Run mouse on 7˚ treadmill at 15 m/min for 5 minutes.
7. On day 6, assess treadmill performance. Place mouse on the unmoving flat treadmill for 2 minutes. Run mouse on 7˚ treadmill at 5 m/min for 5 minutes. Increase speed by 1m/min every 5 minutes. Continuously nudge the mouse to keep it stay on the track. The maximal running capacity for each mouse is defined as the point where the mouse stays on the shocker for 10 seconds without attempting to re-enter the treadmill.

E. EVALUATION AND INTERPRETATION OF RESULTS

1. To calculate the total running distance multiply the time spent at each speed setting by the speed of the treadmill and then add all of these distances together.

F. REFERENCES

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