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# Does open-field exposure during infancy influence open-field behavior of the same adult mice?

José Vidal
*Universitat de Barcelona*

*The goal of this report is to find out whether early exposure of mice to the open-field results in altered behavior of the same adult mice in the same open-field. Early exposure to the open-field was carried out between birth and weaning; two control groups were included: control 2 (mice exposed to a reduced dark space) and control 1 (mice left undisturbed). The (male and female) mice were of the Balb/c and C57Bl/6 strains. Adult C57Bl/6 female mice of the open-field and control 2 groups ambulated to the same extent in the periphery of the open-field, and mice of both groups ambulated more than mice of the control 1 group; no consistent difference between the three groups was noticed on defecation, or ambulation in the center of the field. No effects of early exposure to the open-field were observed in mice of the Balb/c strain. The effect of early exposure to the open-field, or to a dark space, on adult behavior depends on the murine strain, on sex, and on the behavior measured.*
Keywords: *Early handling, open-field, C57Bl/6, Balb/c.*

# ¿Influye la exposición al campo abierto durante la infancia en la conducta del mismo ratón adulto en el campo abierto?

*El objetivo de este artículo es averiguar si la exposición temprana de los ratones al campo abierto se refleja en conducta alterada de los mismos ratones adultos en el mismo campo abierto. La exposición temprana al campo abierto se llevó a cabo entre el nacimiento y el destete; se incluyeron dos grupos control: control 2 (ratones expuestos a un espacio reducido y obscuro) y control 1 (ratones no manipulados). Los ratones, machos y hembras, pertenecían a las cepas Balb/c y C57Bl/6. Las hembras adultas de la cepa C57Bl/6 pertenecientes a los grupos campo abierto y control 2 deambularon lo mismo en la periferia del campo abierto, y los ratones de ambos grupos deambularon más que los ratones del grupo control 1; no se observó ninguna diferencia consistente entre los ratones*

*Correspondencia*: José Vidal, Departament de Personalitat, Facultat de Psicologia, Universitat de Barcelona, Passeig de la Vall d'Hebron, 171, 08035 Barcelona, E-mail: jvidal@ub.edu

*de los tres grupos en la defecación, o en la deambulación en el centro del campo. No se observaron efectos consistentes de la exposición temprana al campo abierto sobre la conducta adulta en los ratones de la cepa Balb/c. El efecto de la exposición temprana al campo abierto, o a un espacio obscuro, depende de la cepa de ratón, del sexo, y de la conducta medida.*

Palabras clave: *manipulación temprana, campo abierto, C57Bl/6, Balb/c.*

## Introduction

Habituation of the mouse in the open-field, assessed by behavior in several sessions on different days, depends on the inbred strain; thus, mice of the C57Bl/6 strain decrease ambulation along sessions (Bolivar, Caldarone, Reilly, & Flaherty, 2000; Bouwknecht, van der Gugten, Groenink, Olivier, & Paylor, 2004; Cabib, Algeri, Perego, & Puglisi-Allegra, 1990), mice of the A/J strain increase ambulation, and mice of the Balb/c strain show no change in ambulation (Bolivar *et al*., 2000). The above results were obtained with adult mice. To my knowledge, no one has exposed mice to the open-field early in life (e.g., between birth and weaning) to check if that event influenced the behavior of the adult mice in the open-field; consequently, this is the goal of this report. The rationale behind this choice was that early stress affects adult behavior (Millstein & Holmes, 2007; Savignac, Dinan, & Cryan, 2011) and it is therefore expected that early exposure to the open-field should influence adult behavior in the open-field.

## Method

### *Subjects*

Male and female mice of the Balb/c and C57Bl/6 strains were purchased from Harlan Iberica (Barcelona, Spain). Eight Balb/c females were mated with eight Balb/c males, and the offspring were the subjects of the experiments reported here (replication 1); the same females were mated a second time with different males, and the offspring were the subjects of replication 2. Similarly, eight C57Bl/6 females were mated twice with different C57Bl/6 males. The males were removed from the females 1 week before parturition.

Adult mice of the same sex were housed 3-5 per cage, at 21±1 ºC, under a 12 h light-dark cycle (lights on at 8:00 hours). Food and water were available ad libitum. The illumination in the center of the mouse room was 227 lux.

The experimental procedures were approved by the University of Barcelona Ethics Committee on Animal Experimentation.

### *Open-field*

The open-field was a rectangular enclosure made of plastic, 39.0 x 32.5 x 25.0 cm, with black walls, and the brownish floor divided by black lines in 49 rectangles. The open field had an inner rectangular zone, 17.0 x 13.5 cm, divided in 9 rectangles; the remaining of the field was the outer zone. The illumination in the center of the open-field was 1554 lux approximately.

### *Procedure*

At birth, each litter was assigned to one of three groups: open-field group, control 1 group, and control 2 group. Pups of the open-field group were exposed to the open-field, for 5 minutes, on days 4, 6, 8, 10, 13, 15, 17, 20 after birth; pups of the control 2 group were kept, for 5 minutes, in a dark cylinder (7.5 cm diameter x 18.5 cm high) on the same days; pups of the control 1 group were left undisturbed.

When adult, the mice were subjected to two sessions in the open-field; the sessions were one week apart. At the time of the first session, the mice were approximately 8 weeks old. Each mouse was placed in a corner of the field and allowed to move freely for 5 minutes. These variables were recorded: ambulation in periphery (number of rectangles crossed in the outer zone), ambulation in center (number of rectangles crossed in the inner zone), and defecation (number of fecal boli). Each session, held between 14:45 and 19:00 hours was videotaped. The field was washed with disinfectant soap between two mouse sessions.

Each experiment was replicated twice. The number of mice in each replication is shown in table 1.

TABLE 1. NUMBER OF MICE.

| | **Replication 1** | | | | **Replication 2** | | | |
|---|---|---|---|---|---|---|---|---|
| | ***C57Bl/6*** | | ***Balb/c*** | | ***C57Bl/6*** | | ***Balb/c*** | |
| **Group** | *Males* | *Females* | *Males* | *Females* | *Males* | *Females* | *Males* | *Females* |
| *open-field* | 6 | 16 | 11 | 8 | 9 | 8 | 4 | 5 |
| *control 2* | 4 | 7 | 5 | 5 | 10 | 11 | 3 | 5 |
| *control 1* | 3 | 8 | 7 | 5 | 9 | 4 | 5 | 14 |

### *Statistical analysis*

For each gender within each strain, and for each of the variables mentioned above, an analysis of variance (ANOVA) of the form replication (2) x treatment (3, open-field, two control groups) x session (2) was performed. A significant treatment effect, or any significant interaction involving the treatment effect, was followed by appropriate contrasts. Ambulation (in the center or in the periphery of the open-field) was graphed (Figures 1 and 3) and the graphs suggested the contrasts between groups. Graphs and contrasts were carried out with the statistical package STATISTICA v6.1 (Tulsa, Oklahoma, USA). An effect size was calculated for the sake of meta-analysis. The effect size was Hedges's *g* (Hedges, 1981; Kline, 2004). When two effect sizes were combined, a weighted mean was calculated, the weight being the reciprocal of the variance (Hedges, 1982; Kline, 2004).

## Results

### *Results in C57Bl/6 mice*

(i) Female mice. An ANOVA of the form "replication(2) x treatment(3) x session(2)", with ambulation in the periphery as the dependent variable, yielded a significant "treatment" effect [$F(2, 48)=13.80$, $p=0.00002$], and a significant "session" effect [$F(1, 48)=81.19$, $p<0.000001$]; the other effects did not reach significance. Figure 1 (next page) suggests that female mice of the open-field group and control 2 group ambulated more in the periphery than female mice of the control 1 group. The contrasts [mean ± standard error (95% confidence interval)] were: open-field group minus control 1 group: 78.65 ± 20.65 (37.14 - 120.17), $t(48)=3.81$, $p=0.0004$; control 2 group minus control 1 group: 112.12 ± 21.48 (68.93 - 155.31), $t(48)=5.22$, $p=0.000004$. The corresponding effect sizes [Hedges's *g* ± standard error (95% confidence interval)] were: open-field group minus control 1 group: 1.15 ± 0.38 (0.39 - 1.90), control 2 group minus control 1 group: 1.87 ± 0.47 (0.95 - 2.79). The same ANOVA with ambulation in the center as the dependent variable yielded a significant "treatment x session" interaction [$F(2, 48)=3.81$, $p=0.029$], a significant "session" effect [$F(1, 48)=28.70$, $p=0.000002$], and a significant "replication" effect [$F(1, 48)=4.81$, $p=0.033$]; the remaining main effects and interaction effects were not significant. In session 2, mice of the control 2 group ambulated more than mice of the control 1 group: 14.24 ± 3.92 (6.36 - 22.11), $t(48)=3.63$, $p=0.0007$. With defecation as the dependent variable, an ANOVA of the form "replication(2) x treatment(3) x session(2)", yielded only a potentially significant "session" effect [$F(1, 48)=4.05$, $p=0.050$].

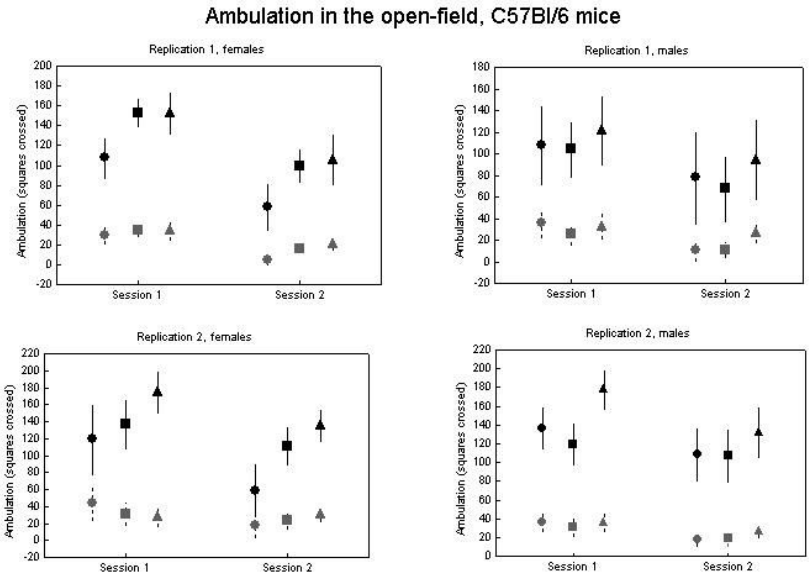


*Figure 1.* Ambulation of C57Bl/6 mice in the open-field. Black circles: control 1 group (ambulation in the periphery of the open-field); gray circles: control 1 group (ambulation in the center of the open-field); black squares: open-field group (ambulation in the periphery of the open-field); gray squares: open-field group (ambulation in the center of the open-field); black triangles: control 2 group (ambulation in the periphery of the open-field); gray triangles: control 2 group (ambulation in the center of the open-field). Bars indicate 95% confidence intervals. Lower confidence limits reaching below zero should be considered zero.

(ii) Male mice. An ANOVA of the form "replication(2) x treatment(3) x session(2)", with ambulation in the periphery as the dependent variable, yielded a significant "treatment" effect [$F(2, 35)=3.61$, $p=0.037$], a significant "session" effect [$F(1, 35)=22.66$, $p=0.00003$], and a significant "replication" effect [$F(1, 35)=10.86$, $p=0.0022$]; the other effects did not reach significance. The contrast control 2 group minus control 1 group (Figure 1) was 47.71 ± 27.25 (-7.62 - 103.04), $t(35)= 1.75$, $p=0.09$. The same ANOVA yielded a significant "session" effect for ambulation in center [$F(1, 35)=38.10$, $p<0.000001$], and no other significant effects. When the dependent variable was defecation, the same ANOVA yielded no significant main effect or interaction effect. Figure 2 (next page) reveals no apparent differences in defecation between the mice of the three groups.

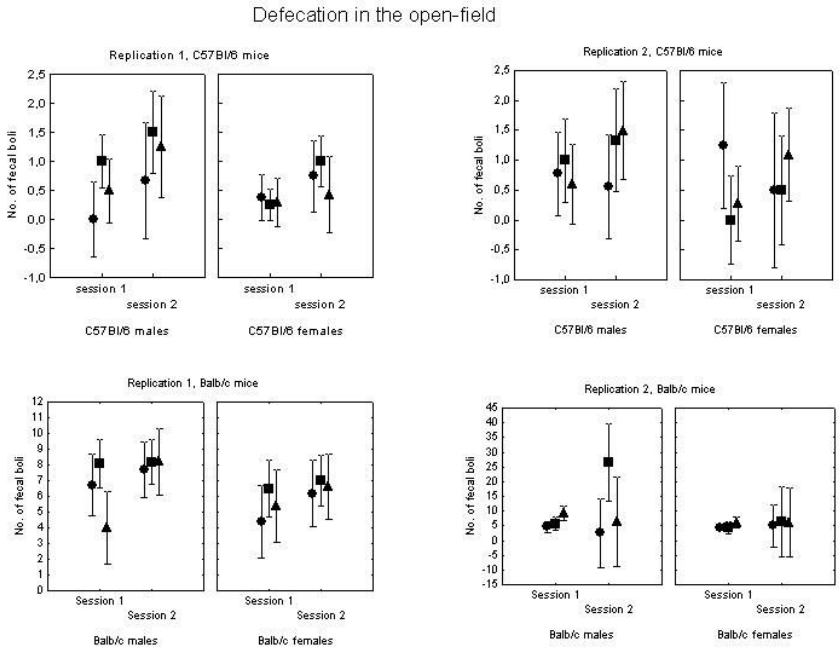


*Figure 2.* Defecation in the open-field. Black circles: control 1 group; black squares: open-field group; black triangles: control 2 group. Bars indicate 95% confidence intervals. Lower confidence limits reaching below zero should be considered zero.

## *Results in Balb/c mice*

In male mice, an ANOVA of the form "replication(2) x treatment(3) x session(2)", yielded no significant main effect, or interaction effect, for ambulation in the periphery or ambulation in the center; in fact, Figure 3 (next page) does not suggest significant and consistent differences in ambulation between groups. For the dependent variable defecation, the above ANOVA yielded a significant "replication x treatment x session" interaction [$F(2, 29)=3.42$, $p=0.046$], and Figure 2 suggest differences between open-field group and control 1 group in session 2 of replication 2 [23.90 ± 8.81 (5.87 - 41.93), $t(29)=2.71$, $p=0.011$], between open-field group and control 2 group in session 2 of replication 2 [20.17 ± 10.04 (-0.36 - 40.69), $t(29)=2.01$, $p=0.054$], between open-field group and control 2 group in replication 1, session 1 [4.09 ± 1.30 (1.43 - 6.75), $t(29)=3.14$, $p=0.004$], and between control 1 group and control 2 group in replication 1, session 1 [2.71 ± 1.41 (-0.18 - 5.60), $t(29)=1.92$, $p=0.065$].

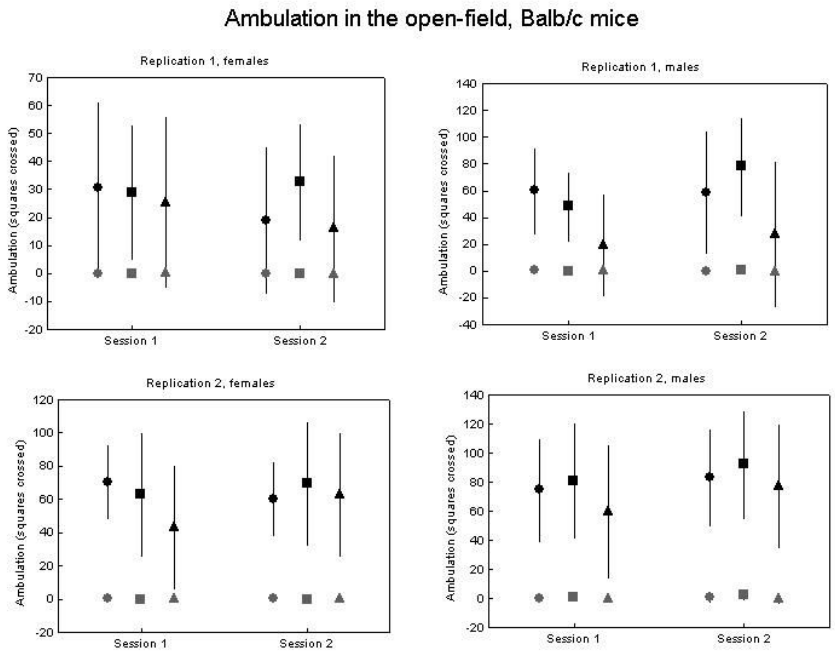


*Figure 3.* Ambulation of Balb/c mice in the open-field. Black circles: control 1 group (ambulation in the periphery of the open-field); gray circles: control 1 group (ambulation in the center of the open-field); black squares: open-field group (ambulation in the periphery of the open-field); gray squares: open-field group (ambulation in the center of the open-field); black triangles: control 2 group (ambulation in the periphery of the open-field); gray triangles: control 2 group (ambulation in the center of the open-field). Bars indicate 95% confidence intervals. Lower confidence limits reaching below zero should be considered zero.

In female mice, an ANOVA of the form "replication(2) x treatment(3) x session(2)", yielded a significant "replication" effect for ambulation in the periphery [$F(1, 36)=11.82$, $p=0.0015$], a significant "session" effect for defecation [$F(1, 36)=5.54$, $p=0.024$], and no other significant main effect or interaction effect. Figure 3 also shows considerable scatter around the mean. In the control 1 group, the coefficient of variation of ambulation of male mice was of the order of 67%, whereas the coefficient of variation for females was of the order of 69%.

## Discussion

The results shown in this report are clear in two respects: (i) exposure to the open-field early in life has no effect on ambulation of adult mice in the same

open-field (Figures 1 and 3 show that the differences between mice of the open-field and control 1 groups are comparable to the differences between mice of the control 2 and control 1 groups; therefore, the mere exposure of infant mice to the open-field cannot explain the difference in ambulation of adult mice), and (ii) mice of the C57Bl/6 strain habituate to the open-field, so that the second test yields lower ambulation scores than the first test (Figure 1 and Results: significant "session" effect), whereas mice of the Balb/c strain do not habituate to the open-field (Figure 3 and Results). These results confirm previous results (Bolivar *et al.,* 2000; Bouwknecht *et al.*, 2004; Cabib *et al.*, 1990).

As to the effect of early handling on adult behavior, some of the results reported here agree with published results. Thus, (i) Millstein and Holmes (2007) found no consistent effect of early handling, or maternal separation, on anxiety-related behavior in several murine strains, among them Balb/c and C57Bl/6; similarly, Savignac *et al.* (2011) found Balb/c and C57Bl/6 mice relatively resistant to the effect of maternal separation on adult anxiety, (ii) early maternal separation of C57Bl/6 mice had different effect, on adult anxiety, on males and females: male mice displayed more anxious behavior in the open-field, whereas female mice in the diestrous phase of the estrous cycle displayed reduced anxious behavior (Romeo, Mueller, Sisti, Ogawa, McEwen, & Brake, 2003). In the experiment reported here, no consistent differences (across replications and sessions) in ambulation in the center of the open-field were evident between mice of the control 2 and control 1 groups (Results and Figures 1, 3); this result agrees with those by Millstein and Holmes (2007) and Savignac *et al.* (2011), although disagrees with results by Romeo *et al.* (2003) with regard to C57Bl/6 male mice.

The only consistent difference (across replications and sessions) emerged in C57Bl/6 female mice: mice of the control 2 and open-field groups ambulated, in the periphery of the open-field, more than mice of the control 1 group (Figure 1 and Results), which suggests an effect of early manipulation, or early stress, on ambulation. This result is in line with results by Savignac *et al.* (2011), who could not reveal any effect of early handling in (Balb/c and C57Bl/6) male mice: according to the results reported here, male mice manipulated before weaning did not display consistently reduced ambulation in the open-field, although C57Bl/6 female mice did. Millstein and Holmes (2007) asked what the robustness of the early-handling effect was; the results reported here reveal a relatively high effect size: Hedges's *g* was 1.87 (for the difference control 2 minus control 1) and 1.15 (for the difference open-field minus control 1).

Early manipulation of Balb/c mice (open-field and control 2 groups) resulted in no consistent alteration of adult behavior (ambulation, defecation) in the open-field (Results and Figures 2, 3). Nevertheless, this result could be due to the large confidence intervals around the means (Figure 3); for instance, Figure 3 suggests that male mice of the control 1 group may ambulate more, across replications and sessions, than male mice of the control 2 group and, accordingly, the effect size

for this difference was 0.62 ± 0.32 (-0.02 - 1.26): this effect size is of moderate strength and shows a trend toward significance ($p$=0.052). It is possible that the same means with smaller standard deviations would have yielded significant results.

In conclusion, manipulation, or stress, of mice before weaning results in altered behavior later in life, but the effect depends on several variables: strain (i.e., genetics; Gariépy, Rodriguiz, & Jones, 2002; Holmes *et al.*, 2005), sex (Romeo *et al.*, 2003), and the behavior measured (e.g., ambulation in the periphery, ambulation in the center).

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