JAINFOUNDATION LGMD2B/R2 | DYSFERLINOPATHY | MIYOSHI
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Assessment of Motor Balance and Coordination in mice using the Balance beam

1. Objective

The balance beam test measures a mouse's ability to traverse a narrow beam between two raised platforms. Fine motor coordination and balance can be assessed by this assay. The goal of this test is for the mouse to walk across an elevated narrow beam to a safe platform without slipping or falling.

The mouse phenotype of dysferlinopathy is mild, which makes it challenging to find a functional readout that adequately tracks the progress of the disease and the effectiveness of potential treatments. The advantage of this task is in the detection of subtle deficits in motor skills and balance that may not be detected by other motor tests, such as the treadmill or rotarod.

2. Scope and applicability

Initial evaluation of the balance beam test in dysferlinopathy was done by the laboratory of Christoph Handschin at the University of Basel. Mice with dysferlinopathy have trouble traversing the balance beam when compared to wild type mice. Dysferlin deficient mice move slower across the beam (time to cross) and their hind feet may occasionally slip off the beam. Gait analysis and MRI evaluations show that hip muscles are highly affected in dysferlin deficient mice, which may lead to difficulty for the mice to correctly position their feet on the beam as their ability to compensate for their hip weakness by using a wider stance is limited by the width of the beam. Thus, the mice struggle to traverse the narrow balance beam, which manifests as a longer time needed to traverse the beam as well as an increased frequency of foot slips from the beam.

Following the adoption of the balance beam evaluation protocol by the Handschin lab, several other labs funded by the Jain Foundation have used the balance beam to test dysferlin-deficient mice. An online workshop was held to compare protocols, experiences, and findings from the different labs. This document summarizes the researchers' findings and recommendations for others interested in using the balance beam test in their studies.

3. General considerations

The following points should be considered when planning experiments.

A. **Age**: Dysferlin deficient mice show symptoms of disease starting at about six months of age. Most researchers have used the balance beam to assess mice between 7 and 12 months of age. The test becomes more challenging as mice become older, due to progression of muscle weakness in the hips as well as the increasing size and weight of the mice.

B. **Width of beam**: Beam width can be used to adjust the level of difficulty of the assessment with participating labs experimenting with widths between 6-30 mm wide and widths of 6 to 12 mm seeming to have the most consistent results. A square beam with sharp corners is preferred, as rounded corners reduce the effective width in ways that are difficult to consistently reproduce between different size beams and complicates comparisons. The optimal width of the beam should be planned while taking into consideration the animals' age. Wide beams (>12mm) are easy to traverse at all ages and may not be challenging enough to evoke a difference in crossing time between WT and dysferlin deficient mice, with the exception that very old or highly affected mice may each require a wider beam to complete the task. In general, mice traverse more slowly on narrower beams, and a beam can be too narrow for mice experiencing advanced symptoms to complete a beam crossing. Also, older

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wild type mice sometimes have difficulty crossing a narrow beam depending on their size and weight. There is a growing consensus that beams between 6mm and 12mm present enough of a challenge to see differences between WT and dysferlin deficient mice between 7 and 12 months while allowing even the most severely dysferlinopathic animals to complete the test.

C. **Beam material**: The surface should allow good traction but not be porous, to facilitate effective cleaning and prevent accumulation of distracting odors. A material that is easy to clean is preferable; metal and plastic are popular options.

D. **Slope and testing distance of beam**: Recommended slope is between 7-10 degrees (upward in the direction the mouse is traveling). Higher slope angles can inhibit the mouse's motivation to traverse the beam. Testing distance is marked by tape and lengths of 70cm-80cm are generally sufficient.

E. **Rewards**: To encourage the mice to traverse the beam two incentives are useful 1) a house at the end of the beam that is dark inside with bedding material combined with bright lighting on the starting platform and balance beam. 2) Food in the house during training runs only. Extensive training usually does not require a reward/incentive.

F- **Operator and Time of day**: Mouse stress and anxiety can affect the assay. Therefore, it is imperative that the operator is comfortable in mouse handling and that the same person performs the assay to minimize inducing behavioral differences in the mice. Training and testing should be performed at a consistent time of day; because mice are nocturnal, testing in the morning (at the end of the mice's waking period) may be preferable.

G- **Cleaning**: Scents from other mice, especially opposite sex, is a distraction that will affect the assessment. Cleaning the beam and platforms with ethanol or hydrogen peroxide should be performed between mice and particularly when switching genders.

H- **Training and Testing** : Familiarizing mice with the apparatus before data collection runs is imperative. Training guidelines: A) Training should commence 2 or 3 days before the test day. B) Mice initially cross on a wider width beam, for three times; rest for 10-15 seconds. C) Second set of training will be on a smaller beam width, for three times; rest for 10-15 seconds. D) 10 minutes rest before changing the beam width. E) Each set of mice and beam width is duplicated or triplicated for practice. Training should be performed by the same operator who will be conducting the test.

I- **Scoring**: On the test day, times to cross each beam are recorded. Two successful trials in which the mouse crossed the beam without stopping or turning around on the beam are averaged. Slip counts can also be recorded. A slip is defined as the foot missing or coming off the top of the beam. In some cases, animals will leave a leg or two hanging off the beam and drag their body along the beam. Researchers have included this behavior in the number of slips or recorded the behavior as an independent measure. The number of slips can be reported directly or in a "Bin" (e.g., no slips, 1-2 slips, 3-5 slips, etc.). A mouse whose feet slip off the beam repeatedly will typically take longer to cross the beam, so counting slips is somewhat redundant with crossing time and often more variable and problematic to record but can offer some additional detail to the data set.

J- **Video recording is highly recommended**: Video recording each crossing can improve the accuracy of timing each crossing and counting slips. If slip counts are to be collected, then Front and Rear view video recording is necessary. Placing a mirror behind the beam can facilitate video recording of both sides of the animal during a crossing.

4. Materials

1. Beam Apparatus
2. Cleaning reagents (70% Ethanol or 0.5% Peroxide)

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3. Timer
4. Video recording device
5. Nesting material from home cage

Most of the material for an apparatus can be found in hardware stores. Commercially manufactured apparatus are available from:
https://conductscience.com/lab/balance-beam-maze/ & https://www.panlab.com/en/products/item/601-beam-balance-test

6. Apparatus Image

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9. References

1) Luong, T. N., Carlisle, H. J., Southwell, A., Patterson, P. H. Assessment of Motor Balance and Coordination in Mice using the Balance Beam. J. Vis. Exp. (49), e2376, doi:10.3791/2376 (2011).