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Temporal analysis of free exploration of an elevated plus-maze in mice

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Abstract

The elevated plus-maze (EPM) is a very common rodent test of anxiety. It is based on an approach/avoidance conflict between secure closed arms and aversive open arms. However, discrepancies remain on the interpretation of animals' behavior in this assay. The purpose of our study was to get a better understanding of the mouse behavior in the EPM. We applied a min-by-min analysis to compare the behavior of mice forcibly exposed to the maze or set free to explore the maze from a familiar box. Three strains of mice (CD1, BALB/c and C57Bl/6) were tested. The combination of our different conditions of test with the min-by-min analysis shows that mice do not avoid open arms during the first 2 minutes of test when they are forcibly exposed to the EPM. Conversely, free exploration of the EPM results in a pattern of behavior characterized by open arm avoidance from the outset, demonstrating that open arm avoidance in mice is unconditioned. These findings generalize across the three mouse strains. These data suggest that rodents enter the open arms to complete spatial information about the apparatus as a whole before their natural tendency to avoid them being expressed. Our data also indicate that a detailed behavioral analysis is needed whenever BALB/c mice are to be exposed by force to the EPM. Further studies are required to fully understand the behavior of rodents in the EPM and avoid false interpretations in the fields of psychopharmacology and behavioral neuroscience.

Keywords: Elevated plus-maze, anxiety, free exploration, emotional reactivity, BALB/c mice, CD1 mice, C57Bl/6 mice.

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Introduction

The elevated plus-maze is certainly the most common device to assess anxiety-like behaviors in rodents in the fields of psychopharmacology and behavioral neuroscience (Carobrez & Bertoglio, 2005; Rodgers, 1997). Up to date, an ISI Web of Knowledge search for the term “elevated plus maze” results in more than 5700 hits, while the same search in the PubMed database answers with more than 4700 hits. Despite the very wide utilization of the elevated plus-maze, the understanding of the rodent behavior in this apparatus is still incomplete and, as for other common device such as the open field, could result in false negative or false positive (Rodgers, 2007). For instance, concerns have been raised about the adequacy of the elevated plus-maze to discriminate measures of anxiety from measures of escape or avoidance responses (Ennaceur, Michalikova, van Rensburg, & Chazot, 2010a; Michalikova, van Rensburg, Chazot, & Ennaceur, 2010). In addition, whether avoidance of the open arms is really an unconditioned process or whether it is learned during exposure to the test is still questioned (Carobrez & Bertoglio, 2005; Holmes & Rodgers, 1999). This is particularly relevant when min-by-min studies are considered because these studies show that animals enter open arms and closed arms equally at the beginning of a trial, but rapidly develop a clear preference for the closed arms (Bertoglio & Carobrez, 2002; Casarrubea et al., 2013; Holmes & Rodgers, 1998; Pereira, da Cunha, Neto, Paschoalini, & Faria, 2005; Rodgers, Johnson, Cole et al., 1996; Rosa, Vandresen, Calixto, Kovaleski, & Faria, 2000). This observation is in contradiction with the idea of an unconditioned approach/avoidance conflict supposed to drive rodent’s behavior in the elevated plus-maze (Pellow, Chopin, File, & Briley, 1985).

In a recent work, the behavior of DA/Han rats that were allowed to freely explore the elevated plus-maze from a familiar box (i.e. “free exploration”) was compared to the behavior of rats that were exposed to the apparatus according to the standard procedure (i.e. “forced exposure”) (Roy, Chapillon, Jeljeli, Caston, & Belzung, 2009). In agreement with the pioneer work by Montgomery (1955), it was demonstrated in the free exploration condition that rats naturally fear open arms and do not need to explore these arms in order to learn about their aversive properties. In addition, it was hypothesized that some of the open-arm entries observed

in the standard version of the elevated plus-maze (i.e. when the animal is forcibly exposed to the apparatus), occurring during the first minutes of the test, could be interpreted as attempts to avoid the whole situation, rather than true indication of anxiety. Indeed, the authors proposed that the approach/avoidance conflict is not clearly set from the beginning of the exposure to the plus-maze (from the animal's point of view); this conflict becoming progressively clearer as the animal grows more familiar with the closed arms. This explanation still has to be demonstrated but seems coherent with studies that have demonstrated a behavioral shift during the course of the initial trial leading to the avoidance of the open arms (i.e. decreasing exploration of open arms) once the entire maze configuration has been learned by mice or rats (Bertoglio & Carobrez, 2000; Casarrubea et al., 2013; Rodgers & Shepherd, 1993; Roy et al., 2009). The existence of such a learning process would be consistent with the one-trial tolerance (OTT) effect, an observation which is characterized by a high avoidance of the open arms in control animals and the inefficiency of anxiolytic compounds during a second trial in the elevated plus-maze (File, Mabbutt, & Hitchcott, 1990; File & Zangrossi, 1993; File, Zangrossi, Viana, & Graeff, 1993; Rodgers, Johnson, Cole et al., 1996; Rodgers & Shepherd, 1993).

In order to address these issues, we compared the free exploration and forced exposure conditions in the elevated plus-maze, through a detailed temporal analysis (i.e. min-by-min) as recommended by Carobrez & Bertoglio (2005), in three strains of mice differing by their anxiety profiles, namely CD1, BALB/cByJ and C57Bl/6 mice. CD1 outbred mice are frequent in the field of pharmacology and their behavior in anxiety tests is well documented (Ennaceur, Michalikova, van Rensburg, & Chazot, 2010b; Michalikova et al., 2010; Shanks, Griffiths, Zalcman, Zacharko, & Anisman, 1990; Wall & Messier, 2000). BALB/cByJ mice have been described as anxious (even as a model of pathological anxiety: Belzung & Griebel, 2001) and stress sensitive while, at the opposite, C57Bl/6 mice have a lower anxiety and more stress resilient profile (e.g. Anisman, Hayley, Kelly, Borowski, & Merali, 2001; Roy, Merali, Poulter, & Anisman, 2007). On the whole, our data improve the current test interpretation.

Materials and Method

Animals

Throughout the experiment, the animals were maintained in an air-conditioned room ($21 \pm 2^\circ\text{C}$) under a 12 hours light-dark cycle (light on at 00h00). All the mice used in this study were test-naïve before the beginning of the experiments. A total of 45 CD1 male mice (2-3 months) from our own breeding colony were used in Experiment 1. Animals were maintained in groups of 7 animals (± 1) in standard polycarbonate cages ($40 \times 28 \times 18$ cm) with free access to food and water. A total of 18 C57Bl/6J (C57) male and 18 BALB/cByJ (BALB/c) male mice (2-3 months) born in our animal facilities were tested in Experiment 2. In this experiment, animals were maintained in groups of 4 animals (± 1) in standard polycarbonate cages ($24 \times 20 \times 14$ cm). In both experiments, the breeders were originated from Janvier (France). Animals were tested during the first part of the dark phase (between 2 and 6 pm).

The research reported in this paper was conducted in accordance with the European council directive for the protection of animals used for experimental and other scientific purposes (EC86/609).

Behavioral testing

Elevated Plus-maze and familiar box

The elevated plus-maze was made of ivory Perspex (IntelliBio, France). The arms were 25 cm long and 5 cm wide, and the apparatus was elevated at a height of 50 cm. The closed arms were surrounded by a 25 cm wall while open arms had 0.5 cm edges in order to maximize open-arm entries through thigmotaxis (Pickles & Hendrie, 2013; Treit, Menard, & Royan, 1993). The maze was illuminated with dim white light: 100 lux on open arms and centre, 50 lux at the extremities of closed arms.

The day before testing, all animals were isolated for familiarization (at least 18h) in beige painted wooden boxes ($20 \times 20 \times 15$ cm). Such boxes were previously used as a “familiar box” for the free exploration paradigm in rats (Roy & Chapillon, 2004; Roy et al., 2009). The floor of each box was covered with sawdust and animals were given free access to food and water. Each box had a small removable door (6×6 cm) on one of its sides and was covered with a removable perforated Plexiglas top.

Experiment 1: Forced exposure vs. free exploration in CD1 mice

Three groups of mice (n = 15) were tested in experiment 1. On the testing day, mice from the first group were taken by hand from their familiar box, placed in the central part of the maze facing an open arm, and allowed to explore the maze for 5 minutes. This condition, later called “forced center”, corresponds to the standard procedure used in most laboratories. In a second group, the “free exploration” group, mice were allowed to freely explore the maze from their familiar box that was positioned next to the elevated plus-maze (Fig. 1). At the time of the test, the experimenter removed the small door of the familiar box and the background panel of the connected closed arm in order to give an access to the maze. The test started as soon as the mouse freely entered the maze. Going back to the familiar box was possible and the test lasted as long as necessary to get 5 minutes of presence in the maze after the initial entrance. In a third group, “forced closed-arm”, mice were taken by hand and placed to the end of a closed arm, facing the end of the arm. This group was set up in order to control the effect of the starting position in the forced condition.

Experiment 2: Forced exposure vs. free exploration in BALB/c and C57Bl/6 mice

In experiment 2, we tested BALB/c and C57Bl/6 mice in two of the previous conditions (forced center vs. free exploration). In this experiment, the beginning of the test in the free exploration condition was set to the first entry of the animal in the central part of the maze instead of the first entry into the closed arm of the maze (experiment 1). This was chosen on the basis of pilot studies in which BALB/c and C57Bl/6 mice had a higher latency to reach the centre platform of the maze than CD1 mice.

In both experiments, animals from the free exposure condition that did not enter the maze within 20 minutes after the door removal were not included in the statistical analyses. Only two mice from experiment 2 (1 C57Bl/6 and 1 BALB/c) were removed from the statistical analyses due to this criteria. In addition 1 CD1 mouse fell off the maze and was discarded from the experiment.

Data recording

All tests were analyzed with ANY-maze® (Stoelting Co, Wood Dale, IL – v4.72, anymaze.com), a video-tracking system dedicated to the analysis of animal behavior assays such as the elevated plus-maze (Walf & Frye, 2007). Times and numbers of entries in the different parts of the maze were automatically recorded by the tracking system. An entrance into an arm was counted when 98% of the animals' area (*or blob*) was detected by the tracking system into this arm. The animal was considered at the center of the maze when 50% of its blob had left the arm. From a pilot study in the lab, these criteria match the usual 4 paws (for entries into an arm) and 2 paws (for exits from an arm) criteria with a trained experimenter. The system also recorded the total distance covered in the maze and closed-arm returns (i.e. going back to the same closed arm after a central part entry, as described by Rodgers, Johnson, Cole et al., 1996). Percent time in the open parts of the maze was calculated by ANY-maze [(time open/session duration) X100]. Some additional measures were manually recorded with the use of the ANY-maze ethological keyboard: rears, protected stretched attend postures (pSAPs) and head scans (the mouse points its head into an open arm with its body still in the centre or in a closed arm of the maze). Protected stretched attend postures are behaviors related to risk-assessment and defined as forward elongation of the head and shoulders followed by retraction to original position (Rodgers, Johnson, Cole et al., 1996). Latency and attempts to enter the maze in the free exploration condition as well as the number of feces in experiment 2 were manually recorded.

Statistical Analyses

Data are presented as mean \pm SEM. Statistical analyses were completed by using ANY-maze and SPSS. In most of the cases, two-way ANOVA were performed with condition of test as a factor (forced center, forced closed-arm or free exploration) and segment of test (min-by-min) as a repeated measure. In experiment 2, Student t tests were ran between BALB/c and C57Bl/6 mice in the free exploration condition only. Confidence intervals (CIs) for partial eta squared and Cohen's d were calculated from the SPSS code kindly provided by Dr. Smithson's on its academic webpage (see also Smithson, 2003). HSD Tukey's tests were used for follow-up comparisons. Infrequent behaviors such as the ethological behaviors (e.g. protected SAPs or closed-arm returns) were compared over 5 minutes with Kruskal-Wallis and/or Mann-Whitney U tests.

Results

Experiment 1

The mean latency to emerge from the familiar box in the free exploration situation was of 18.33 ± 5.53 seconds. The latency to reach the central part of the maze after the 1st maze entrance was 11.87 ± 1.20 seconds. For animal that started at the end of a closed arm, the latency to reach the central platform for the first time was 3.68 ± 0.76 seconds. Since these latencies were quite short, a direct comparison with the mice from the forced groups that started the trial at the center of the maze was possible. The mean number of returns into the home cage in the free exploration group was 10.53 ± 1.17 and the total time spent in this compartment during the test was 55.73 ± 6.37 seconds.

The analysis for number of open arm entries (Fig. 2A) raised a significant main effect of treatment ($F_{2,42} = 7.79$, $p < 0.01$, partial $\eta^2 = 0.271$, 95% CI = 0.078, 0.412), a significant effect for the segment of test ($F_{4,168} = 14.35$, $p < 0.001$, partial $\eta^2 = 0.255$, 95% CI = 0.151, 0.327) and a significant interaction between these two factors ($F_{8,168} = 5.30$, $p < 0.001$, partial $\eta^2 = 0.202$, 95% CI = 0.086, 0.254). The follow-up comparisons showed that open arm entries were less frequent in the free exploration group than in the forced groups on minute 1 (p at least < 0.01). On minute 2, it was only inferior to the forced group exposed to the central platform ($p < 0.05$). In addition, open arm entries in both forced exposure groups were more frequent during minute 1 compared to minutes 3 to 5 (p always < 0.001 for the forced center group and p always < 0.01 in the forced closed-arm group). Interestingly, no difference was found for the min-by-min analysis in the free exploration group.

The analysis for the percent time spent in the open arms (Fig. 2B) raised a significant main effect of treatment ($F_{2,42} = 7.49$, $p < 0.01$, partial $\eta^2 = 0.263$, 95% CI = 0.072, 0.405), a significant effect for the segment of test ($F_{4,168} = 8.44$, $p < 0.001$, partial $\eta^2 = 0.167$, 95% CI = 0.075, 0.235) and a significant interaction between treatment and segment of test ($F_{8,168} = 5.28$, $p < 0.001$, partial $\eta^2 = 0.201$, 95% CI = 0.085, 0.253). Follow-up analyses for the interaction showed that animals from the two forced exposure groups had a higher percent time in the open arms during minute 1 compared to animals from the free exploration group ($p < 0.001$ for the forced center group and $p < 0.01$ for the forced closed-arm group). In addition, this percentage was also significantly higher during minute 1 in the forced center group compared to the forced

closed-arm group ($p < 0.01$). In the forced center group, the percent time in the open arms on minute 1 was higher than during minutes 2 to 5 (p at least < 0.01).

The total distance traveled in the maze (Fig. 2C) only depended on the min-by-min analysis ($F_{4, 168} = 15.10$, $p < 0.001$, partial $\eta^2 = 0.265$, 95% CI = 0.160, 0.337); follow-up comparisons showing that activity was higher during minute 2 than during minutes 1, 3, 4 and 5 (p at least < 0.05).

Number of rears (Table 1) also depended on the min-by-min analysis ($F_{4, 168} = 17.09$, $p < 0.001$, partial $\eta^2 = 0.289$, 95% CI = 0.184, 0.362). Follow-up comparisons showed that rears were less frequent during minute 1 than during other minutes (p always < 0.001).

There was no difference between groups for the number of head scans towards the open arms (Table 1) but this behavior decreased during the test ($F_{4, 168} = 9.45$, $p < 0.001$, partial $\eta^2 = 0.184$, 95% CI = 0.089, 0.253); head scans in minutes 4 and 5 were less frequent than during minute 1 and minute 2 (p at least < 0.05).

Protected SAPs were slightly increased in the free exploration condition (2.60 ± 0.72) compared to the two forced conditions (forced center: 2.00 ± 0.43 and forced closed arm: 1.47 ± 0.27) but the difference was not significant. The number of closed-arm returns was different among groups (Kruskal-Wallis = 6.20, $df = 2$, $p < 0.05$) and follow-up comparisons showed that it was lower in animal forcibly placed at the central part of the maze compared to animal forcibly placed at the end of a closed arm (respectively 0.60 ± 0.16 and 1.67 ± 0.29 ; $U_{15, 15} = 48.50$, $p < 0.05$). It was also lower compared to animals from the free exploration group (1.53 ± 0.42) though not significantly.

Experiment 2

The latency to reach the central position in the elevated plus-maze was of 221.38 ± 68.08 seconds in BALB/c mice and of 424.11 ± 103.78 seconds in C57Bl/6 mice. The number of attempts at the familiar box/maze junction was higher in BALB/c mice (12.63 ± 4.28) than in C57Bl/6 mice (5.67 ± 1.17). These differences were not significant due to data spread. Returns to the familiar box during free exploration of the maze were more frequent in BALB/c mice than in C57Bl/6 mice (respectively 13.75 ± 1.46 and 8.56 ± 1.49 ; $t_{15} = 2.48$, $p < 0.05$, Cohen's $d = 0.62$, 95% CI = 0.074, 1.149) while the total time in the home cage during the test was lower in BALB/c mice than in C57Bl/6 mice (respectively 129.50 ± 27.89 and 249.44 ± 42.61 ; $t_{15} = 2.29$, $p < 0.05$, Cohen's $d = 0.57$, 95% CI = 0.034, 1.095).

The analysis for number of open arm entries in BALB/c mice (Fig. 3A, left) raised a significant main effect of treatment ($F_{1,16} = 12.04$, $p < 0.01$, partial $\eta^2 = 0.429$, 95% CI = 0.111, 0.614) and a significant interaction between treatment and segment of test ($F_{4,64} = 2.68$, $p < 0.05$, partial $\eta^2 = 0.144$, 95% CI = 0.004, 0.234). The follow-up comparisons showed that open arm entries were less frequent in the free exploration group than in the forced group on minute 1 ($p < 0.001$) and minute 2 ($p < 0.01$). In C57Bl/6 mice (Fig. 3A, right), a significant effect for treatment ($F_{1,16} = 17.57$, $p < 0.01$, partial $\eta^2 = 0.523$, 95% CI = 0.197, 0.680), segment of test ($F_{4,64} = 12.52$, $p < 0.001$, partial $\eta^2 = 0.439$, 95% CI = 0.254, 0.529) and the interaction between these two factors ($F_{4,64} = 6.97$, $p < 0.001$, partial $\eta^2 = 0.304$, 95% CI = 0.120, 0.404) was revealed. Follow-up comparisons first showed less open arm entries in the free exploration group compared to the forced group on minute 1 only ($p < 0.001$). In addition, the comparisons for the segments of test showed that open arm entries were more numerous during minute 1 than during other minutes in the forced group (p at least < 0.01) and in minute 2 than in minute 4 ($p < 0.05$).

Consistently with the result for the number of open arm entries, the analysis for the percent time in the open arm in BALB/c mice (Fig. 3B, left) raised a significant main effect of treatment ($F_{1,16} = 25.15$, $p < 0.001$, partial $\eta^2 = 0.611$, 95% CI = 0.298, 0.739) and a significant interaction between treatment and segment of test ($F_{4,64} = 4.90$, $p < 0.01$, partial $\eta^2 = 0.235$, 95% CI = 0.063, 0.335). The follow-up comparisons showed that the percent time in the open arms was significantly lower in the free exploration group than in forced group on minute 1, 2 and 3 (p at least < 0.01). It was also higher in minute 5 than in minute 1 in the free exploration group ($p < 0.05$) while it was lower in minute 4 than in minute 1 in the forced exposure group ($p < 0.05$). In C57Bl/6 mice (Fig. 3B, right), significant effects of test condition ($F_{1,16} = 18.30$, $p < 0.01$, partial $\eta^2 = 0.534$, 95% CI = 0.207, 0.687), segment of test ($F_{4,64} = 6.59$, $p < 0.001$, partial $\eta^2 = 0.292$, 95% CI = 0.109, 0.392) and interaction ($F_{4,64} = 4.07$, $p < 0.01$, partial $\eta^2 = 0.203$, 95% CI = 0.040, 0.301) were observed. Follow-up comparisons showed that animals from the forced group had a higher percent time in open arms ($p < 0.001$) compared to the free exploration group on minute 1 only. In addition, the percent time in the open arms in the forced group was higher in minute 1 than in minutes 4 and 5 (p at least < 0.01) and in minute 2 compared to minute 4 ($p < 0.05$).

The total distance covered in the maze in BALB mice is shown in Fig. 3C, left. No statistical difference was obtained. In contrast, the effect of the segment of test and the

interaction between this factor and the testing condition was significant in C57Bl/6 mice (respectively $F_{4, 64} = 9.54$, $p < 0.001$, partial $\eta^2 = 0.374$, 95% CI = 0.186, 0.470 and $F_{4, 64} = 3.12$, $p < 0.05$, partial $\eta^2 = 0.163$, 95% CI = 0.015, 0.257) (Fig. 3C, right). Follow-up comparisons showed that activity during minute 1 in the forced group was higher than in the free exploration group ($p < 0.01$). Also, activity in the forced group on minute 1 was higher than during minutes 3 to 5 (p at least < 0.05) and activity on minute 2 was higher than during minute 5 ($p < 0.01$).

The analysis for the number of rears (Table 2) resulted in a significant effect for the segment of test in BALB/c mice ($F_{4, 64} = 2.71$, $p < 0.05$, partial $\eta^2 = 0.145$, 95% CI = 0.005, 0.236). There was an increase in the number of rears across the minutes, but the follow-up comparisons showed no difference between the early minutes and the end of the trial. In C57Bl/6 mice, the number of rears was marked by a main effect of the condition of test ($F_{1, 16} = 15.47$, $p < 0.05$, partial $\eta^2 = 0.492$, 95% CI = 0.165, 0.658), mice from the forced group having a higher number of rears.

Analyses for head scans (Table 2) yielded main effects for segment of test ($F_{4, 64} = 3.84$, $p < 0.01$, partial $\eta^2 = 0.498$, 95% CI = 0.171, 0.662), condition of test ($F_{1, 16} = 15.87$, $p < 0.01$, partial $\eta^2 = 0.193$, 95% CI = 0.033, 0.290) and interaction ($F_{4, 64} = 2.95$, $p < 0.05$, partial $\eta^2 = 0.156$, 95% CI = 0.010, 0.248) in BALB/c mice. Follow-up comparisons showed that head scan during minute 1 and 2 were more frequent in mice from the free exploration group than mice from the forced exposure group (p at least < 0.01). In addition, head scans during minute 3 in the free exposure group were less frequent than during minute 1 and 2 ($p < 0.05$). There was a significant effect for segment of test in the number of head scans displayed by C57Bl/6 mice ($F_{4, 64} = 5.23$, $p < 0.01$, partial $\eta^2 = 0.246$, 95% CI = 0.072, 0.347). Follow-up comparisons showed that head scans were more frequent on minute 1 than on minute 4 and 5 (p at least < 0.05).

Results for ethological behaviors are presented in Fig. 4. Statistical analyses show that closed arm returns and protected SAPs were enhanced in BALB/c mice in the free exploration condition (respectively $U_{10, 8} = 5$, $p < 0.001$ and $U_{10, 8} = 12.5$, $p < 0.05$). In C57Bl/6 mice, only protected SAPs were enhanced in the free exploration condition compared to the forced exposure condition ($U_{9, 9} = 16.5$, $p < 0.05$). On the contrary, the number of feces in the free exploration condition was reduced in both strains compared to that number in the forced exposure situation (BALB/c: $U_{10, 8} = 7.5$, $p < 0.01$ and C57Bl/6: $U_{9, 9} = 18$, $p < 0.05$).

General Discussion

The aim of our research was to get a better understanding of the mouse behavior in the elevated plus-maze. To this end, we applied a min-by-min analysis to the behavior of mice exposed under different conditions to the elevated plus-maze. Depending on the experiment, some mice were forcibly placed to the central part of the maze or to the end of a closed arm, while other mice were set free to start their exploration of the maze from a familiar box connected to the end of a closed arm. Three different strains of mice with different anxiety profiles (CD1, BALB/c and C57Bl/6) were tested in order to generalize the results (Anisman et al., 2001; Ennaceur et al., 2010b; Michalikova et al., 2010; Roy et al., 2007; Shanks et al., 1990).

First, we found an effect of the condition of exposure to the elevated plus-maze. Open arm entries and the percent time in the open arms were reduced when a free exploration of the maze started from a familiar box, as opposed to the forced exposure from the central part of the maze. This result applies for CD1 mice in experiment 1 but also for C57Bl/6 and BALB/c mice in experiment 2. Even though non-significant, an intermediate behavior towards open arms was also obtained in CD1 mice when the forced exposure to the elevated plus-maze was realized from the end of a closed arm. This result shows how important could be some methodological aspects such as placing the animal at the central part of the maze versus placing it at the end of a closed arm, or facing an open arm versus a closed arm when the central part option is chosen. The standard methodology (i.e. placement at the central part of the maze, facing an open arm) is made upon initial studies such as the study by Pellow et al. (1985) who found no significant difference for facing an open or a closed arm at the starting of an assay. However, it seems that these methodological aspects should be investigated more systematically.

Back to the previous point, since locomotion in the maze and head scan behaviors towards the open arms were quite similar in the three conditions, it is possible to refute that animals in the free exploration situation did not detect or did not intended to visit the open arms. Thus, forced exposure to an elevated plus-maze, especially from its central platform, results in more frequent visits of open arms than free exploration from a familiar box does.

The min-by-min analysis added relevant results and interpretation. We found that open arms entries in CD1 mice from the two forced conditions mainly occurred during the first two minutes of test. Similarly to other min-by-min studies, we observed thereafter that open arm

entries and the percent time in the open arms dropped throughout the trial (Carobrez & Bertoglio, 2005; Casarrubea et al., 2013; Holmes & Rodgers, 1999; Pereira et al., 2005; Rodgers, Johnson, Cole et al., 1996; Rosa et al., 2000). For instance, the percent time in the open arms in CD1 mice forcibly exposed to the central platform dropped from around 35 percent during the 1st minute to roughly 10 percent during the 3rd, 4th and 5th minutes. A smaller but comparable effect was found in mice initially placed at the end of a closed arm. In comparison, the percent time in the open arms in the free exploration condition was constantly low, around 10 percent from the first minute of the experiment. As a consequence, CD1 mice that were allowed to freely explore the maze entered less frequently the open arms during the two first minutes of test than CD1 mice forcibly exposed to the device. This led to an interaction (exposure condition \times min-by-min) that Roy et al. were not able to demonstrate in their work with the free exploration of a plus maze in rats (2009).

The use of BALB/c and C57Bl/6 mice in experiment 2 allowed us to generalize this result. Even if a small methodological modification was introduced in experiment 2 (i.e. video-tracking started at the first entrance to the central part of the maze whereas it started at the first maze entrance in experiment 1), C57Bl/6 and BALB/c mice forcibly exposed to the central platform also entered the open arms more frequently during the first minute of test than their free exploration counterparts. Small differences between strains were also noticed in particular for the time they needed before avoiding almost completely the open arms. This could be attributed to strains differences, for instance in the speed of the changes in subordinate/dominant status after isolation (Ferrari, Palanza, Parmigiani, & Rodgers, 1998). Nevertheless, the general pattern with immediate open arm avoidance in the free exploration situation and progressive open arm avoidance in the forced exposure situation was still observed in all three strains.

On the whole, these results support a previous hypothesis (Roy et al. 2009). Animals forcibly exposed to the elevated plus-maze first have to explore the whole apparatus before they can behave accordingly to the approach/avoidance conflict. In other terms, the animals must explore both the closed-arms and the open-arms of the apparatus to be able to give them comparative values. Then, they are able to decide that sheltering into the closed arms is the best solution to the approach/avoidance conflict. This is in complete agreement with the hypotheses by Rodgers et al. (Rodgers, Johnson, Cole et al., 1996; Rodgers & Shepherd, 1993) and Bertoglio and Carobrez (2000) that assume respectively that the animal needs to learn about the

spatial configuration of the maze and that the animal needs to experiment both the open and closed arms before behaving accordingly to the approach/avoidance conflict. This interpretation stands in contradiction with a previous hypothesis stating that animals learn specifically the aversive properties of the open-arms during the first exposure to the maze (File & Zangrossi, 1993; File et al., 1993). Since mice from different strains avoided open arms almost completely in the free exploration condition, during the whole test, our results demonstrate that avoidance of open arms is really unconditioned. This is fully consistent with the study in rats by Roy et al. (2009) and with the original work by Montgomery (1955), in which rats avoided open alleys that they were freely allowed to explore.

A hypothesis suggested by our data, though not demonstrated, is that during the early onset of the test, the whole maze could represent a situation to avoid from the animal's point of view. The first open-arm entries thus may represent, to a certain extent, some attempts to escape from the maze. A similar assumption stands for the locomotion in the open field, which can traduce both exploration and attempts to escape the apparatus (Boissier & Simon, 1969; Denenberg, 1969; Misslin, Bouchon, & Ropartz, 1976; Rodgers, 2007; Stanford, 2007). This assumption is somehow provoking regarding the conventional view of open-arm entries meaning a lower anxiety and further research is needed to test this specific hypothesis. Nonetheless, other authors have raised this concern that could explain some puzzling results from the literature (Ennaceur et al., 2010a; Michalikova et al., 2010). For instance, in the work by Brinks et al. (2007), control mice from the stress-reactive/anxious BALB/c strain remarkably entered more frequently and more durably the open arms of an elevated plus-maze than control mice from the stress-resilient/non-anxious C57Bl/6 strain (e.g. Anisman et al., 2001; Belzung & Griebel, 2001; Francis, Zaharia, Shanks, & Anisman, 1995; Roy et al., 2007). However, the authors also pointed out that mice from the BALB/c strain had more frequent SAPs behaviors along with a higher locomotion, defecated more during the test and were also physiologically more reactive in response to a novelty stress (i.e. corticosterone secretion). It can reasonably be assumed, as advanced by the authors, that some of the open-arm entries were intended at escaping from the maze rather than exploring the open arms. The same conclusion was drawn from the observation that C57Bl/6 mice previously stressed by the exposure to a live rat also entered more frequently the open arms of the plus-maze than their respective control while being evidently more anxious (Brinks et al., 2007).

These data emphasize the fact that more attention should be systematically devoted to ethologically relevant behaviors (e.g. Rodgers & Dalvi, 1997; Rodgers, Johnson, Champion, & Mills, 1996; Roy & Chapillon, 2004). Our study adds some contributions to this idea. Indeed, in our second experiment BALB/c mice also entered frequently open arms and spend a high percentage of their time into these arms. In the forced exposure situation, their percentage of time in the open arms was up to eighty percent during the first minute of test and it only dropped around sixty percent at the end of the trial. Taken alone, this result could reflect a low anxiety level in BALB/c mice. These data are strikingly similar to the ones obtained by Brinks et al. (2007) but also by other researchers who concluded to ceiling or floor effects (Trullas & Skolnick, 1993) or to the fact that BALB/c strain was really a non-anxious one (Post et al., 2011; Rogers et al., 1999). However, multiple works have shown that BALB/c mice are very anxious, from behavioral, pharmacological and/or neurobiological aspects (e.g. Anisman et al., 2001; Belzung & Berton, 1997; Belzung & Griebel, 2001; Brinks et al., 2007; Francis et al., 1995; Michalikova et al., 2010; Roy, Belzung, Delarue, & Chapillon, 2001; Roy et al., 2007). As in the study by Brinks et al. (2007), we are able to conclude that BALB/c mice are really anxious by relying on the analysis of ethological behaviors in both conditions of test. Indeed, BALB/c mice in the forced exposure test visited open arms very frequently but also had a high number of feces, which was not the case in C57Bl/6 mice. In the meantime, pSAPs and closed arm returns in BALB/c mice were higher in free exploration situation than in the forced exposure situation. This suggests that these behaviors are not always suitable when anxiety in particularly anxious strains is assessed by means of forced exposure to the plus maze (as in the very majority of reports). On the contrary, the free exploration paradigm makes these behaviors especially relevant by denoting a clear approach/avoidance conflict from the very beginning of the test. This conclusion is particularly consistent with the results obtained by Fonio et al. (2009), Jain et al. (2012) or Welker (1957). These researchers demonstrated that exploration of an open field follows a much more organized and reproducible pattern in the context of a voluntary exploration than if the exposition is forced. In addition, it was found in such a situation that BALB/c mice were much more anxious than C57Bl/6 mice (Fonio et al., 2009). These results indicate that the free exploration paradigm certainly is much appropriated to assess anxiety throughout behavioral elements that highly rely on the approach/avoidance conflict. As a corollary, our results indicate that the forced standard situation is sometimes irrelevant for the

evaluation of anxiety in particular strains (e.g. BALB/c). In that case, a very careful handling of the animals when placing them in the plus-maze certainly reduces the interference between fear of the novel apparatus on one hand and exploration behaviors used to estimate anxiety on the other hand (Hurst & West, 2010).

Conclusion

The use of a free exploration procedure in the elevated plus-maze test combined with a min-by-min analysis allowed us to confirm that rodents forcibly exposed to the test do not behave immediately according to the approach/avoidance conflict. The study also points out that avoidance of the open arms is unconditioned in various strains of mice, as previously observed in rats. Finally, the comparison of the free exploration and forced exposure situations, along with the detailed analysis of ethological measures, could explain some puzzling results of the literature when BALB/c mice are confronted to the elevated plus-maze.

References

- Anisman, H., Hayley, S., Kelly, O., Borowski, T., & Merali, Z. (2001). Psychogenic, neurogenic, and systemic stressor effects on plasma corticosterone and behavior: mouse strain-dependent outcomes. *Behavioral Neuroscience*, *115*, 443-454.
- Belzung, C., & Berton, F. (1997). Further pharmacological validation of the BALB/c neophobia in the free exploratory paradigm as an animal model of trait anxiety. *Behavioral Pharmacology*, *8*, 541-548.
- Belzung, C., & Griebel, G. (2001). Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behavioural Brain Research*, *125*, 141-149.
- Bertoglio, L. J., & Carobrez, A. P. (2000). Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety. *Behavioural Brain Research*, *108*, 197-203.
- Bertoglio, L. J., & Carobrez, A. P. (2002). Behavioral profile of rats submitted to session 1-session 2 in the elevated plus-maze during diurnal/nocturnal phases and under different illumination conditions. *Behavioural Brain Research*, *132*, 135-143.
- Boissier, J. R., & Simon, P. (1969). Evaluation of experimental techniques in the psychopharmacology of emotion. *Annals of the New York Academy of Sciences*, *159*, 898-914.
- Brinks, V., van der Mark, M., de Kloet, R., & Oitzl, M. (2007). Emotion and Cognition in High and Low Stress Sensitive Mouse Strains: A Combined Neuroendocrine and Behavioral Study in BALB/c and C57BL/6J Mice. *Frontiers in Behavioral Neuroscience*, *1*, 1-13. doi:10.3389/neuro.08.008.2007
- Carobrez, A. P., & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neuroscience & Biobehavioral Review*, *29*, 1193-1205. doi:10.1016/j.neubiorev.2005.04.017
- Casarrubea, M., Roy, V., Sorbera, F., Magnusson, M. S., Santangelo, A., Arabo, A. (2013). Temporal structure of the rat's behavior in elevated plus maze test. *Behavioural Brain Research*, *237*, 290-299. doi:10.1016/j.bbr.2012.09.049
- Denenberg, V. H. (1969). Open-field behavior in the rat: what does it mean? *Annals of the New York Academy of Sciences*, *159*, 852-859.
- Ennaceur, A., Michalikova, S., van Rensburg, R., & Chazot, P. L. (2010a). Distinguishing anxiolysis and hyperactivity in an open space behavioral test. *Behavioural Brain Research*, *207*, 84-98. doi:10.1016/j.bbr.2009.09.042
- Ennaceur, A., Michalikova, S., van Rensburg, R., & Chazot, P. L. (2010b). Tolerance, sensitization and dependence to diazepam in Balb/c mice exposed to a novel open space anxiety test. *Behavioural Brain Research*, *209*, 154-164. doi:10.1016/j.bbr.2010.01.039
- Ferrari, P. F., Palanza, P., Parmigiani, S., & Rodgers, R. J. (1998). Interindividual variability in Swiss male mice: relationship between social factors, aggression, and anxiety. *Physiology & Behavior*, *63*, 821-827.

- File, S. E., Mabbutt, P. S., & Hitchcott, P. K. (1990). Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. *Psychopharmacology (Berl)*, *102*, 98-101.
- File, S. E., & Zangrossi, H., Jr. (1993). "One-trial tolerance" to the anxiolytic actions of benzodiazepines in the elevated plus-maze, or the development of a phobic state? *Psychopharmacology (Berl)*, *110*, 240-244.
- File, S. E., Zangrossi, H., Jr., Viana, M., & Graeff, F. G. (1993). Trial 2 in the elevated plus-maze: a different form of fear? *Psychopharmacology (Berl)*, *111*, 491-494.
- Fonio, E., Benjamini, Y., & Golani, I. (2009). Freedom of movement and the stability of its unfolding in free exploration of mice. *Proceedings of the National Academy of Sciences*, *106*, 21335-21340. doi:10.1073/pnas.0812513106
- Francis, D. D., Zaharia, M. D., Shanks, N., & Anisman, H. (1995). Stress-induced disturbances in Morris water-maze performance: interstrain variability. *Physiology & Behavior*, *58*, 57-65.
- Holmes, A., & Rodgers, R. J. (1998). Responses of Swiss-Webster mice to repeated plus-maze experience: further evidence for a qualitative shift in emotional state? *Pharmacology Biochemistry and Behavior*, *60*, 473-488.
- Holmes, A., & Rodgers, R. J. (1999). Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. *Neuroscience & Biobehavioral Reviews*, *23*, 971-980.
- Hurst, J. L., & West, R. S. (2010). Taming anxiety in laboratory mice. *Nature Methods*, *7*, 825-826. doi:10.1038/nmeth.1500
- Jain, A., Dvorkin, A., Fonio, E., Golani, I., & Gross, C. T. (2012). Validation of the dimensionality emergence assay for the measurement of innate anxiety in laboratory mice. *European Neuropsychopharmacology*, *22*, 153-163. doi:10.1016/j.euroneuro.2011.07.001
- Michalikova, S., van Rensburg, R., Chazot, P. L., & Ennaceur, A. (2010). Anxiety responses in Balb/c, c57 and CD-1 mice exposed to a novel open space test. *Behavioural Brain Research*, *207*, 402-417. doi:10.1016/j.bbr.2009.10.028
- Misslin, R., Bouchon, R., & Ropartz, P. (1976). Significance of behavioral parameters in mice in open field. *Physiology & Behavior*, *17*, 767-770.
- Montgomery, K. C. (1955). The relationship between fear induced by novel stimulation and exploratory behaviour. *Journal of Comparative and Physiological Psychology*, *48*, 254-260.
- Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*, 149-167.
- Pereira, L. O., da Cunha, I. C., Neto, J. M., Paschoalini, M. A., & Faria, M. S. (2005). The gradient of luminosity between open/enclosed arms, and not the absolute level of Lux, predicts the behaviour of rats in the plus maze. *Behavioural Brain Research*, *159*, 55-61. doi:10.1016/j.bbr.2004.10.002

- Pickles, A. R., & Hendrie, C. A. (2013). Anxiolytic-induced attenuation of thigmotaxis in the Elevated Minus Maze. *Behavioural Processes*, *97*, 76-79. doi:10.1016/j.beproc.2013.04.006
- Post, A. M., Weyers, P., Holzer, P., Painsipp, E., Pauli, P., Wultsch, T. (2011). Gene-environment interaction influences anxiety-like behavior in ethologically based mouse models. *Behavioural Brain Research*, *218*, 99-105. doi:10.1016/j.bbr.2010.11.031
- Rodgers, R. J. (1997). Animal models of 'anxiety': where next? *Behavioural Pharmacology*, *8*, 477-496; discussion 497-504.
- Rodgers, R. J. (2007). More haste, considerably less speed. *Journal of Psychopharmacology*, *21*, 141-143.
- Rodgers, R. J., & Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neuroscience & Biobehavioral Reviews*, *21*, 801-810.
- Rodgers, R. J., Johnson, N. J., Champion, A. J., & Mills, S. (1996). Modulation of plus-maze behaviour in mice by the preferential D3-receptor agonist 7-OH-DPAT. *Pharmacology Biochemistry and Behavior*, *54*, 79-84.
- Rodgers, R. J., Johnson, N. J., Cole, J. C., Dewar, C. V., Kidd, G. R., & Kimpson, P. H. (1996). Plus-maze retest profile in mice: importance of initial stages of trial 1 and response to post-trial cholinergic receptor blockade. *Pharmacology Biochemistry and Behavior*, *54*, 41-50.
- Rodgers, R. J., & Shepherd, J. K. (1993). Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. *Psychopharmacology (Berl)*, *113*, 237-242.
- Rogers, D. C., Jones, D. N. C., Nelson, P. R., Jones, C. M., Quilter, C. A., Robinson, T. L. (1999). Use of SHIRPA and discriminant analysis to characterise marked differences in the behavioural phenotype of six inbred mouse strains. *Behavioural Brain Research*, *105*, 207-217.
- Rosa, V. P., Vandresen, N., Calixto, A. V., Kovaleski, D. F., & Faria, M. S. (2000). Temporal analysis of the rat's behavior in the plus-maze: effect of midazolam. *Pharmacology Biochemistry and Behavior*, *67*, 177-182.
- Roy, V., Belzung, C., Delarue, C., & Chapillon, P. (2001). Environmental enrichment in BALB/c mice. Effects in classical tests of anxiety and exposure to a predatory odor. *Physiology & Behavior*, *74*, 313-320.
- Roy, V., & Chapillon, P. (2004). Further evidences that risk assessment and object exploration behaviours are useful to evaluate emotional reactivity in rodents. *Behavioural Brain Research*, *154*, 439-448. doi:10.1016/j.bbr.2004.03.010
- Roy, V., Chapillon, P., Jeljeli, M., Caston, J., & Belzung, C. (2009). Free versus forced exposure to an elevated plus-maze: evidence for new behavioral interpretations during test and retest. *Psychopharmacology (Berl)*, *203*, 131-141. doi:10.1007/s00213-008-1378-2
- Roy, V., Merali, Z., Poulter, M. O., & Anisman, H. (2007). Anxiety responses, plasma corticosterone and central monoamine variations elicited by stressors in reactive and

- nonreactive mice and their reciprocal F(1) hybrids. *Behavioural Brain Research*, 185, 49-58. doi:10.1016/j.bbr.2007.07.008
- Shanks, N., Griffiths, J., Zalcman, S., Zacharko, R. M., & Anisman, H. (1990). Mouse strain differences in plasma corticosterone following uncontrollable footshock. *Pharmacology Biochemistry and Behavior*, 36, 515-519.
- Smithson, M. J. (2003). Confidence Intervals, *Quantitative Applications in the Social Sciences Series, 140*. Thousand Oaks, CA: Sage.
- Stanford, S. C. (2007). The Open Field Test: reinventing the wheel. *Journal of Psychopharmacology*, 21, 134-135.
- Treit, D., Menard, J., & Royan, C. (1993). Anxiogenic stimuli in the elevated plus-maze. *Pharmacology Biochemistry and Behavior*, 44, 463-469.
- Trullas, R., & Skolnick, P. (1993). Differences in fear motivated behaviors among inbred mouse strains. *Psychopharmacology*, 111, 323-331.
- Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, 2, 322-328. doi:10.1038/nprot.2007.44
- Wall, P. M., & Messier, C. (2000). Ethological confirmatory factor analysis of anxiety-like behaviour in the murine elevated plus-maze. *Behavioural Brain Research*, 114, 199-212.
- Welker, W. I. (1957). "Free" versus "forced" exploration of a novel situation by rats. *Psychological Reports*, 3, 95-108.

Table 1

Rears and head scan behaviors in CD1 mice in experiment 1

Behavior	Condition	Min1	Min2	Min3	Min4	Min5
Rears	Forced	1.07±0.27	3.27±0.56	4.33±0.61	4.27±0.52	3.40±0.60
	Free	1.40±0.38	3.47±0.61	4.27±0.71	2.73±0.76	3.53±0.60
	Forced CA	1.33±0.21	3.20±0.53	2.53±0.62	3.07±0.65	3.40±0.60
Head Scans	Forced	5.27±0.47	5.73±0.59	5.20±0.72	5.00±0.52	4.33±0.49
	Free	6.33±0.62	5.00±0.75	5.00±0.62	4.20±0.50	3.93±0.47
	Forced CA	6.60±0.88	6.20±0.71	5.00±0.56	3.53±0.41	4.13±0.32

Note. Conditions are Forced exposure in the central part of the maze (Forced), Free exploration and Forced exposure at the end of a closed arm (Forced CA). Rears and head scan behaviors were not different among condition. Significant main effects for the segment of test show that rears were less frequent during minute 1 than during the other minutes (p always < 0.001) and that head scans in minutes 4 and 5 were less frequent than during minute 1 and minute 2 (p at least < 0.05).

Table 2

Rears and head scan behaviors in BALB/c and C57Bl/6 mice in experiment 2

Behavior	Condition	Min1	Min2	Min3	Min4	Min5
Rears	Forced	1.00±0.33	1.50±0.56	1.90±0.55	2.00±0.79	1.80±0.61
	Free	0.25±0.16	0.63±0.32	1.50±0.50	1.75±0.59	2.00±0.63
Head scans	Forced	3.90±0.59	4.70±0.70	4.10±0.78	3.80±0.71	4.40±0.60
	Free	8.75±0.96	8.50±1.15	4.88±0.55	5.75±0.53	6.38±0.94
Rears	Forced	1.89±0.45	3.22±0.78	2.56±0.29	3.11±0.45	3.11±0.31
	Free	0.67±0.37	1.00±0.33	2.00±0.60	1.89±0.42	1.56±0.38
Head Scans	Forced	6.00±0.58	4.89±0.42	4.44±0.53	4.56±0.24	3.89±0.39
	Free	5.89±0.63	5.11±0.63	5.22±0.86	4.11±0.54	3.56±0.44

Note. Rears and head scan behaviors in BALB/c (upper/nonbold) and C57Bl/6 (lower/bold) mice in experiment 2. In BALB/c mice, there was a significant increase in the number of rears over the minutes of test but no follow-up comparison was significant. Head scans in BALB/c mice were more frequent in the free exploration condition than in the forced exposure condition during minute 1 and 2 (p at least < 0.01). In the free exposure condition only, head scans during minute 3 were less frequent than during minute 1 and 2 ($p < 0.05$). In C57Bl/6 mice, the number of rears in the forced condition was higher than in the free exploration condition. Whatever the condition, head scans were more frequent on minute 1 than on minute 4 and 5 (p at least < 0.05).

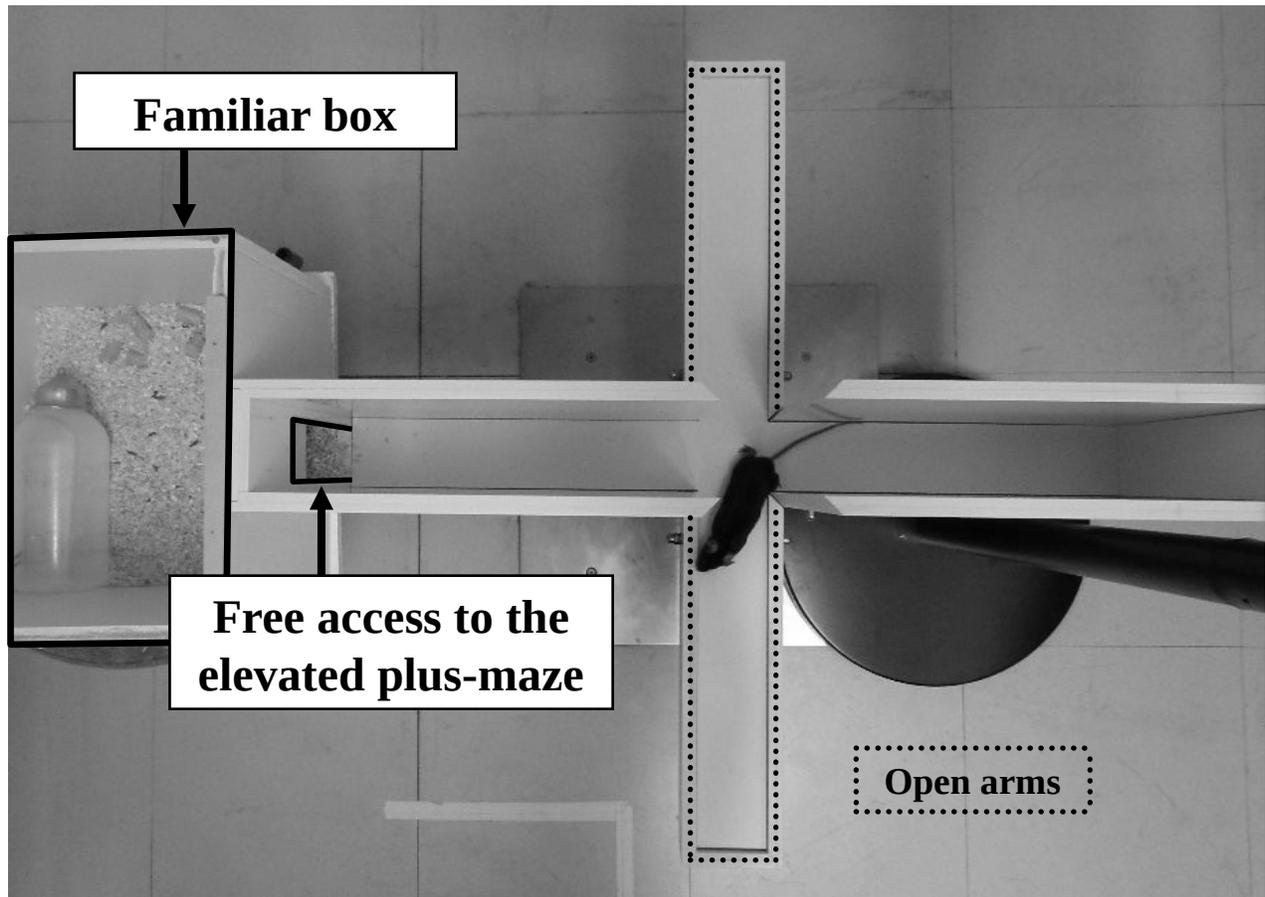


Figure 1. Experimental setup for the free exploration condition. The familiar box (left) is connected to the end of a closed arm of the elevated plus-maze (right). Closed arms are in the extension of the familiar box while the perpendicular open arms are indicated on the figure by dotted lines. At the beginning of the test, the mouse is given free access to the elevated plus-maze when the experimenter removes the door of the familiar box and the background panel of the closed arm.

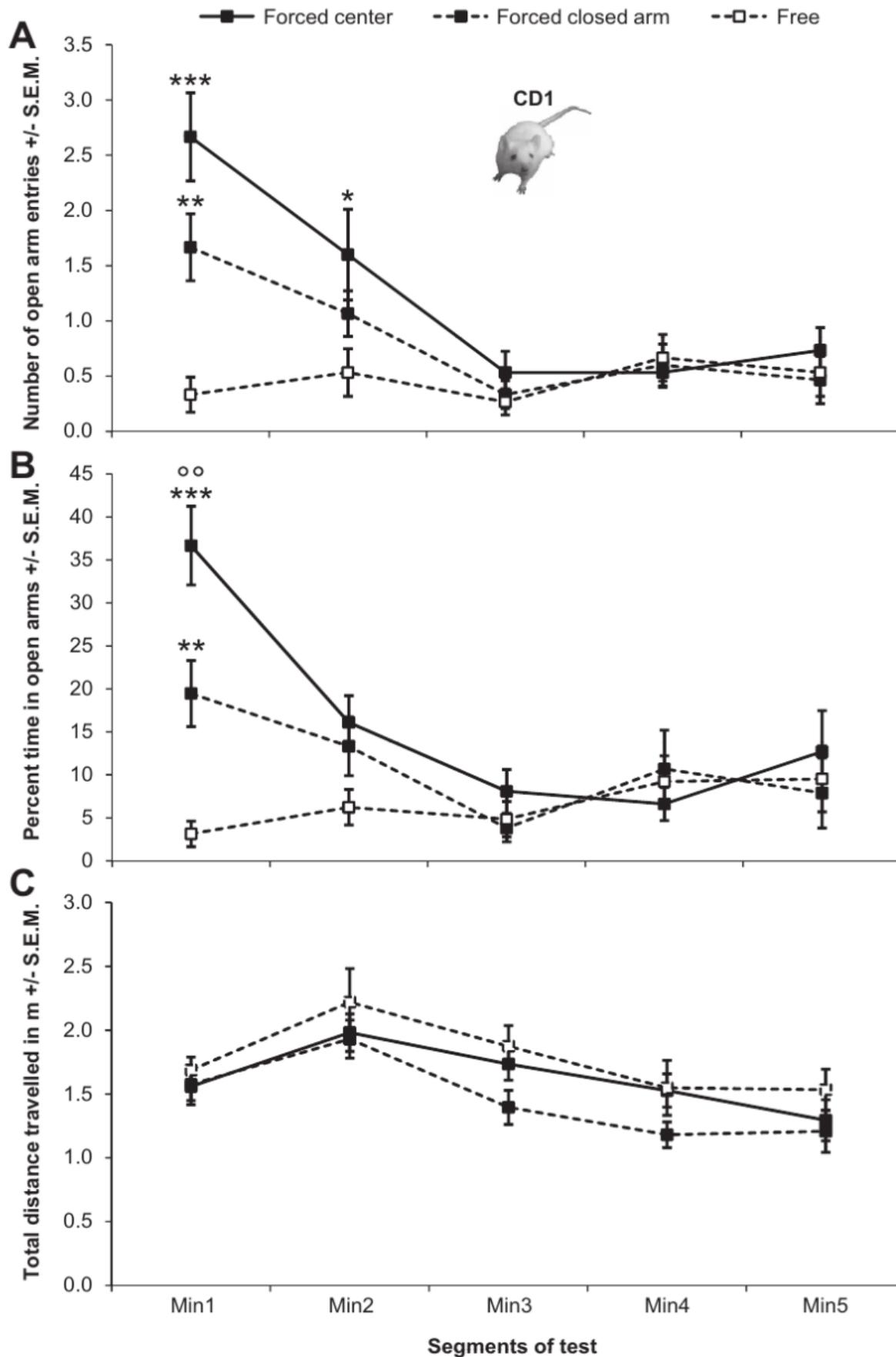


Figure 2. Min-by-min behavior of CD1 mice exposed to the forced center (n=15), to the forced closed arm (n=15) condition or to the free exploration condition (n=15) in the elevated plus-maze. Data are given as Mean \pm SEM. Statistics: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparisons between forced conditions and the free exploration and $^{\circ}$ $p < 0.01$ for comparisons between forced closed arm and free exploration.

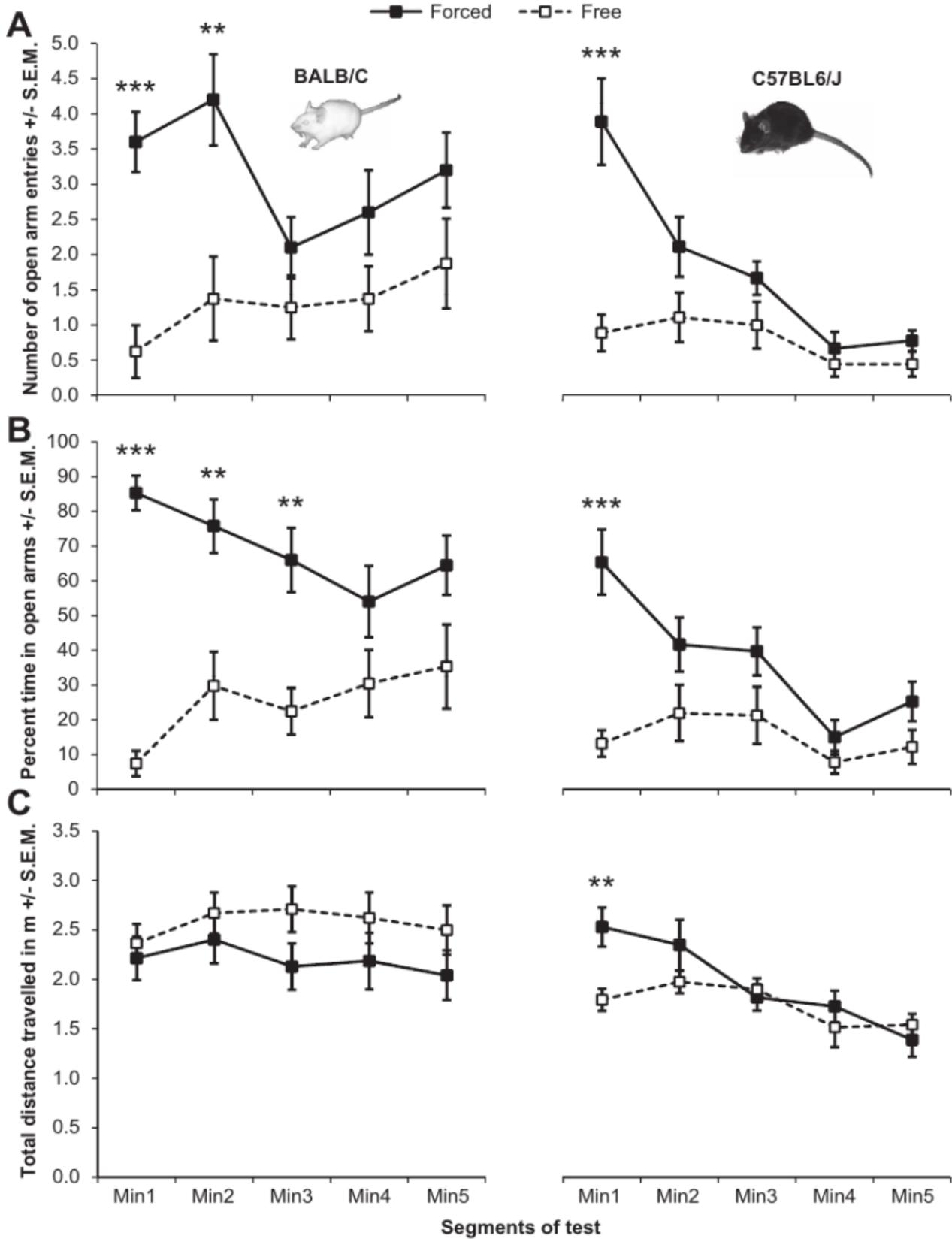


Figure 3. Min-by-min behavior of BALB/c and C57Bl/6 mice in the forced exposure condition (n=10 / n=9) or in the free exploration condition (n=8 / n=9). Data are given as Mean \pm S.E.M. Statistics: ** p < 0.01, *** p < 0.001 for comparisons between forced exposure and free exploration within the same strain.

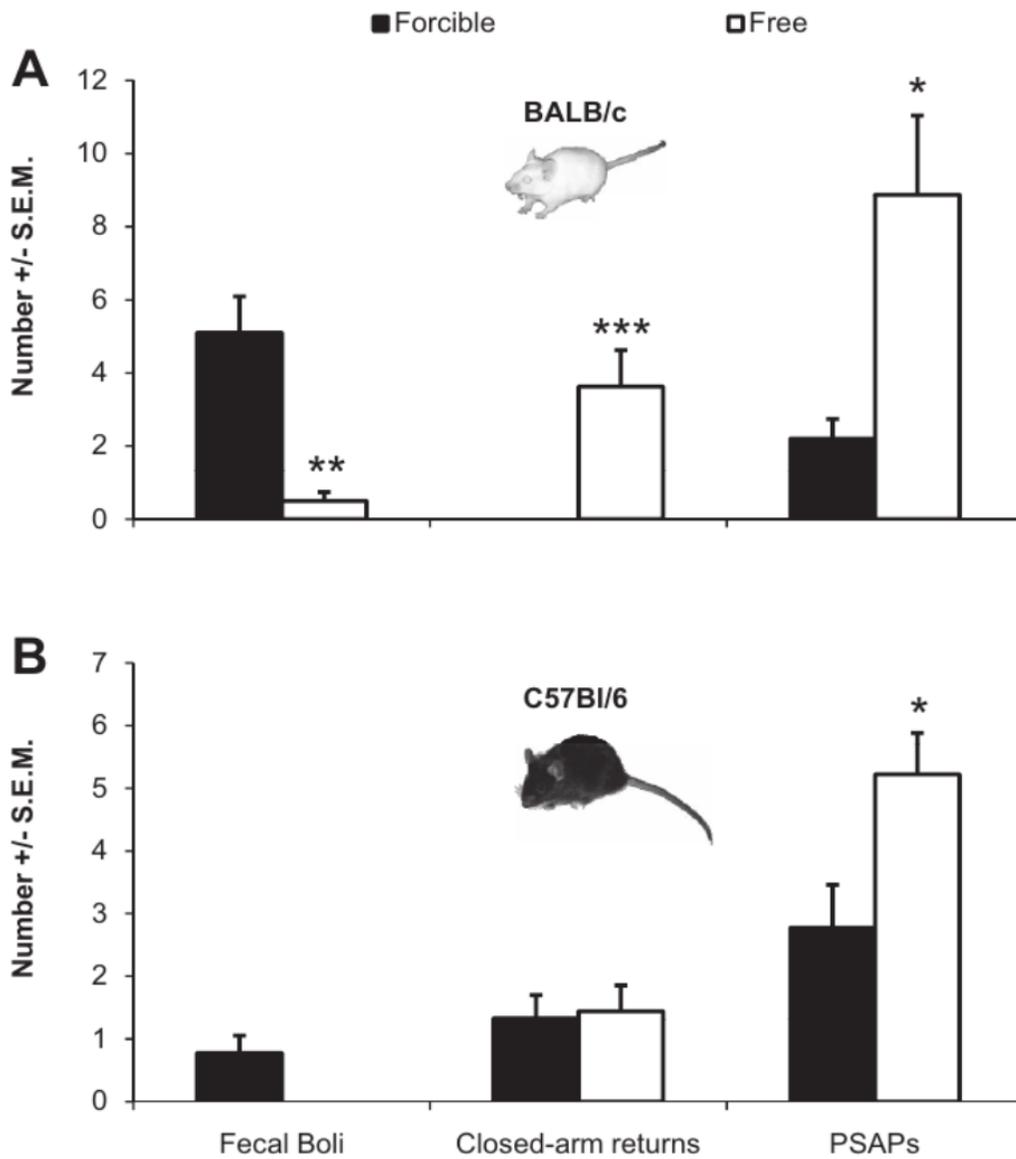


Figure 4. Behavior of BALB/c (A) and C57Bl/6 (B) mice in the forcible exposure vs. free exploration conditions. Data are given as Mean \pm SEM. Statistics: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparisons between forcible exposure and free exploration within the same strain.