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Research report

Slope climbing challenges, fear of heights, anxiety and time of the day

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HIGHLIGHTS

- An elevated platform with steep slopes on two opposite sides is proposed to test anxiety in mice.
- The test apparatus offers a 3-dimensional open-field that compares to a real world navigation space.
- BL6J and CD-1, not BALB/c, crossed onto downward slopes and reached the stands from upward slopes.
- Crossings onto downward slopes decreased with further elevation of the platform in BL6J and CD-1.
- Times of the day did not affect BALB/c behavior in downward shallow and downward steep slopes.

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ABSTRACT

When exposed to an unfamiliar open space, animals experience fear and attempt to find an escape route. Anxiety emerges when animals are confronted with a challenging obstacle to this fear motivated escape. High anxiety animals do not take risks; they avoid the challenge. The present experiments investigated this risk avoidant behavior in mice. In experiment 1, BALB/c, C57BL/6J and CD-1 mice were exposed to a large platform with downward inclined steep slopes attached on two opposite sides. The platform was elevated 75 and 100 cm from the ground, in a standard (SPDS) and in a raised (RPDS) configuration, respectively. In experiment 2, the platform was elevated 75 cm from the ground. Mice had to climb onto a stand at the top of upward inclined slopes (SPUS). In experiment 3, BALB/c mice were exposed to SPDS with steep or shallow slopes either in early morning or in late afternoon. In all 3 test configurations, mice spent more time in the areas adjacent to the slopes than in the areas adjacent to void, however only C57BL/6J and CD-1 crossed onto the slopes in SPDS, and crossed onto the stands in SPUS whereas BALB/c remained on the platform in SPDS and explored the slopes in SPUS. Elevation of the platform from the ground reduced the crossings onto the slopes in C57BL/6J and CD-1, and no differences were observed between BALB/c and C57BL/6J. BALB/c mice demonstrated no difference in anxiety when tested early morning or late afternoon; they crossed onto shallow slopes and avoided the steep one.

1. Introduction

When exposed to an unfamiliar open space, animals experience fear and attempt to find an escape route. Anxiety emerges when animals are confronted with a challenging obstacle to this fear motivated escape. Climbing up or down a steep slope to or from an elevated landing surface is an obstacle that we exploited to assess anxiety in mice. In a previous report [50], we described a novel open space anxiety test, which consisted of a large elevated platform with steep slopes attached on two opposite sides. The test apparatus offers a 3-dimensional open-field, which compares to the real world landscape. In this test, we examined the behavior of three mouse strains [BALB/c, C57BL/6J and CD-1]. We observed that all mice spent more time in the areas adjacent to slopes than in the areas adjacent to the void space, which indicated that both albinos [BALB/c and CD-1] and pigmented [C57BL/6J] mice were able to notice the presence of the hanged slopes. However, C57BL/6J and CD-1 mice crossed onto and explored the slopes whereas BALB/c mice remained the entire 12 min test session on the platform. In that report [50], we described also the behavior of BALB/c and C57BL/6J mice, which were exposed to this novel open space test, in presence or absence of a protected space. In the presence of a refuge, which occupied the central area of the platform, there were

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no significant differences between BALB/c and BL6J mice; they both avoided the slopes. This seems to suggest that both high and low anxiety mouse strains demonstrate a preference for safety, and that a behavioral test with such an option involves fear-induced avoidance/escape, which is distinct from fear-induced anxiety. In the absence of a protected space, animals face ambiguous and risky options; escape or avoidance response does not lead to a reduced or termination of fear – the platform is not less anxiogenic than the slopes. In a subsequent experiment [22], we examined the effect of different doses of amphetamine and diazepam on the behavior of BALB/c mice, and we observed that both drugs produced an inverted-U-shaped dose-dependent facilitation of the number of crossings on the surface of the platform. The increase in locomotor activity produced with amphetamine was at least twice higher than that of diazepam. However, despite such increase, none of the amphetamine treated mice did cross onto the slopes whereas all diazepam treated mice crossed. Hence, unlike in the current tests of unconditioned anxiety [TUA], the effect of diazepam in the present test is not confounded by a change in locomotor activity [16,83].

In the present report, we describe 3 experiments to further validate the present open space anxiety test. In the first experiment, we examined the behavior of separate groups of BALB/c, C57BL/6J and CD-1 mice in two test configurations. In the first configurations (SPDS), the platform was raised 75 cm above the ground, and in the second configuration (RPDS), the platform was raised 100 cm. In both SPDS and RPDS, the slopes were inclined downward. This experiment was intended to confirm previous results obtained in the SPDS in a single session, and examine anxiety responses in these strains of mice over 3 test sessions. It was also intended to examine whether further elevation of the platform from the ground would increase anxiety, and whether this would be observed in all 3 mouse strains. In the second experiment, the platform was raised 75 cm above ground but the slopes were inclined upward (SPUS). However, a preliminary experiment indicated that, in this condition, all mice did not hesitate to climb up and down the upward inclined slopes. Therefore, we introduced a stand that mice need to cross onto when they had reached the top of a slope. We expected here, that mice do not cross onto the stand or, if they do cross, they may not be able to climb down. In the last experiment, we examined the behavior of BALB/c in two platform configurations, one with steep slopes and another with shallow slopes, at two different times of the day, early morning and late afternoon. We expected here that time of the day will not affect anxiety for the simple reason that, unlike in humans, anxiety in animals is not evoked through worries and ruminations. Animals are exposed at different times of the day to an actual anxiety-provoking stimulus.

2. General methods

2.1. Animals

One hundred and twenty one mice [2 months old] were obtained from Charles River [UK]. After their arrival, they were left to acclimatize to local laboratory conditions for two weeks. They were housed in a colony room that was held under a 12:12 h light/dark cycle [light 07:00 to 19:00 h at 80 lx], temperature [21 ± 1 °C] and humidity [50% ±5] controlled conditions. In order to avoid unequal light exposure, the upper shelf was occupied with plastic cages filled with clean sawdust. Mice were housed in a group of 4 or 5 mice per cage. Cage number and individual ear tag code identified individual mice. All mice had *ad libitum* access to food and water. Animal treatment and husbandry were in accordance with approved use of animals in scientific procedures regulated by the Animals [Scientific Procedures] Act 1986, UK.

Table 1
Number of mice per experiment from each mouse strain.

Mouse strains	Experiment 1	Experiment 2	Experiment 3	Total
BALB/c	19	10	32	61
C57BL/6J	20	10		30
CD-1	20	10		30
Total	59	30	32	121

2.2. Apparatus

It consisted of a platform [80 cm × 80 cm wide], which was raised either 75 cm [experiments 1, 2 and 3] or 100 cm [experiment 1] above the ground (dark grey surface). It was made of grey opaque PVC [5 mm thick]. Panels [80 cm × 25 cm] made of rigid wire mesh were attached on two opposite sides of the platform. The angle of inclination of the slopes was ∼77° downward in the first experiment [Fig. 1A], and ∼103° upward in the second experiment [Fig 1B]. The upward inclined slopes ended on a stand [80 cm × 25 cm] that mice needed to climb onto [Fig. 1B]. In the third experiment, the angle of inclination of the slopes was either ∼77° or ∼45° downward. Small ledges [0.5 cm] surrounded the left, right and the bottom sides of the slopes. The platform was divided into a central area covered with a white tile [16 × 16 cm wide and 0.4 cm thick], an inner area surrounding the central area [16 cm wide and 2048 cm^2], and an outer area [16 cm wide and 4096 cm^2]. The outer area was further divided into areas adjacent to the slopes [2048 cm^2] and areas adjacent to void space [2048 cm^2] [Fig. 1A and B]. The surface of the platform was cleaned to minimize the effects of lingering olfactory cues. Any feces and urine were removed with paper towels, then cleaned with antibacterial solution followed by 90% ethanol, and left to dry before the introduction of the next mouse. The illumination on the surface of the elevated platform was ∼40 lx.

2.3. Behavioral testing

The number of mice from each strain in each experiment is shown in Table 1. In the first experiment, mice were exposed to an elevated platform with downward inclined steep slopes. The platform was raised either 75 or 100 cm above the ground level. In the second experiment, mice were exposed to an elevated platform with upward inclined steep slopes, each connected to a horizontal stand. The platform was raised 75 cm above the ground level. In both experiments, mice were tested during the light period of the cycle [0830–1530 h] in 3 consecutive sessions, one session a day. Experiment 3 involved only a single mouse strain, BALB/c mice, which were tested in a single 12 min session. These were allocated randomly to groups that were tested either on a platform with two steep [77°] or with two shallow [45°] downward inclined slopes in the morning [8 am-10am] or in the afternoon [6pm-8pm]. The platform was raised 75 cm above the ground level. There were 4 groups: STEEP AM [n = 8], STEEP PM [n = 8], SHALLOW AM [n = 8], SHALLOW PM [n = 8].

In all experiments, mice were transported in a small bucket, and tipped gently onto the central area of the platform. During the test, mice were observed on a screen monitor connected to a video camera suspended above the test arena. Using an in-house computer program [EventLog] we recorded the number of entries, duration of entries and latency of first entry into the different areas of the test apparatus [see Fig. 1]. The latency of first entry was recorded as the full duration of a test session for mice, which did not cross onto a slope [experiments 1 and 3] or into a stand [experiment 2].

An entry was recorded whenever a mouse crossed with all four paws into an area. A mouse that crossed only once onto a slope in experiment 1 and 3, or onto a stand in experiment 2, will be