Video Article

Elevated Plus Maze for Mice

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Abstract

Although the mouse genome is now completely sequenced, the functions of most of the genes expressed in the brain are not known. The influence of a given gene on a specific behavior can be determined by behavioral analysis of mutant mice. If a target gene is expressed in the brain, behavioral phenotype of the mutant mice could elucidate the genetic mechanism of normal behaviors. The elevated plus maze test is one of the most widely used tests for measuring anxiety-like behavior. The test is based on the natural aversion of mice for open and elevated areas, as well as on their natural spontaneous exploratory behavior in novel environments. The apparatus consists of open arms and closed arms, crossed in the middle perpendicularly to each other, and a center area. Mice are given access to all of the arms and are allowed to move freely between them. The number of entries into the open arms and the time spent in the open arms are used as indices of open space-induced anxiety in mice. Unfortunately, the procedural differences that exist between laboratories make it difficult to duplicate and compare results among laboratories. Here, we present a detailed movie demonstrating our protocol for the elevated plus maze test. In our laboratory, we have assessed more than 90 strains of mutant mice using the protocol shown in the movie. These data will be disclosed as a part of a public database that we are now constructing. Visualization of the protocol will promote better understanding of the details of the entire experimental procedure, allowing for standardization of the protocols used in different laboratories and comparisons of the behavioral phenotypes of various strains of mutant mice assessed using this test.

Video Link

The video component of this article can be found at http://www.jove.com/video/1088/

Protocol

Protocol

1. The apparatus used for the elevated plus maze test is in the configuration of a + and comprises two open arms (25 x 5 x 0.5 cm) across from each other and perpendicular to two closed arms (25 x 5 x 16 cm) with a center platform (5 x 5 x 0.5 cm). The open arms have a very small (0.5 cm) wall to decrease the number of falls, whereas the closed arms have a high (16 cm) wall to enclose the arm. The entire apparatus is 50 cm above the floor (Ohara & Co., Tokyo) and is placed in an empty circular tank (100 cm diameter, 35 cm tall; normally used for the Morris water maze task) to protect the mice that fall or attempt to escape during the experiment. The apparatus is made of plastic materials. The platform is white and the walls are transparent. There is a variation in materials and colors of the apparatus of elevated plus maze.
2. Mice are housed with a 12-h light/dark cycle (lights on at 7:00 AM), as previously described (Takao & Miyakawa, 2006a). Behavioral testing is performed between 9:00 AM and 6:00 PM. All the experimental mice are transferred to the behavior testing room 30 min prior to beginning the first trial to habituate to the condition of the behavior testing room. The order of trials is counterbalanced across genotypes. There are two purposes of the test trial using a practice animal. One is to make sure that everything is fine with the recording system. Another is to keep the testing condition as uniform as possible. That is, the very first mouse in the entire session would experience somewhat different condition with others (i.e., no prior noises made by testing operations and no odor cues from previous trials) without such a practice trial. Animals are maintained according to the guidelines of the Animal Research Committee of Kyoto University.
3. The behavior testing room (170 x 210 x 200 cm, Ohara & Co., Tokyo) is soundproof and the illumination level is maintained at 100 lux. A mouse is placed in the center area of the maze with its head directed toward a closed arm. The elevated plus maze test is recorded using a video camera attached to a computer, which is controlled by a remote device. The number of entries (an entry is defined as the center of mass of the mouse enters the arm) into each arm and the time spent in the open arms are recorded and these measurements serve as an index of anxiety-like behavior.
4. Mice are allowed to move freely about the maze for 10 min. Each mouse receives one trial in our test battery. The application used for acquiring and analyzing the behavioral data (Image EP) is based on the public domain Image J program (developed by Wayne Rasband at the National Institute of Mental Health and available at http://rsb.info.nih.gov/ij/), which was modified by Tsuyoshi Miyakawa (available through O'Hara & Co., Tokyo, Japan).
5. The distance traveled, the number of entries into each arm, the time spent in each arm, and the percent of entries into the open arms are calculated by the Image EP program.

6. After each trial, all arms and the center area are cleaned with super hypochlorous water, that is an efficient odor removal agent and has relatively weak odor of itself compared to other cleaning solutions, to prevent a bias based on olfactory cues. Thus we can conduct the tests under controlled condition regarding olfactory cues.

Discussion

Although the mouse genome has been sequenced, the functions of most of the genes are not known. Genetic modification techniques allow for deletion or other manipulation of a specific gene in mice (Austin et al., 2004; Aiba et al., 2007). The influence of a given gene on a specific behavior can then be determined by conducting behavioral analyses of the mutant mice (Takao and Miyakawa, 2006b; Takao et al., 2007).

The elevated plus maze test is one of the most popular tests of all currently available animal models of anxiety (Rodgers and Dalvi, 1997; Crawley, 2007). This test for anxiety-like behavior has been used for screening and phenotyping transgenic and knockout mice (Crawley, 1999) and for drug discovery (Hogg et al. 1996; Crawley, 2007). The elevated plus maze test has a strong predictive validity for screening anxiolytic drugs (Rodgers and Dalvi, 1997; Mechiel Korte and De Boer, 2003; Crawley, 2007); anxiolytic drugs specifically increase, and anxiogenic drugs specifically decrease, the number of entries into the open arms and the time spent there. The total entries score and total distance are considered a useful index of general activity. Total entries score is also an index of anxiety, and the percentages of entries and time spent in each arm constitute the index of primary anxiety (Rodgers and Dalvi, 1997, Mechiel Korte and De Boer, 2003). The open and closed arms are considered to evoke the same exploratory drive, therefore avoidance of the open arms is considered to be a result of the induction of higher levels of fear (Rodgers and Dalvi, 1997). It is thought that the aversion of mice to explore the open arms of the maze is caused by fear of open and elevated spaces.

In 1984, Handley and Mithani reported on preliminary work with the elevated X (plus) maze test described above. The original test apparatus was raised 70 cm above the floor, and comprised two closed and two open arms, each of which measured 45 cm long by 10 cm wide. In their initial studies, they reported the ratio of open/total arm entries (Handley and Mithani, 1984). Subsequently, other indices were developed that included the number of entries into the closed and open arms and the time spent in the closed and open arms for rats (Pellow et al, 1985; Pellow and File, 1986) and mice (Lister, 1987).

Changes to the elevated plus maze test included lengthening of both the open arms (50 x 10 cm) and closed arms (50 x 10 x 40 cm) with tall surrounding walls and an open roof on the closed arms, and the entire maze was elevated to a height of 50 cm (Pellow et al, 1985; Pellow and File, 1986). Presently, in our laboratory, the elevated plus maze test apparatus is configured in the shape of a +, with two open arms (25 x 5 cm, with a very slight, 0.5-cm, wall) across from each other and perpendicular to two closed arms (25 x 5 x 16 cm), and is raised 50 cm above the floor (Miyakawa et al, 1996; Manabe et al, 2000; Miyakawa et al, 2001; Seeger et al, 2004; Morishima et al, 2005; Miyamoto et al, 2005; Arrow et al, 2006; Hattori et al, 2007; Niemann et al, 2007; Sano et al, 2008; Horii et al, 2008; Fukuda et al, 2008; Ikeda et al; 2008). The mouse is placed in the center of the + (5 cm x 5 cm) and is allowed to explore the maze freely. Though 5 min recording is common, the behavior is recorded for 10 min in our protocol to increase the opportunity to detect the phenotype. The open and closed elevated arms induce an exploration conflict (Mechiel Korte and De Boer, 2003; Crawley, 2007).

The measures of the elevated plus maze test are recorded by an observer during the experiment. In our laboratory, the test is recorded with a video camera connected to a computer and the behavioral data (Image EP) are acquired and analyzed using the Image EP program. Number of entries onto the open arms versus number of total arm entries, and time spent on the open arms versus closed arms, provide the measures of anxiety-like behavior.

We assessed more than 90 strains of genetically engineered mutant mice using the protocol shown in the movie and have a large set of raw data for more than 5000 mice (including wild-type and mutant mice). In our test battery, wild-type littermates are usually used as a control. As a background strain, C57BL/6J mice are widely used. We collected the data of C57BL/6J mice in the behavioral tests. The values obtained from C57BL/6J mice in our elevated plus maze test are as follows (n=914; mean SEM); total distance traveled: 1547.55 14.27 cm; duration of time spent: 56.49 2.42 s (open arms), 384.02 3.13 s (closed arms), 161.90 2.17 s (maze center); ratio of time spent in the open arms: 9.19 0.36%, ratio of time spent in the closed arms: 63.82 0.52%; number of entries: 7.64 0.21 (open arms), 24.32 0.28 (closed arms); % open arm entries: 21.9 0.05, % closed arms entries: 78.1 0.05. C57BL/6J mice spend less time in open arms than in closed arms ($p<0.0001$, n=914, paired-t test). This indicates that C57BL/6J mice tend to avoid the open arms, and that the time spent in the open arms is a valid index of anxiety-like behavior. Additionally, the order of trials is counterbalanced across genotypes, because the trial number affects the time spent in the open arms and the center platform. That is, the indices all increase during the 3rd and 4th mice compared to the 1st mice ($p=0.0089$, n=476) (unpublished data). With our protocol, analysis of more than 1661 mice showed that the sequential order of the mice tested in a cage does not significantly affect the open arm stay time (unpublished data). That is, the performances of the first mice taken from the cage do not significantly differ from those of the second, the third or the last mice.

Although the elevated plus maze test and the light/dark transition test are both used for assessing anxiety-like behavior, the results are not always consistent between them (Holmes et al, 2000; Tujimura et al, 2008; Nakajima et al, in press) For example, forebrain-specific calcineurin-knockout mice spend a decreased amount of time in the light chamber in the light/dark transition test, but an increased amount of time in the open arms in the elevated plus maze test (Miyakawa et al., 2003). Factor analysis of our behavioral test battery indicates that the elevated plus maze test and the light/dark transition test assess different aspects of anxiety-like behavior, such as bright-space anxiety in the light/dark transition test and open-space anxiety-like behavior in the open field test (Takao and Miyakawa, 2006b; Yamasaki et al, 2006). Accordingly, both the light/dark transition test and the elevated plus maze test are included in our behavioral test battery.

Crabbe and colleagues reported that uncontrolled variables and experiments characterizing mutants may yield results that are specific to a particular laboratory (Crabbe et al., 1999). The procedural differences that exist between laboratories make it difficult to replicate or compare the results among them. Establishing visual documentation of the protocol will promote better understanding of experimental procedures, allowing for standardization of the protocols used across laboratories and for comparisons of the behavioral phenotypes of various strains of mutant mice assessed using these tests. We previously published a movie protocol of the light/dark transition test (Takao and Miyakawa, 2006a). Likewise,

movies of other protocols, such as for the open field test, the porsolt forced swim test, and fear conditioning tests that we use in our behavioral test battery are presently being made for publication as future video journal articles.

Disclosures

All procedures were approved by the Animal Use and Care Committee of Kyoto University.

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