

## **Rotarod 小鼠跑步測試**

### **1. Purpose**

1.1 The Rota-rod test is designed to evaluate the motor coordination and balance of the mouse by forcing the subject to run. Researchers can program various training methods for their experiments. For example: fixation speed and acceleration, rock by setting angle...etc. This test is applicable to study motor activity change, anti-fatigue drug and neural degeneration diseases. Researchers perform multiple experiments and discuss the results regarding the subject's ability of motor learning and motor memory.

### **2. Scope**

- 2.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure strictly.
- 2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the Neurobehavioural Genetics Project Leader.
- 2.3 Any deviances from this protocol must be reported to the Neuroscience Project Leader.

### **3. Safety Requirements**

3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

### **4. Notes**

- 4.1 The validity of results obtained from behavioural phenotyping is largely dependent on methods of animal husbandry. It is of vital importance that individuals following this procedure are experienced and aware of the animal's welfare, and are familiar with the animal being tested. This will reduce the anxiety levels of the animal prior to testing.
- 4.2 The majority of mouse behavioural studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.
- 4.3 Environmental factors may contribute to the levels of anxiety within the mouse. The temperature, humidity, ventilation, noise intensity and lighting intensity must be maintained at levels appropriate for mice. It is essential that the mice be kept in a uniform environment before and after testing to avoid anomalous results being obtained.
- 4.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day.
- 4.5 Where particular mutants or aged subjects are used, the procedure may require some additional training before the test phase. For example: one 60 sec trial at 0rpm and two 60 sec trials at 4rpm constant speed.

### **5. Equipment**

5.1 Commercially available Rota Rod apparatus (47600 Rota-Rod, Ugo Basile, Italy)

modified as described: Dimensions of the apparatus: Rotating rod diameter is ca. 5 cm made of hard plastic material covered by grey rubber foam (cut from insulation material to cover water pipes); lanes width is ca. 5 cm. The apparatus must allow an accelerating speed from 4rpm to 40rpm in 300 sec.

## 6. Supplies

- 6.1 Pens
- 6.2 Marker pen
- 6.3 Datasheet
- 6.4 Gloves
- 6.5 Paper mask
- 6.6 Ethanol 70%
- 6.7 Hand towels
- 6.8 Absorbent bench top
- 6.9 Brush
- 6.10 Detergent (Windex)
- 6.11 Rotarod trip plate

## 7. Procedures

- 7.1 Minimum n=10 mice per experimental group.
- 7.2 Mice are to be housed three per cage at least one week before the test.
- 7.3 On the day before testing mice should be individually marked to be easily identified on the test day. One suggestion is to mark their tails as 1, 2, 3 in each cage. Use a non-toxic, no odor black marker to make 1 or 2 or 3 stripes at the base of the tail.
- 7.4 On the day of testing, mice should be kept in their home cages and acclimate to the testing room for at least 15 min.
- 7.5 Turn on the Rotarod apparatus.
- 7.6 Training phase
  - 7.6.1 Training trial 1 (t1): place three mice from the same cage/strain on lanes 1, 3 and 5 (i.e. leaving an empty lane between two mice). Try to have the mice on the rod walking forward to keep their balance. The rod is initially rotating at 4rpm constant speed to allow positioning of all the mice in their respective lanes. Keep their balance at 4 rpm constant speed for 60 sec.
  - 7.6.2 Clean apparatus with 70% EtOH, detergent and wipe it dry.
  - 7.6.3 It consists of three trials separated by 10 min inter-trial intervals (ITI).
  - 7.6.4 Training trial 2 (t2): repeat procedure as t1.
  - 7.6.5 Training trial 3 (t3): repeat procedure as t1.
  - 7.6.6 Repeat trial 3 once if a mouse falls off the rod before 60 sec cut off (not more than 4 trials at maximum).
  - 7.6.7 All mice have to stay on rod at 4 rpm for 60 secs before moving on to the test.
  - 7.6.8 Leave at least a 30 min break between training and test phases.
- 7.7 Test phase

It consists of three trials separated by 15 min inter trial intervals (ITI). It is possible to run a total of nine mice (three cages, 3 mice per cage) consecutively in one trial before moving to the next.

- 7.7.1 Set apparatus to accelerating mode from 4 to 40rpm in 300 sec. The apparatus will indicate “acceleration waiting” of 4rpm constant speed until the start button is pressed.
- 7.7.2 Test trial 1 (T1): place three mice from the same cage/strain on lanes 1, 3 and 5.  
Once all the mice are “ready” push the start button and the rod will be accelerating from 4rpm to 40rpm in 300 sec.
- 7.7.3 Record the latency and the rpm at which each mouse falls off the rod.
- 7.7.4 If a mouse is clinging on the rod and completes a full passive rotation stop the timer for that mouse by pushing down the lever and record the latency. Remove the mouse and place it back in its home cage. Be very careful not to disturb the other mice that are still running in the adjacent lanes. Also take note of passive rotation on the data sheet.
- 7.7.5 Clean apparatus with 70% EtOH, detergent and wipe it dry. Test the next two sets of three mice, repeating for both sets the procedure for trial 1 as in 7.7.2 – 7.7.5.
- 7.7.6 Leave a 15 min inter trial interval (ITI) between the beginnings of consecutive trials (e.g. T1-ITI-T2-ITI-T3).
- 7.7.7 Test trial 2 (T2): repeat procedure as trial 1 (T1).
- 7.7.8 Test trial 3 (T3): repeat procedure as trial 1 (T1).
- 7.7.9 Also note on the data sheet, any observations during the test, including occurrence of jumping, passive rotations etc.

