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# ASSESSMENT OF MOTOR FUNCTION IN MICE MODEL OF PARKINSON'S DISEASE

Authors – **GEETHA.S, VINUTHA SHANKAR**

Affiliation - Department of Physiology, Sri Devaraj Urs Medical College, Tamaka, Kolar
Address – Department of Physiology, I floor, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka – 563 101
Telephone number – Geetha.S – 9964015609, Vinutha Shankar – 9845065374
Email of corresponding author – drgeetha.shivanna@gmail.com

**Abstract**

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. There is degeneration of the dopaminergic neurons in the basal ganglia leading to manifestation of motor symptoms of rigidity, resting tremors, bradykinesia and postural instability.

Several studies have been conducted to evaluate the behavioral changes in mice model of PD to validate a PD model, study the etiology and pathogenesis of PD, identify potential therapeutic targets, and develop drugs as a cure for this disease.

This article gives a summary of six, established tests of motor function including locomotion test, rotarod test, narrow beam walking test, pole test, adhesive dot removal test and hanging test.

This article provides information regarding the apparatus required for the tests, procedure for conducting these tests, expertise required in handling the animals to conduct the test, variables tested and ease of conduct of the test.

This article aims to provide guidance to budding researchers working with mice models of PD in selecting the appropriate tests of motor function for preclinical research.

Keywords - Parkinson's disease, mice model, motor function tests

Animal models are of immense value in studying diseases related to humans.
Three animal groups are commonly used in research related to human diseases. They are: rodents, non-human primates (NHP), and non-mammalian species. Among these groups, rodents are extensively used to model diseases afflicting humans, to develop a cure or treatment. Also, rodents are less expensive, smaller in size, require less space and are easy to care for in laboratory conditions, have well established experimental protocols including administration of different drugs and assessments of behavior. [1]

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Some of the human diseases for which mice have been used as models include diabetes mellitus, hypertension, obesity, Parkinson's disease, Alzheimer's disease, different types of cancers, stroke, etc**.** [2]

Parkinson's disease (PD) is a neurodegenerative disease that progresses gradually and is progressive and irreversible in nature. PD causes degeneration of the dopaminergic neurons in the substantia nigra of the basal ganglia. PD patients present with several symptoms including the manifestation of the four characteristic motor symptoms of resting tremor, muscular rigidity, akinesia and postural instability. [3] Presentation of these symptoms by the patients forms the basis of most behavioral testing in mouse models of PD.
Currently the etiology of PD is not clearly understood, and a lot of research is being conducted to understand the etiopathogenesis of PD. Presently, there is no cure for PD and hence, a suitable PD model is necessary to study its motor and non-motor symptoms, and to identify the potential therapeutic targets to aid in development of novel drugs.
Two ways of developing a model for PD is by use of neurotoxins or genetics based approach. Neurotoxin based models are easier to develop and less expensive.
The neurotoxins used are 6-hydroxydopamine (6-OHDA), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), and pesticide rotenone, the herbicide paraquat, and the fungicide maneb. [1]

Rats are resistant to MPTP toxicity [4] and differences in MAO activity have been proposed as the reason for their low susceptibility. The C57BL/6 mouse strain is most sensitive to MPTP [5,6]
A battery of tests exists to assess various behavioral parameters including locomotor activity and motor coordination. These tests include the rotarod test, pole climbing test, actophotometer test, stride test, stair climbing test, narrow beam walking test and adhesive dot removal test.
The most commonly used tests to assess motor function in mice are the rotarod test and the locomotor test.
As part of a larger study involving the C57BL6 mice model of Parkinson's disease, a panel of behavioral tests was conducted by the authors as a baseline study of motor function. The tests conducted were actophotometer test, rotarod test, hanging test, narrow beam walking test, pole test and adhesive dot removal test.
The study has been approved by the institution animal ethics committee of Sri Devaraj Urs Medical College, Kolar.
32 male, C57BL6 mice, weighing 22-24 g were purchased from Biogen laboratory animal facility, Anekal, Bangalore.
All the tests were conducted after one week of acclimatization of the animals. The experiments were conducted in the central animal house of Sri Devaraj Urs Medical College, Kolar.

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Animals were housed in standard mice cages with ad libitum access to food and water. Four mice were housed together in a cage. Temperature was maintained at 20-21 ˚C. Housing conditions were maintained under 12 hour light: 12 hour dark cycle.

**Locomotion test**

The locomotor activity was measured using an Actophotometer. An actophotometer consists of a solid metallic cage. At the bottom, the instrument has six lights and six photocells in the outer periphery. They are situated such that a mouse blocks only one beam of light at a time. When light falls on the photocells, they get activated. When a mouse crosses a light beam, the beam is interrupted. The number of such interruptions is recorded for 10 minutes. [7]

**Rotarod test**

Motor coordination was tested using a Rotarod apparatus. The apparatus consists of a motorized, circular rod that rotates at a constant or accelerating speed. It has 2-3 vertical barriers which divides the apparatus into 3-4 lanes or compartments and separates the animals from one another. At the bottom, each lane contains timers with fall sensors. These sensors stop the timers when the animal is no longer able to run on the rotating rod and falls down and record the time for which the animal was on the rotating rod.

The animals were first conditioned on a stationary rod for 30 s. Any animal that fell during this time was placed back on the rod. The animals were next placed on the rod set to rotate at a constant speed to 10 rpm for a period of 10 minutes. Animals that failed the first training were given two additional trials. For the test, the animals were placed on the rod, rotating at a constant speed of 30 rpm. The time for which they remained on the rod was noted. The test was repeated thrice, and the average was calculated. [8]

**Hanging Test**

The hanging test makes use of the natural instinct of the mice to hang on to a support to avoid falling. This test assesses the muscle strength and coordination ability of the animals.

In this test, the mice were placed on a lid of the mouse cage and allowed to grip the lid. The lid was then inverted and suspended above the mouse cage. The time taken for the animal to fall was recorded. Each animal was tested thrice and the average of the three trials was calculated. [9]

**Narrow Beam Walking Test**

Fine motor coordination and balance can be assessed by the narrow beam walking test. This test records the time taken for a mouse to stay upright and walk across an elevated narrow beam from one end to another.

A narrow wooden beam (100 cm long and 1 cm wide) was placed at a height of 100 cm above the ground. At one end of the beam, a bright light was fixed to encourage the animals to move towards the opposite end which contained a small, dark, wooden box.

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The animals were trained to move from the light end of the beam to the opposite end. All animals received three trials with an inter-trial time of 5 minutes. The animals were then tested thrice on the beam and the average was calculated. [10]

**Pole test**

The pole test tests the ability of a mouse to grasp a wooden pole and maneuver itself in order to descend to its home cage.

The apparatus consisted of a vertical, wooden pole (length= 548 mm, diameter= 8mm) placed inside a mouse cage. The time taken by the animal to turn itself and descend from the top of the pole to the bottom was measured.

All animals were trained in the procedure three times. The animals performed this test thrice during the test and the average of the tests was calculated.[11]

**Adhesive dot removal test**

This test is used to evaluate fine motor function.

Small adhesive stimuli (quarter inch round stickers) were placed on the snout of each mouse using a pair of forceps. The mice raise their forepaws and attempt to remove the stickers from their snouts. The time taken to remove the stimuli was recorded. The adhesive was manually removed if the mouse failed to remove the adhesive within 60 s. The test was conducted three times and the average was calculated. Each animal was given three trials and the average was calculated. [12]

| Test | Apparatus required | Cost of the apparatus – expensive/ can be sourced or constructed locally | Training of the animal required – yes / no | Variable measured | Requires experienced animal handler – yes / no |
|---|---|---|---|---|---|
| Locomotion test | Actophotometer | Expensive | No | Number of crossings of the light beam | No |
| Rotarod test | Rotarod | Expensive | Yes | Duration for which the animal stays on the rotating rod | Yes |

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|---|---|---|---|---|---|
| Hanging test | Mouse cage lid | Can be sourced or constructed locally | No | Duration for which the animal hangs onto the cage lid by its paws | No |
| Narrow beam walking test | Narrow wooden beam of 100 cm length and 1 cm width place 100 cm above the floor | Can be sourced or constructed locally | Yes | Time taken by the animal to traverse the beam from one end to another | Yes |
| Pole test | Wooden pole, 50 cm in height, 0.5 cm in diameter | Can be sourced or constructed locally | Yes | Time taken by the animal to turn around and climb down an erect pole | Yes |
| Adhesive dot removal test | Forceps and circular adhesive dots of 4 mm diameter | Can be sourced or constructed locally | No | Time taken by the animal to remove the adhesive dot from its snout using its forepaws | Yes |

**Table 1: tests of motor function**

## Conclusion

PD patients manifest both motor and non-motor symptoms. Testing for motor impairment in Parkinson's model mice is relatively easy. A panel of behavioral tests exists to test motor functions. Researchers can choose the tests they conduct depending on the feasibility, ease of conducting the experiments and their experience in handling the animals.

However, it is not enough to choose one best test of the selection available. Researchers must bear in mind that different tests assess different aspects of motor function and more than one test would be required to evaluate all the aspects of motor function.

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**Conflict of Interest statement**

The authors have no conflicts of interest to declare. The co-author has seen and agrees with the contents of the manuscript and there is no financial interest to report. We certify that the submission is an original work and is not under review at any other publication.