

Passive Avoidance Test
IACUC Standard Procedure
Effective Date: May 2025

Description of Procedure:

This procedure evaluates learning and memory through fear-motivated associations. The shock used in this task should be the minimum intensity and duration needed to elicit a reaction from the animal, which may vary according to strain. This standard procedure is specifically for tests using a 0.5-2 second, 0.2-0.5 mA foot shock, which is the minimum required to elicit flinching, running, jumping, and/or vocalization. Foot shocks of greater intensity and duration must be described in the IACUC protocol and may be Category E. Mice exhibiting any abnormalities indicative of locomotor disabilities or pain perception (e.g., hyperalgesia) must be excluded from testing. To avoid introducing bias when conducting multiple behavior tests in the same animal, tests should be conducted in order from least to most invasive and approved latency in between tests should be respected.

Supplies:

- Dual compartment testing apparatus
- Electroshock generating device
- Environment: Testing room should be dimly lit with either red or white light

Procedure Steps:

Habituation:

Habituation is typically not recommended for this assay. Preventing the animals from building a context association where they start exhibiting a fear response to the room where the shock test takes place.

Training Trial:

1. The testing apparatus is a closed arena divided into two distinct compartments that are separated by a sliding door. The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment is equipped with shock capability.
2. The apparatus is cleaned before use, per the cleaning SOP that must be approved and attached to the protocol.
3. The training trial begins by placing the animal in the bright compartment facing the door.
4. After a pre-determined latency, the door is opened to allow access to the dark compartment.
5. The latency to enter the dark compartment is recorded.
6. When the animal steps into the dark compartment with all four paws, the door is closed and a 0.5-2 second foot shock is delivered (0.2-0.5 mA shock, minimum required to elicit flinching, running, jumping, and/or vocalization).
7. The animal remains in the dark compartment for an additional 10-30 seconds after the termination of the aversive stimulus before being removed and placed back into a clean, temporary cage until all cage mates have finished testing, after which animals are returned to the home cage.
8. The apparatus is cleaned (e.g., with 70% ethanol) in between animals.

Test Trial:

1. At the time of the test trial (usually 1-14 days after training), the animal is placed inside the bright compartment and the door is raised to allow access to the dark compartment.
2. The latency to enter the dark compartment is recorded; however, there is no aversive

- stimulus applied to animal upon re-entry into the dark compartment during testing.
3. This test session can be conducted multiple times to assess the extinction of the learned association.

Protocol requirements:

RIO Documents Section

- Email a completed "[LARC Behavioral Equipment Sanitation SOP](#)" form to LARC (ORRodentNurses@ucsf.edu) for review and to request ATP testing. Once sanitation procedure is approved, upload the completed form to Documents section of RIO protocol and post the form in the procedure room.