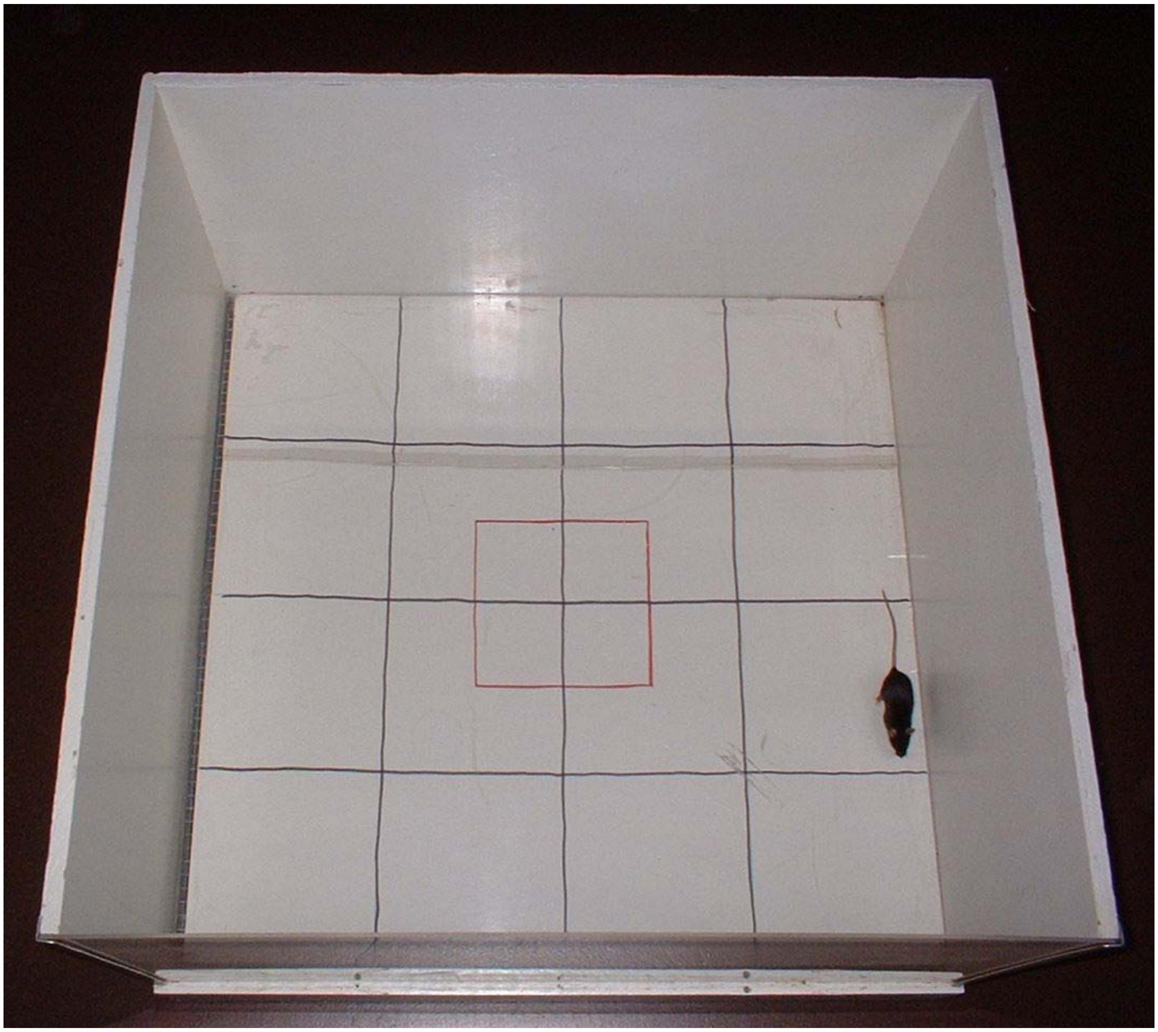
The Open Field Test



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The open field can be of different sizes; small (38 x 38 cm), or large (72 x 72 cm). The small open field can also serve as a hole board and as a test chamber for the novel object recognition task. The large open field is used for measuring anxiety and exploration as well as locomotion as it has a large center arena.

Apparatus

The open field apparatus was constructed of white plywood and measured 72 x 72 cm with 36 cm walls. One of the walls was clear Plexiglas, so mice could be visible in the apparatus. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field (Brown, Corey, & Moore, 1999). The central square is used because some mouse strains have high locomotor activity and cross the lines of the test chamber many times during a test session. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations (Carrey, McFadyen, & Brown, 2000).

The maze was located in a 1.8 x 4.6 m test room and lit by a 60-watt red lamp for background lighting. The open field maze was cleaned between each mouse using 70 % ethyl alcohol. Behavior was scored with Hindsight for MS-dos (ver 1.5), and each trial was recorded for latter analysis, using a video camcorder (Hitachi, VM-7500LA) positioned above the apparatus. Measures of line crosses were obtained with an automated camera-based computer tracking system (Limelight, Actimetrics) on an IBM PC computer with the camera fixed to the ceiling, 2.1 m above the apparatus.

Procedure

Mice were carried to the test room in their home cages and were handled by the base of their tails at all times. Mice were placed into the center, or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, mice were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and permitted to dry between tests. To assess the process of habituation to the novelty of the arena, mice were exposed to the apparatus for 5 minutes on 2 consecutive days.

Behaviours scored

The behaviours scored (Brown et al, 1999) included:

1. Line Crossing: Frequency with which the mice crossed one of the grid lines with all four paws.
2. Center Square Entries: Frequency with which the mice crossed one of the red lines with all four paws into the central square.
3. Center Square Duration: Duration of time the mice spent in the central square.
4. Rearing: Frequency with which the mice stood on their hind legs in the maze.
5. Stretch Attend Postures: Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
6. Grooming: Duration of time the animal spent licking or scratching itself while stationary.
7. Freezing: Duration with which the mouse was completely stationary.
8. Urination: number of puddles or streaks of urine.
9. Defecation: number of fecal boli produced.

Each animal was then given a score for total locomotor activity that was calculated as the sum of line crosses and number of rears.

What is Measured in the Open Field Test?

The Open Field Test (Walsh & Cummins, 1976) provides simultaneous measures of locomotion, exploration and anxiety. The number of line crosses and the frequency of rearing are usually used as measures of locomotor activity, but are also measures of exploration and anxiety. A high frequency of these behaviours indicates increased locomotion and exploration and/or a lower level of anxiety. The number of central square entries and the duration of time spent in the central square are measures of exploratory behaviour and anxiety. A high frequency/duration of these behaviours indicates high exploratory behaviour and low anxiety levels.

Stretch attend postures are “risk-assessment” behaviours which indicate that the animal is hesitant to move from its present location to a new position (Blanchard, Griebel & Blanchard, 2001) and thus a high frequency of these postures indicates a higher level of anxiety. Grooming behaviour is a displacement response and is expected to be displayed in a novel environment (Espejo, 1997). Therefore grooming behaviour should decrease with repeated exposure to the testing apparatus. Defecation and urination are often used as measures of anxiety, but the validity of defecation as a measure of anxiety has been questioned (Lister, 1990). Hall (1934) describes defecation and urination as indices of anxiety in rodents. He argues that the animal will have reduced locomotion in a novel environment but the autonomic nervous system will be activated which will increase defecation in this noxious arena. However, Bindra & Thompson (1953) argue that there is no significant relation between fearfulness and urination and defecation as measured in the Open Field test. Nevertheless, Bindra & Thompson (1953) agree that defecation and urination in a novel environment are signs of emotionality, which is not to be equated with fearfulness or timidity.

Factor Analysis

Several dependent variables measured in the open field correlate significantly with one another, such as: line crosses and rearing, as well as line crosses and central square activity (Walsh & Cummins, 1976).

Factor analysis of open field behaviour generally yields 3 factors but the names of these factors vary. Jahkel et al. (2000) identified 3 factors: **Activity** (53.5% of the variance) (line crosses, time active); **Exploration** (15.5% of the variance) (Center squares crossed, time in center); and **Irritation** (9.5% of the variance) (time passive). Ramos et al. (1997) also identified 3 factors: **Anxiety or Approach/Avoidance** (36.6% of variance) (locomotion in center squares); **Locomotor Activity** (30.3% of variance) (total lines crossed, lines crosses in outer squares) and **Defecation** scores (18.3% of variance). Crusio & Schwegler (1987) also found 3 factors: **Locomotion** (rearing, lines crossed); **Grooming** (grooming frequency and duration); and **Defecation** (sniffing, defecates). These studies did not always measure the same behaviours and the different factors identified indicate that the definitive study of the open field test has yet to be conducted.

Repeated Exposure

Repeated exposure to the open field apparatus results in time dependent changes in behaviour (Choleris, et al., 2001). At first, when the apparatus is novel to the animals, more fear-related behaviours (such as stretch attends and activity in the corners and walls of the open field) are displayed. However, with repeated trials, more exploration and locomotor activity (such as rearing and line crosses as well as more central square activity) is observed. There are, however, strain differences in behaviour after repeated testing in the open field. With repeated exposure, some strains show increased activity while others show habituation and decreased activity levels and others show no change (Bolivar et al. 2000).

7.5 Statistical analysis

The statistical analysis will be completed using Statview v5.0.1 (SAS instruments) for windows. Data collected from the open field will be analyzed using a repeated measures ANOVA (between strain and sex). Post hoc comparisons were made using the Student-Newman-Keuls design during both days of exploration.

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