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

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Summary: 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.

Keywords: Fear; Anxiety; Affordance; Attention bias; Climbing; Decision-making.

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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 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

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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.

2.2. Apparatus

It consisted of a platform [80 cm x 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 x 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 x 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 x 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].

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

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 recorded as having made one entry if it did leave the slope or the stand. However, a mouse that made a single entry onto a slope or a stand and remained there until the end of the test session will be recorded as having made no entry. We did not observe such behavior in our experiments but it can occur with different mouse strains, or as a result of an experimental manipulation.
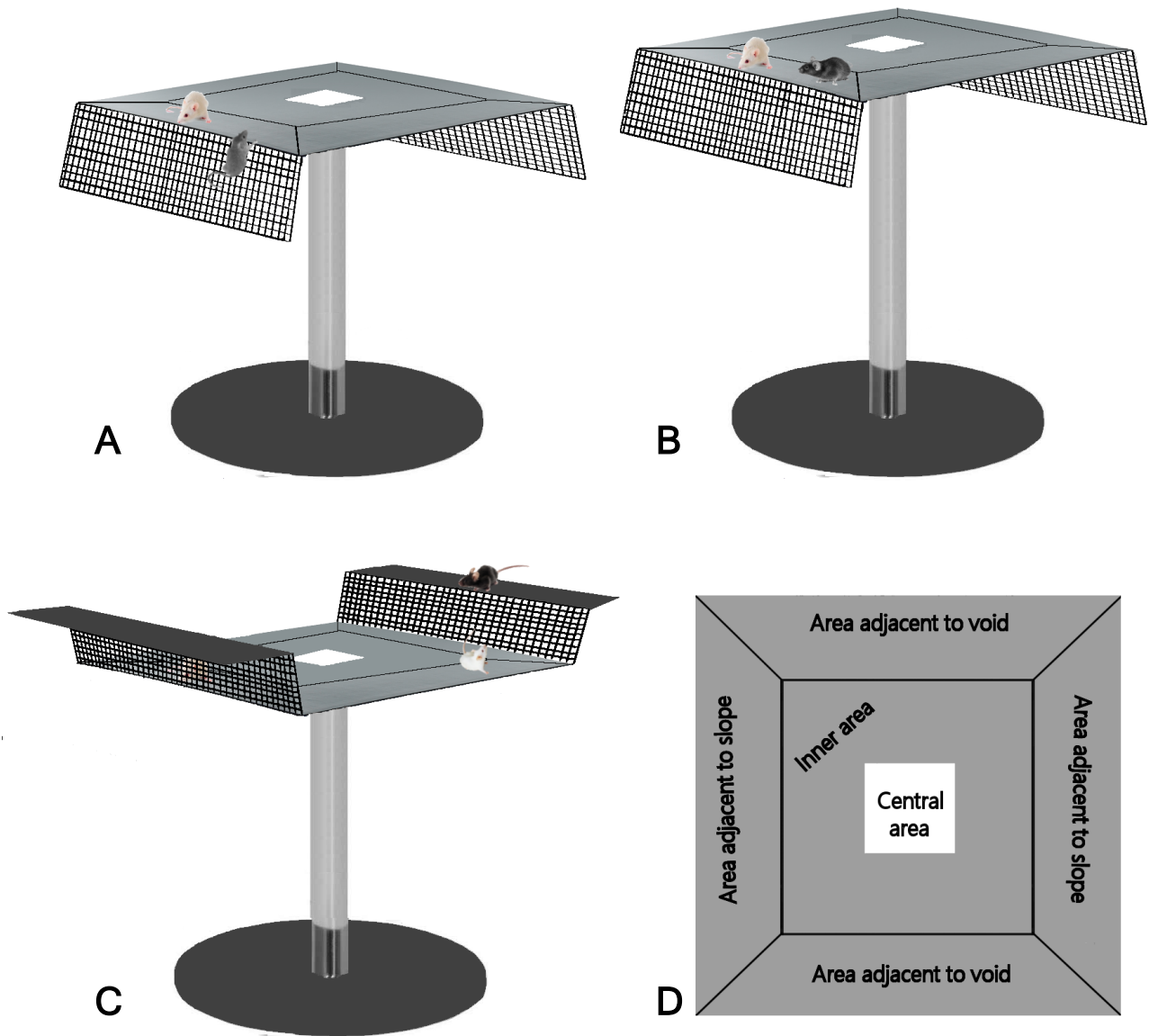
Fig. 1: (A) SPDS configuration with platform elevated 75 cm above the ground; (B) RPDS configuration with platform elevated 100 cm above the ground; (C) SPUS configuration with platform elevated 75 cm above the ground; (D) Area divisions on the surface of the platform. Mice were tested singly. Here, mice are shown in pairs for illustrations.

2.4. Statistical analysis

This was performed using Statistica 6 [Statsoft, Tulsa, OK]. All data were expressed as mean ± s.e.m. Differences among group mean values for each measurement were tested for significance with one-way ANOVA (experiment 3), and with two-way (experiment 2) or three-way (experiment 1) ANOVA repeated measures. These were followed up with Newman-Keuls post-hoc comparisons. Results were considered significant when p≤0.05. When p>0.05 and p≤0.10, the p value was reported as non-significant, and rounded up to the nearest value.

3. Results

3.1. Experiment 1 – Standard platform (SPDS) vs. raised platform (RPDS) configurations

3.1.1. Number of crossings on the platform

There were significant differences between groups $[F_{2,53}=31.77, p<0.0001]$ and between sessions $[F_{2,106}=12.36, p<0.0001]$, but not between conditions ($F_{1,53}=0.01, p>0.10$). There were also significant interactions between groups and conditions $[F_{2,53}=9, p<0.0004]$, between groups and sessions $[F_{4,106}=2.86, p<0.0004]$, but not between conditions and sessions $[F_{2,106}=1.95, p>0.10]$. There were however significant interactions between groups, conditions and sessions $[F_{4,106}=10.74, p<0.0001]$.

In SPDS *(Figure 2A)*, BALB/c made few crossings on the platform compared to BL6J and CD-1 in each test session $[p<0.0005]$. There were no significant differences between BL6J and CD-1 $[p>0.10]$. The number of crossings was significantly low in BALB/c and high in BL6J and CD-1 in session 1 compared to sessions 2 and 3 $[p<0.01]$. In RPDS *(Figure 2B)*, BALB/c and BL6J made few crossings compared to CD-1 in sessions 2 and 3 $[p<0.03]$, and BALB/c made few crossings compared to BL6J in session 3 $[p<0.02]$. In BALB/c, the number of crossings was significantly high in session 1 compared to sessions 2 $[p<0.05]$ and 3 $[p<0.005]$, and in session 2 compared to session 3 $[p<0.05]$. In BL6J, it was significantly high in session 1 compared to sessions 2 $[p<0.005]$ and 3 $[p<0.0003]$. There were no significant differences between sessions in CD-1 $[p>0.10]$.

The number of crossings on the platform was significantly high in BALB/c exposed to RPDS compared to BALB/c exposed to SPDS in session 1 $[p<0.0001]$ and 2 $[p<0.03]$. However, it was significantly low in BL6J exposed to RPDS compared to BL6J exposed to SPDS in sessions 1 $[p<0.001]$ and 2 $[p<0.05]$ but not in session 3 $[p<0.08]$. CD-1 demonstrated a decrease in crossings in RPDS, in session 1 only $[p<0.03]$.

3.1.2. Number of crossings into the central area (Figure 2C and D)
There were significant differences between groups $[F_{2,53}=39.97, p<0.0001]$ but not between conditions $[F_{1,53}=0.673, p>0.10]$ and between sessions $[F_{2,106}=0.48, p>0.10]$. There were, also, no significant interactions between groups, conditions and sessions $[p>0.10]$. In both SPDS and RPDS, BALB/c made few crossings into the center compared to BL6J and CD-1 in each session $[p<0.001]$.
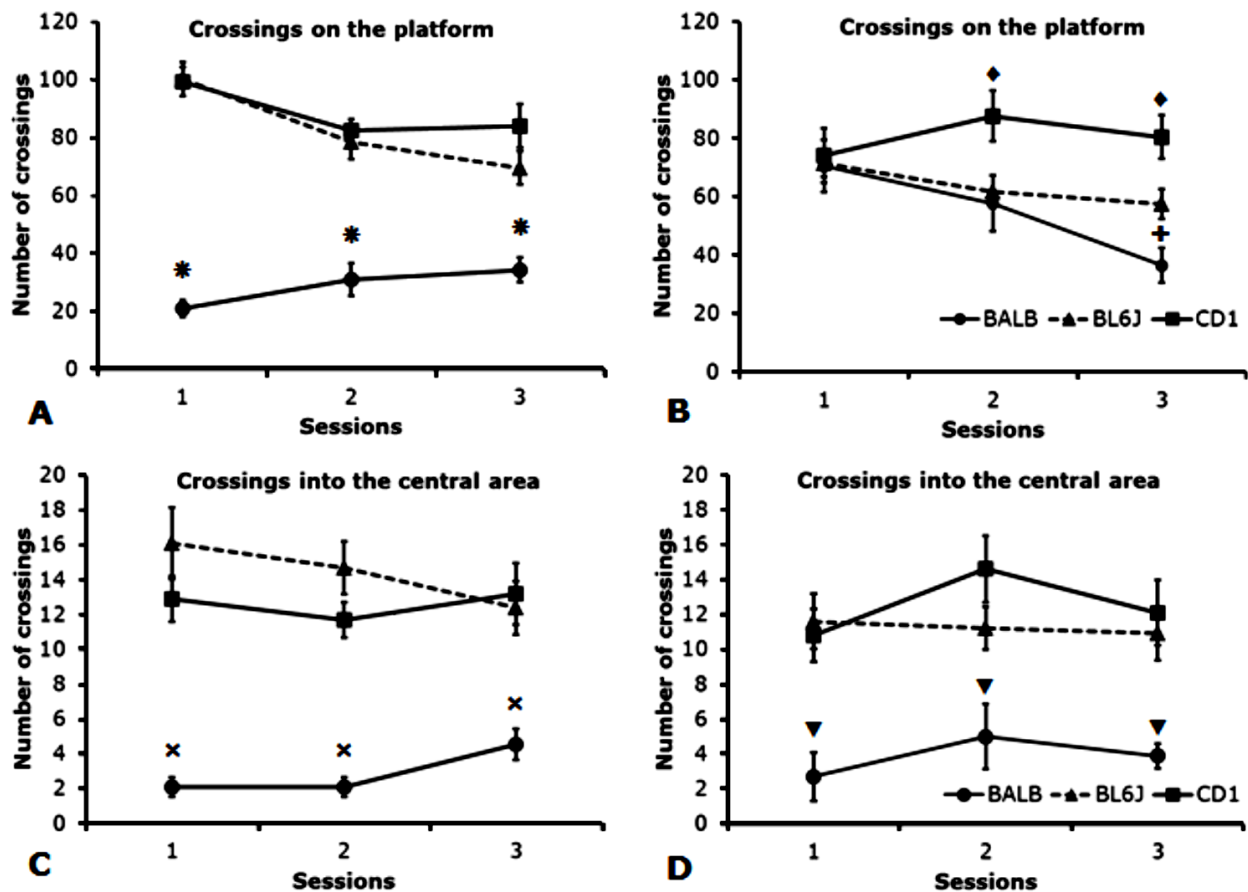
Figure 2: Standard **P**latform **D**ownward **S**lopes, left column, and **R**aised **P**latform **D**ownward **S**lopes, right column. (**A**)✶BALB/c compared to BL6J and CD-1 [p<0.0005]; (**B**) ▼BALB/c compared to BL6J [p<0.02]; ♦CD-1 compared to BALB/c and BL6J [p<0.03]; (**C**)✕BALB/c compared to BL6J and CD-1 in each session [p<0.001]; (**D**)✚BALB/c compared to BL6J and CD-1 [p<0.001].

3.1.3. Number of crossings onto the areas adjacent to slopes [AS] and areas adjacent to void [AV]

In both SPDS and RPDS, there were significant differences between groups [$F_{2,53}$= 31.81 and 3.16 respectively, p<0.05] and between sessions [$F_{2,106}$=25.47 and 22.44 respectively, p<0.0001], but not between conditions [$F_{1,53}$=0.6 and 1.55 respectively, p>0.10]. There were also significant interactions between groups and conditions [$F_{2,53}$=15.06 and 15.24 respectively, p<0.0001], and between groups and sessions in RPDS [$F_{4,106}$=4.21, p<0.003] but not in SPDS [$F_{4,106}$=1.68, p>10]. There were no significant interactions between conditions and sessions [$F_{2,106}$=0.81 and 1.83 respectively, p>0.10]. There were however, significant interactions between groups, conditions and sessions [$F_{4,106}$=17.34 and 15.75 respectively, p<0.0001].

In SPDS *(Figure 3A and C)*, BALB/c made fewer crossings onto AS than BL6J and CD-1 [p<0.0005], and BL6J made fewer crossings than CD-1 [p<0.04] in each test session. BALB/c made also fewer crossings onto AV compared to BL6J and CD-1 in session 1 [p<0.0001], and both BALB/c and BL6J made fewer crossings than CD-1 in session 3 [p<0.04]. The number of crossings onto AV was not significant between groups in session 2 [p>0.10]. In addition, the

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number of crossings onto AS and AV was significantly low in BALB/c [p<0.05] and high in both BL6J [p<0.0001] and CD-1 [p<0.05] in session 1 compared to sessions 2 and 3.

In RPDS *(Figure 3B and D)*, BL6J made few crossings onto AS compared to BALB/c in session 1 [p<0.05] and to CD-1 in sessions 2 [p<0.008] and 3 [p<0.0003]. They also made few crossings into AV compared to BALB/c in sessions 1 [p<0.0005] and 2 [p<0.003], and to CD-1 in sessions 1 [p<0.04] and 3 [p<0.03]. However, BALB/c made few crossings onto AS compared to CD-1 in session 2 [p<0.05] and 3 [p<0.0001], but made more crossings into AV compared to CD-1 in session 1 [p<0.0004]. In addition, in BALB/c, the number of crossings into AS and AV was significantly high in session 1 compared to session 2 [p<0.01] and in sessions 1 and 2 compared to session 3 [p<0.01]. In BL6J, it was significantly high in session 1 compared to sessions 2 and 3 [p<0.03]. In CD-1, there were no significant differences between sessions [p>0.10].

In BL6J and CD-1, the number of crossings into AS was significantly higher than into AV in each test session, and in both test configurations [p<0.002]. In BALB/c, it was significantly higher than the number of crossings into AV in sessions 1 [p<0.004] and 2 [p<0.03] but not in session 3 [p<0.06] in SPDS. There were no significant differences between AS and AV in RPDS [p>0.10].

The number of crossings onto AS was significantly increased in BALB/c exposed to RPDS compared to BALB/c exposed to SPDS in sessions 1 [p<0.0001] and 2 [p<0.03]. It was, however, significantly decreased in BL6J exposed to RPDS compared to BL6J exposed to SPDS in all 3 test sessions [p<0.03]. CD-1 demonstrated a decrease in crossings in RPDS, in session 1 only [p<0.006].

The number of crossings onto AV was significantly increased in BALB/c exposed to RPDS compared to BALB/c exposed to SPDS in sessions 1 [p<0.0001] and 2 [p<0.02]. It was, however, significantly decreased in BL6J and in CD-1 exposed to RPDS compared, respectively, to BL6J and CD-1 exposed to SPDS in sessions 1 [p<0.0001 and p<0.05].

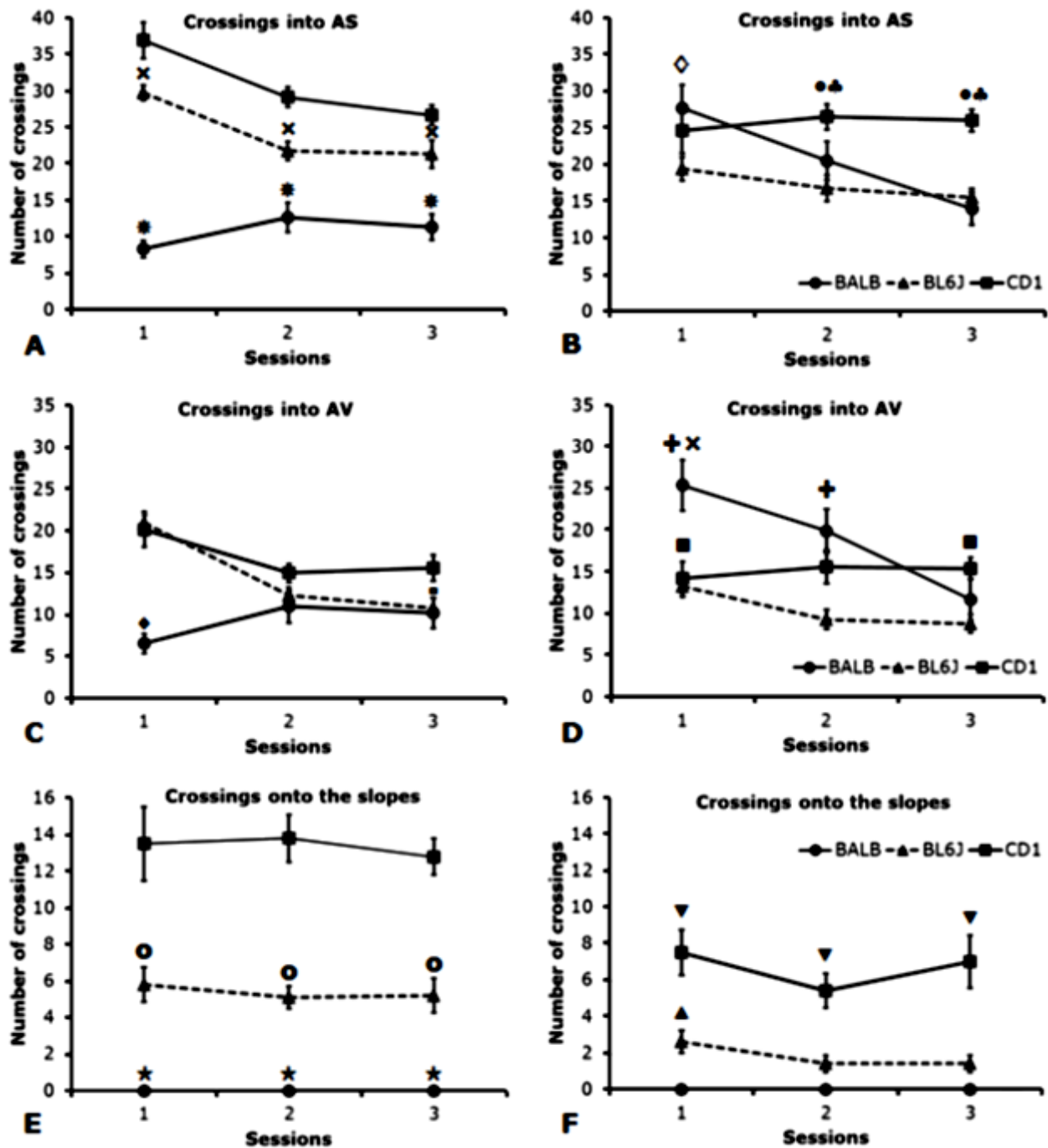**Figure 3: S**tandard **P**latform **D**ownward **S**lopes, left column, and **R**aised **P**latform **D**ownward **S**lopes, right column. (**A**) ✳BALB/c compared to BL6J and CD-1 [p<0.0005]; ✗BL6J compared to CD-1 [p<0.04]; (**B**) ◊ BALB/c compared to BL6J [p<0.05]; ●CD-1 compared to BALB/c [p<0.05]; ♣ CD-1 compared to BL6J [p<0.008]; (**C**)♦BALB/c compared to BL6J and CD-1 [p<0.0001], ▪BALB/c and BL6J compared to CD-1 [p<0.04]; (**D**)✚BALB/c compared to BL6J [p<0.003]; ✗BALB/c compared to CD-1 [p<0.0004]; ▪BL6J compared to CD-1 [p<0.04]; (**E**)✳BALB/c compared to BL6J and CD-1 [p<0.0001]; ○BL6J compared to CD-1 [p<0.0004]. (**F**) ▼CD-1 compared to BALB/c [p<0.0002] and to BL6J [p<0.0007]; ▲BL6J compared to BALB/c [p<0.03].

3.1.4. Number of crossings onto the slopes

There were significant differences between groups [F2,53= 112.56, p<0.0001], between conditions [F1,53= 38.89, p<0.0001] but not between sessions [F2,106=1.15, p>0.10]. There were also significant

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interactions between groups and conditions [F2,53=12.36, p<0.0001] but no between groups or conditions and sessions [p>0.10].

In SPDS *(Figure 3E)*, BL6J and CD-1 crossed onto the slopes while BALB/c did not. BL6J made few crossings compared to CD-1 [p<0.0004]. In RPDS *(Figure 3F)*, BALB/c did not cross onto the slopes in all 3 test sessions whereas BL6J made very few crossings [Fig. 4], which were greater than in BALB/c in session 1 only [p<0.03]. However, CD-1 made significantly more crossings than BALB/c [p<0.0002] and BL6J [p<0.0007] in all 3 sessions.

The number of crossings onto the slopes was significantly decreased in BL6J and CD-1 exposed to RPDS compared, respectively, to BL6J and CD-1 exposed to SPDS in sessions 1 [p<0.02], 2 [p<0.0002] and 3 [p<0.004].

3.1.5. Time spent in the central area
There were significant differences between groups [F2,53= 20.37, p<0.0001], between conditions [F1,53= 7.13, p<0.01] and between sessions [F2,106=8.55, p<0.0004]. There were also significant interactions between groups and conditions [F2,53=4.19, p<0.02], and between groups and sessions [F4,106=3.90, p<0.005]. There were, however no significant interactions between conditions and sessions [F2,106=2.12, p>0.10], and between groups, conditions and sessions [F4,106=2.12, p<0.08].

In SPDS *(Figure 4A)*, BL6J spent more time in the center compared to BALB/c [p<0.03] and CD-1 [p<0.04] in all 3 sessions. In RPDS *(Figure 4B)*, BL6J spent more time in the center compared to BALB/c in sessions 1 [p<0.001] and 2 [p<0.0006], and compared to CD-1 [p<0.03] in all 3 sessions. CD-1 spent less time in the center compared to BALB/c [p<0.05] in session 3. In addition, the time spent in the center was significantly low in session 1 compared to sessions 2 [p<0.05] and 3 [p<0.01], and in session 2 compared to session 3 [p<0.05] in BALB/c. It was significantly low in session 1 compared to session 2 [p<0.04] and 3 [p<0.05] in BL6J. There were no significant differences between sessions in CD-1 [p>0.10].

The number of crossings onto the center was significantly increased in BL6J exposed to RPDS compared to BL6J exposed to SPDS in all 3 sessions [p<0.04]. There were no significant differences between test configurations in BALB/c and in CD-1 groups [p>0.10].
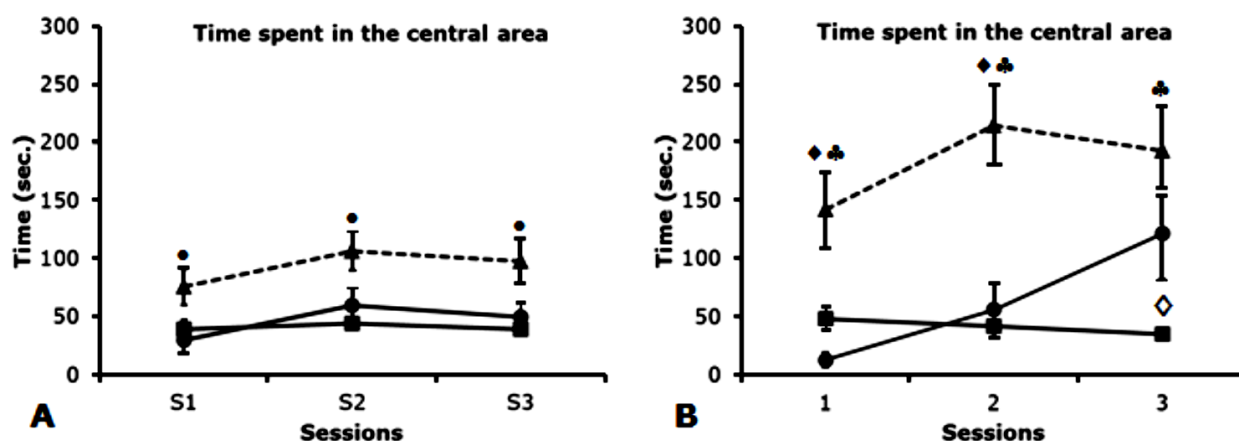
Figure 4: Standard **P**latform **D**ownward **S**lopes, left column, and **R**aised **P**latform **D**ownward **S**lopes, right column. (**A**) •BL6J compared to BALB/c [p<0.03] and CD-1 [p<0.04]; (**B**) ♦BL6J compared to BALB/c [p<0.001]; ♣BL6J compared to CD-1 [p<0.03]; ◊CD-1 compared to BALB/c [p<0.05].

3.1.6. Latency of first entry into the outer area

There were significant differences between groups [F2,53= 4.89, p<0.01] but not between sessions [F2,106= 2.89, p<0.06] and conditions [F1,53= 0.2, p>0.10]. There were, however, significant interactions between groups and sessions [F4,106=4.04, p<0.004].

In the SPDS , BALB/c took a long time to cross onto the outer area compared to BL6J and CD-1 in session 1 [p<0.04] and 3 [p<0.005] and compared to CD-1 in session 2 [p<0.05]. There were no significant differences between sessions in any mouse strain [p>0.10]. In RPDS, there were no significant difference between groups [p>0.10]. There were also no significant difference between sessions [p>0.10] except in CD-1 which took longer time to cross onto the outer area in session 1 compared to session 2 [p<0.02] and 3 [p<0.0009].

3.1.7. Time spent in AS and AV

In both AS and AV, there were significant differences between groups [F2,53=11.41 and 18.78 respectively, p<0.0001] and significant interactions between groups and conditions [F2,53=4.21 and 3.81 respectively, p<0.03]. In AS *(Figure 5A and 5B)*, there were significant differences between conditions [F1,53=3.89, p<0.05] but not between sessions [F2,106=0.70, p>0.10]. There were also significant interactions between groups and sessions [F4,106=4.29, p<0.003] but not between conditions and sessions [F2,106=0.31, p>0.10] and between groups, conditions and sessions [F4,106=0.67, p>0.10]. In AV *(Figure 5C and 5D)*, there were significant differences between sessions [F2,106=4.38, p<0.01], and significant interactions between conditions and sessions [F4,106=3.83, p<0.02], and between groups, conditions and sessions [F4,106=2.57, p<0.04].

In SPDS *(Figure 5A and 5C)*, BALB/c spent more time in AS compared to BL6J and CD-1 in session 1 [p<0.0001] and 2 [p<0.01]. They also spent more time in AV compared to BL6J and CD-1 in session 2 [p<0.001] and session 3 [p<0.02] but not in session 1 [p>0.10]. In addition, the time

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spent in AV was significantly high in session 2 compared to session 1 in BALB/c [p<0.05], and it was low in session 2 and 3 compared to session 1 in BL6J [p<0.001]. In RPDS *(Figure 5B and 5D)*, CD-1 spent more time in AS compared to BL6J in session 2 [p<0.04]. They also spent more time in AV compared to BL6J in session 2 [p<0.05] and 3 [p<0.01]. BALB/c spent more time in AV compared to BL6J in all 3 sessions [p<0.02], and compared to CD-1 in session 2 [p<0.0002]. In addition, the time spent in AV was significantly low in session 3 compared to session 1 and 2 in BALB/c [p<0.01]. It was also significantly low in sessions 2 and 3 compared to session 1 in BL6J [p<0.003]. There were no significant differences between sessions in CD-1 [p>0.10].

The time spent in AS was significantly low in BALB/c exposed to RPDS compared to BALB/c exposed to SPDS in sessions 1 [p<0.01] and 2 [p<0.05]. There were no significant differences between test configurations in BL6J and in CD-1 groups [p>0.10]. However, the time spent in AV was significantly high in BALB/c exposed to RPDS compared to BALB/c exposed to SPDS in sessions 1 [p<0.003] and it was significantly low in BL6J exposed to RPDS compared to BL6J exposed to SPDS in sessions 1 [p<0.04]. There were no significant differences between test configurations in CD-1 [p>0.10]. In both SPDS and RPDS, the 3 mouse strains spent significantly more time in AS than in AV [p<0.001].

3.1.8. Time spent on the slopes

There were significant differences between groups [F2,53=51.50, p<0.0001], between conditions [F1,53=15.88, p<0.0002] but not between sessions [F2,106=2.87, p<0.06]. There were also significant differences between groups and conditions [F1,53=3.94, p<0.03] and between groups, conditions and sessions [F4,106=3.50, p<0.01] but not between groups and sessions [F4,106=2.10, p<0.09] and between conditions and sessions [F2,106=1.20, p>0.10]. In SPDS *(Figure 5E)*, BL6J and CD-1 crossed onto the slopes while BALB/c did not. BL6J spent less time on the slopes compared to CD-1 in sessions 1 and 2 [p<0.0003] but not in session 3 [p<0.09]. In RPDS *(Figure 5F)*, BALB/c did not cross onto the slopes and BL6J made very few crossings. Hence, the time spent on the slopes was not significantly different between BL6/J and BALB/c in all 3 sessions [p>0.10]. However, all CD-1 crossed onto the slopes in each test session. They spent more time on the slopes compared to BALB/c [p<0.002] and BL6J [p<0.005].

The time spent on the slopes was significantly low in BL6J exposed to RPDS compared to BL6J exposed to SPDS in all 3 sessions [p<0.02]. It was also low in CD-1, but only in session 1 [p<0.008].
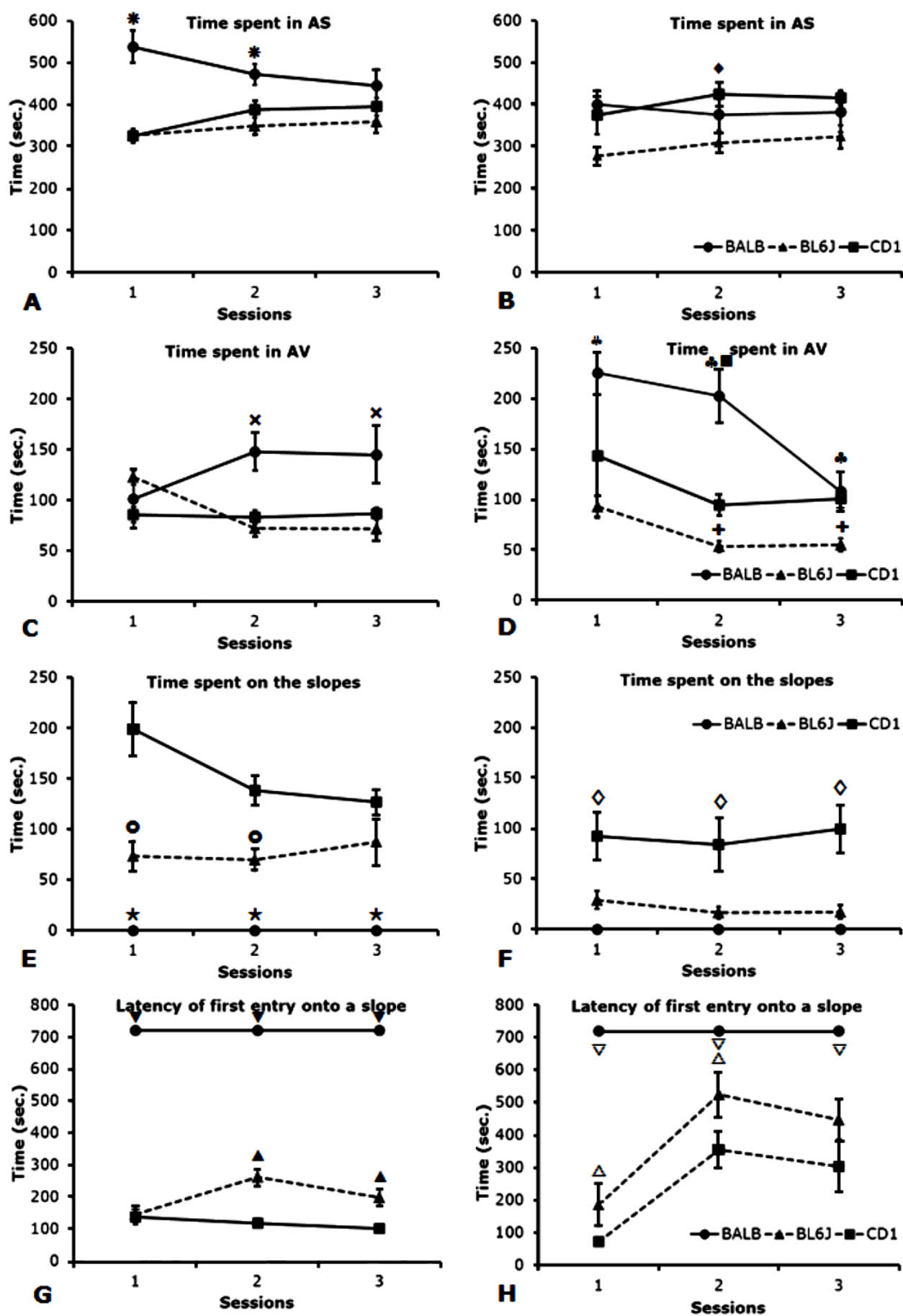
Figure 5: Standard **P**latform **D**ownward **S**lopes, left column, and **R**aised **P**latform **D**ownward **S**lopes, right column. (**A**) ✱BALB/c compared to BL6J and CD-1 [p<0.01]; (**B**) ♦CD-1compared

to BL6J [p<0.04]; (**C**) ✖BALB/c compared to BL6J and CD-1 [p<0.02]; (**D**) ♣BALB/c compared to BL6J [p<0.02]; ∎BALB/c compared to CD-1 [p<0.0002]; ✚BL6J compared to CD-1 [p<0.05]; (**E**) ★BALB/c compared to BL6J [p<0.001] and CD-1 [p<0.0001]; ⬤BL6J compared to CD-1 [p<0.0003]; (**F**) ◊CD-1 compared to BALB/c [p<0.002] and BL6J [p<0.005]; (**G**) ▼BALB/c compared to BL6J and CD-1 [p<0.0002]; ▲BL6J compared to CD-1 in sessions 2-3 [p<0.001]; (**H**) ▽BALB/c compared to BL6J and CD-1 in sessions 1-3 [p<0.003]; △BL6J compared to CD-1 in sessions 1-2 [p<0.04].

3.1.9. Latency of first entry onto a slope

There were significant differences between groups [F2,53=209.05, p<0.0001], between conditions [F1,53=20.96, p<0.0001], and between sessions [F2,106=20.80, p<0.0001]. There were also significant interactions between groups and conditions [F2,53=5.73, p<0.006], groups and sessions [F4,106=6.20, p<0.0002], conditions and sessions [2,106=12.74, p<0.0001] and groups, conditions and sessions [F4,106=3.32, p<0.01]. In both SPDS and RPDS, BALB/c did not cross onto the slopes in any of the 3 sessions, hence they recorded the total test duration. In SPDS *(Figure 5G)*, BL6J did cross but took longer time than CD-1 in sessions 2 and 3 [p<0.001]. They also took longer time to cross onto a slope in session 2 compared to sessions 1 [p<0.01] and 3 [p<0.04] whereas CD-1 took longer time in session 1 compared to session 3 [p<0.01]. In RPDS *(Figure 5H)*, BALB/c took longer time to cross onto a slope compared to BL6J and CD-1 in all 3 sessions [p<0.003], and BL6J took longer time than CD-1 in sessions 1-2 [p<0.04]. Both BL6J and CD-1 took less time to cross onto a slope in session 1 compared sessions 2 [p<0.04] and 3 [p<0.02].

3.2. Experiment 2 - Standard platform with upward slopes (SPUS)

3.2.1. Number of crossings on the platform *(Figure 6A)*

There were significant differences between groups [F2,27=33.89, p<0.0001] but not between sessions [F2,54= 1.52, p>0.10], and significant interactions between groups and sessions [F4,54=3.13, p<0.02]. BALB/c did fewer crossings than the other mouse strains in all 3 sessions [p<0.005], and CD-1 made significantly more crossings than BL6J in sessions 1 [p<0.0003] and 2 [p<0.01].

3.2.2. Number of crossings into the central area *(Figure 6B)*

There were significant differences between groups [F2,27=10.10, p<0.0005] and between sessions [F2,54=8.23, p<0.0008] but no significant interaction between groups and sessions [F4,54=1.97, p>0.10]. In all 3 sessions BALB/c made significantly less crossings onto the central area compared to the other strains of mice [p<0.01]. The number of crossings was significantly high in session 2 compared to session 1 in CD-1 [p<0.03] but not in BALB/c [p>0.10] and BL6J [p<0.08]. It was significantly high in session 3 compared to session 1 in BALB/c [p<0.02] and BL6J [p<0.002] but not in CD-1 [p>0.10]. It was also significantly high in session 3 compared to session 2 in BALB/c [p<0.02] but not in BL6J [p<0.06] and CD-1 [p>0.10].
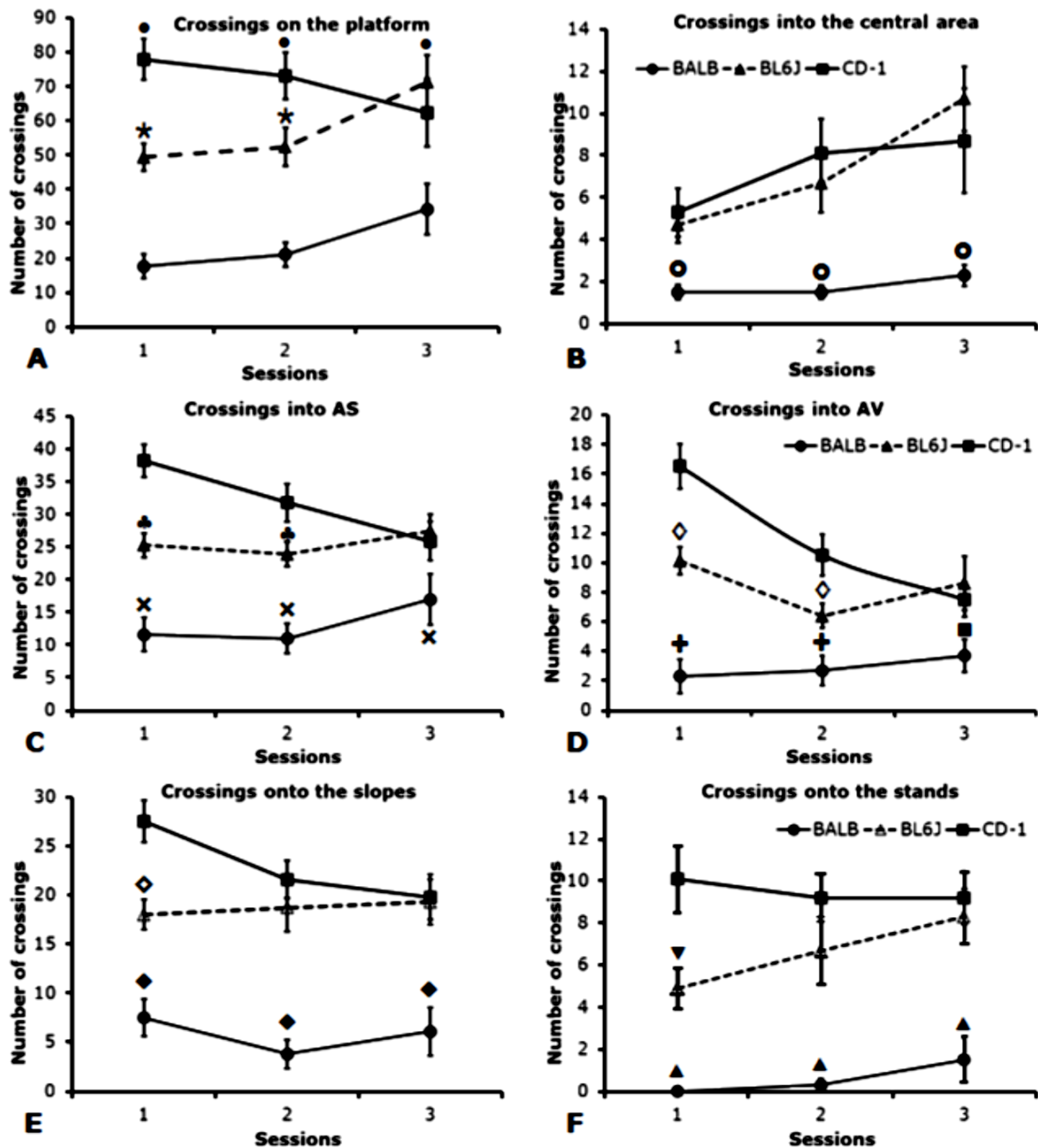
Figure 6: Standard Platform Upward Slopes. [**A**] ●BALB/c compared to BL6J and CD-1 [p<0.005], and ★BL6J compared to CD-1 [p<0.01]; [**B**]◐BALB/ compared to BL6J and CD-1 in session 1-3 [p<0.01]; [**C**] ✗BALB/c compared to BL6J and CD-1 [p<0.05]; ♣BL6J compared to CD-1 [p<0.02]; [**D**] ✚BALB/c compared to BL6J and CD-1 [p<0.02]; ■BALB/c compared to BL6J [p<0.05]; ◇BL6J compared to CD-1 [p<0.01]; [**E**] ◆BALB/c compared to BL6J and CD-1 [p<0.001]; ◇BL6J compared to CD-1 [p<0.001]; [**F**] ▲BALB/c compared to BL6J and CD-1 [p<0.003]; ▼BL6J compared to CD-1 in session 1 [p<0.002].

3.2.3. Number of crossings into AS (Figure 6C) and AV (Figure 6D)

There were significant differences between groups [F2,27=34.56 and 34.11 respectively, p<0.0001] and significant interactions between groups and sessions [F4,54=3.44 and 5.09 respectively, p<0.01]. There were however significant differences between sessions in AV [F2,54= 6.15, p<0.004] but not in AS [F2,54= 0.88, p>0.10] entries. BALB/c made few crossings onto AS compared to BL6J and

CD-1 in the 3 test sessions $[p<0.05]$, and BL6J made few crossings compared to CD-1 in the first 2 sessions $[p<0.02]$. BALB/c made also few crossings onto AV compared to the other groups in the first 2 sessions $[p<0.02]$, and compared to BL6J in session 3 $[p<0.05]$. In addition, BL6J made few crossings onto AV compared to CD-1 in sessions 1 $[p<0.001]$ and 2 $[p<0.01]$. CD-1 made few crossings onto AV in sessions 2 and 3 compared to session 1 $[p<0.002]$ and BL6J made few crossings onto AV in session 2 compared to session 1 only $[p<0.006]$. There were no differences between sessions in BALB/c $[p>0.10]$. In all 3 mouse strains, the number of entries in AS was significantly higher than in AV $[p<0.001]$.

3.2.4. Number of crossings onto the slopes (Figure 6E) and onto the stands (Figure 6F)

There were significant differences between groups $[F_{2,27}=37.08$ and 27.97 respectively, $p<0.0001]$ but no significant differences between sessions $[F_{2,54}=2.40$ and 1.67 respectively, $p>0.10]$ and no significant interactions between groups and sessions $[F_{2,54}=1.88$ and 1.42 respectively, $p>0.10]$. The number of crossings onto the slopes and onto the stands was significantly low in BALB/c compared to BL6J and CD-1 in all 3 sessions $[p<0.003]$. It was also significantly low in BL6J compared to CD-1 in the first session $[p<0.002]$.

3.2.5. Time spent in the central area (Figure 7A)

There were significant differences between groups $[F_{2,27}=7.91, p\leq0.002]$, but no differences between sessions $[F_{2,54}=0.58, p>0.10]$ and no significant interactions between groups and sessions $[F_{4,54}=0.69, p>0.10]$. BALB/c spent less time in the center compared to BL6J in each test session $[p<0.02]$, and compared to CD-1 in session 3 $[p<0.03]$. CD-1 spent also less time in the center compared to BL6J in session 1 $[p<0.02]$.

3.2.6. Latency of first entry onto the outer area

There were no significant differences between groups $[F_{2,27}=0.93, p>0.10]$ but significant differences between sessions $[F_{2,54}=3.36, p\leq0.04]$, and no significant interactions between groups and sessions $[F_{4,54}=1.45, p>0.10]$.

3.2.7. Time spent in AS (Figure 7B) and in AV (Figure 7C)

In AS, there were significant differences between groups $[F_{2,27}=44.01, p\leq0.0001]$ and between sessions $[F_{2,54}=0.34, p>0.10]$ but no significant interactions between groups and sessions $[F_{4,54}=0.96, p>0.10]$. In AV, there were significant differences between groups $[F_{2,27}=16.57, p\leq0.0001]$, between sessions $[F_{2,54}=6.09, p<0.004]$ and significant interactions between groups and sessions $[F_{4,54}=3.31, p<0.02]$. Post-hoc comparisons indicates that BALB/c spent more time in AS compared to the other mouse strains in each test session $[p<0.0002]$, and BL6J spent significantly more time compared to CD-1 in session 1 $[p<0.01]$. It indicates also that BALB/c spent less time in AV compared to the other groups in session 1 $[p<0.0002]$, and compared to CD-1 in session 2 $[p<0.001]$. BL6J spent less time in AV compared to CD-1 in session 2 $[p<0.01]$.

The time spent in AS was not significantly different between sessions in any group. The time spent in AV was also not significantly different between sessions in BALB/c [p>0.10], however it was significantly low in sessions 2 and 3 compared to session 1 in CD-1 [p<0.02], and in session 2 compared to session 1 in BL6J [p<0.0003]. In all 3 mouse strains, the time spent in AS was significantly higher than in AV [p<0.0001].

3.2.8. Time spent on the slopes and on the stands
There were significant differences between groups [F2,27= 7.59 and 24.43 respectively, p≤0.002] and between sessions [F2,54= 21.44 and 10.51 respectively, p≤0.0001]. There were, however, no significant interactions between groups and sessions [F4,54= 1.96 and 1.66 respectively, p≥0.10].

The time spent on the slopes *(Figure 7D)* was significantly low in BALB/c compared to BL6J in sessions 1 [p<0.03] and 2 [p<0.0007], and compared to CD-1 in session 1 [p<0.02]. The time spent on the slopes was significantly high in session 1 compared to sessions 2 and 3 in CD-1 [p<0.0008]. It was significantly high in sessions 1 and 2 compared to session 3 in BL6J [p<0.02]. There were no significant differences between sessions in BALB/c [p>0.06].

The time spent on the stands *(Figure 7E)* was significantly low in BALB/c compared to BL6J and CD-1 in each of the 3 test sessions [p<0.001], and in BL6J compared to CD-1 in the first 2 sessions [p<0.01]. The time on the stand was significantly high in session 3 compared to session 1 in BL6J [p<0.002], and compared to session 2 in BALB/c [p<0.04].

3.2.9. Latency of first entry on a slope and on a stand
There were significant differences between groups [F2,27=27.09 and 51.19 respectively, p<0.0001] and between sessions [F2,54= 3.51 and 4.91 respectively, p<0.04]. There were also significant interactions between groups and sessions in the slope latency [F4,54= 5.33, p<0.001] but not in the stand latency [F4,54= 1.08, p>0.10]. The latency of first entry on a slope *(Figure 7F)* and on a stand *(Figure 7G)* was significantly high in BALB/c compared to the BL6J and CD-1 in all 3 sessions [p<0.0002]. The slope latency was not significant between sessions within each group [p>0.10], except in BALB/c which demonstrated a short latency in session 1 compared to sessions 2 and 3 [p<0.01]. However, the stand latency was low, but not significant, in session 3 compared to session 1 [p<0.08] and 2 [p<0.06] in BALB/c, and it was significantly low in sessions 2 and 3 compared to session 1 [p<0.02] in CD-1.

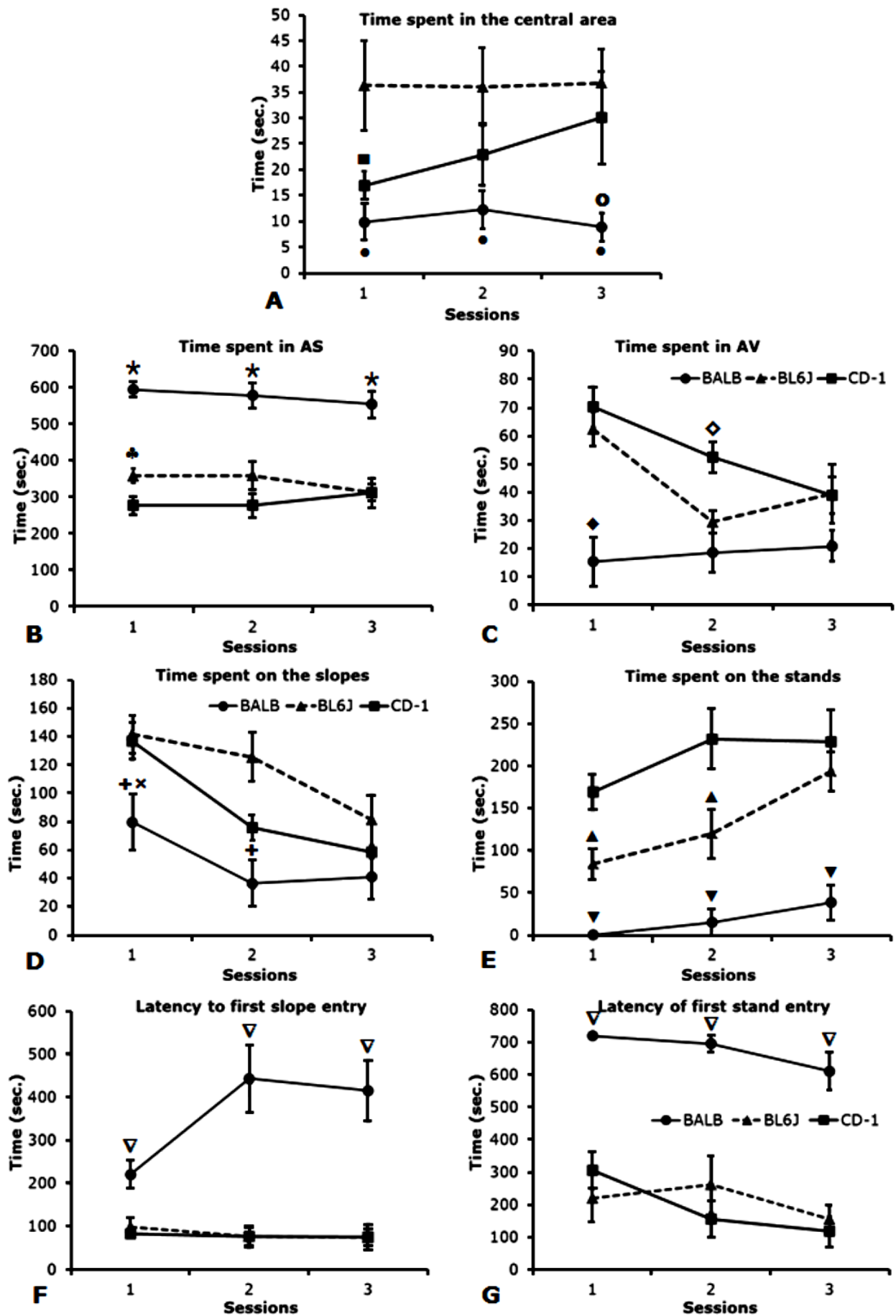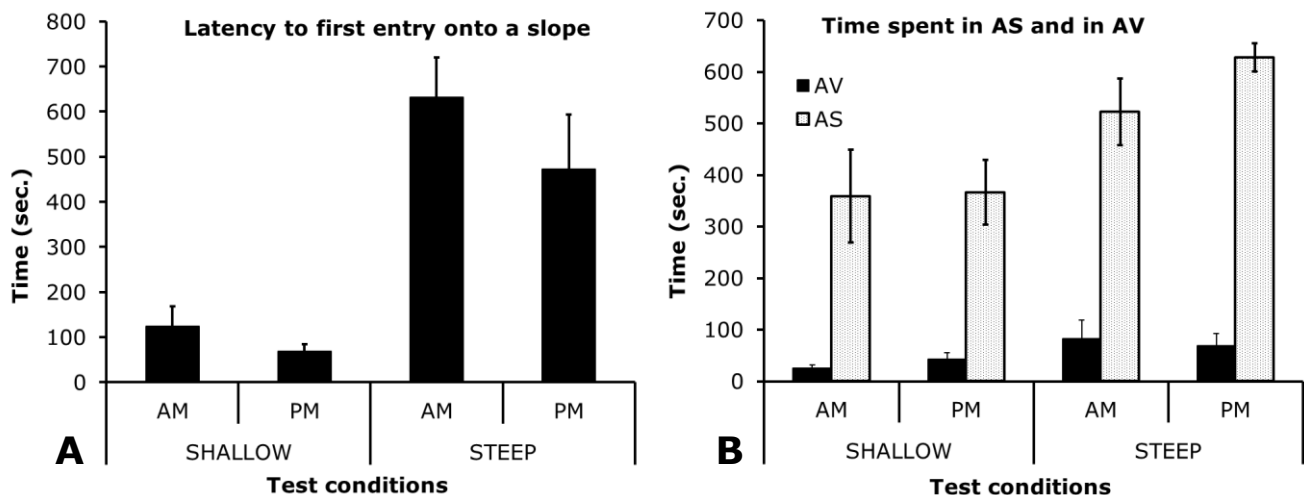**Figure 7:** Standard Platform Upward Slopes. [**A**] ●BALB/c compared to BL6J [p<0.02]; ○BALB/c compared to CD-1 [p<0.03]; ■CD-1 compared to BL6J [p<0.02]; [**B**] ★BALB/c compared to BL6J

and CD-1 [p<0.0002]; ♣BL6J compared to CD-1 [p<0.01]; [**C**] ◆BALB/c compared to BL6J and CD-1 [p<0.0002]; ◇CD-1 compared to BALB/c [p<0.001] and BL6J [p<0.01]; [**D**] ✚BALB/c compared to BL6J [p<0.03]; ✖BALB/c compared to CD-1 [p<0.02]; [**E**] ▼BALB/c compared to BL6J and CD-1 [p<0.001]; ▲BL6J compared to CD-1 [p<0.01]; [**F; G**] ▽BALB/c compared to the BL6J and CD-1 [p<0.0002].

3.3. Experiment 4 – Diurnal cycle

There were no significant differences between the times of the day for any behavior measures [F1,28<1.41, p>010]. There were however significant differences between test configurations in the latency of first entry [F1,28=33.54, p<0.0001], number of entries [F1,28=36.80, p<0.0001] and duration of entries [F1,28=24.11, p<0.003] onto the slopes. There were also significant differences between test configurations in the time spent in AS [F1,28=10.71, p<0.002]. There were, however, no significant interactions between times of the day and test configurations [F1,28<2.78, p>010]. Post-hoc analysis revealed that the latency of first entry onto a slope was significantly low in the shallow morning and afternoon conditions compared to both steep morning and afternoon conditions [p<0.004; Fig. 8A]. It revealed also that the number of entries onto and the time spent on the slopes was significantly high in the shallow morning and evening conditions compared to the steep morning and evening conditions [p<0.008; Figs. 8C and 8D].

The time spent in AS was significantly high in the evening steep condition compared to both morning and afternoon shallow conditions [p<0.03, Fig. 8B]. The time spent in AS was significantly higher than the time spent in AV within each test condition [p<0.007].

A Latency to first entry onto a slope — Time (sec.) vs Test conditions (SHALLOW AM/PM, STEEP AM/PM)

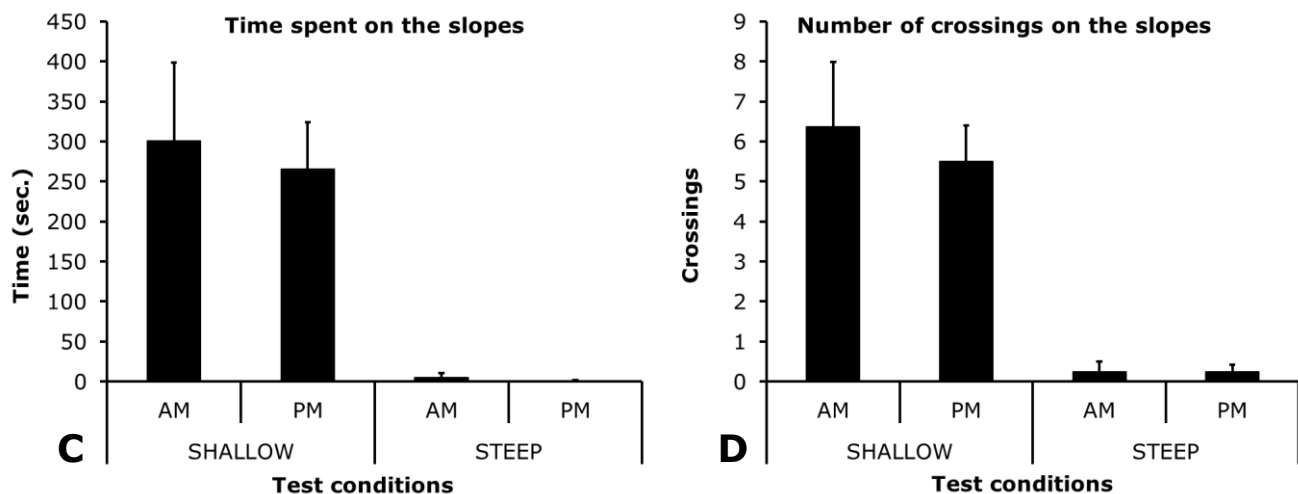**B** Time spent in AS and in AV — Time (sec.) vs Test conditions (SHALLOW AM/PM, STEEP AM/PM); ■AV, □AS

Fig. 8: Standard Platform Downward Slopes. [A] AM and PM Steep compared to AM and PM shallow [p<0.004]; [B] AM and PM Steep compared to AM and PM shallow [p<0.03]; [C] AM and PM Steep compared to AM and PM shallow [p<0.008]; [D] AM and PM Steep compared to AM and PM shallow [p<0.001]. One AM and one PM mouse crossed onto the steep slopes once for 12 and 5 sec, respectively.

4. DISCUSSION

The present experiments demonstrated that BALB/c mice, unlike BL6J and CD-1 mice, avoided crossing onto the slopes in the standard (SPDS) and in the raised (RPDS) platform configurations, and they avoided crossing onto the stands in the platform with upward inclined slopes (SPUS). These avoidance responses were observed over 3 test sessions. An increase in the elevation of the platform from the ground reduced significantly the number of crossings onto and the time spent on the slopes as well as it increased significantly the latency of first entry onto a slope in both BL6J and CD-1 mice; there were no differences between BL6J and BALB/c mice. All 3 mouse strains were able to climb the slopes when presented upward but only BL6J and CD-1 crossed onto the stands.

The number of crossings onto the slopes in SPDS and RPDS, and the number of crossings onto the stands in SPUS have been used to determine anxiety in mice. High anxiety mice avoided crossing onto the downward slopes in the former, and avoided crossing onto the stands in the latter. In SPDS, BALB/c mice demonstrated high level of anxiety in comparisons to BL6J and CD-1 mice. These confirm our previous results observed in a single test session with these mouse strains [50].

BALB/c mice were unable to take a risk and cross onto the slopes except when these were shallow or inclined upward. They were also unable to take a risk and cross onto the stands. This risk avoidant behavior has been associated with anxiety in humans [17, 32-33, 47-48, 51]. In the elevated platform, mice face uninformative or ambiguous stimuli, and the outcome from the choice between these stimuli is uncertain. High anxiety animals demonstrate impaired decision-making under these conditions; this is indicated by the prolonged time spent in AS. In

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humans with high anxiety, decision-making is also impaired in presence of ambiguous choices [33, 51, 88]. The elevated platform with downward slopes, unlike the plus-maze, light/dark box, and the open-field, does not provide a refuge or a shelter, which promote safety and security over risk taking [24, 21]. The presence of protected/unlit spaces may reduce fear and anxiety [50]; the decision-making is simplified by the availability of the choice between an apparent safe place and an apparent aversive place. While the forced exposure to a plus-maze, light/dark box or an open-field may produce fear and anxiety, these tests do not allow to determine anxiety in animals that preferred the enclosed/unlit space. Indeed, fear from an aversive stimulus is indicated by escape or avoidance response, and it does not always lead to anxiety. In the real world, both high and low anxiety animals may show preference for safety and security when these options are present. Indeed, our previous study [50] demonstrated clearly that C57BL/6 mice, a low anxiety strain, were comparable to BALB/c mice, a high anxiety strain, when a refuge was available in the center of the platform; they avoided the slopes. Other studies did also indicate that rats show a preference for a refuge or a shelter placed within an open-field [27, 55, 84], and foraging behavior depends mostly on safety needs rather than food availability. In presence of a threat animals show preference for a refuge [3, 19, 37, 45, 69, 75].

In SPDS, anxious animals seem unable to decide whether to endure the aversiveness of the open platform space or take a risk and venture onto the downward inclined slopes. In SPUS, BALB/c mice seem unable to cross onto the stand. This may be due the fact that the stands were not accessible to direct sensory perception. However, most of these mice did reach the top of the slopes; hence, they would have become aware of the presence of the stands. It can be argued that the avoidance of the downward slopes could be due to the inability of BALB/c mice to see and appreciate the elevation of the platform from the ground, or that it can be due to their physical inability to afford crossing onto and/or climbing down the slopes. However, both arguments could be challenged by the fact that CD-1 are also albinos, and that BL6J have the same body size as BALB/c. In addition, BALB/c mice were able to climb up and down the upward slopes, which are of the same steepness as the downward slopes. Furthermore, our previous study conducted over 3 or more sessions demonstrated that BALB/c mice were able to cross onto the downward slopes when treated with diazepam [22-23].

When exposed to the unfamiliar open spaces in SPDS or SPUS configurations mice experience fear and attempt to escape. The slopes are perceived as possible escape routes, which are attended to but avoided by animals with high anxiety, and attempted and explored by animals with low anxiety. An increase in the height of the platform decreased these attempts in BL6J and CD-1 mice. The downward slopes may appear more challenging than the upward one. In the former, mice may overestimate the distance from the top of the platform; the bottom end of the downward slopes may appear distant or undistinguishable from the grey dark floor

surface. Human studies suggest that vertical distances are overestimated when looking down from the top [38, 70-72], and this overestimation of distance is associated with fear of heights [71, 73]. The falling cost from downward slope would appear much higher than from upward slopes [38-39, 43].

Comparable challenges exist when mice try to move from the platform to a downward slope or from an upward slope to a stand. These challenges require sensory-motor skills, which are more complex than climbing up and down. Human studies suggest that anxiety alters the patterns and execution of movements [14, 30, 56, 65, 81-82], particularly during climbing [25, 59-61, 66-68]. People with high anxiety demonstrated longer climbing durations and less fluent displacement of the body's center of gravity in comparison to people with low anxiety. In SPDS condition, mice walked back and forth along the edges of AS with the snouts oriented toward the slopes, and they appeared to test different body orientations and reaching strategies when trying to cross onto the slopes. Such gauging of affordances is difficult to achieve when at the top of an upward inclined steep slope. The movements of the body and the limbs need to be synchronized in order to minimize the risk of fall when releasing the grip of the hind paws from the surface of a slope (wire mesh). Heightened anxiety can emerge from the attempt to execute the first steps onto a slope in SPDS or onto the stand in SPUS configurations.

Human studies suggest that anxiety is associated with the tendency to selectively attend to threat or negative stimuli [4, 18, 20, 46, 77]. This attentional bias could be inferred from the time spent in the outer areas (AS and AV), which represents more than 80% of a test session in BALB/c mice. In all 3 mouse strains, and in each test configuration, the time spent in AS was significantly greater than in AV. In addition, the time spent in AS was greater in BALB/c than in BL6J and CD-1 mice in SPDS and SPUS configurations, and it was comparable between strains in RPDS configuration. In BALB/c, the time spent in AS represented 75%, 66% and 62% of sessions 1 to 3 respectively in SDPS and 83%, 80%, and 77% in SPUS. In comparison, BL6J and CD-1 did not spend more than 55% of the test duration in AS. In RPDS configuration, the time spent in AS was comparable between the 3 test sessions. It represented 55%, 45% and 59% (maximum) of a test session in BALB/c, BL6J and CD-1 mice, respectively. The preference for and the large amount of time spent in AS suggest that all 3 mouse strains demonstrated high interest (attention) in the slopes, but this interest was more pronounced in BALB/c than in BL6J and CD-1 in SPDS and SPUS configurations. In SPDS, BALB/c mice appear to appraise the escape options that are available to them, and seem to demonstrate a selective attentional bias towards the downward slopes that they were unable to explore. In SPUS, it is not possible to determine whether appraisal and attention bias were present. The behavior of BALB/c mice compares to that observed in open-fields in which animals appear to seek refuge against walls [74, 80, 85]. In RPDS, BALB/c did demonstrate attentional bias toward the slopes as they spent more time in AS than in AV. However, the time spent in AS was significantly

reduced and the time spent in AV was significantly increased in comparison to the time spent in AS and AV in SPDS configuration. The behavior of BALB/c mice, in RPDS, may be due to a reduction of the difference in the perceptual estimation of the distance to the ground from AS and AV; this decreased the attractiveness of the intimidating slope option. The elevation of the platform, did also lead BALB/c mice, in session 3, and BL6J mice, in all 3 sessions, to spend more time in the center of the field than in the SPDS configuration.

BALB/c mice demonstrated no difference in anxiety when tested early morning or late afternoon. They crossed onto the shallow slopes and avoided the steep one. A number of studies demonstrated no effect of time of the day on anxiety in the plus-maze [5, 13, 40], the light/dark box [13, 52], the staircase [62], and the open-field [5, 40, 52, 76]. However, there are other studies, which reported an effect of time of the day in these tests [2, 29, 31, 41, 78, 86-87].

Time of the day is one of the many variables that have been reported to account for the conflicting results in the study of anxiety in animals [5, 31, 36, 63]. It was implicated in human studies, mostly in patients with depression, and/or with some forms of anxiety disorders [8-9, 35, 54, 57]. These patients have been reported to experience a circadian fluctuation of their anxiety symptoms [11-12; 15, 53; see 35]. However, a number of studies were unable to demonstrate a clear association between time of the day and anxiety in patients with anxiety disorders [28, 35]. In addition, healthy subjects do not appear to demonstrate fluctuations in anxiety throughout the day [11].

The effect time of the day on anxiety in animals has been based on reports from studies on humans diagnosed with anxiety disorders. In these studies, humans had to report their state of anxiety without being exposed at any time to an anxiogenic stimulus that would have evoked their emotional responses [11-12, 15, 28, 53]. This approach, which relies on verbal feedback, is facilitated by the fact that humans with anxiety worry [1, 7, 10, 58, 64], and may ruminate for hours or days about potential failure or threat [6, 26, 34, 42, 44, 49, 58, 79]. In animal studies, normal animals are exploited for differences in their level of emotionality and anxiety. Unlike humans, they cannot be assessed for their anxiety without exposure and/or re-exposure to an anxiogenic stimulus. Hence, anxiety in animals is a direct response to the actual anxiogenic stimulus. It is not clear why such a direct exposure to an anxiogenic stimulus is expected to produce anxiety at certain times of the day, and not at another time in animals. If an anxiogenic stimulus appears effective to produce anxiety in animals at (a) particular time(s) of the day; there should be a question mark about the anxiogenicity of that stimulus. These animals may show, as well, changes in performance in tasks unrelated to anxiety at this (these) specified time(s).

The results of the present experiments provide further support to the validity of our open space anxiety test, the elevated platform with downward slopes. They confirmed previous reports, which indicated high anxiety in BALB/c mice as compared to BL6J and CD-1 mice, and demonstrated that further elevation of the platform increased anxiety in BL6J mice. Hence, it appears that height is a significant factor in producing anxiety, and that it can be manipulated to increase anxiety in mouse strains with low anxiety in the standard configuration. In the configuration where the slopes were upward inclined, all mice were able to climb up, but only BL6J and CD-1 did reach and crossed onto the stands. This configuration confirmed that BALB/c mice represent a high anxiety mouse strain. The behavior of mice in the elevated platform with downward and upward slopes is not dissimilar from that of humans facing a climbing challenge at the foot of a steep slope or a descending challenge from the top of a cliff.

The elevated platform with slopes offers a 3-dimensional open field, which has a close resemblance to the navigation space in the real world. This promotes the natural animal's behavioral repertoire, which is highly restricted in the current boxed open-field. In the elevated platform with slopes, low and high anxiety mice do explore (move around) and investigate all parts of the test apparatus, except the slopes, which are avoided by high anxiety mice. They do not just cross over the central area; they stop and spend time there. By comparison, in the traditional open-field test, which is in fact a box with an open top, motor exploration is restricted to the center of the field and the walled outer area. This restriction is aggravated by the small size of the field, which is, in most research studies, ≤60 cm in width. The open-field from Med Associates Inc. (St Albans, Vt., USA), which has been used in behavioral phenotyping by Jackson Laboratory (USA) and The Wellcome Trust Sanger Institute (UK), is even much smaller (width 27.31 x 27.31 cm for mice and 43.18 x 43.18 cm for rats); this width is about 3 times the body length of a mouse or a rat, respectively. In these open-fields, avoidance of the center is used as an index of anxiety. However, this is represented by a cumulative recording of very briefs crossing time of the central area. There is no evidence that low anxiety animals stop and linger for some amount of times investigating this center.

We propose SPDS configuration for neurobiological studies of anxiety in mice or rats because, unlike in SPUS configuration, it is possible to manipulate the elevation of the platform from the ground, and therefore modify anxiety response. Manipulation of the platform elevation can be used in behavioral phenotyping of transgenic and knockout mice or in screening for novel anxiolytic drugs. In SPDS, a high anxiety mouse strain is required to determine an anxiolytic effect of a treatment, and a low anxiety mouse strain is required to determine an anxiogenic effect. A mouse strain, which had demonstrated low anxiety in SPDS, can also be selected to investigate the anxiolytic effect of a treatment, but the platform needs to be raised further above the ground.

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