Defensive responses of Brandt's voles (Lasiopodomys brandtii) to stored cat feces

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HIGHLIGHTS

• Fresh cat feces induce highest behavioral, physiological, c-fos mRNA responses in Brandt's voles.
• Behavioral, endocrine and molecular responses are concurrent.
• Waning of all defensive responses happened with old predator feces.

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ABSTRACT

Predator odors are non-intrusive natural stressors of high ethological relevance. Animals are daily challenged with stressors of varying intensity and it is essential for their survival to respond to a wide range of threats. Behavioral and hormonal responses and changes in the level of medial hypothalamic c-fos mRNA were examined in Brandt's voles (Lasiopodomys brandtii) exposed to the feces of a domestic cat (Felis catus) stored for different periods. One hundred voles were tested in the defensive withdrawal apparatus. The voles showed an aversion to freshly collected cat feces, indicated by high levels of flight-related behaviors, increased freezing behavior, and more vigilant rearing compared to old feces. The serum levels of adrenocorticotropic hormone and corticosterone significantly increased when the voles were exposed to fresh cat feces. The level of c-fos mRNA in the medial hypothalamic region was highest in the individuals exposed to fresh cat feces. All of these behavioral, endocrine and c-fos-mRNA responses were lower when voles were subjected to older cat feces. We conclude that these responses depend on volatile chemical constituents of cat feces rather than their physical characteristics and that this accounts for the lower responses to feces stored for longer periods.

1. Introduction

Predation is a strong selective force in the evolution of prey species [45]. Most animals must deal with predators during their lifetime and failure in this task may lead to their death. Consequently, prey species have developed adaptations at several levels (e.g., morphological, behavioral, physiological, molecular) to decrease the risk of being preyed upon [28]. A large number of field and laboratory studies have highlighted anti-predator defensive responses, including changes in behavior [35,42], hormone levels [28,40], and neuronal activation of specific regions of the brain [34]. Although anti-predator strategies are essential for survival, they may be costly. Cessation of regular feeding activities and increased investment in defensive strategies reduce energy income and may also interfere with mating behavior [33]. Hence, animals would be expected to modulate their anti-predator responses according to the perceived risk of predation [16,18].

In predator–prey interactions, early perception is the key step for prey animals to avoid being preyed on. The information that the prey animals are able to acquire from predators varies qualitatively and quantitatively with the type of cues presented. Therefore, it is important to know which stimuli or cues are better perceived by the prey animals: visual, auditory or chemical [15]. It has already been shown that chemical cues are of superior importance to others because they can provide comprehensive information to the prey regarding predation risk [22]. In particular, the strength of the chemical cue may provide information on the distance from the predator, the number of predators nearby [18], or what the predator has recently been feeding on [27].

The idea that prey animals assess and respond flexibly to different degrees of predation threat is known as the threat-sensitive predator avoidance hypothesis [16]. It predicts that prey animals use predator cues to evaluate danger and respond in a manner appropriate to the level of threat. This type of strategy minimizes the costs of mistakenly
responding to predator activities and avoids interfering with foraging opportunities, territorial defense, or mate search when there is actually no risk [10]. Several studies have examined the threat-sensitive predator avoidance hypothesis by changing the concentrations of predator odors in aquatic organisms [17,41,26]. Brandt’s vole (Lasiopodomys brandtii), a typical herbivorous rodent, mainly inhabits the grasslands of the Inner Mongolia Autonomous Region, China, as well as Mongolia and the Baikal region of Russia. The voles live in social groups and dig complex burrow systems with densities as high as 5616 holes/ha [48]. To test the threat-sensitive predator avoidance hypothesis in rodents, we used a novel approach in this study that mimicked a naturalistic situation when an animal is confronted with old feces of a predator. Brandt’s voles were subjected to cat feces stored for different periods, and then we measured the changes of behavior, endocrine and c-fos gene expression. We hypothesized that the strongest anti-predator responses would occur at these three levels when the voles were confronted with fresh feces and that they would exhibit flexible graded responses with increased storage time. The data presented here expand our understanding for the ethology of predator odors and highlight their potential usefulness in damage control of the Brandt’s voles.

2. Material and methods

2.1. Animals

Our experiments were conducted in the Laboratory of Animal Behavior, College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, Jiangsu, China, between September and December 2012. The wild Brandt’s voles were trapped using live-capture cage traps (YZ-LA: Shanghai Sinokil Environmental Service Co., Ltd., Shanghai, China) in the grassland of Inner Mongolia, transported to Laboratory of Animal Behavior, Yangzhou, and housed in plastic-bottomed wire cages (15 × 22 × 18 cm) in male/female pairs. The individuals of first generation (G1) were weaned on the 21st day after birth and housed in a large cage (45 × 30 × 20 cm) with 12 same gender individuals in each cage. Then two or three same-gender individuals were housed in small cage (15 × 22 × 18 cm) when they were at the age of 60 days. Animals were provided with water and food ad libitum with commercial diet supplied by Science and Technology Co. Ltd. Anritsu, Nanjing, Jiangsu, China. Wood shavings were used as bedding materials and changed every two weeks. Cages were washed every four weeks. Animals were kept on a constant 16L:8D light cycle (light on 6:00 am) at 21–23 °C. 50 male and 50 female individuals of first generations (three-month-old, 40–70 g weight) were used in this study.

2.2. Odor and animal grouping

Feces of a one-year-old domestic male cat (Felis catus) were collected and used for the experiment. The cat was captured on the Wenhu campus of Yangzhou University and housed in a wire cage (120 × 40 × 30 cm high) with a wire-mesh bottom and provided with water and food ad libitum. The cat was kept on a meat diet during the whole period of feces collection. The cat cage was monitored every 2 h and as soon as feces were found they were collected and immediately stored at −70 °C. The 100 voles were randomly allocated to 5 groups of 20 (10 males and 10 females in each). The five groups are referred to as: the Control group (Distilled water group); the First Day group, the Second Day group, the Fourth Day group, and the Eighth Day group (see Procedures section below).

2.3. Testing apparatus

The testing apparatus (75 cm × 37 cm × 40 cm) is shown in Fig. 1. An opaque Plexiglas® wall divided the box into two compartments. The ‘testing arena’ consisted of a rectangular area (60 cm × 37 cm × 40 cm) divided into 12 smaller areas marked with black lines. The second compartment, termed the ‘hide box’, was constructed from black Plexiglas®. A small square hole (6 cm × 6 cm) in the front wall of the hide box was just wide enough for the tested vole to enter the arena. The testing apparatus was mounted with two video cameras; one was situated on a tripod directly above the center of the apparatus and the other on the side wall of the testing arena for better recording and scoring of behaviors. Voles were transferred from the housing room to the testing room by a familiar person. A computer was connected to the cameras and located outside the testing room for live viewing and recording the session.

2.4. Procedures

All procedures were conducted between 9:00 am and 2:00 pm and all voles were handled identically for five days prior to the start of the experiment. Handling included weighing voles, holding them for 1 min, releasing them into cages, and then transporting them to the room in which testing was to be carried out without placing them in the test apparatus. Voles were always handled with protective rubber gloves and metal forceps. The test procedure was divided into two phases: familiarization and testing sessions. At the start of the experiment, each vole received a familiarization session on two consecutive days before the experiment, during which they were placed in a test apparatus for 10 min with no odor present. Voles in the Control group were tested for 10 min with distilled water. Collected cat feces were

![Fig. 1. Defensive withdrawal apparatus.](image-url)
then taken out from −70 °C and left to defrost. One gram of feces was weighed and placed on a clean filter paper and immediately presented for 10 min to each animal in turn in the First Day group. After that, filter papers carrying feces were taken from the testing room to an adjacent storage room, where the temperature and conditions were identical to those in the testing room (21–23 °C). The feces were stored for 24 h and then re-presented to the Second Day group. After this exposure, the feces were again collected and stored for 48 h and offered to the Fourth Day group. Then the feces were stored for a further 96 h and finally used to test the Eighth Day group. Extra feces were prepared on filter papers on the first day and then the filter paper was shredded or the feces were removed by the voles, the odor source was replaced by another piece that had been left the same number of days as the original. After each animal test, the apparatus was swabbed using 5% alcohol.

2.5. Measurements of behavioral responses

The following behavioral responses were considered in this experiment: 1) Avoidance: the vole spends most of the time in the square in front of the hide box [8]; 2) Jumping: the vole stands on its rear legs, raises the forelimbs and then jumps; 3) Contact: the vole makes direct tactile contact with the stimulus, including chewing [8]; 4) Concealing: the vole retreats into the hide box [45]; 5) Freezing: the vole is immobile except for respiration with body in laying posture and all limbs supporting body weight [2]; 6) Locomotion: any movement from one marked section to another, commenced by line crossing [8]; 7) Head out: the vole has its head or both the head and shoulders outside the entrance of the hide box with most of its body concealed inside the hide box [8]; and 8) Vigilant rearing: the vole stands on its rear legs with forelimbs raised (i.e., not placed on anything for support; [8]). The durations of all of these behaviors were recorded in seconds.

2.6. Measurement of ACTH and CORT

At the end of the 10-min exposures, 12 voles from each group (6 males and 6 females) were removed from the testing box and quickly euthanized by decapitation, taking great care to avoid imposing stress on the voles. The bleeding procedure was completed within 3 min. Blood was collected, left to clot, and centrifuged to obtain serum that was subsequently stored at −70 °C until use. We used the serum to measure the level of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) the major glucocorticoid in voles [46]. The serum concentrations of ACTH and CORT were measured using a vole-specific enzyme-linked immunosorbent assay kit (R&D Systems China Co. Ltd., Shanghai, China) following manufacturer's procedures. These kits had been previously validated for Brandt's voles [37]. The prepared samples and the standards were placed in separate plate wells and incubated for 1 h at 37 °C. The plate was then washed three times with wash solution, horseradish peroxidase conjugated reagent was added, and the solution was incubated for 1 h at 37 °C. The plate was washed three more times and Chromogen solutions A and B were added. After 30 min of incubation at 37 °C, the reaction was stopped using a stop solution. The optical density of the samples was determined at 450 nm using a Metertech microplate reader (BioTek Instruments, Winooski, VT, USA), after zeroing on a blank well. The sensitivities of the analyses were 1.0 pg/ml for ACTH and 1 ng/ml for CORT. The intra- and inter-assay coefficients of variation for ACTH were 5.5% and 4.6%, respectively, and those for CORT were 5.0% and 7.2%, respectively. All samples were tested in duplicate.

2.7. Real-time polymerase chain reaction (RT-PCR) assay of medial hypothalamic gene expression of c-fos

After blood collection, skulls of Brandt’s voles were rapidly opened and brains were dissected to extract the hypothalamus. Two coronal cuts were made, the first just behind the optic chiasma and the second 1 mm in front of the mammillary bodies. The hypothalamus was removed by a horizontal cut passing through the peri-hypothalamic sulcus (a groove in the lateral wall of the third ventricle marking the boundary between the thalamus and hypothalamus) and two horizontal cuts separating the hypothalamus from the cortex. After dissecting out the hypothalamus, incisions were made on both sides of the excited portion and the major parts of the lateral hypothalamic regions were then removed, retaining most of the medial hypothalamic region, which is principally located on either side of the third ventricle. The lateral hypothalamic region plays an essential role in ingestion behavior, which was not considered important for this investigation, while the medial hypothalamic region is critically involved in the expression of anti-predator defensive responses [5]. Total RNA was extracted using TRIZOL according to the manufacturer’s instructions (RNAiso Plus, Takara Biotechnology Co., Ltd., Dalian China). RNA was eluted in nuclease-free water and was subject to DNase treatment to remove genomic DNA (Recombinant DNase II, Takara). Following DNase treatment, RNA was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE, USA). All samples had A260/A280 values greater than 1.8, indicating high quality RNA. Samples were stored at −70 °C until reverse transcription was performed. We reverse-transcribed 3 μg of RNA per sample using PrimeScript 1st strand (Takara) following the manufacturer’s instructions. The resulting cDNA was stored at −20 °C until analysis.

c-fos sequences of mouse (Mus musculus), rat (Rattus norvegicus), and Chinese hamster (Cricetulus griseus) were aligned using Lasergene software (DNASTAR, Inc., Madison, WI USA) to pick up degenerate primers from only the conserved regions among the three sequences. c-fos cDNA was amplified by PCR using Taq DNA Polymerase® (Takara). PCR solutions were prepared (2.5 μl 10× PCR Buffer, 2 μl dNTP, 0.4 μl Taq, 0.5 μl each of primers, and 2 μl of cDNA in a final volume of 25 μl) following the manufacturer’s instructions. Cycling conditions were: initial denaturation at 95 °C for 5 min, 35 cycles at 95 °C for 30 s; 57 °C for 30 s; 72 °C for 30 s, and final extension at 72 °C for 5 min. The amplified product was 497 bp in length (Table 1). PCR products of the predicted size were extracted from the agarose gel and cloned into pCR4-TOPO vectors according to manufacturer’s directions (Tiangen, Beijing, China). Recombinant plasmids from isolated colonies were extracted from overnight liquid cultures and sequenced using a commercial sequencing service (GenScript, Nanjing, China). In the next step, a species-specific primer set for the c-fos gene was designed based on the cloned gene sequence using Primer Premier 5th software (Premier Biosoft, Palo Alto, CA, USA).

The cDNA was subjected to real-time PCR amplification using the Syber® premix EX Taq II (Takara) in the 7500 Real-Time PCR System (Applied Biosystems China Ltd., Shanghai, China). Real-time PCR (qPCR) was conducted in 20 μl of reaction mixture composed of 10 μl SYBR Premix EX II, 2 μl cDNA templates and 0.2 μl of each primer, made up of 20 μl water. Each sample was analyzed in triplicate. Thermal cycling conditions were: 50 °C for 2 min, 95 °C for 30 s, 40 cycles at 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 30 s. Melting curve analysis showed a single PCR product after amplification of c-fos and β-actin. We made a standard curve for each gene with five-fold serial dilutions of cDNA. Analysis of the standard curves of the target gene and β-actin [7] showed that they had similar amplification efficiency, which ensures the validity of comparative quantitative method. No amplification was detected in the absence of template or in the no-RT control. The fold change for gene expression was calculated using the relative quantification method (2−ΔΔCt) with β-actin as an endogenous control. The average ΔCt for the control group was used as a calibrator for each sample. ΔCt normalized target = c-fos Ct − β-actin Ct; ΔΔCt = ΔCt normalized target − ΔCt normalized calibrator; from which the n-fold (2−ΔΔCt) was calculated. Therefore, the n-fold value represented the hypothalamic gene expression for each sample in each vole relative to the samples of the control group, normalized to the endogenous control β-actin [36]. No amplification was detected in the absence of template or in the no-RT control.
Table 1  
Nucleotide sequences of primers and cycling conditions used for PCR and qPCR amplification.

| Gene       | PCR type | Forward primer                           | Reverse primer                           | Product size |
|------------|----------|------------------------------------------|------------------------------------------|--------------|
| c-fos      | Traditional | 5-CCATAATTGACCCACACAGA-3 | 5-CCTCCTGCTACCTGTA-3 | 497          |
| c-fos      | qPCR     | 5-TACGGAAACCGGAAACCCTCG-3 | 5-TGACTCCTTCCCTACTGTC-3 | 208          |
| β-Actin    | qPCR     | 5-TGTGCGGCATGACAAAGAG-3 | 5-ATGCCAGAAATGTCATCCACC-3 | 200          |

All procedures were approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Yangzhou University.

2.8. Data analysis

Focal sampling and continuous recording techniques were used to score the behavior of the tested subjects for the 10 min recording session [31]. Each testing session was analyzed using The Observer XT 7.0 analysis software (Noldus Information Technology, Wageningen, The Netherlands) for the behavioral parameters. For molecular changes, statistical analyses were performed on the ΔCt values, processed into ΔΔCt, and converted to n-fold ($2^{-\Delta\Delta Ct}$) for data presentation. SPSS 16.00 software was used for all analyses. Two-way analysis of variance was used with data sets that met the assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test). The behavioral, physiological and gene expression variables were log-transformed, if necessary. Values are presented as means ± SE. Fecal age and sex were used as fixed factors. The behavioral, physiological and gene expression variables were used as dependent variables. The post hoc least significant difference (LSD) test was used to identify specific differences among the treatment groups. The level of significance at which the null hypothesis was rejected was $\alpha = 0.05$.

3. Results

3.1. Behavioral responses upon exposure to cat feces of different storage periods

Our analysis showed that neither sex (except for locomotion) nor (Period × Sex) interaction had significant effects on any of the measured parameters. Therefore, data from males and females were combined.

The first day group showed strong defensive behavioral responses toward the freshly collected cat feces (Fig. 2). During the 10 min odor presentation, animals tested with freshly collected feces (First day group) showed significant flight-related behaviors (avoidance $F_{4,91} = 8.014$, $P = 0.001$, jumping $F_{4,91} = 3.042$, $P = 0.022$, contact time $F_{4,91} = 3.029$, $P = 0.022$, and concealing $F_{4,91} = 3.709$, $P = 0.008$). Freezing behavior was significantly suppressed when the used feces became older over time ($F_{4,91} = 7.604$, $P = 0.001$). A non-significant locomotion behavior ($F_{4,91} = 1.688$, $P = 0.161$) occurred with a significant sex effect ($F_{1,91} = 5.972$, $P = 0.05$) that males showed longer boots of locomotion than females did ($128.93 \pm 9.838$ and $99.80 \pm 8.214$ respectively). Head out behavior showed a non-significant difference ($F_{4,91} = 0.288$, $P = 0.885$) between groups. The first day group displayed the highest duration of vigilance rearing ($F_{4,91} = 5.634$, $P = 0.001$) compared to other treatment groups.

3.2. Hormonal responses upon exposure to cat feces of different storage periods

The serum levels of ACTH and corticosterone significantly increased when animals were exposed to freshly collected feces ($F_{4,91} = 16.513$, $P = 0.001$ and $F_{4,91} = 19.151$, $P = 0.001$, respectively). The first day group showed a distinctive higher endocrine responses than others which subsequently decreased when the stored feces came to be older (Fig. 3).

3.3. c-fos mRNA expression level upon exposure to cat feces of different storage periods

The specificity of the primers and the absence of non-specific products and primer dimers were tested by analyzing the reactions in a 1% agarose gel stained with ethidium bromide. Only the expected amplification bands were shown in the electrophoresis image and no other bands were visible. The specificity of the primers was also tested using a melting-curve analysis. Amplified DNA segment showed consistent peak Tm values (Fig. 4). Two-way ANOVA and post hoc test disclosed that exposure to freshly collected cat feces significantly increased c-fos mRNA expression in the medial hypothalamic region of Brandt’s voles ($F_{4,80} = 46.126$, $P = 0.001$). The level of c-fos mRNA declined upon exposure to old cat feces (Fig. 5).

4. Discussion

This study was conducted to emulate a natural situation under laboratory conditions by exposing Brandt’s voles to either fresh cat feces or to old feces that had been stored for differing periods. Freshness of the feces was considered a critical factor and the ability of voles to discriminate between them may be important for their safety. For example, old feces may mean that a predator was in the area but has since left, or is too far away to make a successful strike, and hence poses no real risk [27].

Our results clearly demonstrated that Brandt’s voles showed distinctive behavioral and hormonal responses and changes in the CNS of c-fos expression upon exposure to freshly collected cat feces. These defensive responses suggest that cat feces may contain aversive chemo-signals which constitute a generalized meat-eater cue [11] or a predator “leitmotif” [44,38]. Although the chemical nature of this leitmotif remains obscure, one possibility is that it features odoriferous constituents that reflect the dietary composition of the predator. Such odors might include sulfur-containing metabolites of protein digestion [24,30,1] that are present in very low quantities in the feces of carnivores [47]. Once the cat has deposited the feces, they may undergo changes, including evaporation of the sulfur-containing metabolites [39], which in turn may terminate the interspecific communication between the cat and voles.

A positive correlation between the strength of anti-predator responses and that of the chemical cue received has been documented in many studies and termed signal detection theory [13,10]. The theory predicts that a prey evaluates a signal, such as a predator chemical cue, against a predetermined threshold level. When the signal reaches the threshold, it is positively identified and the subject responds accordingly. Likewise, in our study, the level and concentration of the chemical signals in fresh feces were appropriate to induce a robust defensive response in Brandt’s voles. Later, these responses diminished possibly because the level of the sulfur-containing metabolites decreased below the threshold by evaporation. Our results are also consistent with the threat-sensitive predator avoidance hypothesis [16]. The old cat feces may be perceived as a low predation risk that does not deserve investment in energy-consuming defensive displays or responses. Conversely, fresh cat feces imply a high predation risk and thus the voles respond differently with anti-predator strategies. The signal detection theory and threat-sensitive predator avoidance hypothesis are not necessarily mutually exclusive. According to the first scheme, if the stimulus reaches the predetermined threshold, the animal will respond in an all-or-nothing manner. However, both theories are complementary to each other. Animals may first detect the level
Fig. 2. Behavioral responses of Brandt's voles after a 10-min exposure to cat feces stored for different time. Different letter labels over the bars indicate significant differences ($P < 0.05$) between the groups.
were signiﬁcant differences (P < 0.05) between the groups.

\[\text{c-fos mRNA expression levels in animals exposed to fresh cat feces were significantly higher than in the other groups.} \]

A strong endocrine response of Brandt’s voles to fresh cat feces was illustrated by the increase in the levels of serum ACTH and CORT, which subsequently decreased as storage time increases. ACTH and CORT are routinely used as indicators of HPA activation and they are important components of the stress response [23]. ACTH is a polypeptide tropic hormone secreted by and released from the anterior pituitary gland. It is produced in response to a variety of stressors [6]. Under stress, circulating ACTH is a key regulator of corticosterone secretion by the adrenal cortex [14]. Corticosterone participates in the control of whole body homeostasis and the organism’s response to stress through energy mobilization, which in turn is used in the display of behavioral responses [29]. It also plays a key regulatory role in the basal activity of the HPA and in the termination of the stress response [42]. Observations consistent with this scheme have also been reported in other rodents species exposed to predator odors [32,20,28].

We propose that activation of the medial hypothalamic zone triggers a series of neuronal stimulations that lead to display of various defensive behaviors directly and/or indirectly through activation of HPA. That endocrine status and behavioral outputs are strongly linked as shown by observations that the corticosterone level measured in the blood of rats is correlated with the strength of the behavioral signs of fear in response to a predator cue [43]. The link between the medial hypothalamic and stress hormones was also demonstrated by Filaretov and Rakitskaya [12], who showed that destruction of the medial hypothalamus induced a fall in the corticosterone level in experimental animals. Furthermore, subjecting rats to bilateral ibotenic acid lesions in the dorsal premammillary nucleus in the medial hypothalamic zone conspicuously eliminated the expression of speciﬁc defensive behavioral responses during the predatory encounter [4]. Voles displayed ﬂight-related behaviors (avoidance, jumping, concealing, and reduced contact) toward fresh cat feces, which progressively waned as the feces aged. These behaviors would tend to keep the vole away from the source of danger [25] and would avoid potential predator–prey interactions. Freezing behavior was previously shown to be highest in voles exposed to weasel and cat feces. This is a general defensive response adapted to minimize the chance of being detected by the predator.

\[\text{Fig. 3. Mean serum concentrations of ACTH (pg/ml ± SE) and corticosterone (ng/ml ± SE) in Brandt’s voles following a 10-min exposure to cat feces stored for different times. Different letter labels over the bars indicate significant differences (P < 0.05) between the groups.} \]

\[\text{Fig. 4. Melt Curve} \]

\[\text{Fig. 5. Effect of exposure to cat feces stored for different periods on the expression of c-fos mRNA in the hypothalamus of Brandt’s voles. Different letter labels over the bars indicate significant differences (P < 0.05) between the groups.} \]
In our study we did not observe any changes in locomotion activity patterns between the different groups of voles. Likewise, a parallel study conducted on free ranging European rabbits (Oryctolagus cuniculus) revealed that locomotion did not decrease in response to predator feces [28]. In the wild, rabbits build a complex burrow system providing shelter against most predator attacks. In our study, the hide box represented a safe shelter corresponding to the burrow, which might make reduction in locomotion unnecessary. Head out and vigilant rearing are considered risk-assessment behaviors and serve primarily to scan the environment and gather information [9,3]. We observed that Brandt’s voles generally increased their vigilant rearing when fresh cat feces were presented. However, head out behavior did not change significantly between groups. We assume that both head out and vigilant rearing are adapted to the collection of information about the surrounding environment. However, it appears that when additional information must be collected in the absence of direct physical contact with the predator, vigilant rearing is more frequently performed than head out behavior.

5. Conclusion

Overall, our study showed that Brandt’s voles displayed strong defensive reactions on different levels (behavioral, hormonal and neurobiological) toward fresh cat feces. Interestingly, these reactions subsequently faded with the storage time prolonged. We therefore conclude that the defensive behavioral, endocrine and molecular responses are odor-threshold dependent and are linked to chemical constituents in cat feces which evaporate over time. Obviously, the neuronal, endocrine and behavioral responses to predator stress are closely integrated and deserve further investigation.

Acknowledgments

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