

Description of procedure:

This test provides quantitative gait analysis as an objective assessment of walking ability. Parameters collected include stride pattern, coordination, stance duration, and paw swing speed. As this is a general description of the standard materials, test settings and procedures, variations may be required to fit specific needs.

Procedure Steps:

Subjects require training and should be acclimated to the testing environment, the apparatus, and the experimenter before testing. The apparatus consists of an illuminated glass walkway on a frame with a camera mounted underneath the walkway. Gait analysis software (e.g., CatWalk by Noldus, license and dongle required) is used. The animal should traverse the walkway in a brisk, even stride with no hesitation, rearing, or reversing. To achieve this, the home cage (retrofitted with a ramp or step to make the cage accessible from the walkway) or a dark-colored shelter with some bedding from the home cage can be placed at the end of the walkway. Treats can also be used to increase motivation.

1. Test environment: The room needs to be very dark. The computer screen should be dimmed as much as possible, and all other lights should be turned off.
2. Acclimation: subjects in the home cages are placed in the testing room for at least 30 minutes before testing to minimize the effects of stress on behavior during testing.
3. After acclimation, suggestions for training the animal to traverse walkway without hesitation, rearing, or reversing:
 - Place the animal at the edge of the walkway closest to the home cage/escape box and allow it to enter and rest in the target area for up to 1 minute.
 - Then, place the animal about halfway down the walkway, facing the target area. If the animal easily traverses and enters the target area, then training can stop. Use these steps to train the animal to enter the target area, but don't allow the animal to habituate to the walkway, as the novelty of the walkway, and the desire to enter the target area should remain high. Habituation will prevent proper implementation of the test due to the animal potentially stopping to groom, etc.
4. Testing procedures:
 - The animal is placed at the far end of the walkway and allowed to traverse the walkway toward the target escape area.
 - Repeat this until three compliant runs (defined by a minimum of 4 complete sequence steps with no interruptions while traversing walkway) are recorded. Animals unable to complete three compliant runs are returned to their home cage and attempted again at end of day.
 - After three compliant runs are obtained, the animal is returned to their home cage and apparatus is cleaned with at least 70% ethanol, so no paw prints

are visible.

5. Data analysis can include the following parameters: step sequence, initial & max contact, stride length, print area, base of support, print position, swing mean intensity, and swing speed.

Any animals showing signs of distress will be removed from the apparatus and allowed to rest in the home cage. Animals consistently showing signs of distress over multiple days will be removed from the study. Researchers should contact LARC veterinary team if they have questions about signs of pain or distress.