	Title: Rotarod test	
Standard Operating Procedure	Doc. Number: ESLIM_010_001 Rev No. 2	Date Issued: 06/08/2008

1.0 Purpose:

1.1 The rotarod test is used to assess motor coordination and balance in rodents. Mice have to keep their balance on a rotating rod. It is measured the time (latency) it takes the mouse to fall off the rod rotating at different speeds or under continuous acceleration (e.g. from 4 to 40rpm).

2.0 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 Head of Phenotyping Facility.

2.3 Any deviances from this protocol must be agreed by the Head of Phenotyping Facility.

3.0 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.

4.0 Associated Documents:

5.0 Notes

5.1 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 is familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.

5.2 The majority of mouse behavioural studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

5.3 Body weight might influence performance in this test. Thus, consideration of body weight is essential for better evaluation of the data.

5.4 Surface and diameter of the rod are major parameters affecting performance in this test. These parameters should be rigorously controlled.

5.5 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.

5.6 There is no training period prior to the test phase.

5.7 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.

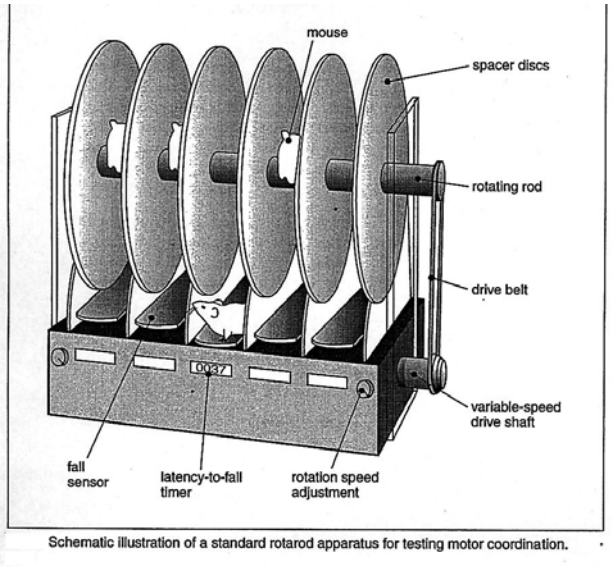
6.0 Quality Control:

7.0 Equipment:

7.1 Commercially available Rota Rod apparatus modified as described below:

7.2 Dimensions of the apparatus: Rotating rod diameter is ca. **5cm** made of hard plastic material **covered by grey rubber foam** (cut from insulation material to

cover water pipes); lanes width is ca. 5cm. The apparatus must allow an accelerating speed from 4rpm to 40rpm in 300 sec.



Schematic illustration of a standard rotarod apparatus for testing motor coordination.

From Current Protocols in Neuroscience, 2001

7.3 Stop watch

8.0 Supplies:

8.1 EtOH 50%
8.2 Paper towels

9.0 Procedure:

9.1 On the day of testing, mice should be kept in their home cages and acclimate to the testing room for at least 15 min (*acclimation phase*).

9.2 For ease of identification at later trials, mark the mice, using non-toxic ink, with respective stripes at the base of the tail before testing.

9.3 Replace the grey rubber foam prior to the start of testing each new cohort.

9.4 Turn on the Rotarod apparatus.

9.5 *Test phase*
It consists of **three** trials separated by **15 min** inter-trial intervals (**ITI**). It is possible to run the next batch(es) of mice consecutively in one trial before moving to the next. There is no training period prior to the test phase.

9.5.1 Set apparatus to accelerating mode from 4 to 40rpm in 300sec. The apparatus will indicate "acceleration waiting" of 4rpm constant speed until the start button is pressed.

9.5.2 Test trial 1 (T1): place the mice on the lanes (if handling the mice is difficult leave an empty lane between two mice and measure only 3 mice per run)**.**

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. Once all the mice are "ready" (i.e. check that they are able to walk forward for a few seconds at 4 rpm) push the start button and the rod will be accelerating from 4 rpm to 40 rpm in 300 sec.

9.5.3 Record the latency at which each mouse falls off the rod.

9.5.4 If a mouse clinging on the rod completes a **full passive rotation** stop the timer for that mouse by pushing down the lever and record the latency. In this case, passive rotation is considered a failure in performance like falling. 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 make a note of passive rotation on the data sheet.

9.5.5 Clean apparatus with water then with 50% EtOH, wipe it dry. Test the next set of mice repeating the procedure for trial 1 as in 9.4.2- 9.4.4.

9.5.6 Leave a **15 min** inter trial interval (ITI) between consecutive trials of the same batch of mice (e.g. T1-ITI-T2-ITI-T3).

9.5.6.1 At the end of trial 3, weigh each mouse and make a note of the body weight on the data sheet.

9.5.7 Note on the data sheet any observations during the test, including occurrence of jumping, passive rotations etc.

9.5.8 The whole test for three sets of mice, excluding the acclimation phase (at least 15 min), will require approx. **45min**

10.0 Data Records and Reports:

10.1. Data recorded:
Latency to fall -t1
Passive rotation -t1 (yes/no)
Latency to fall -t2
Passive rotation -t2 (yes/no)
Latency to fall -t3
Passive rotation -t3 (yes/no)
Latency to fall -mean
Body weight
Comments

11.0 Supporting information

11.1 Brooks SP, Pask T, Jones L, Dunnet SB (2004) Behavioural profiles of inbred mouse strains used as transgenic backgrounds. I: motor tests. Genes, Brain and Behavior 3: 206-215

11.2 Carter RJ et al. (1999) Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci 19:3248-3257

11.3 Carter RJ, Morton AJ, Dunnet SB (2001) Motor coordination and balance in rodents. Current Protocols in Neuroscience, unit 8.12.1-14. John Wiley & Sons, Inc.

11.4 Caston J, Jones N, Stelz T (1995) Role of preoperative and postoperative sensorimotor training on restoration of the equilibrium behaviour in adult mice following cerebellectomy. Neurobiol Learn Mem 64:195-202

11.5 Caston J, Vasseur F, Stelz T, Chianale C, Delhaye-Bouchaud N, Mariani J (1995) Differential roles of cerebellar cortex and deep cerebellar nuclei in the learning of the equilibrium behaviour: studies in intact and cerebellectomized Lurcher mice. Devel Brain Res 86:311-316

11.6 Crawley JN (1999) Behavioural phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioural tests. Brain Res 835:18-26

11.7 Dunham NW, Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharmac Assoc Sci Ed 46:208-209

11.8 Hilber P, Caston J (2001) Motor skills and motor learning in Lurcher mutant mice during aging. Neuroscience 102:615-623

11.9 McFadyen MP, Kusek G, Bolivar VJ, Flaherty L (2003) Differences among eight inbred strains of mice in motor ability and motor learning on a rotorod. Genes, Brain and Behavior 2: 214-219

11.10 Rozas G, Guerra MJ, Labandeira-Garcia JL (1997) An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism. Brain Res Prot 2:75-84

11.11 Rustay NR, Whalsten D, Crabbe JC (2003) Assessment of genetic susceptibility to ethanol intoxication in mice. Proc. Natl. Acad. Sci. USA 100: 2917-2922.

12.0 History review

In this version, only the latency is recorded. Passive rotations are recorded for each trial for potential subsequent analysis.
In the previous versions:

10_009, Version 1: the speed of rotation was also recorded and the foam rubber cover was standardised.

10_009, Version 0: there was no homogeneous foam rubber cover between all partners. Pre-training and 4 testing trials were performed.