Supplementary data

Each lesser anteater was moved from its enclosure to the evaluation room. This movement took less than a 1 min, and was performed by the same person, holding the animal by the tail as the keepers usually do to check body weight. There were no visitors during the evaluations.

In table 1 we summarized the timeline (year 2017) of the evaluations performed to support Study 1. Between days -12 and -11, the lesser anteaters remained in their enclosures without any experimental intervention. November 30 corresponded to day -1 (not indicated in the table), in which the lesser anteaters were individually taken to the evaluation room and exposed to the empty feeders used in the food stimuli evaluations (Study 1).

Table S1. Timeline of lesser anteater behavioral evaluations to support the main experimental design (Study 1)

Date	Evaluation day	Number of evaluations and session time	Behavioral evaluations
November 13	-18 to -13	3 (30 min)	Novel environment
November 21	-10 to -3	2 (32 min)	Odor discrimination
December 1	0 to +2	3 (10 min)	Food stimuli (Study 1)

Novel environmental evaluation

JUSTIFICATION

In order to reduce the influence of the evaluation room stimuli on the lesser anteaters, we exposed them individually (session: 30 min) to the novel environment. In total, each lesser anteater entered the room 3 times to record their activities. Based on the duration of each session and the time slot of the evaluations (between 17:00 – 20:00 h), we randomly divided the lesser anteaters into two groups (n = 4 and n= 3 anteaters), alternating the days of entrance of each group. In this way, we evaluated 4 or 3 anteaters each day.

METHODS

We analyzed lesser anteaters' activities in the evaluation room through videos recorded on a DVR (HIKVISION Turbo HD DVR-DS 7200 Series). We used the focal sampling method for all lesser anteaters. As in the studies on other species [53], prior to the evaluations we divided the evaluation room in a 36-square grid using adhesive tape (Figure 1 b, main document). We used squares of 50 x 50 cm, according to the size of the lesser anteater [1] and the evaluation room. We marked the squares firstly in the evaluation room and secondly on the monitor in which we analyzed the videos. Before beginning the evaluation, we removed the tapes used from the evaluation room. We analyzed the activities, as indicated in section 2.3.3 (Main document: Methodology and variables for the behavioral evaluations):

- Ambulatory latency (s): time elapsed between placing the lesser anteater in the evaluation room with its four legs contacting the ground (see for example the cross in Figure 1) and the complete head leaving the first square.
- Location register (counting): we registered the location of the lesser anteater every 30 s in the different zones of the evaluation room. The results were expressed as cumulative proportions.

We statistically analyzed ambulatory latency by a one-way analysis of variance with the lesser anteaters as the factorial structure. Since the variable was not normally distributed according to the Shapiro-Wilks test, we transformed the data (log10) and then applied a Fisher LSD test. For the location register, we adjusted a mixed linear model for each zone, where the fixed effect was the sessions and the random effect was the lesser anteaters (Figure 1).

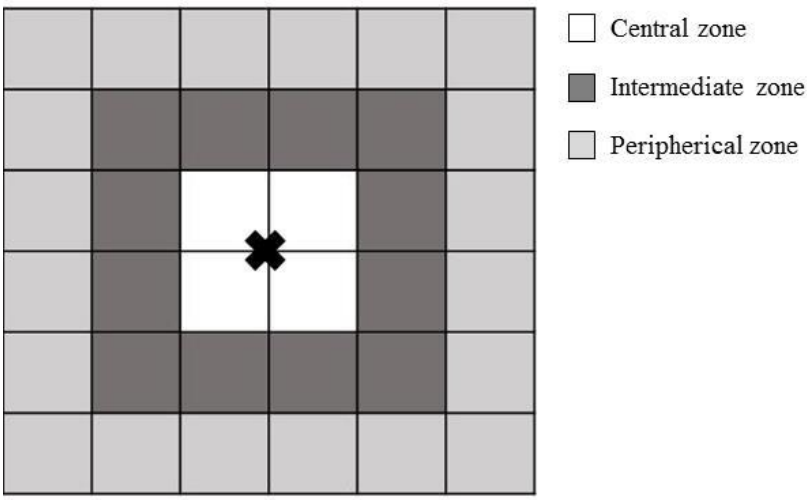

Figure S1: Diagram of evaluation room divided into 36 squares for the purpose of analysis. The cross indicates the initial position of the lesser anteater in each session for evaluation novel environment.

RESULTS

Ambulatory latency (seconds) among lesser anteaters did not differ between sessions (Session 1 = 16.1 ± 5.6, Session 2 = 14.7 ± 5.0, Session 3 = 10.9 ± 0.7; $F_{2, 12} = 0.4$, $p = 0.6817$). For the location register (counting), we detected differences between sessions in the peripheral zone ($F_{2, 12} = 25.32$, $p < 0.0001$). The Fisher LSD *a posteriori* test showed the following response pattern: Session 1> Session 2> Session 3 (Figure 2). In the central and intermediate zones, we detected differences between the sessions, with a response pattern different from that observed in the peripheral zone ($F_{2,12} = 7.88$, $p = 0.0065$ and $F_{2,12} = 12.02$, $p = 0.0014$, respectively). For these two zones, we observed the following patterns: intermediate, session 1 = session 2 <session 3, and central, session 1≤ session 2 ≤session 3.

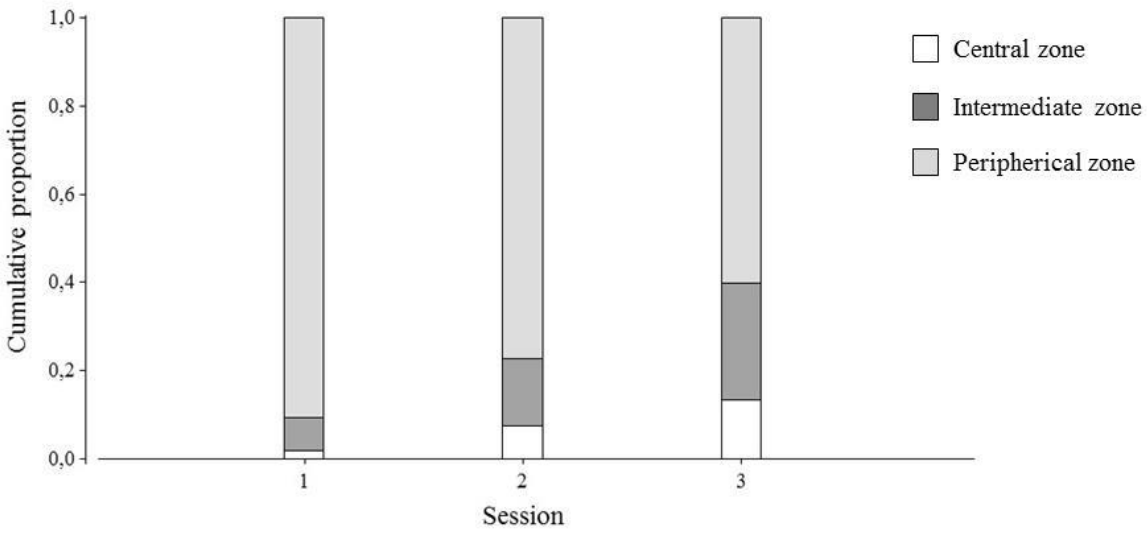

Figure S2. Cumulative proportions of the location register of the lesser anteaters during the novel environmental evaluation sessions. Each session lasted 30 minutes. Statistical differences for the response pattern according to the zone: peripheral, session 1> session 2> session 3; intermediate, session 1 = session 2 <session 3, and central, session 1≤ session 2 ≤session 3.

DISCUSSION

The results observed in the novel environment evaluation provide useful insight for the experimental design of studies on lesser anteater feeding behavior in the presence of ants and termites. Previous studies on rodent species evaluated in open-field tests have shown that an increase in animal records in non-peripheral zones might indicate an anxiolytic effect [53]. In the present evaluation, the lesser anteaters showed changes in the activity levels between zones during the novel environment, perhaps indicating a proclivity for more exploratory activities in response to novelty. This would suggest that the evaluation room did not negatively affect the exploratory behavior of the lesser anteaters, and this result positively supports the food stimuli evaluation.

Odor discrimination evaluation

JUSTIFICATION

We carried out an odor discrimination evaluation according to the procedure proposed by [54] in order to assess the response of lesser anteaters to odors so as to include them or not in the main study depending on their ability to react to them. We used the followings aromatic descriptors: water, almond essence and banana essence. We additionally added a fourth descriptor to the evaluation, jaguar faeces (*Panthera onca*¸ a natural predator *Tamandua* species [55]). We evaluated just one individual per day (32 min) in order to reduce residual odors in the room from both the aromatic descriptors and the other lesser anteaters.

METHODS

For each evaluation, we first placed an empty plastic cup (200 mL, no aromatic descriptor) in the room and then removed it after 5 minutes through a sliding door (35 x 20 cm). We then began presenting the aromatic descriptors one after the other, also in plastic cups (200mL), with a 1-minute pause between them. Each descriptor was in the room for 3 periods of 2 minutes.

The cup was removed for 5 seconds between periods. The order of presentation of the descriptors was the same for all lesser anteaters: water, almond essence, banana essence and jaguar faeces. Each lesser anteater was therefore evaluated for a daily total of 32 minutes. We placed the plastic cups in a fixed location at ground level in a corner of the room.

Preparation of the aromatic descriptors: 7 h before each evaluation, we placed 5mL of each aromatic descriptor on different units of compact cotton (3x3 cm) in sterile glasses (50mL). The descriptors were: water (distilled), almond essence (Pura Química Laboratory, Córdoba, Argentina, food grade) and banana essence (Pura Química Laboratory, Córdoba, Argentina, food grade). We obtained the descriptor jaguar faeces from fresh faeces of a male specimen housed at Córdoba Zoo. We placed 10 g of faeces in a sterile glass and added distilled water to form a mixture of 60mL that was left for 24 h before the first separation. As with the other descriptors, we placed 5mL of the faeces mixture in sterile glasses (50mL). The compact cotton units remained in individual sterile glasses, sealed until their presentation.

For the evaluation, we employed instantaneous sampling, registering the location of the lesser anteater in the evaluation room every 30 seconds, giving 60 sample points. We proceeded as in Arbuckle et al. [54], recording the number of times that the lesser anteater visited the olfactory zone during each presentation of the different aromatic descriptors (Figure 3).

For statistical analysis: we adjusted a mixed linear model for each aromatic descriptor (fixed effect), evaluating the variation in the number of records between the habituation period (5 min with the plastic cup) and the 3 periods of 2 minutes per descriptor.

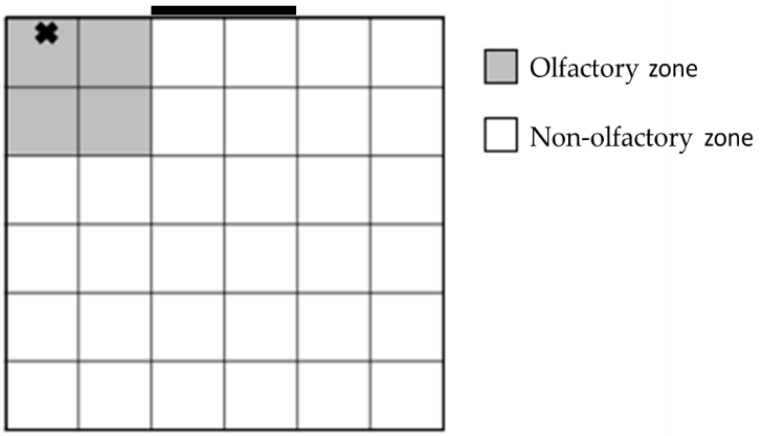

Figure S3. Diagram of the room divided into 36 squares for the evaluation room zones during odor discrimination evaluation. In order to analyze the lesser anteater´s activity, we divided the room in two zones: olfactory and non-olfactory. The cross indicates the location of the plastic cup with the aromatic descriptor. The black rectangle represents the access door. We worked on the analysis as indicated above, marking the squares on the monitor where we analyzed the videos and then removing the tapes used from the evaluation room.

RESULTS

The results showed no differences between habituation and the water ($F_{3,18}$ = 2.69, p= 0.0773) or jaguar faeces ($F_{3,18}$ = 2.98, p = 0.0590; Figure) descriptors. In contrast, we detected statistical differences between habituation and the almond ($F_{3,18}$= 7.69, p = 0.0016) and banana essence ($F_{3,18}$= 12.44, p = 0.0001; Figure 4) descriptors.

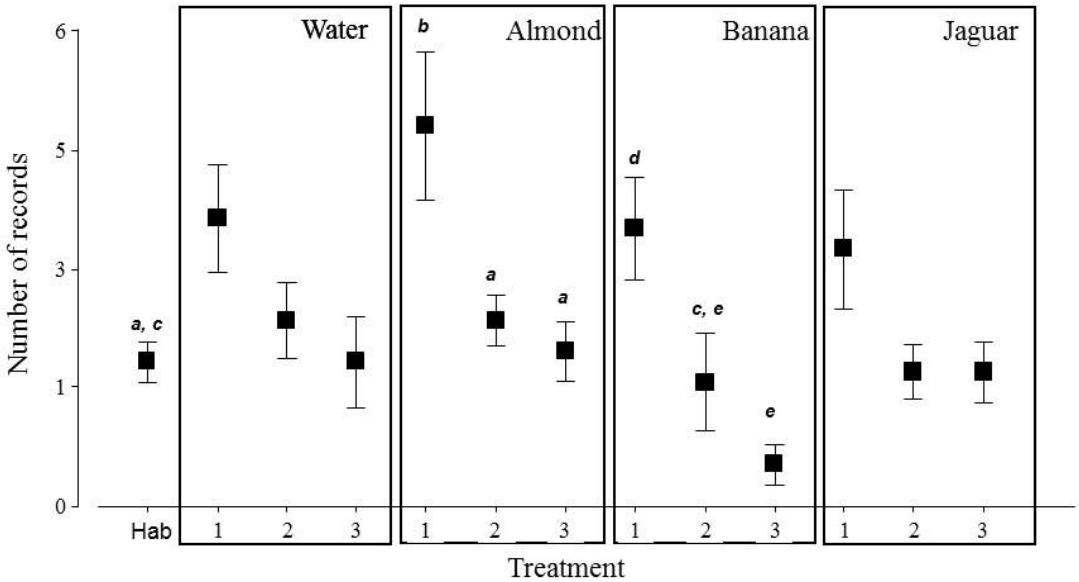

Figure S4. Location register (counting) of the lesser anteaters in the olfactory zone during the odor discrimination evaluation. Each session lasted 32 minutes. Hab: habituation period to the plastic cup; we presented the water, almond, banana and jaguar (fresh faeces) descriptors 3 times per session (1-3 values on X axis). The statistical analysis was partitioned by descriptor, evaluating the differences between habituation and each descriptor presentation (treatment). Different letters (a vs b within Almond treatment, and c vs d vs e within Banana treatment) indicate statistical differences (p <0.05).

DISCUSSION

Our results clearly indicated that lesser anteaters react to odor stimuli and that they showed olfactory discrimination during this evaluation. Similar results were conducted on mice for this type of assessment [55]. Therefore, this supports the main study and presumably these lesser anteaters were able to detect other odor stimuli such as the volatile compounds (pheromones) released by ants and termites.

Monitoring of respiratory frequency during the evaluations

JUSTIFICATION

In order to monitor how lesser anteaters were responding to the evaluations, we measured their respiratory frequencies as a simple indicator of anxiety [56]. Variations in respiratory rates provided information on how lesser anteater cope with the evaluation room. This would help us to monitor lesser anteaters' welfare and obtain reliable results during behavioral evaluations.

METHODS

We measured the respiratory frequency of each lesser anteater by counting the number of chest movements per minute (this procedure was performed by a veterinary). We carried out the respiratory monitoring in the Novel environment evaluation and in Study 1 during 3 occasions. The occasions were: pre-evaluation (in the enclosure, before the lesser anteater was taken by the tail), post-evaluation (in the evaluation room, once the session was over) and post-resting (in the enclosure, after a fixed period of free time). For the novel environment evaluation, we also

measured the respiratory frequency of those lesser anteaters that stayed in their enclosure (not evaluated) while the others were being evaluated. Given that the sessions were of different durations, different post-evaluation and post-resting periods were determined for monitoring the respiratory frequency: for a novel environment, 30 minutes (post-evaluation) and 60 minutes (post-resting); and for food stimuli evaluation, 10 minutes (post-evaluation) and 20 minutes (post-resting).

For respiratory frequency (counting/minute), we counted the number of chest movements per 30 seconds. Results were expressed as register/minute. For statistical analysis, we used an analysis of variance adjusted to a mixed linear model, where the pre-evaluation, post-evaluation and post-rest were fixed factor levels and the lesser anteater was the random effect. the random effect was the lesser anteater.

RESULTS

The results of the respiratory frequency evaluation are shown in Figure 5 (panels A-B). There were significant statistical differences in the respiratory rate when we exposed the lesser anteaters to the first evaluation (novel environment evaluation, Panel A, $F_{2, 33} = 10.09$, $p = 0.0004$) but not for those lesser anteaters that stayed in their enclosure (not evaluated). For the food stimuli evaluation (panels B), the statistical analysis did not detect differences.

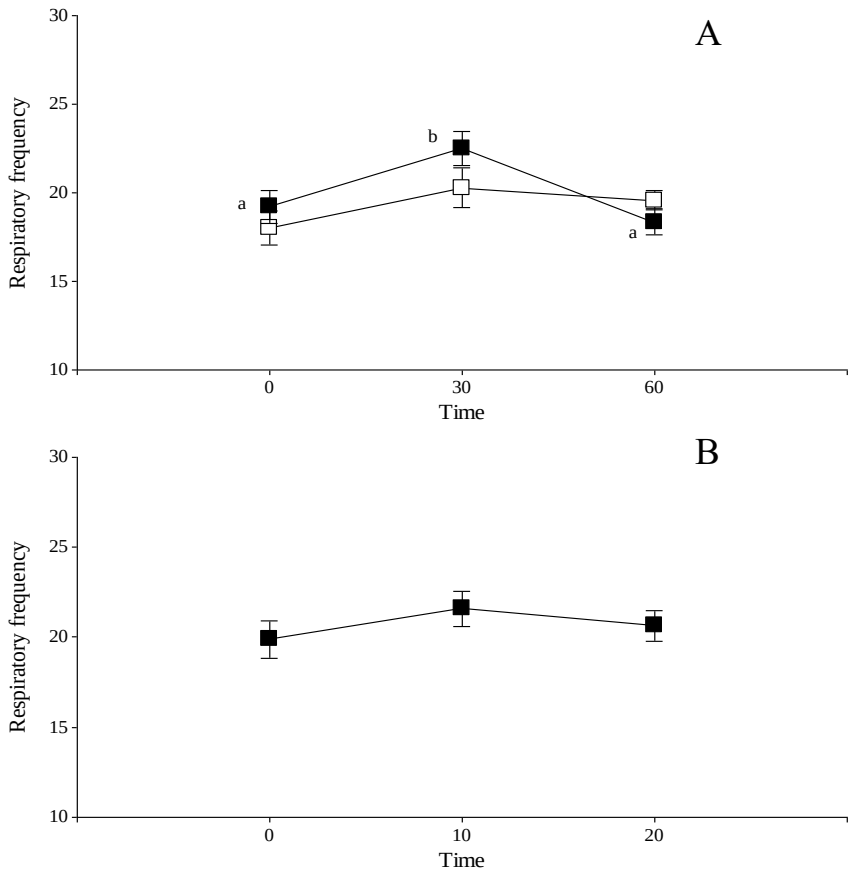

Figure S5. Respiratory frequency (registers/minute) in the lesser anteaters during novel environmental and food stimuli evaluations. The registration times (X-axis) correspond to pre-evaluation, post-evaluation and post-resting for evaluations. Panel A: The measurements of the

lesser anteaters exposed to the evaluation room are represented in black and those of animals not exposed to the evaluation room are represented in white. Panel B: evaluation in presence of food stimuli (10 minutes). Different letters indicate statistical differences (p <0.05).

DISCUSSION

We monitored respiratory frequency as a simple indicator of reaction to the environment. The lesser anteaters showed differences only when they were exposed to the evaluation room for the first time (novel environmental evaluation); the highest value was observed in the post-evaluation time (tachypnea). Lesser anteaters reacted one out of three times, so most of them showed signs of stability. Although the evaluation room could be considered equivalent to the open field for testing purposes, we nonetheless expanded the experimental timescale in order to reduce the possibility that evaluations would be affected by room stimuli. We are satisfied that the lesser anteaters reached the food stimuli evaluation without any significant alteration in this parameter, which indicates that they were in good condition to be tested, supporting the feeding behavior assessment.