Avoidance behaviour in laboratory house mice (*Mus musculus*) and Norway rats (*Rattus norvegicus*) towards predator odours

Luciana B. Adduci¹, Vanina A. León¹, Annika Schloettleburg², María Busch¹, Jimena Fraschina¹*

¹ Facultad de Ciencias Exactas y Naturales, Facultad de Ciencias Exactas y Naturales, Departamento de Ecología, Genética y Evolución, Universidad de Buenos Aires and Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEB), UBA-CONICET, Universidad de Buenos Aires, Intendente Güiraldes 2160—Ciudad Universitaria—C1428EGA, Ciudad Autónoma de Buenos Aires, Argentina, ² Division of Land Use Systems, Humboldt-University of Berlin, Faculty of Life Science, Institute of Agriculture and Horticulture, Albrecht-Thaer-Weg, Berlin, Germany

* jfraschina@ege.fcen.uba.ar

Abstract

*Mus musculus* and *Rattus* sp. are considered pest species because they reach high densities in urban areas, crop fields and food storage and productive systems such as breeding farms and orchards. Their control relies mainly on rodenticide application, but the effectiveness of this application is reduced due to behavioural responses and resistance. Novel methods are based on the use of chemical signals as odours that may be attractants, repellents or may reduce the reproductive success of pest species. The aim of this paper is to study the aversive effect of TMT, cat urine and cat body odour on predator-inexperienced *Mus musculus* and *Rattus norvegicus* under laboratory conditions. The experimental apparatus comprised three boxes connected by PVC pipes in a linear arrangement. In lateral boxes, odour sources or distilled water were introduced, while animals were placed in the central box at the beginning of the experiment. Rats showed freezing behaviour, reduced visits in the presence of TMT and cat fur. Mice reduced their visits with cat body and cat urine. This study provides evidence of the usefulness of using fear responses as a way to control rodent pests, which must be adapted to the environment and species to be applied.

1. Introduction

Human activity causes environmental changes that have large effects on many animal species. While in many cases these effects are negative, many rodent species benefit from anthropogenic changes because of an increase in food sources or refuges (in agricultural or urban areas) or a decrease in predator density [1]. These species may reach pest densities in anthropized habitats, causing several damages through the consumption of food, contamination, damaging building structures, reducing distribution of some endangered species and transmitting diseases to both humans and domestic animals [2–6].
Among the main rodent pest species, *Mus musculus* and *Rattus* sp. are cosmopolitan species reaching high densities in urban areas, crop fields, and food storage and productive systems such as breeding farms and orchards [7]. Their control relies mainly on the application of anticoagulant rodenticides, but it involves some environmental risk by poisoning of non-target species [8,9]. Furthermore, the effectiveness of rodenticide application can decrease over time because rodent populations can develop aversive behaviours and genetic resistance [10].

Recent advances in ecological research and analytical technology have led to novel methods that use chemical signals to create effective attractants and repellents for pest species [11]. This potential use of chemical signals for managing pest species is based on the behavioural and physiological responses to intraspecific or predator odours [12–15], to plant secondary metabolites and to toxic substances [16,17].

Many studies explored the effect of odours on reproduction [13], aversive behaviours or food intake [16] and suggested their potential use as alternative methods for rodent control [18]. Predator odours, such as feline urine and 2,3,5-trimethylthiazoline (TMT), which is a component of red fox (*Vulpes vulpes*) faeces, have an aversive and reproductive effect on rodents [19,20]. Cat collars, cloth rubbed on cats and cat fur were also demonstrated to have an aversive effect on rats [19,21]. The response to predator odours may elicit innate reactions in rodents, including stereotyped avoidance behaviours, but the future fear response can be modulated by experience [18]. Behavioural responses associated with the perception of predation risk were considered avoidance to move towards the odour source, short permanence in the vicinity of the odour source, and fear and alert responses such as freezing, sniffing, escape attempts and exploration [21–24]. Some authors consider grooming to be a non-defensive behaviour [19,21], while others assume that it is a response to stress stimuli that reflects the process of deearousal due to the termination of a stressful situation [25,26].

In rural habitats of central Argentina, rats and domestic mice reach high densities on breeding farms, where they cause economic damage through food consumption and contamination, damaging building structures, and, in poultry farms, kill chicks [27,28]. They can also transmit diseases to both humans and domestic animals [2–4,28]. In spite of rodenticide application, most poultry farms are infested with rodents [27], probably because of recolonization after control and the presence of individuals with low sensitivity to anticoagulants [29,30]. In previous works, we explored the potential of different odours as alternative methods for their control in both laboratory and semicaptivity conditions. In laboratory conditions, the reproductive success of *M. musculus* females was affected by unfamiliar males, cat urine and TMT odours [31], while domestic and Geoffroy’s cat odours did not produce avoidance in wild *M. musculus* in semicaptivity conditions [28].

The aim of this paper is to study the aversive effect of TMT, cat urine and cat body odour on predator-inexperienced *M. musculus* and *R. norvegicus* individuals under laboratory conditions. Our hypothesis was that individuals of both species will avoid TMT, cat urine or cat body odours and will display “alert behaviours” in their presence.

### 2 Materials and methods

The procedures conducted in this work were approved by the Institutional Comittee for Care and Use of laboratory animals (CICUAL, FCEN, Universidad de Buenos Aires), protocol number 88.

#### 2.1. Subjects

Many studies have used laboratory albino animals [21,23,32,33] to assess the effect of chemical signals on rodent behaviour. The use of laboratory animals has the advantage of the facility to
obtain sufficient animals of both sexes, with known age, similar genetic composition and life histories, thus decreasing potential heterogeneities in responses. On the other hand, the use of laboratory animals to preselect potential products for use in rodent control may reduce the number of wild animals used in experiments, in accordance with EU Directive 2010/63.48. Although laboratory animals have been isolated from environmental cues for many generations, they show generalized responses to odours from historical predators [34]. In consequence we used, as a first step prior to experiments with wild animals, laboratory mice and rats from domestic strains.

The subjects (n = 128) were male and female *M. musculus* (CrlFcen: CF 1 mice) and *R. norvegicus* (HsdFcen:WI) obtained from the breeding colony of the animal husbandry unit of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. Both males and females were sexually inexperienced and between 9 and 10 weeks old. Prior to the experiments, rodents were housed individually for 7 days for acclimatization in metal cages (41 x 36 x 17 cm) with softwood shavings, cotton and cardboard tubes as nesting material, and food (commercial food pellets, Cooperación ACA Nutrición Animal) and water *ad libitum*. They were kept at a temperature of 23˚C on a 12:12 h light–dark cycle.

### 2.2. Odour sources

The tested odours included TMT, cat urine, cat body odour and distilled water as a control. TMT (97.5%) was obtained from SIGMA ALDRICH (now Merck KGaA, Darmstadt, Germany). Because of logistic reasons, we could use only urine from one domestic, castrated female cat, which frequently hunts wild rodents and was fed with meat. This cat was trained to urinate in a container allowing urine collection. For the cat body odour, a piece of cloth (5 x 5 cm) was rubbed vigorously against male and female domestic cats (n = 4) for 5 min, according to Muñoz Abellán et al. and File et al [35,36]. Urine samples and cloth pieces were frozen (-18˚C) until experiments were performed and were unfrozen at least 30 min before they began. For liquid odours and distilled water, a volume of 1 ml was applied to a tissue paper. The use of distilled water as a control was decided according to Fendt et al., [20] and Horii et al., [23].

### 2.3. Apparatus

The test apparatus comprised three transparent plastic boxes (19 x 30 x 23 cm) where both mice and rats could stand on their hind legs, connected by two opaque PVC pipes (50 cm length; 7 cm diameter) in a linear arrangement. This device was placed in a room with evenly distributed light of 40 watt intensity to avoid differences in light intensity among boxes.

### 2.4. General procedure

The experiments were conducted in the facilities of the Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora, Argentina (34.77˚ South, 58.45˚ West), from November 2017 to August 2018.

To allow habituation to the testing environment all animals received a daily 10-min habituation session over four consecutive days [37]. They were moved from the animal room to the test apparatus with blank stimuli (Petri dishes with tissue papers without odour). Odour exposure tests began on the fifth day. After tested, animals did not return to the vivarium where other animals waited to be taken to the experimental room.

On the exposure days, we placed Petri dishes covered with metal mesh (to prevent direct contact of animals with the odour source) in the right (odour source) and left (distilled water as control) boxes. We decided to maintain the box with odour (right box) along the different trials to prevent remaining odours from affecting the results, even though the experimental
device was thoroughly cleaned after each trial. Rodents were individually placed in the central box of the experimental apparatus. We also conducted trials with distilled water in both lateral boxes. We renewed the tissue papers every three trials while the cloths with cat body odour were renewed after every trial. The test apparatus, the petri dishes and the metal mesh were vigorously cleaned at the end of each observation with a 70% ethanol solution. We assessed the effect of each different odour on different days, and after the sessions of one day, the test apparatus was intensely cleaned.

Animals were randomly assigned to the type of trial (treatment): TMT- Control (TMT), Cat Urine- Control (Cat Urine), Cat body- Control (Cat Body) and Control- Control (Control). Each odour was tested with 16 house mice (8 females, 8 males) and 16 rats (8 females, 8 males). All sessions started with the rodent placed at the central compartment of the experimental device. Behavioural responses were recorded over 10 min by two observers placed 2 meters from the experimental apparatus. We did not observe behaviours suggesting that animals were influenced by the presence of observers. We recorded at each second the location of the individual when the entire body was inside the compartment: central, odour, control box or connecting pipes (left and right), and the occurrence of the following behaviours: freezing, sniffing and grooming.

2.5. Data analyses

We conducted analyses separately for each species using the statistical program R (version 3.5.1, RCore Team 2018). To assess the effect of odours on avoidance behaviour, we compared the proportion of individuals who visited the odour box at least once for treatments with odours with respect to the Control by means of a one-sided test of difference between proportions [38]. We constructed models in which we considered as response variables the total number of visits to the odour box (Visits) and the number of seconds (out of 600) in which the animal stayed at the odour compartment (Duration), considering the total time as a sum of the duration in each visit.

The effect of odours on “alert behaviours” was assessed by the frequency (time intervals in which the behaviour was displayed with respect to the total time of observation) of sniffing (Sniffing) and grooming (Grooming). We did not conduct models for freezing behaviour because it was displayed by few individuals. We examined the effect of explanatory variables using generalized linear mixed models (GLMM) with the R package “lme4” [39], Matrix [40] and GLMM TMB [41]. Models were fitted by maximum likelihood (Laplace approximation) and selected according to AIC values.

For Visits, we first ran models including sex and treatment and their interactions as fixed explanatory variables. The time of the experiment was included as a random factor due to logistic limitations because we were not able to perform all replicates at the same time. For both species, we found significant interactions between the effect of treatment and sex, and in consequence, we conducted Tukey Multiple comparisons, with – “emmeans” package [42]. The best models for mice were ZAP- Zero-altered Poisson models. For rats, we adjusted a GLMM with a Poisson distribution of errors and a log-log link function.

For the variable Duration, we adjusted GLMM with a binomial distribution of errors. For both species, the models included the sex, treatment and their interactions as fixed explanatory variables and the time of the experiment and the individual (to avoid overdispersion) as random factors [43]. For mice, we did not find a significant interaction between the treatment and sex, while for rats there was a significant interaction, and in consequence we conducted Tukey multiple comparisons to test for differences among treatments according to sex with the emmeans package [42].
For the variables Sniffing and Grooming we ran GLMM including the sex and treatment (TMT, Cat Urine, Cat Body and Control) as fixed factors and the individual as a random factor. We assumed a hurdle Poisson distribution of errors. In the GLMM we compared odour treatments with the Control (intercept).

3. Results

3.1. Visits

The number of mice that visited at least once the odour box did not differ significantly (p>0.050 in all cases) between odour treatments and the Control. For rats, fewer animals visited the odour box for the Cat Body treatment than the Control treatment (p = 0.039; Table 1).

The number of visits of female mice to the odour box was lower for the Cat Body and Cat Urine treatments than for the Control treatment (p = 0.00314, p = 0.012, respectively, Tukey test, df = 50), while for male mice there were no significant differences between odour treatments with respect to the Control in the number of visits to the odour box (Fig 1 and Tables A.2 and A.3). Female rats visited the odour box less frequently in the Cat Body and TMT treatments than in the Control treatment (p = 0.0358 and p < 0.001 respectively, Tukey test, df = 55, Fig 1 and Tables A.4 and A.5). Male rats visited the odour box less frequently in the Cat Body than in the Control treatment (p = 0.001, Fig 1 and Tables A.4 and A.5).

3.2. Duration

For both species the best model for the duration of visits to the odour box according to treatment was the null model (lowest AIC), (Fig 2 and Tables A.6 and A.7).

3.3. Behaviours

From all possible behavioural responses, we only observed rats freezing, sniffing and grooming and mice sniffing and grooming, probably because of the confined conditions within the test chamber [20], especially for rats. Freezing was only observed in rats in the TMT treatment; one female in the control box and one female and one male in the central box.

3.3.1. Sniffing. The mean frequency of sniffing was 4.54±1.45 for mice and 12.27±2.19 for rats. Mice did not show a treatment effect on the frequency of sniffing neither an effect of sex. For rats there was an effect of treatment and sex, but not an interaction between them. Sniffing was more frequent in both Cat Urine and TMT treatments with respect to the Control (p<0.0001 and p = 0.01, respectively, df = 55 Fig 3). Females showed a higher frequency of sniffing than males (14.9±1.50 versus 9.45±1.47, p<0.01, Tukey test, df = 55).

3.3.2. Grooming. In comparison, rats display more frequently the grooming behavior than mice (mean grooming = 0.72±0.05 and 0.24±0.11, respectively, p<0.0001). Both species showed a lower frequency of grooming than sniffing (mean sniffing = 4.54±0.57 and 12.34±1.54, for mice and rats, respectively). For mice, the analysis could not be done due to the

Table 1. Number of animals that visited the odour box at least once according to the species and treatment.

| Treatment          | TMT | Cat Urine | Cat Body | Control |
|--------------------|-----|-----------|----------|---------|
| Mus musculus       | 7   | 8         | 7        | 10      |
| Rattus norvegicus  | 12  | 16        | 11*      | 15      |

* indicates p value<0.05.
excess of zeros and the low frequency in which they display this behaviour. For rats, the best model did not include the effect of the treatment (AIC null model: 152.61, df = 4, AIC model: 164.9, df = 14).

4. Discussion

We investigated whether cat urine, cat body or TMT odours elicited avoidance and alert behaviours in laboratory mice and rats. We considered the number and duration of visits to odour boxes in relation to control boxes with distilled water and alert behaviours as sniffing, grooming and freezing as evidence of avoidance to an odour source.

We found evidence of aversive behaviour in rats to TMT and cat body odours. TMT was the only odour that caused freezing behaviour in this species and its effect was also expressed in a
lower number of female’s visits to the odour box and a higher frequency of sniffing of both sexes in this treatment with respect to the Control. The effect of Cat body odour was expressed in a lower proportion of individuals who visited the odour box than in the Control treatment and in a lower number of visits of both sexes to the odour box. The only significant effect of Cat Urine in rats was a higher frequency of sniffing with respect to the control treatment. Female mice showed an aversive behaviour to Cat body and Cat Urine odours, reducing the number of visits to the odour box in these treatments, while males did not show significant effects.

There were no effects of odour treatments on the duration of visits for any species, suggesting that the aversive effect is not maintained once the rodent registers that there is no predator in the box, and in consequence it ignores the odour. This behaviour shows that the innate response to odours may be modified according to experience, as suggested by Bedoya-Pérez et al [18]. Differences in the effect of odours between rats and male mice may be related to differences in risk assessment behaviour, while mice actively approached and investigated possible dangers, this behaviour was not observed in rats [32].

The absence of an effect of treatments on the frequency of grooming suggests that this behaviour, in our experimental conditions, was not a response to the odours, according to the idea that it is a non-defensive behaviour. Grooming can occur once the animal considers that the surroundings are safe [44].

Our results must be interpreted taking in account the particular conditions of the experiment, the distance from the central and lateral boxes was only 50 cm, and the total length from the odour to the control box was 130 cm (50 cm of each tube plus 30 cm of the central box). This size may have caused an odour effect in all the apparatus, but we consider that animals reacted identifying the source of odour. We cannot discard, however, an apparatus effect, especially in rats who display freezing behaviour in the TMT treatment. It would have been more adequately to use species specific experimental apparatus, with higher size for rats. According to Blanchard et al. [32], results may also be interpreted taking in account potential effects of the origin (laboratory or wild) and strain of the animals used, as well as species and gender effects. In consequence, when considering the potential use of odours as pest repellents in field conditions, our results must be interpreted taking in account the characteristics of the experiment and the test apparatus, in which the small size allowed animals to explore and discard the presence of any predator. In field conditions, the “uncertainty” may last longer. On the other hand, odours are concentrated in the laboratory while in the field they diffuse more readily.

Fig 3. Mean frequency of Sniffing in the different treatments for rats.
https://doi.org/10.1371/journal.pone.0245441.g003
5. Conclusion

In conclusion, we found a potential aversive effect of TMT and cat body odour on rats, and cat body and cat urine in female mice. The effect of TMT was expressed in the number of visits and in the freezing behaviour that was only observed in rats in the TMT treatment. The use of cat body odour as a repellent in field conditions may be limited because it is not volatile and because animals may have an effect near or in contact with the source of odour [45]. Despite the fact that some authors [46,47] consider that TMT lacks some specific qualities of cat body/skin odour, this does not preclude the usefulness of its aversive effect for rodent control because it also has a detrimental effect on mouse reproduction [31]. We expect more effects on rats, which cause more damage in farm buildings than mice, including chicken mortality (Noriega com. pers.).

Our work provides evidence of the usefulness of using fear responses as a way of managing rodent pests, but, as pointed by Bedoya-Pérez et al [18], it is not easy, because anti predator responses are embedded in complex ecological systems and rely on complex contextual clues.

Appendix

Table A.2. Generalized linear mixed model (GLMM) results for the number of visits to the odour box per treatment for mice, considering the experiment as a random effect, with ZAP model (Zero-inflation Poisson distribution). Signif. codes: 0 ‘***’, 0.001 ‘**’, 0.01 ‘*’, 0.05 ‘.’.

| Explanatory variable | Estimate | SE | Z  | P-value   |
|----------------------|----------|----|----|-----------|
| Intercept (Control)  | 2.2772   | 0.1602 | 14.217 | <2e-16 *** |
| Cat Body             | -0.9671  | 0.2893 | -3.343 | 0.000830 *** |
| Cat urine            | -1.1627  | 0.3152 | -3.689 | 0.000225 *** |
| TMT                  | -0.9109  | 0.4018 | -2.267 | 0.023372 * |
| Sex: Male            | -0.4332  | 0.2286 | -1.895 | 0.058141 |
| Odour Cat Body:SexMale| 1.0680  | 0.4269 | 2.502 | 0.012348 * |
| OlorCat urine:SexMale| 0.6849  | 0.4653 | 1.472 | 0.141003 |
| OlorTMT:SexMale      | 1.4277   | 0.4549 | 3.139 | 0.001697 ** |

SE = Standard error. Z = parameter estimated. Total number of observations: 64. Df.resid = 50.

Table A.3. Tukey comparison for all possible pairs for the model in Table A.2. Signif. codes: 0 ‘***’, 0.001 ‘**’, 0.05 ‘.’.

| Contrast                           | Estimate | SE  | df | t.ratio | p.value   |
|------------------------------------|----------|-----|----|---------|-----------|
| Control Female—Cat body Female     | 0.9671   | 0.289 | 50 | 3.343   | 0.0314 *  |
| Control Female—Cat urine Female    | 1.1627   | 0.315 | 50 | 3.689   | 0.0120 *  |
| Control Female—TMT Female          | 0.9109   | 0.402 | 50 | 2.267   | 0.3315    |
| Control Female—Control Male        | 0.4332   | 0.229 | 50 | 1.895   | 0.5609    |
| Control Female—Cat body Male       | 0.3322   | 0.312 | 50 | 1.064   | 0.9613    |
| Control Female—Cat urine Male      | 0.9109   | 0.341 | 50 | 2.673   | 0.1553    |
| Control Female—TMT Male            | -0.0836  | 0.211 | 50 | -0.396  | 0.9999    |
| Cat body Female—Cat urine Female   | 0.1956   | 0.363 | 50 | 0.539   | 0.9994    |
| Cat body Female—TMT Female         | -0.0561  | 0.440 | 50 | -0.128  | 1.0000    |
| Cat body Female—Control Male       | -0.5339  | 0.291 | 50 | -1.835  | 0.6002    |
| Cat body Female—Cat body Male      | -0.6349  | 0.360 | 50 | -1.761  | 0.6482    |
| Cat body Female—Cat urine Male     | -0.0561  | 0.385 | 50 | -0.146  | 1.0000    |
| Cat body Female—TMT Male           | -1.0507  | 0.277 | 50 | -3.788  | 0.0090    |

(Continued)
### Table A.3. (Continued)

| Contrast                                    | Estimate | SE   | df | t.ratio | p.value  |
|---------------------------------------------|----------|------|----|---------|----------|
| Cat urine Female—TMT Female                | -0.2517  | 0.458| 50 | -0.550  | 0.9993   |
| Cat urine Female—Control Male              | -0.7295  | 0.317| 50 | -2.303  | 0.3123   |
| Cat urine Female—Cat body Male             | -0.8304  | 0.382| 50 | -2.177  | 0.3829   |
| Cat urine Female—Cat urine Male            | -0.2517  | 0.405| 50 | -0.621  | 0.9984   |
| Cat urine Female—TMT Male                  | -1.2463  | 0.304| 50 | -4.096  | 0.0036   |
| TMT Female—Control Male                    | -0.4778  | 0.403| 50 | -1.186  | 0.9323   |
| TMT Female—Cat body Male                   | -0.5787  | 0.456| 50 | -1.270  | 0.9055   |
| TMT Female—Cat urine Male                  | 0.0000   | 0.476| 50 | 0.000   | 1.0000   |
| TMT Female—TMT Male                        | -0.9946  | 0.393| 50 | -2.529  | 0.0273   |
| Control Male—Cat body Male                 | -0.1010  | 0.314| 50 | -0.322  | 1.0000   |
| Control Male—Cat urine Male                | 0.4778   | 0.342| 50 | 1.396   | 0.8548   |
| Control Male—TMT Male                      | -0.5168  | 0.213| 50 | -2.423  | 0.2531   |
| Cat body Male—Cat urine Male               | 0.5787   | 0.403| 50 | 1.436   | 0.8361   |
| Cat body Male—TMT Male                     | -0.4158  | 0.301| 50 | -1.380  | 0.8618   |
| Cat urine Male—TMT Male                    | -0.9946  | 0.331| 50 | -3.007  | 0.0733   |

### Table A.4. Generalized linear mixed model (GLMM) results for the number of visits to the odour box per treatment for rats, considering the experiment as a random effect, with Poisson distribution. Signif. codes: 0 ‘***’; 0.001 ‘**’; 0.01 ‘*’; 0.05 ‘.’.

| Explanatory variable       | Estimate       | SE   | Z    | P-value  |
|----------------------------|----------------|------|------|----------|
| (Intercept)                | 2.110e+00      | 1.231e-01| 17.143| < 2e-16 *** |
| Cat Body                   | -6.633e-01     | 2.111e-01| -3.142| 0.00168 ** |
| Cat urine                  | 6.281e-15      | 1.741e-01| 0.000 | 1.00000 |
| TMT                        | -1.194e+00     | 2.552e-01| -4.678| 2.9e-06 *** |
| Sex: Male                  | -5.521e-01     | 2.036e-01| -2.711| 0.00671 ** |
| Olor Cat Body: Sex Male    | -1.028e+00     | 4.623e-01| -2.224| 0.02612 *  |
| Olor Cat urine: Sex Male   | 2.336e-01      | 2.783e-01| 0.839 | 0.40123 |
| Olor TMT: Sex Male         | 8.522e-01      | 3.585e-01| 2.377 | 0.01744 *|

SE = Standard error. Z = parameter estimated. Total number of observations: 64. Df.resid = 55.

### Table A.5. Tukey comparison for all possible pairs for the model in Table A.4. Signif. codes: 0 ‘***’; 0.001 ‘**’; 0.05 ‘.’.

| Contrast                                    | Estimate | SE   | df | z.ratio | p.value  |
|---------------------------------------------|----------|------|----|---------|----------|
| Control Female—Cat Body Female              | 0.663    | 0.211| 55 | 3.142   | 0.0358   |
| Control Female—Cat urine Female             | 0.000    | 0.174| 55 | 0.000   | 1.0000   |
| Control Female—TMT Female                   | 1.194    | 0.255| 55 | 4.678   | 0.0001 **|
| Control Female—Control Male                 | 0.552    | 0.204| 55 | 2.711   | 0.1191   |
| Control Female—Cat body Male                | 2.244    | 0.398| 55 | 5.645   | < .0001  |
| Control Female—Cat urine Male               | 0.318    | 0.190| 55 | 1.679   | 0.7012   |
| Control Female—TMT Male                     | 0.894    | 0.228| 55 | 3.913   | 0.0023   |
| Cat body Female—Cat urine Female            | -0.663   | 0.211| 55 | -3.142  | 0.0358   |
| Cat fur Female—TMT Female                   | 0.531    | 0.282| 55 | 1.883   | 0.5627   |
| Cat body Female—Control Male                | -0.111   | 0.236| 55 | -0.471  | 0.9998   |
| Cat body Female—Cat body Male               | 1.580    | 0.415| 55 | 3.808   | 0.0035   |
| Cat body Female—Cat urine Male              | -0.345   | 0.224| 55 | -1.538  | 0.7866   |
| Cat body Female—TMT Male                    | 0.231    | 0.258| 55 | 0.894   | 0.9867   |

(Continued)
Table A.5. (Continued)

| Contrast                     | Estimate | SE  | df  | z.ratio | p.value |
|------------------------------|----------|-----|-----|---------|---------|
| Cat urine Female—TMT Female  | 1.194    | 0.255 | 55  | 4.678   | 0.0001  |
| Cat urine Female—Control Male| 0.552    | 0.204 | 55  | 2.711   | 0.1191  |
| Cat urine Female—Cat body Male| 2.244    | 0.398 | 55  | 5.645   | <.0001  |
| Cat urine Female—Cat urine Male| 0.318    | 0.190 | 55  | 1.679   | 0.7012  |
| Cat urine Female—TMT Male    | 0.552    | 0.204 | 55  | 2.711   | 0.1191  |
| TMT Female—Cat fur Male      | 1.050    | 0.439 | 55  | 2.391   | 0.2458  |
| TMT Female—Cat urine Male    | -0.875   | 0.266 | 55  | -3.289  | 0.0224  |
| TMT Female—TMT Male          | -0.300   | 0.295 | 55  | -1.017  | 0.9720  |
| Control Male—Cat body Male   | 1.692    | 0.411 | 55  | 4.113   | 0.0010  |
| Control Male—Cat urine Male  | -0.234   | 0.217 | 55  | -1.076  | 0.9619  |
| Control Male—TMT Male        | 0.342    | 0.252 | 55  | 1.358   | 0.8762  |
| Cat body Male—Cat urine Male | -1.925   | 0.405 | 55  | -4.759  | 0.0001  |
| Cat body Male—TMT Male       | -1.350   | 0.424 | 55  | -3.183  | 0.0316  |
| Cat urine Male—TMT Male      | 0.575    | 0.241 | 55  | 2.392   | 0.2452  |

Table A.6. GLMM models with different distributions for the variable duration in the odour box per treatment for mice. All models included the experiment as a random factor.

| Treatment | Individual (random factor) | Distribution | AIC      | df |
|-----------|----------------------------|--------------|----------|----|
| M0        | +                          | Binomial     | 608.841  | 9  |
| M1        |                           | Binomial     | 272.053  | 10 |
| M2 (Null) |                           | Binomial     | 265.440  | 3  |

Table A.7. GLMM models with different distributions for the variable duration in odour box per treatment for rats. All models included the experiment as a random factor.

| Treatment | Individual (random factor) | Distribution | AIC      | df |
|-----------|----------------------------|--------------|----------|----|
| M0        | +                          | Binomial     | 642.9638 | 9  |
| M1        |                           | Binomial     | 467.8241 | 10 |
| M1 Null   |                           | Binomial     | 463.2132 | 3  |

Supporting information
S1 Data.
(XLSX)

Acknowledgments
We want to thank Bernardo Rimoldi and Candela Encina for help in the experimental work and Lucía Babino for statistical advice. We also want to thank University Extension Secretary Ernesto Benavidez and the Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora (UNLZ), for providing experimental facilities.

Author Contributions
Conceptualization: Luciana B. Adduci, Vanina A. León, María Busch, Jimena Fraschina.
Formal analysis: Luciana B. Adduci, Vanina A. León, Annika Schlötelburg, Jimena Fraschina.

Funding acquisition: María Busch.

Investigation: Luciana B. Adduci, Vanina A. León, María Busch, Jimena Fraschina.

Methodology: Luciana B. Adduci, Vanina A. León, Annika Schlötelburg, María Busch, Jimena Fraschina.

Writing – original draft: Luciana B. Adduci, Vanina A. León, Annika Schlötelburg, María Busch, Jimena Fraschina.

Writing – review & editing: Luciana B. Adduci, Vanina A. León, Annika Schlötelburg, María Busch, Jimena Fraschina.

References
1. Macdonald DW, Fenn MGP, Gelling M. The natural history of rodents: preadaptations to pestilence. Rodent pests and their control. CAB International Oxon, UK; 1994. pp. 1–21.
2. León VA, Fraschina J, Busch M. Population subdivision of house mice (Mus musculus) in an agrarian landscape: consequences for control. Can J Zool. 2010; 88: 427–435.
3. Lovera R, Fernández MS, Jacob J, Lucerno N, Morici G, Brihuega B, et al. Intrinsic and extrinsic factors related to pathogen infection in wild small mammals in intensive milk cattle and swine production systems. PLoS Negl Trop Dis. 2017; 11: e0005722. https://doi.org/10.1371/journal.pntd.0005722 PMID: 28665952
4. Miño MH, Cavia R, Gómez Villafañe IE, Bilenca DN, Busch M. Seasonal abundance and distribution among habitats of small rodents on poultry farms. A contribution for their control. Int J Pest Manag. 2007; 12: 1–6.
5. Stenseth NC, Leirs H, Skonhoft A, Davis S, Pech RP, Andreassen HP, et al. Mice, rats and people: the bio-economics of agricultural rodent pests. Front Ecol Environ. 2003; 1: 367–375.
6. Timm RM. Commensal rodents in insulated livestock buildings. Control mammal pests/edited by CGJ Richards TY Ku. 1987.
7. Buckle AP, Smith RH. Rodent pests and their control. CAB International Wallingford, UK; 1994.
8. Brakes CR, Smith RH. Exposure of non-target small mammals to rodenticides: short-term effects, recovery and implications for secondary poisoning. J Appl Ecol. 2005; 42: 118–128.
9. Geduhn A, Jacob J, Schenke D, Keller B, Kleinschmidt S, Esther A. Relation between intensity of bio-icide practice and residues of anticoagulant rodenticides in red foxes (Vulpes vulpes). PLoS One. 2015;10.
10. Pelz H-J, Rost S, Hünenerberg M, Fregin A, Heilberg A-C, Baert K, et al. The genetic basis of resistance to anticoagulants in rodents. Genetics. 2005; 170: 1839–1847. https://doi.org/10.1534/genetics.104.040360 PMID: 15879509
11. Parsons MH, Apfelbach R, Banks PB, Cameron EZ, Dickman CR, Frank ASK, et al. Biologically meaningful scents: a framework for understanding predator—prey research across disciplines. Biol Rev. 2018; 93: 98–114. https://doi.org/10.1111/brv.12334 PMID: 28444848
12. Apfelbach R, Blanchard CD, Blanchard RJ, Hayes RA, McGregor IS. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. Neurosci Biobehav Rev. 2005; 29: 1123–1144. https://doi.org/10.1016/j.neubiorev.2005.05.005 PMID: 16085312
13. Feoktistova NY, Naidenko SV, Voznessenskaia AE, Krivomazov GJ, Clark L, Voznessenskaya V V. The influence of predator odours and overcrowded mouse odours on regulation of oestrous cycles in house mice (Mus musculus). In: Singleton GR, Hinds LA, Krebs CJ, Spratt DM, editors. Rats, mice and people: Rodent biology and management. AUSTRALIAN CENTRE FOR INTERNATIONAL AGRICULTURAL; 2003. pp. 173–175.
14. Voznessenskaya VV, Naidenko SV, Feoktistova NY, Krivomazov GJ, Miller LA, Clark L. Predator odours as reproductive inhibitors for Norway rats. USDA Natl Wildl Res Center-Staff Publ. 2003; 251.
15. Voznessenskaya V, Klinov A, Kvasha I. Responses to Domestic Cat Chemical Signals are Modulated by Early Olfactory Experience in the House Mouse. CHEMICAL SENSES. 2015. p. 283.
16. Hansen SC, Stoltzer C, Jacob J. The smell to repel: the effect of odors on the feeding behavior of female rodents. Crop Prot. 2015; 78: 270–276.
17. Schlötelburg A, Bellingrath-Kimura S, Jacob J. Development of an odorous repellent against common voles (Microtus arvalis) in laboratory screening and subsequent enclosure trials. J Pest Sci (2004). 2019; 92: 677–689.

18. Bedoya-Pérez MA, Smith KL, Kevin RC, Luo JL, Crowther MS, McGregor IS. Parameters that affect fear responses in rodents and how to use them for management. Front Ecol Evol. 2019; 7: 136.

19. Dielenberg RA, McGregor IS. Defensive behavior in rats towards predatory odors: a review. Neurosci Biobehav Rev. 2001; 25: 597–609. https://doi.org/10.1016/s0149-7634(01)00044-6 PMID: 11801285

20. Fendt M, Endres T, Lowry CA, Apfelbach R, McGregor IS. TMT-induced autonomic and behavioral changes and the neural basis of its processing. Neurosci Biobehav Rev. 2005; 29: 1145–1156. https://doi.org/10.1016/j.neubiorev.2005.04.018 PMID: 16099043

21. Staples LG, McGregor IS, Apfelbach R, Hunt GE. Cat odor, but not trimethylthiazoline (fox odor), activates accessory olfactory and defense-related brain regions in rats. Neuroscience. 2008; 151: 937–947. https://doi.org/10.1016/j.neuroscience.2007.11.039 PMID: 18201833

22. Bolbroe T, Jeppesen LL, Leirs H. Behavioral response of field voles under mustelid predation risk in the laboratory: more than neophobia. Annales Zoologici Fennici. 2000. pp. 169–178.

23. Horii Y, Nikaido Y, Nagai K, Nakashima T. Exposure to TMT odor affects adrenal sympathetic nerve activity and behavioral consequences in rats. Behav Brain Res. 2010; 214: 317–322. https://doi.org/10.1016/j.bbr.2010.05.047 PMID: 20595033

24. Osada K, Kurihara K, Izumi H, Kashiwayanagi M. Pyrazine analogues are active components of wolf urine that induce avoidance and freezing behaviours in mice. PLoS One. 2013; 8: e61753. https://doi.org/10.1371/journal.pone.0061753 PMID: 23639701

25. Bindra D, Spinner N. Response to different degrees of novelty: The incidence of various activities. J Exp Anal Behav. 1958; 1: 341. https://doi.org/10.1901/jeab.1958.1-341 PMID: 16811232

26. Spruijt BM, Van Hooff JA, Gispen WH. Ethology and neurobiology of grooming behavior. Physiol Rev. 1992; 72: 825–852. https://doi.org/10.1152/physrev.1992.72.3.825 PMID: 1320764

27. Gómez Villaña IE, Bilencia DN, Cavia R, Miño MH, Cittadino EA, Busch M. Environmental factors associated with rodent infestation in Argentine poultry farms. Br Poult Sci. 2001; 42: 300–307. https://doi.org/10.1080/00071660120055241 PMID: 11469547

28. Busch M, Burrone NE. Foraging activity of commensal Mus musculus in semi-captivity conditions. Effect of predator odours, previous experience and moonlight. Pest Manag Sci. 2015; 71: 1599–1604. https://doi.org/10.1002/ps.3962 PMID: 25492030

29. Guidobono JS, León V, Gómez Villaña IE, Busch M. Bromadiolone susceptibility in wild and laboratory Mus musculus L. (house mice) in Buenos Aires, Argentina. Pest Manag Sci Former Pestic Sci. 2010; 66: 162–167.

30. León VA, Frauschina J, Busch M. Bromadiolone susceptibility in Mus musculus (house mice) of Argentina. Int J Pest Manag. 2020; 66: 7–12.

31. Adduci LB, León VA, Busch M, Frauschina J. Effects of different odours on the reproductive success of Mus musculus as an alternative method of control. Pest Manag Sci. 2019; 75. https://doi.org/10.1002/ps.5498 PMID: 31140683

32. Blanchard DC, Griebel G, Blanchard RJ. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci Biobehav Rev. 2001; 25: 205–218. https://doi.org/10.1016/s0149-7634(01)00009-4 PMID: 11378177

33. Morrow BA, Redmond AJ, Roth RH, Elsworth JD. The predator odor, TMT, displays a unique, stress-like pattern of dopaminergic and endocrinological activation in the rat. Brain Res. 2000; 864: 146–151. https://doi.org/10.1016/s0006-8993(00)02174-0 PMID: 10793199

34. Williams JL, Rogers AG, Adler AP. Prolonged exposure to conspecific and predator odours reduces fear reactions to these odors during subsequent prod-shock tests. Anim Learn Behav. 1990; 18: 453–461.

35. Muñoz-Abellán C, Armario A, Nadal R. Do odors from different cats induce equivalent unconditioned and conditioned responses in rats? Physiol Behav. 2010; 99: 388–394. https://doi.org/10.1016/0031-9384(93)90333-3 PMID: 20006964

36. File SE, Zangrossi H Jr, Sanders FL, Mabbutt PS. Dissociation between behavioral and corticosterone responses on repeated exposures to cat odor. Physiol Behav. 1993; 54: 1109–1111. https://doi.org/10.1016/0031-9384(93)90333-3 PMID: 2895949

37. Blanchard DC, Griebel G, Blanchard RJ. Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion. Prog Neuro-Psychopharmacology Biol Psychiatry. 2003; 27: 1177–1185.

38. Zar JH. Biostatistical analysis. Pearson Education India; 1999.
39. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. 67: 48. arXiv Prepr arXiv14065823. 2015.
40. Bates D, Maechler M. Matrix: Sparse and Dense Matrix Classes and Methods. R package version 1.2–14. 2018.
41. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, et al. Modeling zero-inflated count data with glmmTMB. BioRxiv. 2017; 132753.
42. Lenth R. emmeans: Estimated Marginal Means, aka Least-Squares Means. 2020. Available: https://cran.r-project.org/package=emmeans.
43. Harrison XA. A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. PeerJ. 2015; 3: e1114. https://doi.org/10.7717/peerj.1114 PMID: 26244118
44. Vernet-Maury E, Polak EH, Demael A. Structure-activity relationship of stress-inducing odorants in the rat. J Chem Ecol. 1984; 10: 1007–1018. https://doi.org/10.1007/BF00987509 PMID: 24318845
45. Dielenberg RA, Hunt GE, McGregor IS. ‘When a rat smells a cat’: the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. Neuroscience. 2001; 104: 1085–1097. https://doi.org/10.1016/s0306-4522(01)00150-6 PMID: 11457592
46. McGregor IS, Schrama L, Ambermoon P, Dielenberg RA. Not all ‘predator odours’ are equal: cat odour but not 2, 4, 5 trimethylthiazoline (TMT; fox odour) elicits specific defensive behaviours in rats. Behav Brain Res. 2002; 129: 1–16. https://doi.org/10.1016/s0166-4328(01)00324-2 PMID: 11809490
47. Blanchard DC, Markham C, Yang M, Hubbard D, Madarang E, Blanchard RJ. Failure to produce conditioning with low-dose trimethylthiazoline or cat feces as unconditioned stimuli. Behav Neurosci. 2003; 117: 360. https://doi.org/10.1037/0735-7044.117.2.360 PMID: 12708532