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Home cage activity and activity-based measures of anxiety in 129P3/J, 129X1/SvJ and C57BL/6J mice

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Abstract

We investigated the home cage activity and emotional behavior in mouse strains used as background for many studies of altered genes [C57BL/6J (B6, *n*=20), 129X1/SvJ (X1, *n*=20) and 129P3/J (P3, *n*=19)]. In their home cages, X1 and P3 mice exhibited less locomotion than did B6 mice, and the X1 mice showed significantly greater rearing than B6 and P3 mice did. A battery of three tests conducted in an open field (open field, emergence and novel object) revealed strain rankings of B6>X1>P3 or B6>X1=P3 in most activity variables. Significant correlations were found between home cage activity and activity in each of three tests, but not in all observation periods. Strain rankings on the elevated zero maze test were B6=X1>P3 in the number of stretched-attend body postures (SAPS) during the initial 6-min exposure for naïve mice. Naïve and nonnaïve mice showed significantly different behaviors on the elevated zero maze. The results suggest that rankings on anxiety are P3>X1>B6 and that B6 mice have greater exploration in a novel environment compared with X1 and P3 mice. However, anxiety-like behaviors differed among strains in open-field-based tests and in the zero maze, and testing experience impacted performance on the zero maze. The findings illustrate that test variations and experience can influence performance and suggest the need for the consideration of how these factors interact with background strains in assessing gene-altered mice.

Keywords: 129X1/SvJ; 129P3/J; C57BL/6J; Open field; Emergence; Novel object; Spontaneous locomotor activity

1. Introduction

Mice of the 129 "family" are widely used in genetic research and typically supply embryonic stem cells for the creation of targeted mutations [1–3]. Mice of a 129 strain are also often crossed with C57BL/6J (B6) mice in producing the background strain for gene-altered animals. However, it is recognized that the characteristics of the background strain may have considerable relevance for interpreting results in gene-altered animals [3] and that there is the possibility that abnormal behaviors in knockout mice might be determined by their genetic background rather than by the loss of gene function [2,4–6].

Recent studies have consistently reported that many 129 mouse lines show less locomotor activity and more anxiety than do B6 mice in the open field and other behavioral tests [5–15]. However, a critical question for many behavioral tests is whether differences in performance results from greater or lesser anxiety or from differences in locomotor activity [16,17]. For instance, the behaviors of rodents in the open field are complex and may include components of arousal, novelty seeking, fear responses, stereotypy, and reactions to handling [18–20]. These behaviors may be associated with fear or anxiety in response to the novel environment and/or to the natural aversion of rodents to open spaces [21]. Nevertheless, locomotion in the open field also has been used as an index of general locomotor activity or exploratory behavior [2,22–29], as well as anxiety [30–37]. This suggests that spontaneous activity should also be measured in situations such as the home cage that would not involve potentially increased arousal or emotion.

In this study, we compared a steel-derived strain [129X1/SvJ (X1)], a parental strain [129P3/J (P3)] and B6 mice on activity in the home cage and on activity-based behavioral tests. Spontaneous activity was monitored continuously in

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the home cage activity over 3 days. We then examined behavior and activity in the open field and in two other tests based on open field performance, an emergence test [21,32,37] and a novel object test [30,32,38]. These tests are thought to vary in the levels of anxiety and exploration that they elicit. We also compared the rankings of the strains on activity in the home cage and in each test. Lastly, we tested naïve and previously tested mice of each strain on the elevated zero maze. This maze is thought to produce anxiety related to the aversion to heights and open spaces in rodents [39]. Defecation was measured in each test as an indicator of anxiety that is independent of locomotion [24,40].

2. Methods

2.1. Subjects

The subjects were male mice (B6, n=20; X1, n=20; P3, n=19). All animals were obtained from The Jackson Laboratory (Bar Harbor, ME). They were 8 to 9 weeks of age at the start of the study and weighed 20–30 g over the course of the experiment. The mice were individually housed with food and water available ad libitum. The experimental room was kept on a 12:12 light–dark cycle, with lights on from 0700 to 1900 h. Light intensity during the light period was 100 to 110 lx and less than 1 lx during the dark period. Room temperature was maintained at 24±1 °C.

2.2. Activity and behavioral measures

2.2.1. Home cage activity

For recording home cage activity, a standard polycarbonate shoebox cage (28.5×17.5×12 cm) was placed inside a set of photobeam sensor panels, and photobeam interruptions produced by movements of the mouse were detected and analyzed by a computerized analysis system [AccuScan, Digiscan Model RXYZCM(16)CCD]. Home cage activity variables included locomotion, rearing and single-beam breaks. Locomotion (travel distance in centimeters) was obtained from the horizontal sensors and calculated taking into account the path taken by the mouse. Rearing was obtained by counting the number of beam interruptions in the bank for vertical sensors during 1-min intervals. Single-beam breaks (typically produced by behaviors such as grooming and head bobbing) were determined when a mouse repeatedly broke the same beam (or set of the beams).

Cages were freshly changed before beginning the recording. Following a habituation period of 4 h to the newly changed cage, activity was monitored for 72 consecutive hours. To eliminate potential strain differences due to the cage change, data from the last two recording days were used in the analyses. The activity measures were collapsed into 60-min bins to examine the hourly distribution of the activity. The data were then summed across days and averaged to obtain the 2-day mean levels of activity for the12-h light and dark periods and the total 24-h recording period. These means were used in the strain comparisons.

2.2.2. Behavioral tests in the open field

The photobeam apparatus was also used for monitoring activity in three behavioral tests: open field, emergence and novel object. In the open field, the mice were placed in the center area of the open field chamber (41×41×30 cm), which fit within the banks of photobeam sensors, and were monitored for 30 min. In the emergence test, the mice were placed in a rectangular, dark plastic container that had a transparent strip to allow the photobeams to function properly. The container was then positioned facing the center of the open field and activity was monitored for 15 min. In the novel object test, the mice were allowed to explore the open field for 30 min, and then a novel plastic cylinder was placed in the center of the open field. Afterwards, activity was monitored for an additional 30 min.

Three activity-based variables were obtained in 5-min time bins for each test situation: (1) locomotion: travel distance (cm); (2) rearing: total number of beam interruptions detected by the vertical movement sensors; and (3) center time: time spent (s) away from the walls of the cage margin (more than 1 cm) as determined by the sensors and software.

2.2.3. Elevated zero maze test

The zero maze consisted of a circular Perspex platform (5-cm width with a 40-cm inner diameter) that was equally divided into four quadrants (AccuScan Model ZMA-8). Two quadrants on opposite sides of the platform were enclosed by Perspex walls (30 cm high); for testing, these were covered with dark paper. The other two quadrants were open and were bordered by a Perspex lip (0.5 cm high), which served as a security and tactile guide on the open quadrants. The maze was elevated 100 cm above the floor. Between the tests, the maze was cleaned with 5% alcohol.

For testing, the mice were placed at the entrance of a closed quadrant and were monitored for 12 min. Behavior on the elevated zero maze was assessed using visual observation, in combination with activity measurements made utilizing infrared photobeam sensors and a computerized analysis system (AccuScan Digiscan Model ZM-CU). The computerized system recorded the time in the open arm and the number of transitions between the open and closed arms. Visually scored variables included the number of head dips (the frequency of head dips over the edge of the platform when the subjects were located in either the open or the end of closed quadrants) and the number of stretched-attend body postures (SAPS: an elongated body stretch forward posture with at least the snout passing over the closed–open divide from the end of a closed to an open quadrant). These data were analyzed in 6-min time bins.