Video Article

Light/dark Transition Test for Mice

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Abstract

Although all of the mouse genome sequences have been determined, we do not yet know the functions of most of these genes. Gene-targeting techniques, however, can be used to delete or manipulate a specific gene in mice. The influence of a given gene on a specific behavior can then be determined by conducting behavioral analyses of the mutant mice. As a test for behavioral phenotyping of mutant mice, the light/dark transition test is one of the most widely used tests to measure anxiety-like behavior in mice. The test is based on the natural aversion of mice to brightly illuminated areas and on their spontaneous exploratory behavior in novel environments. The test is sensitive to anxiolytic drug treatment. The apparatus consists of a dark chamber and a brightly illuminated chamber. Mice are allowed to move freely between the two chambers. The number of entries into the bright chamber and the duration of time spent there are indices of bright-space anxiety in mice. To obtain phenotyping results of a strain of mutant mice that can be readily reproduced and compared with those of other mutants, the behavioral test methods should be as identical as possible between laboratories. The procedural differences that exist between laboratories, however, make it difficult to replicate or compare the results among laboratories. Here, we present our protocol for the light/dark transition test as a movie so that the details of the protocol can be demonstrated. In our laboratory, we have assessed more than 60 strains of mutant mice using the protocol shown in the movie. Those data will be disclosed as a part of a public database that we are now constructing.

Visualization of the protocol will facilitate understanding of the details of the entire experimental procedure, allowing for standardization of the protocols used across 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 https://www.jove.com/video/104/

Protocol

1. The apparatus used for the light/dark transition test consisted of a cage (21x42x25 cm) divided into two sections of equal size by a partition with door (Ohara & Co., Tokyo).
2. Mice are housed three to four per cage in a room with a 12 hr light/dark cycle (lights on at 7:00 A.M.) with ad libitum access to food and water. Ideally, two controls and two mutants are housed together, by re-organizing the mice as soon as they are genotyped at the time of weaning. Behavioral testing is performed between 9:00 A.M. and 6:00 P.M. All the cages containing mice are transferred to the behavior testing room 30 min before the first trial begins.
3. One chamber is brightly illuminated by white diodes (390 lux), whereas the other chamber is dark (2 lux). Mice are placed into the dark side and the door is opened automatically 3 seconds after the mouse is detected by the infrared camera. The door is used so that the mice do not enter the light chamber immediately after the release with their motivation to escape from experimenter, since the latency to enter the light chamber may serve as an index of anxiety-like behavior.
4. Mice are allowed to move freely between the two chambers with door open for 10 min. The application used for acquiring and analyzing the behavioral data (Image LD4) is based on the public domain Image J program (developed byWayne 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 in each chamber, the total number of transitions, the time spent in the each chamber, and the latency to enter the light chamberare recorded by Image LD4 program.
6. After each trial, all chambers are cleaned with super hypochlorous water to prevent a bias based on olfactory cues.

Discussion

Although all of the mouse genome sequences have been determined, the functions of most of these genes are not known. Gene-targeting techniques, however, can be used to delete or manipulate a specific gene in mice(Aiba et al., 2007; Austin et al., 2004). 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, 2006; Takao et al., 2007). As a test for behavioral phenotyping of mutant mice, the light/dark transition test is one of the most widely used tests to measure anxiety-like behavior in mice(Crawley, 2000). The test is based on the natural aversion of mice to brightly illuminated areas and on their spontaneous exploratory behavior in novel environments(Crawley, 1985). The test is sensitive to anxiolytic drug treatment(Crawley, 1985).

The apparatus consists of a dark chamber and a brightly illuminated chamber. A restricted opening, 3 cm high by 5 cm wide, connects the two chambers. Mice are allowed to move freely between the two chambers. The number of entries into the bright chamber and the duration of time spent there are used as indices of bright-space anxiety in mice.

The light/dark transition test was originally developed by Crawley and colleagues (Crawley and Goodwin, 1980). There are two differences between their original version and our test. First, the light chamber is larger than the dark chamber in the original version, whereas the size of the two chambers is the same in our version of the test. Second, in the original version, the light chamber had no ceiling and the walls of the light chamber were transparent(Crawley and Goodwin, 1980), while we use opaque white plastic for the ceiling and walls of the light chamber. These differences, namely the size and openness of the light chamber, allow for the simultaneous detection of bright-space anxiety as well as open-space anxiety in the original version of the test. In our laboratory, however, open-space anxiety-like behavior in mice is tested in an elevated plus maze. The opaque walls and ceiling, size of the light chamber, and our specific light/dark transition test protocol is more specialized for detecting bright-space anxiety compared to the original version.

Although the light/dark transition test and elevated plus maze are both used for assessing anxiety-like behavior, the results are not always consistent. For example, forebrain-specific calcineurin knockout mice spend an decreased amount of time in the light chamber in the light/dark transition test, but a increased amount of time in the open arms in the elevated plus maze(Miyakawa et al., 2003). We hypothesized that our light/dark transition test and elevated plus maze tests assess different aspects of anxiety-like behavior, such as bright-space anxiety and open-space anxiety-like behavior. To test this hypothesis, we used a large data set collected using the same mice in both tests. In our laboratory, we conduct behavioral test batteries covering many domains to screen for the behavioral significance of genes. The test battery requires several weeks to complete. During the testing period, mouse cages are kept on a rack in our facility. If mice are transported to our laboratory from other institutions, they are habituated to the environment of our facility for at least 1 week. After the habituation period, mice are subjected to a general health check and neurologic screening (Miyakawa et al., 2003), and after these simple examinations, mice are subjected to the light/dark transition test. The test is conducted in a sound proof experimental room after 30 min of habituation to the experimental room, as shown in the movie.

We have assessed more than 60 strains of genetically engineered mutant mice using the protocol shown in the movie and have a large set of raw data for more than 3000 mice (including wild-type and mutant mice). We conducted factor analyses to take advantage of the large set of raw data. Indeed, we detected different factors from each test (unpublished data), although there were some significant correlations between indices (unpublished data).. Those data will be disclosed as part of a public database that we are now constructing.

In our test battery, wild-type littermates are usually used as a control. As a background strain, C57BL/6J mice are commonly used. We collected the data of C57BL/6J mice used as a control of mutant mice in the test battery. The indices obtained from C57BL/6J mice in our light/dark transition test were as follows (n=795; mean SEM); distance traveled: 846.5 8.88 cm in the light chamber, 1478.5 9.53 cm in the dark chamber ; duration of time spent:215.8 1.85 s in the light, 395.8 1.86 s in the dark; number of transitions between compartments: 30.52 0.43; latency to enter the light chamber: 64.94 2.40 s. Movement of mice around the entrances to the chambers is detected by the camera and is counted as time spent in both chambers. Because of this, the sum of the mean duration of time spent in the light and that in dark compartments was greater than that of the entire duration of the test (600 s).

In our protocol, a mouse is first placed in the dark chamber. Three seconds after placing the mouse in the dark chamber, the door between the chambers automatically opens and the mouse can move freely between the two chambers. C57BL/6J mice spend less time in the light chamber than in the dark chamber ($p<0.0001$, duration if time spent in light: 215.8 1.85 s, duration of time spent in dark: 316.3 2.11 s [the time spent in the dark does not include the initial time spent in the dark prior to entering the light chamber for the first time], n=795, paired-t test). This indicates that C57BL/6J mice tend to avoid the light chamber, and that the time spent in the light is a good index of anxiety-like behavior.

The results obtained from various strains of mutant mice are easily compared, because all data are obtained using the same protocol. To obtain phenotyping results of a strain of mutant mice that can be readily reproduced and compared with data of other mutants, the behavioral test methods should be as identical as possible between laboratories. Crabbe and colleagues tested the interaction between mouse behavior and laboratory environment using exactly the same inbred strains and one null mutant strain. In their study, the apparatus, test protocols, and many environmental variables were rigorously standardized. Despite the standardization, they found systematic differences in behavior across laboratories, although they also detected strain differences in all behaviors(Crabbe et al., 1999). They reported that uncontrollable variables and experiments characterizing mutants may yield results that are idiosyncratic to a particular laboratory. The procedural differences that exist between laboratories make it difficult to replicate or compare the results among laboratories. Here, we present our protocol for the light/dark transition test as a movie so that the details of the protocol can be demonstrated.

Establishing visual documentation of the protocol will facilitate better understanding of the details 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 this test. Movies of other protocols, such as for the elevated plus maze, open field test, porsolt forced swim test, fear conditioning, that we use in our behavioral test battery are currently being made for publication as future video journal articles.

Disclosures

All procedures were approved by the Animal Use and Care Committee of Kyoto University. Tsuyoshi Miyakawa is associated with BIRD, Japan Science and Technology Agency, Saitama, Japan.

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