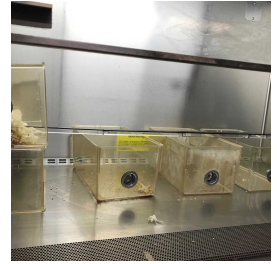


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Beam traversal

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We use this protocol and it's working

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Abstract

This test measures the time for mice to cross a 1-meter plexiglass beam with segments decreasing in width (3.5 cm, 2.5 cm, 1.5 cm, 0.5 cm). Mice were trained for two days before testing. On testing day, mice were placed at the start of the 3.5 cm segment for three trials and time to cross the beam was recorded. Time was recorded when hindlimbs reached the home cage, with a maximum time of 60 seconds.

Protocol has been approved by the California Institute of Technology's Institutional Animal Care and Use Committee (IACUC).



Information

- 1 Experiments involving GF and GF x SPF comparisons were conducted inside germ-free isolators. For comparisons involving only SPF animals, experiments were completed in a biological safety cabinet. All experiments were performed between ZT6 and ZT10 of the light phase and started at the same time each day.

Beam traversal set-up

- 2 The Beam transversal consist in 1-meter plexiglass beam with segments decreasing in width (3.5 cm, 2.5 cm, 1.5 cm, 0.5 cm).
- 3 All the beam segments are connected (from larger to smaller) and placed on top of 4 empty mouse cages.
- 4 Mice are transferred to a new cage, and their home cage containing the used bedding will be used for training and testing (see image at description for set up).
- 5 Wipe the beam and empty cages with 70% EtOH before beginning any trials and between each mouse.

Acclimation

- 6 On each training and assessment day bring the mice up from the vivarium to the behavior room at least 1h prior to assessment to allow for acclimation to the environment prior to assessment.

Training Day 1

- 7 Determine the order of assessment that you will use for the length of the training and assessment, record this in lab notebook.
- 8 Mice are transferred to a new cage, and their home cage containing the used bedding is placed at the end of the 0.5 cm segment (see image at description).
- 9 Training day 1 will then consist of five trials for each mouse.
- 10 Trial 1: Place the mouse at the beginning of the 3.5 cm segment, with the home cage positioned at the end of this segment. Allow the mice to freely move from the beginning of the



segment to the home cage.

- 11 Trial 2: Place the mouse at the beginning of the 2.5 cm segment, with the home cage positioned at the end of this segment. Allow the mice to freely move from the beginning of the segment to the home cage.
- 12 Trial 3: Place the mouse at the beginning of the 1.5 cm segment, with the home cage positioned at the end of this segment. Allow the mice to freely move from the beginning of the segment to the home cage.
- 13 Trial 4: Place the mouse at the beginning of the 0.5 cm segment, with the home cage positioned at the end of this segment. Allow the mice to freely move from the beginning of the segment to the home cage.
- 14 Trial 5: Place the mouse at the beginning of the 3.5 cm segment, with the home cage positioned at the end of this segment of 0.5 cm segment. Allow the mice to freely move from the beginning of the segment to the home cage.

Training Day 2

- 15 Determine the order of assessment that you will use for the length of the training and assessment, record this in lab notebook.
- 16 Mice are transferred to a new cage, and their home cage containing the used bedding is placed at the end of the 0.5 cm segment (see image at description).
- 17 Place the mouse at the beginning of the 3.5 cm segment, with the home cage positioned at the end of this segment of 0.5 cm segment. Allow the mice to freely move from the beginning of the segment to the home cage.

Test Day

- 18 Determine the order of assessment that you will use for the length of the training and assessment, record this in lab notebook.
- 19 Mice are transferred to a new cage, and their home cage containing the used bedding is placed at the end of the 0.5 cm segment (see image at description).
- 20 Place the mouse at the beginning of the 3.5 cm segment, with the home cage positioned at the end of this segment of 0.5 cm segment. Allow the mice to freely move from the beginning of the segment to the home cage.
- 21 Begin the stopwatch/timer as soon as you place the mouse at the beginning of the 3.5 cm segment. Stop timing once all 4 paws are touching the home cage.



- 22 Repeat this 2 more times (total of 3 trials) for each mouse.
- 23 Allow the mouse to rest for 30 sec between trials.
- 24 After each mouse complete 3 trials they are moved to a new cage.
- 25 Clean the beam and cages with 70% EtOH between each mice.
- 26 After all mice that belong to the same cage complete 3 trials, they are moved back to their home cage.
- 27