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**Environmental enrichment in BALB/c mice:  
Effects in classical tests of anxiety and exposure to a predatory odor**

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## **Abstract**

Early stimulation by environmental enrichment generally leads to improved learning abilities in rodents. However, the effects of environmental enrichment on emotional reactivity remain more questionable and were mostly studied by using classical tests of anxiety based on confrontation with a novel environment. The main goal of our study was to use different tests of anxiety to compare BALB/c mice reared in either a standard (SC) or enriched (EC) condition. Exposure to cat feces was used to assess anxiety according to an ethoexperimental approach and a comparison was made with the elevated plus maze and the open field as classical tests of anxiety. In accordance with previous works, our results show that EC mice were more active than SC mice in the elevated plus maze and the open field. Thus, possibly as a direct consequence of frequent changes in their breeding conditions, reactivity to a novel environment was reduced in EC mice. However, the cat odor test revealed no intergroup differences for behavior, although corticosterone levels were reduced in EC mice. These results indicate that classical (*i.e.* reaction to novel environments) and ethoexperimental based tests (*i.e.* exposure to predator cues) measure different aspects of emotional reactivity. Further studies using enriched conditions should be useful for the delineation of the neurobiological substrates of these different reactions.

*Keywords:* Enriched Environment; BALB/c mice; Emotional reactivity; Defensive behaviors; Cat odor; Corticosterone level.

## 1 Introduction

Early stimulation by an enriched environment leads to many behavioral changes that have been described in many studies (for a review refer to [37]). However, whereas animals from enriched conditions (EC) clearly have better learning abilities than animals from standard conditions (SC), the effects of environmental enrichment on emotionality are less documented and remain more questionable. While some authors report no or inconsistent effects [16,22,37], others have shown a decreased emotionality in EC animals [10,11,15,29].

A recent study from our laboratory demonstrated that EC mice were less emotional than SC mice in the elevated plus maze and the free exploration test [11]. Moreover, in accordance with previous reports [30,31], we found that the effects of environmental enrichment depended on the strain used. The anxious BALB/c strain, characterized as a possible model of neophobia [5,19], was more affected than the non-anxious C57BL/6 strain. EC significantly decreased state anxiety of BALB/c mice in the elevated plus maze and reversed their neophobic reactions in the free exploration test of trait anxiety.

However, the beneficial effects were found in tests measuring confrontation with novel environments. This remark applies to nearly all the studies that demonstrate positive effects of EC on emotionality. Depending on the test, the novel environment can be freely entered, as in the free exploration paradigm, or imposed, such as the open field [10,22,29] or the elevated plus maze [11], in addition to avoidance tasks, where learning is initiated in a non-familiar place [15]. Thus, emotionality was mainly assessed by exploration (*i.e.* activity in the open field or arm entries in the elevated plus maze) and habituation to novel environments. As environmental enrichment implies many changes in reactions to novelty and exploration, it is not surprising that EC animals detect novelty more rapidly and are faster to explore new environments than SC mice [23,43,45]. Therefore, the emotionality decrease observed in EC animals in classical tests could possibly be explained more parsimoniously by reduction in neophobia (*i.e.* an increased motivation to explore) and not necessarily by a general decrease in emotional reactivity. This point of view has already

been discussed for the open field [22,37] and for the elevated plus maze [16], according respectively to Archer's and Dawson's reservations about the validity of these apparatus to measure anxiety [4,12].

Given the above considerations, the main goal of our study was to measure anxiety in EC reared BALB/c mice by using an ethoexperimental approach. This paradigm is based on defensive behaviors displayed by rodents when confronted to a predator but also to its odor [8,9]. This confrontation can be measured in the animal's home cage or in a familiar apparatus, permitting the observation of several parameters such as hiding [14], sheltering [46,47] and risk assessment or avoidance of the aversive stimulation [6].

Our experiment compared BALB/c mice reared in SC or EC on two classical tests of emotional reactivity: the elevated plus maze and the open field. These tests involve a forced confrontation with a novel environment and give rise to behavioral [13,28,35,38] and physiological reactions [18,36] related to anxiety. According to previous work in our laboratory, we expected EC mice to be less reactive in these tests, indicated by an increased level of exploration.

Afterwards, SC and EC mice were exposed, in their home cage, to the introduction of a novel odorant object. Mouse reactions were observed either to cat odor or a neutral odor (modeling clay). Cat feces were chosen among different possibilities (urine, fur or collar) because of their efficacy in producing emotional responses in a preliminary experiment we carried out. We predicted that EC mice would be less reactive to a predator odor than SC mice in view of the results of Klein *et al.* [26], who found that EC rats engaged in less defensive behavior than SC rats when exposed to a cat in a two-chamber apparatus.

In addition to behavior, corticosterone levels were measured following the novel odor test. This physiological variable has often been found to correlate with the intensity of the anxiogenic situation [21,36] and risk assessment in the elevated plus maze test [39]. Corticosterone levels in rats were also increased during the initial exposure to cat odor [17].

## 2 Materials and Methods

### 2.1 Animals and rearing conditions

Sixty-six male mice of the BALB/cByJ strain whose progenitors were purchased from IFFA Credo, France were raised in either standard (n=36) or enriched (n=30) conditions. Throughout the experiment, the animals were maintained in an air-conditioned room with an ambient temperature of 22°C ( $\pm 1$ ) and a 12: 12 light / dark cycle (light on at 19h30).

When obviously pregnant, the dams were isolated under standard conditions consisting of a 22 x 12 x 12 cm polycarbonate cage with food and water ad lib. On day 12, day 0 being the date of birth, the females were randomly assigned to SC or EC. The litters were not culled, as the number of pups and the male / female ratio were very similar in each condition (number of SC pups =  $5.8 \pm 0.4$ ,  $3.2 \pm 0.3$  males and number of EC pups =  $6 \pm 0.6$ ,  $3.1 \pm 0.4$  males). SC females remained in their cages with their pups, while EC females were placed in an enriched environment with their litter. This procedure, based on the Paylor *et al.* study [34], was used for two reasons: 1) to avoid possible differences in maternal behavior during the first days of life, and 2) to allow interactions with environmental enrichment before weaning, during an important period for central nervous system maturation [44].

Our enriched environment was composed of two colored plastic boxes, respectively  $36 \times 25 \times 30$  cm and  $22 \times 18 \times 20$  cm, joined by tunnels. Each enriched environment contained a running wheel and some colored objects. In order to stimulate the pup's exploratory activities, the boxes and tunnel patterns were changed every three days. In parallel, the SC cages were also cleaned every three days. On day 23, the pups were weaned, marked in each litter and identified as to sex. Only males were kept for this experiment. SC pups stayed in standard cages in groups of 3 or 4, while EC pups remained in the enriched environment in groups of 10 ( $\pm 2$ ). At this time, some litters were combined in order to obtain a sufficient number of mice per environment. Therefore, as expressed by Renner and Rosenzweig [37], our environmental enrichment included both physical and social complexity.

On day 52, EC animals were placed in standard conditions. This 10-day period of standard conditions before behavioral testing was used to avoid short-term effects of EC and particularly hyperactivity [11]. Body weight was measured on day 60.

The research reported in this paper was conducted in accordance with the Guide for care and Use of laboratory Animals established by the National Institute of health of the United States of America and with applicable guidelines from the Ministère de l'Agriculture, France.

## **2.2 Behavioral testing**

The testing schedule was the following: elevated plus maze on day 62, open field on day 72 and cat odor exposure on day 82. Since it has been shown that exposure to a predator or to its odor has lasting effects on other tests of anxiety [3,46], the testing order was not counterbalanced. All tests were performed at the beginning of the dark phase between 9h00 and 11h00.

### **2.2.1 Elevated plus maze**

The apparatus was composed of a central part (5 x 5 cm), two opposing open arms (20 x 5 cm) and two opposing enclosed arms (5 x 5 x 15 cm). The maze was made of beige painted wood, elevated at a height of 50 cm and the open arms were illuminated by two 40 W white bulbs providing a 250 lux illumination on their extremities. Those lighting conditions were chosen to provide mild anxiogenic conditions and to allow a better discrimination between mice from the two rearing conditions. The test lasted 5 min long and began with the placement of mice in the center of the maze, with their head facing an enclosed arm. A camera video mounted above the maze was used to record each trial and to allow a later analysis. The number of entries and the time spent in open and enclosed arms were recorded as well as the number of rears and fecal boli. A four-paw criterion was used for arm entries. More ethological measures, like stretched attends postures (SAP), head scan toward the open arms and head dipping were also noted [40].

### 2.2.2 Open field

The apparatus was composed of a white circular Plexiglas floor (40 cm in diameter) surrounded by a gray PVC wall (30 cm in height). The floor was divided into 1 central and 6 peripheral parts of equal surface. A 100 W white bulb placed 70 cm above the open field provided a 500 lux illumination at floor level. At the beginning of the test, the mice were placed in a peripheral part of the open field and video recorded for 5 min. Latencies before leaving the initial part was noted, together with the total number of entries into peripheral and central parts. As in the elevated plus maze test, SAP, rearing and defecation were counted.

### 2.2.3 Cat odor exposure

Approximately 20 hours before the test, the mice were isolated in standard cages and randomly allocated to one of the three following group: control (no odor), neutral odor (modeling clay) and predator odor (cat feces). SC mice were divided in groups of 12 subjects while EC mice were divided in groups of 10 subjects. Cat feces were obtained from a two-year old male domestic cat, collected outdoors, quickly after defecation. The cat was fed with domestic food, but was frequently observed catching and eating wild mice and shrews. The neutral odor was provided by a 2 cm diameter ball of blue modeling clay of the same volume as cat feces. On the test day, the mice were individually brought in their home cage to the testing room dimly lit with a 40 W red bulb (15 lux on the testing location). After the removal of the grid, and depending on the group, an odorant stimulus was placed on the sawdust, at the opposite location of the food compartment. The same procedure was followed for the control groups except that no stimulus was given. The grid was then replaced and the behavior of each mouse was recorded from the side during 5 min. At the end of the exposure, the odorant stimulus was removed and the subject was carried to an adjacent room. In order to avoid any disruption between odors, the mice from the control group were always tested first, followed by those from the neutral odor group and the predator odor group. As all mice from the same testing day were behaviorally recorded and sacrificed in less than 90 min, we could be reasonably certain that no time of day confound was introduced by our procedure.

The time spent at the opposite location of the odorant stimulus (*i.e.* under the food compartment), the number of entries into the two parts of the cage (*i.e.* under the food compartment or stimulus location), and the number of rears were measured in the six groups differing in rearing condition and odor exposure. Moreover, the number of contacts with the odorant stimulus, the number of SAP, and the number of burrows toward the stimulus were used to compare more precisely SC and EC animals exposed to modeling clay or cat feces.

### **2.3 Measurement of plasma corticosterone level**

Ten minutes after the end of the novel odor exposure, the mice were carried to a third room specially allocated for blood collection. The mice were weighted and killed by decapitation within a minute. Trunk blood was then collected in a tube with 50 $\mu$ l of EDTA added and kept in ice. The plasma was recovered after centrifugation (5000 rpm during 20 minutes, 4°C) and frozen until the corticosterone assay. After extraction in absolute ethanol, the corticosterone concentration was determined by radioimmunoassay as previously described by Leboulenger *et al.* [27]. The sensitivity threshold of the assay was 20 pg. and the recovery was 100 %.

### **2.4 Data collection and statistics**

Data were collected from video analysis by using Etholog software version 2.25 [33]. All data are given as mean values  $\pm$  SEM and were analyzed with STATISTICA (version 5.1). Unpaired Student's *t*-tests were performed to compare SC and EC mice for weight measures, for the elevated plus maze, and the open field. A 2  $\times$  3 factorial ANOVA (examining rearing condition and odor group) was applied for behavioral and physiological parameters in the odor exposure test. Note that for behaviors such as SAP or burying, observed only in the presence of an odorant stimulus, a 2  $\times$  2 factorial ANOVA was applied. Planned comparisons were used for post hoc comparisons.

### 3 Results

#### 3.1 *Body weight*

The results of body weight measurements are shown in figure 1. Measures from day 60 and 82 show that EC mice were significantly heavier than SC ones on each day [respectively  $t(64) = 6.19$ ,  $p < 0.001$  and  $t(64) = 6.15$ ;  $p < 0.001$ ].

#### 3.2 *Elevated plus maze*

Significant behavioral differences were observed according to rearing conditions in the elevated plus maze (see table 1). The number of enclosed arm entries was higher in EC mice [ $t(64) = 2.88$ ,  $p < 0.01$ ] as well as the number of head scans in the open arm and of total rearing [respectively  $t(64) = 2.09$ ,  $p < 0.05$  and  $t(64) = 3.91$ ,  $p < 0.001$ ]. On the contrary, the number of SAP and of fecal boli were significantly reduced in EC mice [respectively  $t(64) = 2.22$ ,  $p < 0.05$  and  $t(64) = 2.59$ ,  $p < 0.05$ ]. Although SC mice appeared to spend more time in the open arm and less time in enclosed arms than EC mice, these results did not reach significance [respectively  $t(64) = 1.66$ ,  $p = 0.10$  and  $t(64) = 1.83$ ,  $p = 0.07$ ]. Finally, rearing conditions did not modify the number of head dipping.

As the number of enclosed arm entries was significantly different between the two groups, the open / total arm entries ratio was not used, in accordance with a previous work that demonstrated the inadequacy of this parameter to illustrate anxiety in those conditions [12].

#### 3.3 *Open field*

Behavioral changes were also observed in the open field (see table 2). EC mice left the initial part faster than SC mice [ $t(64) = 2.18$ ,  $p < 0.05$ ] and subsequently made more entries in peripheral parts of the apparatus [ $t(64) = 2.48$ ,  $p < 0.05$ ]. As in the elevated plus maze, more SAP were observed in SC mice [ $t(64) = 4.14$ ,  $p < 0.001$ ], but other variables did not differ significantly [ $p > 0.28$ ].

### **3.4 Cat odor exposure**

Neither the rearing condition factor nor the rearing condition  $\times$  odor group interaction reached significance for the behavioral parameters observed. A significant main effect of the odor was observed for locomotion between the two parts of the home cage, for time at the opposite location of the odorant stimulus, and for rearing behaviors [respectively  $F(2, 60) = 16.03, p < 0.001$  ;  $F(2, 60) = 73.36, p < 0.001$  and  $F(2, 60) = 14.47, p < 0.001$ ]. Post hoc comparisons (see table 3) revealed that the cat odor group had a smaller locomotion level compared to the control and the modeling clay groups [ $p < 0.001$ ]. In addition, mice from the cat odor group spent more time at the opposite location to the odorant stimulus [ $p < 0.001$ ], and showed less rearing behaviors [ $p < 0.001$ ] than the two other groups. The modeling clay group did not differ from the control group for these three measures.

Statistical analysis of modeling clay and cat odor groups only, revealed significant effects of the odorant stimulus for contacts with the stimulus, SAP, and burying [respectively  $F(1, 40) = 56.64, p < 0.001$  ;  $F(1, 40) = 55.68, p < 0.001$  and  $F(1, 40) = 8.27, p < 0.01$ ]. There were no significant effects on the rearing condition factor and on the rearing condition  $\times$  odor group interaction. Post hoc analysis showed that mice engaged in fewer contacts with the cat boli than with the modeling clay ball [ $p < 0.001$ ]. In addition, SAP and burying were significantly more frequent in the case of cat odor exposure [respectively  $p < 0.001$  and  $p < 0.01$ ].

### **3.5 Corticosterone assay**

Corticosterone levels following the third test are shown in figure 2. The results obtained from the rearing condition  $\times$  odor condition ANOVA show significant main effects of the rearing condition and the odor condition [respectively  $F(1, 60) = 4.62, p < 0.05$  and  $F(2, 60) = 3.32, p < 0.05$ ]. The rearing condition  $\times$  odor condition interaction was not significant.

Planned comparisons first show that the plasma corticosterone level was not significantly different between SC and EC mice from the no-odor groups. For each rearing condition, although a slight augmentation in plasma corticosterone was observed when a modeling clay ball was presented, no significant effect was noted. A significant increase was observed in SC animals exposed to the cat odor [ $p < 0.01$ ] when compared to control mice while their EC counterparts did not differ from their control counterpart. Plasma corticosterone was significantly higher in SC mice from the cat odor group when compared to EC mice exposed to the same stimulus [ $p < 0.01$ ].

## **4 Discussion**

### **4.1 *Body weight***

In accordance with previous studies [20,30,42], we found that EC mice had a greater body weight. A possible explanation for this observation could be an improved regulation of body temperature in EC mice [42] and observations we made of mice in the enriched environment seem to confirm this hypothesis, since the occurrence of groups resting inside tunnels or objects was frequent, which may reduce heat loss.

### **4.2 *Classical tests of anxiety***

The results obtained in the elevated plus maze and the open field emphasize that EC mice were less reactive than SC mice in reaction to a forced confrontation with a novel environment. In the elevated plus maze, EC mice explored the apparatus by making more frequent entries into the enclosed arms and more frequent rears. This tendency was confirmed by the open field session, in which EC mice left more quickly the initial part and showed a higher locomotion level. Our results confirm previous ones in EC rats [22] or EC mice [10,29,30] in the open field. Likewise, EC mice showed significantly less SAP in both apparatus. This behavior was described as an approach-avoidance conflict [41] and was expressed as part of a risk assessment factor in the elevated plus

maze [40]. Fewer SAP in EC mice thus reinforced the idea that their motivation to explore was increased, along with a decrease in novelty avoidance as previously observed in our laboratory [11]. However, the number of entries and the time spent in open arms of the maze were not higher in EC mice. This result contrasts with our previous study [11], and may be attributed to the use of white as opposed to red bulbs. White bulbs were chosen herein to provide a more anxiogenic condition and thus to allow a better separation of EC and SC mice. It appears that the opposite effect was obtained, due to a ceiling effect. This explanation seems plausible, since EC mice pointed their head into the open arms more frequently. This risk assessment behavior can be considered as an attempt to enter open arms and this observation is in accordance with our initial report. These observations are strengthened by the fact that defecation, an autonomous reaction proposed to characterize a high level of emotionality [13], was more frequent for SC mice in the elevated plus maze. The number of fecal boli in the open field was not different between the two groups but for each group was higher than in the elevated plus maze. This certainly justifies the consideration that the brightly lit open field was much more anxiogenic than the elevated plus maze, in which enclosed arms could provide an escape location. As for open arm entries in the elevated plus maze, the high anxiogenic level may be responsible for the finding that EC mice did not make more entries into the central part of the apparatus.

In conclusion, reactivity to novel environments was reduced in EC mice, mostly by an increased level of exploration [40]. This may result from the frequent changes of condition in the enriched environment, but, as pointed out in the introduction, do not necessary imply a general decrease in anxiety.

### **4.3 *Cat odor exposure***

There were no significant differences in spontaneous activity (locomotion and rearing) between SC and EC mice from the no odor groups. This result gives validity to our interpretation that the higher locomotion level of EC mice in forced situation tests reflected an increased motivation for

exploration. Moreover, as previously described in the same strain [42], there was no difference in basal corticosterone level between the two rearing condition groups.

The modeling clay exposure clearly demonstrates that not every novel odorant object introduced in the home cage is aversive. Even if the modeling clay ball initially provoked some SAP, those behaviors were quickly replaced by proximal contact (sniffing or gnawing) and defensive burying was rarely observed. In addition, SC and EC mouse reactions toward the modeling clay were not different. This result can appear to contrast with the idea that EC mice are more exploratory [23,37,43,45], but a likely explanation emerges from studies which demonstrated that exploration in EC animals varies more in quality than in quantity [37,45]. The corticosterone level after exposure to modeling clay did not differ between the two rearing conditions and the slight increase with regard to the no-odor groups probably results from exploration activities.

A strong effect of the cat odor condition was found. Animals exposed to this stimulus showed many signs of repulsion. Locomotion was reduced, as well as the large amount of time spent sheltering at the opposite location of the stimulus. These results are in agreement with previous studies [6,14,17,47], where animals exposed to a predator odorant cue typically freeze, avoid the threatening source, and hide whenever possible. Moreover, behaviors related to rodent defensive reactions, like SAP and defensive burying, were observed almost exclusively in this situation. Hence, even if the cat odor need not necessary imply any ecological validity [24,25], it appears evident that the present situation is highly anxiogenic and therefore measures a strong emotional response in BALB/c mice.

It is important to consider that no behavioral differences were observed between SC and EC mice in this situation. This observation contrasts with classical tests of anxiety sessions and does not support the differences found by Klein *et al.* between SC and EC rats during cat exposure [26]. A potential explanation is that possible reactions were limited in our testing situation. There was no possibility to avoid the stimulus (*i.e.* escape from its location) except by active avoidance, in particular by burying the threatening source. The predatory odor threat may have been too strong to

separate SC and EC mice. A solution to this problem would be to allow mice to be passive and / or active in front of the threatening source, for example by presentation of the cat odor in a larger apparatus [6,8]; on the other hand, this would certainly raise interactions between anxiety and exploration. However, our behavioral results suggesting different effects of EC according to the kind of test are in agreement with a recent study demonstrating that exposure to cat odor in mice may not measure the same phenomenon that classical tests of anxiety [5a]. Nevertheless, differences in corticosterone level were observed between SC and EC mice. Although caution concerning the corticosterone and stress responses is necessary [7,32], this result may indicate that EC mice may be able to cope better with the threatening stimulus, by different levels of regulations of their physiological reactions to stress (*e.g.* HPA axis functioning).

## **5 Conclusion**

The results obtained in classical tests reinforced the idea that environmental enrichment has beneficial effects on emotionality in mice. However, behavioral responses to cat odor failed to confirm this idea, and only the corticosterone level following exposure indicates that EC mice were less affected in this test. Thus, a difference seems to appear between classical tests (*i.e.* reaction to novel environments) and ethoexperimental tests (*i.e.* exposure to predator or relative cues). Further studies should investigate the comparison between those tests, that certainly measure different aspects of emotional reactivity, and that were recently found to be controlled by different neurobiological substrates [1,2].

Table 1. Behaviors in the elevated plus maze

	SC mice	EC mice	
Open arm entries	1.69 ± 0.4	1.77 ± 0.4	
Closed arm entries	11.97 ± 0.8	16.80 ± 1.5	**
Time in open arms	19.63 ± 5.2	9.57 ± 2.4	
Time in closed arms	136.14 ± 9.4	164.20 ± 12.4	
Rearing	15.53 ± 1.1	23.30 ± 1.7	***
SAP	9.50 ± 0.8	7.07 ± 0.8	*
Head scan	11.56 ± 0.8	14.67 ± 1.3	*
Head dipping	12.69 ± 1.6	11.53 ± 1.5	
Defecation	6.14 ± 0.6	3.83 ± 0.6	*

Data are given as mean values ± SEM. SC mice (n=36) and EC mice (n=30). Statistic: unpaired Student's *t*-test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

Table 2. Behaviors in the open field

	SC mice	EC mice	
Time to leave initial part	59.92 ± 15.0	20.33 ± 8.4	*
Peripheral parts entries	34.56 ± 3.8	49.23 ± 4.7	*
Central part entries	2.83 ± 0.6	1.97 ± 0.6	
Rearing	6.97 ± 1.1	8.83 ± 1.3	
SAP	6.47 ± 0.6	3.17 ± 0.5	***
Defecation	7.83 ± 0.4	8.17 ± 0.5	

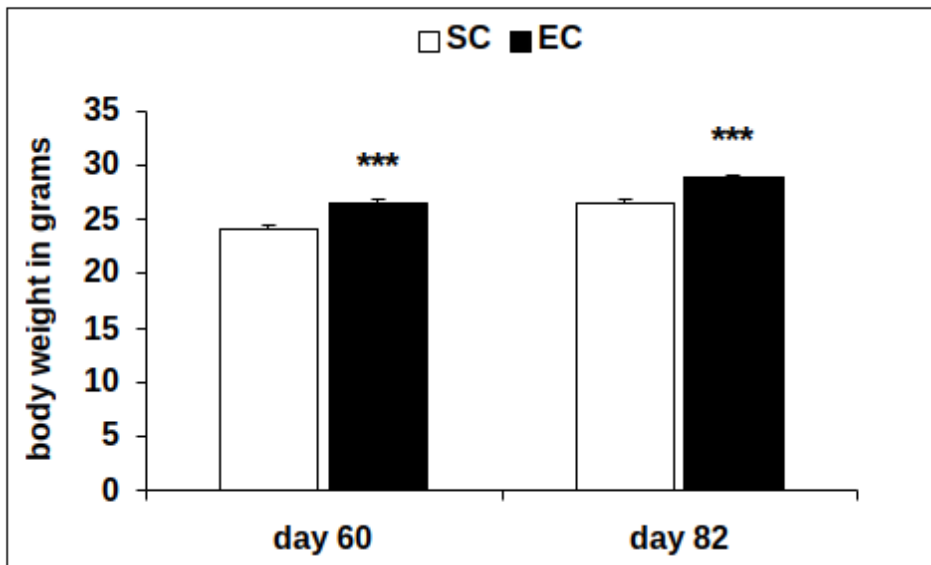
Data are given as mean values ± SEM. SC mice (n=36) and EC mice (n=30). Statistic: unpaired Student's *t*-test (\*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ).

Table 3. Behaviors during exposure to an odorant stimulus

	No odor	Modeling Clay	Cat Odor	
Locomotion	15.18 ± 1.40	17.77 ± 1.04	8.45 ± 1.04	*** ●●●
Time at opposite location	77.09 ± 4.81	79.45 ± 6.52	208.77 ± 12.69	*** ●●●
Rearing	45.18 ± 2.08	41.59 ± 1.76	31.27 ± 1.70	*** ●●●
Contact	---	26.64 ± 1.32	10.91 ± 1.56	●●●
Sap toward	---	1.23 ± 0.17	18.41 ± 2.28	●●●
Burying	---	0.14 ± 0.08	3.00 ± 1.02	●●

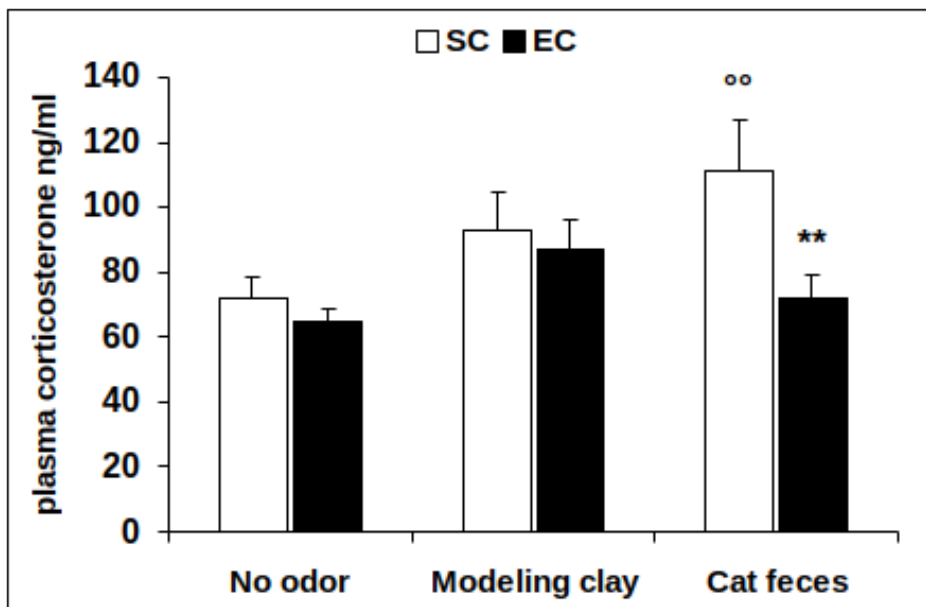
SC and EC mice are grouped for each odorant stimulus since no significant effects were observed on the rearing condition factor. For control, modeling clay and cat odor group n = 22 (12 SC and 10 EC mice). Data are given as mean values ± SEM. Planned comparisons were used for post hoc analysis (\*\*\*  $p < 0.001$ , cat odor compared to no odor; ●●  $p < 0.01$ , ●●●  $p < 0.001$ , cat odor compared to modeling clay).

Figure 1.



Graph 1. Body weights in grams at days 62 and 80 for SC mice (n=36) and EC mice (n=30). Data are presented as mean values (+ SEM) and unpaired Student's *t*-test were used (\*\*\*)  $p < 0.001$ .

Figure 2.



Graph 2. Plasma corticosterone levels for each odorant stimulus group of the two rearing conditions: SC groups (n = 12) and EC groups (n = 10). Data are presented as mean values (+ SEM). Planned comparisons were used as post hoc analysis (<sup>oo</sup>  $p < 0.01$ , compared to no odor group of same rearing condition; \*\*  $p < 0.01$ , compared mice to SC mice from the same odorant stimulus group).

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