Open Field 開放空間移動軌跡分析

1. Purpose

1.1 This test measures the level of locomotor activity of the tested mice and their patterns of movement when the subjects are placed in a slightly large and open space that may cause anxiety and fear. Open field test can be done repeatedly without interfering with other behavioral study and is applicable to study the locomotion and anxiety levels of mice before and after compound administration.

2. Safety Requirements

2.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

3. Notes

3.1 The validity of results obtained from behavioral phenotyping is largely dependent on methods of animal husbandry. It is important that individuals following this procedure are experienced and aware of the animal's welfare, and be familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.

3.2 The majority of mouse behavioral studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

3.3 Environmental factors may contribute to the levels of mouse anxiety. The temperature, humidity, ventilation, noise intensity and light intensity must be maintained at levels appropriate for mice. It is essential that the mice be kept in a uniform environment before and after testing to avoid anomalous results being obtained. In particular, background noise and illumination levels should be measured and documented for each room. Ideally, all mice should be exposed to the same illumination levels in the holding room. For example, in conventional housing, mice housed on the top of the racks may have up to 10 times more Lux than mice on the bottom of racks. No additional experiments which are either noisy or emit odors should be performed during acclimation and testing in the antechamber and the testing room. Ensure that during the test animals are not exposed to any distracting visual signals.

3.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day. The ideal testing time for all animals is during the first half of the lights-on period (in the morning until early afternoon). If animal groups are tested at different times of the day it is necessary to perform subsequent tests analogous to the initial test in the experimental design.

3.5 Light is an important anxiogenic factor that will strongly influence the ambulation in a U-shaped way. Experiments under various illumination intensities and factor-analysis have shown that locomotion under dim light is a measure of activity rather than of fear (e.g. Trullas & Skolnik 1993). Most

pharmacological studies have shown that anxiolytic agents are more likely to have an effect on the area of the activity rather than on the amount of activity itself (Crawley & Paylor 1997; Choleris et al. 2001).

4. Quality Control

4.1 Before each experiment, measure illumination with a Luxmeter and make a record. To obtain evenly distributed light, indirect lighting should be employed. Alternatively, an illumination system for simple and even illumination can be employed. It is most important that light is not significantly darker in the corners (avoid shadows), to prevent bias in the locomotor activity of the animal.

5. Equipment

5.1 Illumination system, Luxmeter, EthoVision video tracking system.

5.2 The equipment is constructed as following:

5.2.1 The open field is a square arena no smaller than 50 x 50 cm.

5.2.2 Walls should be opaque (so that animals cannot see the room) and approximately 35 cm high.

5.2.3 The base color of the open field is white, when C57BL/6 mice are used as subjects and a video-tracking system is used.

5.3 Video-tracking system. The EthoVision video tracking system is employed to analyze data with different zones/parameters.

6. Supplies

6.1 Pens
6.2 Marker pen
6.3 Datasheet
6.4 Gloves
6.5 Facial mask
6.6 Ethanol 70%
6.7 Detergent (Windex)
6.8 Hand towels
6.9 Kimwipes
6.10 Color paper
6.11 Battery

7. Procedures

7.1 Minimum n=6 mice per experimental group.

7.2 On the day before testing mice should be individually marked to be easily identified on the test day. One suggestion is to mark their tails.

7.3 Transport animals for testing and leave undisturbed for 30 minutes before the test in the home cage in testing room.

7.4 Wipe the open box clean with detergent (Windex) and 70% ethanol; allow time for it to dry.

7.5 Ensure that the light conditions are set appropriately and that all equipment is working correctly.

7.6 Switch on the computer and record mouse activity by EthoVision video tracking system for a period of time (5, 10, 30 or 60 minutes) according to the experimental requirement.

7.7 Remove a mouse from its home cage, gripping the tail between the thumb and the forefinger and place it into the middle of one side of the arena facing the wall. If more than one mouse can be tested in parallel, in adjacent open field arenas and mice are video-tracked, it is important to ensure that the tracking of each mouse starts as soon as the mouse is released to make data comparable.

7.8 Following the experimental session, remove the mouse carefully from the open field arena, gripping the tail between the thumb and the forefinger, and place in its home cage.

7.9 Wipe the open box clean with detergent (Windex) and 70% ethanol after each experimental session to avoid olfactory cueing. Allow time for it to dry.

7.10 Set up the appropriate database and arena range in the program so that mouse tracking can be analyzed by EthoVision system software.

7.11 Save data from the experimental sessions onto a disc and analyses.

7.12 The results are presented in 1 excel file and 1 track image file.