Results of the Open Field Test at different light intensities in C57 mice

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Introduction

There is widespread agreement on the need for standardization of parameters that might affect behavioral data generated in phenotyping experiments in different laboratories in order to minimize the variability of results and waste of animals. The Open Field Test, as well as other tests for measuring anxiety and locomotion behaviors in mice, is difficult to standardize between laboratories and the results obtained are often contradictory. However, this should not prevent us from making an effort to control and delimitate conditions that could potentially increase the variability obtained from these tests in order to reduce the use of animals. Even though the level of illumination is generally considered to be an important factor that may influence the mood of animals when confronted with a novel space, particularly in nocturnal animals such as mice, there is a considerable lack of information on light intensities used in such studies throughout the bibliography. In the cases where information is provided, variation of light levels used is surprisingly broad (Table 1).

Most of the articles do not mention the light intensity that they are using to carry out the experiments.	67
Light intensity indicated (but ranged between 2 and 657 lux).	19
The light intensity is not indicated but the setup used to test mice is described (which varied widely: 1, 2 or 3 bulbs, power, color and different distances from the arena).	14

Table 1: Review of 100 recent articles (from the past few years) in relevant journals using Open Field or Plus Maze tests.

We tried to address whether and to what extent light intensity influenced the anxiety and exploratory related behaviors of C57 mice in our Open Field Test in control conditions and after anxiolytic or anxiogenic treatment.

Materials and methods

Experiments were conducted in accordance with approved IACUC protocol (CEEA- University of Seville) and performed following the welfare standards of the European Union.

Light intensities chosen: 40 lux, approximately the light that reaches the interior of the animal cages measured with a calibrated luxometer. 250 lux, approximately the light intensity in the animal room. The animals are usually only exposed to this light when transferring from cages or during manipulation. 600 lux, animals are not exposed to this light. In some experiments is used intentionally as an aversive stimulus [4], but some studies are performed under this and even higher levels of illumination [5].

Animals: 180 Adult (9-10 weeks old) male and female C57BL/6 mice bred in our facility were used; they were housed in a room with a 12-hour light/dark cycle (7:00-19:00). Room temperature was fixed at 21 ± 2ºC. Food and water were available ad libitum in all cases. Animals were randomly distributed in 9 groups (Control-Epinephrine-Diazepam, at the 3different light intensities chosen).

Open field test: The open field apparatus used in this study measured 50x50x50 cm., made of grey plexiglass, and was illuminated by 4 bulbs of 60 W placed at 1,8m above the arena; a potentiometer allowed us to adjust the intensity of light reaching the bottom of the maze as desired.

Variables studied: Selected parameters were collected during the test. N entries (frequency of mouse entry in the arena center), Ptime periphery (permanence time in the periphery), Dist periphery (distance travelled in the peripheral zone), Total dist travelled (total distance travelled in the test) and Resting time. Ethological variables were also observed and taken into consideration.

Drugs: Epinephrine (EP) 1mg/Kg as anxiogenic treatment [6], or Diazepam (DZ) 5mg/Kg as anxiolytic treatment were injected intraperitoneally 30 min before the behavioral test [4]. The same volume of saline was injected into the control groups.

Procedure: Prior to the experiment mice were taken to the test room to acclimate them to the test room conditions. Mice were treated with EP, DZ or saline 30 min before the test and were placed anywhere in the open field. The arena was divided in two zones, the peripheral zone next to the open field's walls and the central area which were similar for all groups tested. Behaviors were recorded for 30 min using a video camera and video tracker. Results were exported to the statistical package SPSS for calculations. The open field was carefully cleaned after every test with a non-odor detergent.

Statistics: Data from the behavioral assays were subjected to analyses of variance, ANOVA, Tukey-Dunnet test (homogeneus variances or not), Kruskall-Wallis followed by Mann-Whithney U-test when distributions were no normal.

Results and discussion

As shown in table 2, highly significant differences($p<0.01$) were observed when the maze was brightly illuminated (600 Lux) compared to 250 lux and 40 lux both in the number of entries and in the relative time spent in the periphery of the arena in both sexes. Interestingly, in females distance travelled was statistically different between 600 and 250 Lux, but not when comparing 600 and 40 lux (Table 3); this seemed to be related (figure 1 and 2), to the exploration becoming similarly reduced (and resting time increased) when animals are either relatively calm or subjected to aversive stimuli although due to opposite mood states (resting versus freezing) and could mean that females are more susceptible to the increase in the intensity of light.

MALES	CONTROL			EPINEPHRINE			DIAZEPAM		
	40-250	250-600	40-600	40-250	250-600	40-600	40-250	250-600	40-600
N entries	-	▼	▼	-	-	-	-	-	-
P time periphery	-	▲	▲	-	△	-	-	-	-
Dist periphery	-	-	-	-	-	-	-	-	-
Total Dist travelled	-	-	▼	-	-	-	-	-	-
Resting time	△	-	▲	-	-	-	-	-	-

Table 2: Effects of light intensity within control and treatment groups. ▲▼($p<0.01$), ▽△($p<0.05$), - (No significant)

FEMALES	CONTROL			EPINEPHRINE			DIAZEPAM		
	40-250	250-600	40-600	40-250	250-600	40-600	40-250	250-600	40-600
N entries	-	▼	▼	-	-	-	-	-	-
P time periphery	-	▲	▲	-	-	-	-	-	-
Dist periphery	-	-	-	-	-	-	-	-	-
Total Dist travelled	-	▽	-	-	-	-	-	-	-
Resting time	-	▲	-	-	-	-	-	-	-

Table 3: Effects of light intensity within control and treatment groups. ▲▼($p<0.01$),▽ ($p<0.05$), - (No significant)

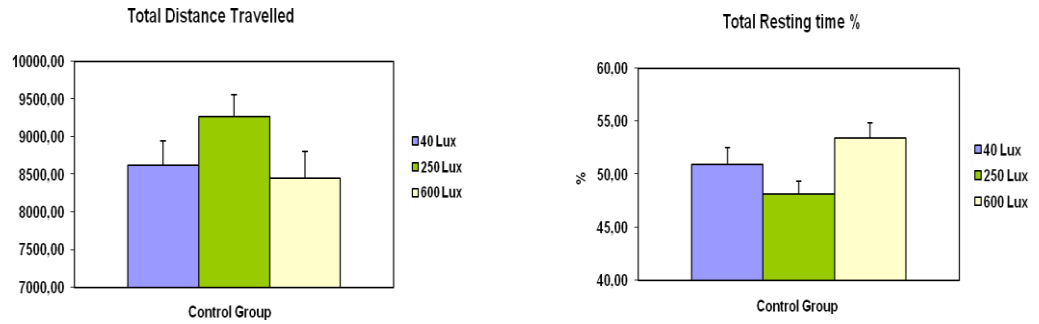

Figure 1 and 2: Total distance travelled in each light intensity (left) and total Resting Time (%) (right) in each light intensity in females within control group.

Within treatment groups no differences were found at any of the light intensities used, denoting the injected drugs exceeded the effects of the illumination of the arena. A slight increase in thigmotactic behavior seems to appear in males when combining anxiogenic treatment and a bright arena.

We then analyzed the same data with a different approach to try to address whether the differences in the parameters tested in treated versus untreated mice could be better appreciated at a specific light intensity, which could be extrapolated to the study of lines expected to have anxiety or exploratory related alterations (tables 4-5)

MALES	40 lux	250 lux	600 lux	40 lux	250 lux	600 lux	40 lux	250 lux	600 lux
	Cont- EP	Cont- EP	Cont- EP	Cont-DZ	Cont- DZ	Cont- DZ	EP-DZ	EP-DZ	EP-DZ
N entries	▼	▼	▼	▼	▼	-	△	△	△
P time periphery	▲	▲	△	-	-	▽	▼	▽	▼
Dist periphery	▼	▼	▼	▼	▽	▽	-	-	-
Total Dist travelled	▼	▼	▼	▼	▼	-	-	-	-
Resting time	▲	▲	▲	▲	△	△	-	-	▽

Table 4: Comparison between treatments at a fixed light intensity. ▲▼($p<0.01$), ▽△($p<0.05$), - (No significant).

FEMALES	40 lux	250 lux	600 lux	40 lux	250 lux	600 lux	40 lux	250 lux	600 lux
	Cont- EP	Cont- EP	Cont- EP	Cont-DZ	Cont- DZ	Cont- DZ	EP-DZ	EP-DZ	EP-DZ
N entries	▼	▼	▼	▼	▼	▼	-	-	-
P time periphery	▲	△	-	-	△	-	▼	-	-
Dist periphery	▼	▼	▼	▼	▼	▼	-	▲	-
Total Dist travelled	▼	▼	▼	▼	▼	▼	-	△	-
Resting time	▲	▲	▲	▲	▲	▲	-	▽	-

Table 5: Comparison between treatments at a fixed light intensity. ▲▼($p<0.01$), ▽△ ($p<0.05$), - (No significant).

In agreement with the results shown above, changes in female anxiety related behavior between animals stimulated with epinephrine versus control animals were dependent on illumination level. They were best seen under a dimmer light; significant differences were still present at 250 lux, but a brightly illuminated arena (600 lux) rendered statistically similar results compared to untreated animals. In males the effect was similar but less intense, supporting they may be slightly less influenced by bright arenas.

Some differences between sexes were observed when anxiolytic treated and control animals were analyzed under different brightness. Surprisingly the time spent in the periphery of the arena, considered usually as one of the main indicators of anxiety did not vary or varied in opposite direction than anticipated. Both in males and females anxiolytic treatment produced a decrease in thigmotactic behavior at 40 and 600 lux. (significantly reduced in diazepam treated males at 600 lux) as could be expected. However at 250 lux a significant increase in this parameter in females was observed; this increase could be seen in males too (although not statistically significant). These results were in agreement with a

previous report on the Open Field Test in C57 mice using different doses of anxiolytic (ketamine) at 165 lux [1]. Whether said effect is specific to anxiolytic treated animals at intermediate light intensities should be confirmed.

In exploratory variables, less significant differences in distance travelled in the periphery of the arena or total resting time were seen at 250 lux in males; at 600 lux, data from diazepam treated and untreated males appeared even more uniform, while female results were unaffected.

Whether these results from exploratory and anxiety variables in males have any biological meaning (i.e. only when aversively stimulated the effect of a tranquilizing is detected by decreasing anxiety and attenuating differences in exploratory activity) or it is just an artifact of our conditions (i.e. periphery area chosen, or number of animal analyzed) should be further addressed.

The most streaking differences between male and female outcomes were observed when comparing the results of Epinephrine versus Diazepam treated animals under different illuminations. In males diazepam induced a marked reduction in the percentage of time spent in the periphery of the arena compared to epinephrine injected mice, while leaving unchanged the exploratory variables at the three lights studied. On the other hand, a very bright arena rendered statistically similar results between drug treated females in a very bright arena. However, as mentioned above, profound differences were seen when the behavior of the animals was carefully monitored. Diazepam treated female mice were calm during the test while the epinephrine injected animals were nervous and freezing behavior was present. In both cases, the "resting time" was high and the program rendered similar results. This highlights the importance of ethological analysis when performing the Open Field Test [3]. Differences in exploratory behavior between drugs were only seen at 250 Lux in females and the percentage of time spent in the periphery was only seen when analyzing data obtained at 40 lux.

Taken together our results seem to favor the use of a light intensity similar to the light the animals are exposed to, for Open Field studies. At 40 lux, all differences observed between EP or DZ treated and between treated and control animals were maximum, and almost identical between sexes. Nonetheless we cannot rule out the hypothesis that a slightly brighter arena could be better for detecting mutant lines with an expected exploratory divergence.

On the other hand there seems to be some divergences in the combined effects of light and treatment between male and female C57 mice, being the results obtained using males generally more consistent and predictable.

Finally, our results emphasize the need to perform ethological analysis when studying animals in this maze, as some values obtained in the study could become statistically similar when the animals are in fact experiencing opposite mood states.

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