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Maternal-Neonatal Pheromone/Interomone Added to Cat Litter Improves Litter Box Use and Reduces Aggression in Pair-Housed Cats

John J. McGlone[a], Arlene Garcia[a], William G. Thompson[b], and Glenna M. Pirner[a]

[a]Department of Animal and Food Sciences, Texas Tech University, Lubbock, Texas, USA; [b]Operations, Animal Biotech, Dallas, Texas, USA

ABSTRACT

Introducing a new cat into a household with one or more resident cats can be a significant source of stress for the cats involved. These studies sought to determine if rabbit maternal-neonatal pheromone (2-methyl-2-butenal [2M2B]) in litter impacted cat social behaviors and litter box use. Study 1 determined that cats preferred to eliminate in litter containing 2M2B; other semiochemicals tested did not change litter box use. Cats prone to aggression were identified in an intermediate pilot study, and eight pairs of these cats were selected for Study 2. In Study 2, cat pairs were provided litter containing either vehicle or 2M2B for 24 hours. Cats experiencing control litter displayed more aggression during the first 6 hours ($p < .01$) and spent more time using the litter box 12 hours and 18 hours after pairing compared with cats experiencing litter with 2M2B ($p = .02$). These results suggest 2M2B-infused cat litter may act as an interomone in cats housed domestically to prevent initial occurrences of aggression and may improve cat welfare in multicat households.

KEYWORDS
Behavior; feline; interomone; pheromone; stress

Introduction

More than 36 million homes in the United States have cats, with most having two or more cats (American Veterinary Medical Association, 2012). Introduction of a new cat into a household with a resident cat can result in significant stress for the cats. A survey of caregivers (owners) with two or more cats revealed that if aggression occurred upon introduction of a new cat into a household with a resident cat, fighting was more likely to occur during the subsequent 12-month period compared with cats of owners who did not observe aggression upon initial introduction (Levine, Perry, Scarlett, & Houpt, 2005). Problems between new and resident cats, aggression toward other nonhuman animals, and being fearful cumulatively accounted for approximately 35% of the reasons cats were relinquished to animal shelters (Salman et al., 2000). Intercat aggression was the underlying cause for 42% of feline behavior cases presented to the University of Georgia Veterinary Behavior Service during a three-year period (Amat et al., 2008). Intercat aggression is a welfare concern not only on a physical level, but also on a psychological level. Currently, few studies have examined the physiological and psychological effects of social stress in cats. Stella, Croney, and Buffington (2012) found that healthy cats exposed to a variety of environmental stressors showed an increase in sickness behaviors such as vomiting, diarrhea, and inappropriate elimination.

Inappropriate elimination is a concern in multicat households. House soiling is the leading single-behavior cause of cats being relinquished to animal shelters (Salman et al., 2000). Inappropriate elimination use in cats housed indoors may be caused by medical or behavioral issues; this complex

issue is known as Pandora syndrome (Buffington, Westropp, & Chew, 2014; Hart, Eckstein, Powell, & Dodman, 1993). Stressed or anxious cats may urine-mark to increase their own comfort level; on the other hand, confident cats may mark to establish territory and announce their presence (Carney et al., 2014; Neilson, 2004). Although communication between cats does occur by vocalization and body language, olfactory communication is important when cats are not in immediate spatial or temporal contact (Fox, 1975).

Olfactory communication is facilitated via semiochemicals, molecules that convey information within and between species. Pheromones are molecules produced by an individual of a species and are then received by a second individual of the same species in whom the pheromones elicit a specific physiological and/or behavioral response (Karlson & Luscher, 1959). Conservation of metabolic processes responsible for production of semiochemicals as well as conservation of olfactory receptor gene families across species increase the likelihood that chemical signals may be interpreted across species; these molecules are termed interomones (Ache & Young, 2005; McGlone, 2011). The first pheromone shown to have an interomone effect was the pig sexual pheromone, androstenone (AN). When applied in spray form, AN reduced barking in dogs housed domestically (McGlone, Thompson, & Guay, 2014). The rabbit maternal-neonatal pheromone 2-methyl-2-butenal (2M2B) is secreted by rabbit dams to calm and attract pups to the nipple for efficient nursing (Coureaud, Langlois, Sicard, & Schaal, 2003; Schaal et al., 2003). The conservation of molecules and receptor pathways as well as the potent calming effects of 2M2B make it an attractive candidate for reducing anxiety in other species.

Limited pharmaceutical treatment options are available for multicat households experiencing intercat aggression or inappropriate elimination (Crowell-Davis, Curtis, & Knowles, 2004). Medications such as amitriptyline and buspirone have variable success rates and may have undesirable side effects such as sedation or hepatotoxicity (Lindell, Erb, & Houpt, 1997; Overall, 1992). Feliway[TM], a derivative of Feline Facial Pheromone (Ceva Animal Health, Lenexa, KS), has been shown to reduce urine marking, aggression, and fear in domestic cats when administered in spray and diffuser forms (Gunn-Moore & Cameron, 2004; Mills & Mills, 2001; Ogata & Takeuchi, 2001). However, these studies have had variable success rates and sprays require daily reapplication (Gunn-Moore & Cameron, 2004; Mills, Redgate, & Landsberg, 2011). Additionally, Feliway[TM] increased urine spraying in some multicat households; therefore, investigation of more effective pheromonal treatments is warranted (Mills et al., 2011).

The purpose of this study was to determine if 2M2B acts as an interomone in domestic cats and, if so, to determine if it reduces aggression, anxiety-like behavior, and inappropriate elimination in pair-housed cats. Pheromone detection in cats and other species takes place in the vomeronasal organ (VNO) and the main olfactory epithelium (MOE). During investigation of a new scent, particularly a pheromone odor, a cat will typically sniff, thereby drawing odors over the MOE, and then open its mouth slightly in what is known as the Flehmen reaction/response, facilitating passage of odor molecules into the VNO (Estes, 1972). Prior to using the litter box, many cats will show sniffing behaviors; thus, by adding pheromones into the litter, the cats will be readily exposed to the odor. We hypothesized that the inclusion of 2M2B (or other semiochemicals) in cat litter may reduce aggression and anxiety-like behaviors and increase the frequency of litter box use during the first 24 hours after introduction. This hypothesis was based on research showing the calming effects elicited by 2M2B in rabbits and dogs, combined with knowledge of the conservation of olfactory receptor genes among mammals and common metabolic pathways (Ache & Young, 2005; Coureaud et al., 2003).

Methods

General

All research was conducted after approval from the Texas Tech University Institutional Animal Care and Use Committee (IACUC) and the IACUC of the contract research facility (Protocol #14,009–01). Research was conducted at a contract research facility. Space, management, and care

of cats were consistent with the US Animal Welfare Act. All animal rooms were provided with 100% fresh air intake and exhaust with 10 to 15 exchanges per hour. Treatments were tested in separate rooms to prevent cross-contamination. Each kennel received food once daily and water was available ad libitum. Kennels were cleaned by research facility personnel every 24 hours with a commercial-grade kennel disinfectant. Litter boxes were plastic, open boxes measuring 57.2 cm in length × 42.4 cm in width × 17.0 cm in height. Research facility personnel and Texas Tech University research assistants were blind to treatments.

Study 1: Preference study

The objective of this study was to determine the relative preference or aversion of cats to various semiochemicals in litter compared to a control litter.

Litter

A clumping litter derived from dried, processed corn cobs was used. Because cats were accustomed to a clumping clay litter, the corn cob litter was designed such that particle size was similar. Four treatment litters were compared to a control litter (CON) to determine cat preference. All pheromone and interomone formulas were prepared by dilution with isopropyl alcohol prior to mixing with litter. Isopropyl alcohol was chosen as the diluent based on solubility of the molecules. The control litter was mixed with isopropyl alcohol at 100 mL per 136.4 kg of litter. Androstenone or 2M2B was mixed into litter at 10 µg/kg of litter each. The cat urinary semiochemical (Miyazaki et al., 2006) 3-Mercapto-3-methylbutan-1-ol (3M3M) was mixed into litter at 50 µg/kg of litter. 3-Mercapto-3-methylbutan-1-ol and 2M2B were mixed into litter at 50 µg 3M3M + 10 µg 2M2B/kg of litter. These concentrations were selected based on pilot studies conducted in this laboratory when examining doses of test molecules, and they are similar to those used in commercial products currently marketed. Litters were separated into 1.36 kg aliquots and were labeled as A, B, C, or D before being sent to the research facility.

Animals

Forty randomly selected intact male and intact female cats aged one year to three years old were used in this study. Cats were of mixed genetics and weighed 4.0 ± 0.2 kg. Cats were group-housed in their home kennels prior to being enrolled in this study.

Procedure

Cats were moved from their home kennels to 1.5-m × 2.1-m kennels with concrete floors, stainless steel walls, and chain-link ceilings and doors, where they were housed individually for 24 hours. Figure 1 (a) illustrates the kennel layout. Cats were provided with one litter box with CON and one litter box with odorant-infused litter that was assigned using a random number table (n = 10 cats/ treatment). Each litter box contained 1.36 kg of the designated litter, and litter boxes with litter/waste were weighed at the beginning and end of the 24-hour period. Based on final litter box weights, a preference index (PI) was calculated to determine preference or avoidance of litter types. The PI has been used to describe preference/avoidance in several animal models (McGlone & Morrow, 1987; Morrow-Tesch & McGlone, 1990). The PI was calculated by the following formula: PI = 100 × A / (A + B), where A is the treatment litterbox weight and B is the CON litterbox weight. Values of PI were compared to an expected PI of 50% using a one-sample t test. At a significance level of p = .05, litter box use with a PI significantly greater than 50% indicated preference, while litter box use significantly less than 50% indicated aversion.
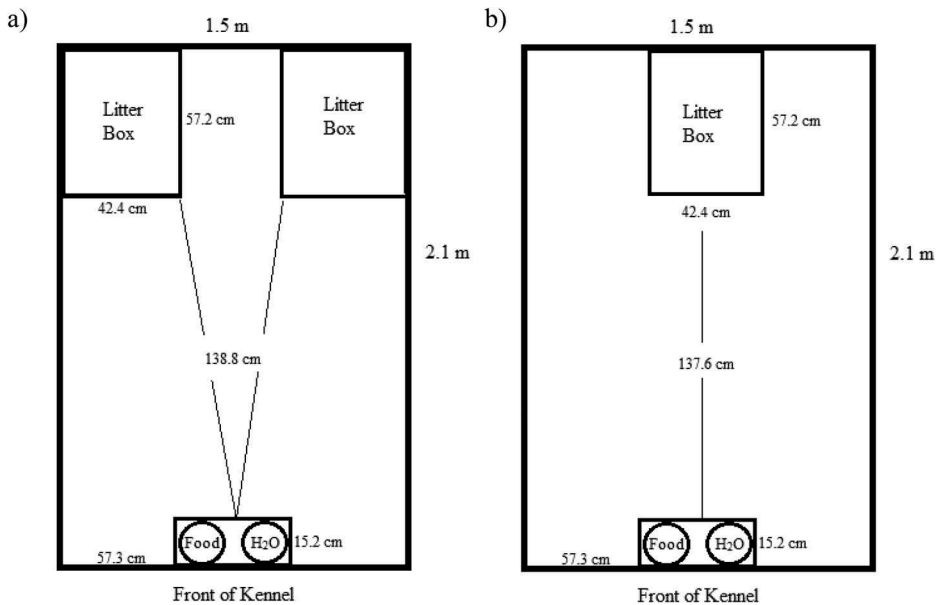
Figure 1. Layout and size of kennels used in Study 1 (a) and Study 2 (b).

Study 2: 2-methyl-2-butenal litter efficacy

The specific objective of this study was to determine if 2M2B, selected from Study 1, reduced aggression and anxiety-like behavior and improved litter box use in newly introduced cat pairs.

Litter

A clumping litter derived from processed corn cobs as described in Study 1 was used. Plastic collars impregnated with 2M2B and placebo collars provided by Perrigo Animal Health (Omaha, NE) were processed into beads approximately 1.6 mm long. Placebo beads were mixed into the CON litter, and 2M2B beads were mixed into the 2M2B litter at 3% of the total litter weight. Litter was separated into 1.36 kg aliquots, labeled A or B, and delivered to the contract research facility.

Animals

An intermediate pilot study using 48 mixed genetic cats (n = 23 intact males, 1 castrated male, and 24 intact females) was conducted prior to Study 2 to identify cat pairs who displayed elevated levels of aggression. Cats were removed from their home kennels and paired with unfamiliar cats of the same sex for 24 hours, and aggression was measured. After this pilot testing, cats returned to their home kennels with familiar kennel mates for 12 weeks until this study began.

Eight pairs of intact males (N = 16) were chosen from the pilot study for Study 2. Male cat pairs were chosen because intercat aggression is more common between male-male pairs than between female-female pairs or mixed-sex pairs (Landsberg, Hunthausen, & Ackerman, 1999; Lindell et al., 1997). Average body weight of cats enrolled in the study was 4.4 ± 0.2 kg.

Procedure

Each of the eight pairs of aggressive cats were assigned to receive either CON or 2M2B litter using a random number table, with four pairs per treatment. Each pair was placed together in a 1.5-m × 2.1-m kennel as described earlier for 24 hours with one litter box containing the designated litter. Kennel

layouts are illustrated in Figure 1 (b). At the end of the 24 hours, facility employees noted any soiling outside the litter box. Litter boxes were weighed at the beginning and end of the study.

Behavior observations

Cat behavior was captured on video for the duration of the study (Sony® DCR-SR85 camcorders; Sony, San Diego, CA). Lights were on a 12:12 light:dark cycle. Red lights were used at night to allow for continuous video observation. Trained and validated observers blind to the treatments reviewed the videos at Texas Tech University. Observers were first trained to become familiar with each behavior (Table 1) and the type of behavior scoring used in the study and were then given a behavior scoring test. Observers were considered validated when the coefficient of variation for each behavior was less than 5% compared to a standardized key prepared by the principal investigators. Videos were analyzed in five-minute scan samples, which provided a suitable estimation of behavior (Altmann, 1974). At each five-minute period, cats were categorized as anxious (anxiety-like behaviors; defined in Table 1) or relaxed (not showing anxiety-like behaviors; Paws in Training, 2015). Aggression and litter box usage occurring between the five-minute periods were noted.

Data analyses

The number of occurrences of each behavior listed in Table 1 was totaled in 2-hour periods, which provided a score for each behavior within each period. Two-hour periods were chosen because they not only provide a strong estimate of behavior frequency but also allow for visualization of behavior patterns throughout the 24-hour period (Altmann, 1974). The maximum score for any behavior was 24 per period, with the exception of aggression and litter box use, which are often brief and can be difficult to capture using scan sampling techniques; these behaviors are reported as average number of occurrences per period. Anxiety-like behaviors were measured as a percent of total time in each period. All data were analyzed using analysis of variance (ANOVA) within the General Linear Models procedure of SAS 9.3 (SAS Institute, Cary, NC). Data were tested for homoscedasticity and normality and met all assumptions. The model included effects of treatment, cat pair within treatment, period, and treatment by period, where period represented each 2-hour period. Least squares means were calculated and post-hoc comparisons were used to assess treatment differences within each 2-hour period where indicated as appropriate by significant ANOVA. Overall aggression was analyzed first, and then aggression in the first 6 hours was categorized as near (within one body length) or away from (more than one body length) the litter box to determine if the proximity to the

Table 1. Ethogram[1] of Cat Behaviors.

Behavior	Definition
Head in food/water dish	Cat is actively eating or drinking; or cat is showing significant interest in food or water.
Sitting or Lying—not touching	Cat is sitting or lying with no body part in direct contact of another individual.
Sitting or Lying—touching	Cat is sitting or lying with some part of body in direct contact of another individual.
Aggression	Cat is actively engaged in fighting, hissing, or dominance behavior.
In litter box—not using	Cat is sitting, standing, or lying inside litter box, but is not actively eliminating.
In litter box—using	Cat is urinating or defecating inside litter box.
Grooming self	Cat is grooming own body.
Grooming other	Cat is grooming body of other individual.
Sleeping	Cat is lying in a relaxed state with eyes closed.
Neutral social interaction	Cat is engaged in nonaggressive interaction with other individual, such as nose touching or rubbing.[2]
Locomotion	Cat is walking, pacing, running, or climbing.
Play	Cat is engaged in activity for sake of amusement, either alone or with other individual.
Anxiety-like behaviors	Cat is displaying behaviors typically associated with stress, such as hiding, pacing, aggression, excessive vocalization, and obvious tension.[3]

[1]Definitions adapted from Thompson et al. (2013), unless noted otherwise.
[2]This definition adapted from Crowell-Davis et al. (2004).
[3]Behaviors identified using Paws in Training infographic, 2015.

2M2B odor had an effect. Litter box weights in Study 2 were not evaluated due to significant amounts of litter being spilled from seven of the eight litter boxes by the cats.

Results

Study 1: Preference study

Based on litter box weights, 50% of cats preferred to use AN litter compared with CON litter, indicating no difference in odor preference. Cats avoided litter with 3M3M and 3M3M+2M2B with PI values of 40% and 12%, respectively ($p < .01$, both). The PI value for 2M2B was 78%; additionally, litter boxes containing 2M2B weighed 138% more than CON litter boxes, suggesting that cats have a strong preference for 2M2B-containing litter ($p < .01$).

Study 2: 2-methyl-2-butenal litter efficacy

The average percent of time cats spent engaged in each behavior and aggression (reported as number of occurrences) is shown in Table 2. No differences were observed in sitting or lying not in direct contact/touching, sitting or lying in direct contact/touching, not using the litter box, grooming another, or locomotion.

There was a significant Treatment × Period effect on litter box use, $F(11, 168) = 2.15$, $p = .020$; however, average time spent in the litter box only differed between treatments in Periods 12 and 18, with the CON group spending more time using the litter box than the 2M2B group ($p < .001$ and $p = .019$, respectively; Figure 2). There was a significant Treatment × Period effect on aggression, $F(11, 168) = 2.53$, $p = .006$. Cats experiencing CON litter displayed 7.50, 5.75, and 4.75 more occurrences of aggression during the first three periods, respectively, than did cats experiencing 2M2B litter ($p < .05$). After the first six hours, there were no differences in occurrences of aggression between the two treatments. During the first six hours, occurrences of aggression were categorized as near or away from the litter box; aggression patterns during this time are shown in Figures 3 (a) and 3(b). Cats experiencing CON litter had higher occurrences of aggression both near and away from the litter box in Period 0 ($p < .01$). These cats also had significantly higher occurrences of aggression away from the litter box during Period 2 ($p < .01$).

There was a significant effect of period on head-in-food/H$_2$O dish, grooming self, sleeping, neutral social interactions, playing, and anxiety. Post-hoc tests revealed no differences between periods in eating behavior but did reveal that eating behavior for both groups peaked between 4

Table 2. Average percent of time cats spent engaged in each behavior when exposed to control/placebo litter (CON) or litter infused with 2-methyl-2-butenal (2M2B).

Behavior	CON	2M2B	SE	Period p Value	Treatment p Value	Treatment-Period p Value
Head in food/H2O dish (%)	3.12	2.27	.01	.0470	.1061	.1907
Lying/sitting—not touching (%)	57.86	61.14	.08	.3015	.3097	.6276
Lying/sitting—touching (%)	2.94	2.47	.03	.2388	.6652	.0881
In litter box—not using (%)	5.80	8.99	.06	.9738	.1933	.8802
Grooming self (%)	2.14	1.94	.01	< .0001	.6685	.2342
Grooming other (%)	0.12	0.00	.01	.5151	.1823	.5151
Sleeping (%)	2.92	1.77	.02	< .0001	.242	.1819
Neutral social interaction (%)	0.40	0.51	.01	.0094	.6019	.3366
Locomotion (%)	21.37	17.34	.06	.1365	.0830	.9944
Play (%)	3.33	3.57	.02	.0038	.7879	.9291
In litter box—using (%)*	1.18	0.78	.01	.0068	.1212	.0197
Anxiety-like behaviors (%)*	17.09	25.10	.08	< .0001	.0179	.5012
Aggression (#)*	3.61	2.01	.06	.0020	.0096	.0056

Note. Behaviors of interest (denoted by *) are not included in total day averages. In litter box—using is reported as the percent during total 24-hour period. Aggression data are reported in number of occurrences rather than percentage. n = cats/treatment.
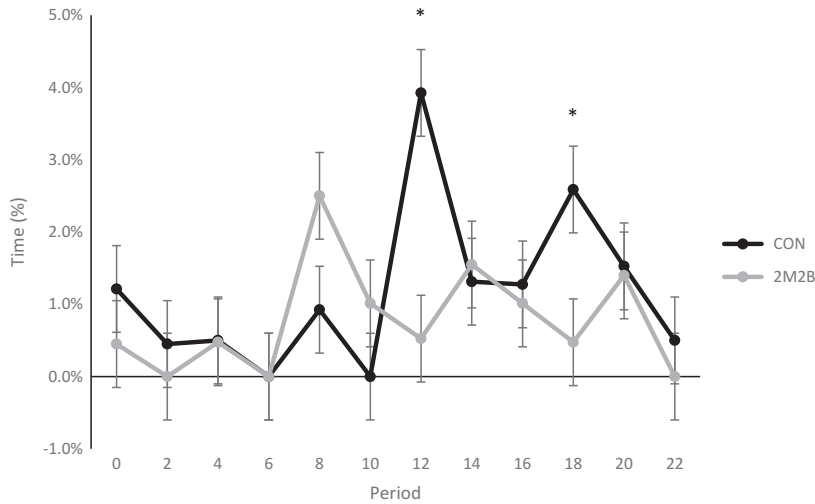
Figure 2. Average percent of time cats receiving control litter (CON) or litter infused with 2-methyl-2-butenal (2M2B) spent using the litter box. Each period represents two hours. $n = 8$ cats/treatment. SEpooled = .006. * Within period, denotes difference in Least Squares (LS) means, $p < .05$.

hours and 6 hours and then decreased throughout the remainder of the trial. Time spent self-grooming in both groups was highest between 4 hours and 8 hours, and then again between 10 hours and 12 hours ($p = .017$, $p < .001$, and $p = .001$, respectively). Time spent engaged in neutral social interactions and playing was extremely variable during the 24-hour trial, and although post-hoc tests revealed some period differences, no distinct, significant patterns were discernable for these behaviors. Sleeping behavior accounted for less than 2.0% of each period during the first 12 hours of the trial, and then it increased, with peaks in Periods 14 and 22 ($p = .041$ and $p < .001$, respectively). Anxiety for both groups decreased from an average of 63.6% at the beginning of the trial to approximately 20.0% between 4 hours and 6 hours, which was significantly lower than the initial level ($p < .001$; Figure 4). Anxiety remained between 10% and 20% until around 20 hours; thereafter, anxiety decreased again to less than 10% for the remainder of the trial.

Discussion

With limited background information, no directional hypotheses could be made about the preference for or aversion to the odors used in Study 1. The aversion to 3M3M and 3M3M + 2M2B may be related in part to the fact that 3M3M is a constituent of cat urine and may be associated with a soiled litter box. This evidence would support the proper cleaning of litter boxes to remove urine odors and encourage proper use (Olm & Houpt, 1988). The preference for 2M2B and the indifference to AN may be attributed to the conservation of olfactory transduction mechanisms and semiochemical production mechanisms observed between species (Ache & Young, 2005). According to this theory of conservation, there are common features of both olfactory and metabolic processes across a number of phylogenetically separated animals; thus, it is highly probable that a biologically active molecule in one species may elicit effects in a different species. In the current study, this theory would suggest that 2M2B may act as an interomone in cats, while AN does not.

Aggression in pair-housed cats is a serious concern for cat welfare. Chronic stress resulting from aggression and social anxiety can cause gastric ulcers, immunosuppression, and marked alteration of the hypothalamic-pituitary-adrenal axis (Biondi, 2001; De Goeij, Dijkstra, & Tilders, 1992; Filaretova, Filaretov, & Makara, 1998).
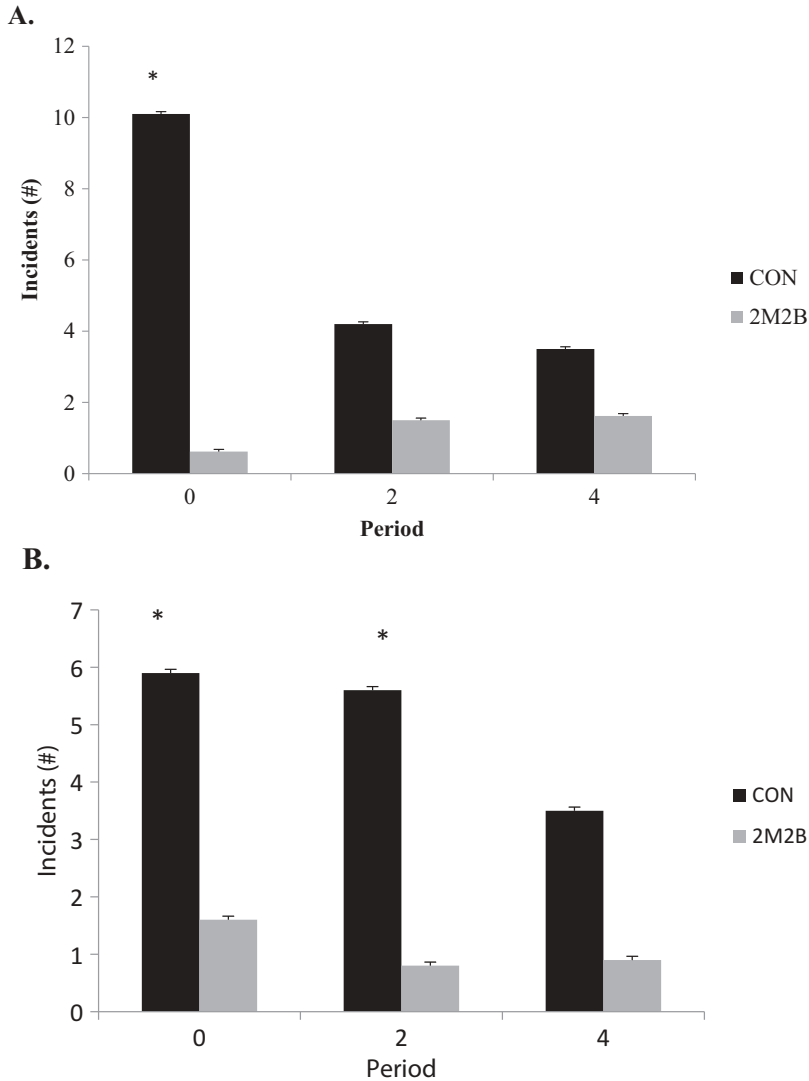
A.

B.

Figure 3. Incidents of aggression in pair-housed cats receiving either control litter (CON) or litter infused with 2-methyl-2-butenal (2M2B) within the first six hours after introduction. Each period represents two hours. (A) Incidents of aggression near (within one body length) of the litter box. (B) Incidents of aggression away (more than one body length) from the litter box. $n = 8$ cats/ treatment. *Within period, denotes a difference in LS means, $p < .05$.

In a home with a resident cat, introduction of a novel cat is known to cause stress and displays of aggression in some cases (Landsberg et al., 1999; Levine et al., 2005). Upon adoption of a new cat, owners who described the first encounter between their cats as aggressive also reported higher incidences of fighting during the 12 months following introduction (Levine et al., 2005). Results of Study 2 support this finding; the pair of cats exhibiting the most incidents of aggression in the first two hours also had the most incidents of aggression in the final two hours of the study. In contrast, the least aggressive pair of cats exhibited no incidents of aggression during the final two hours. The cats receiving 2M2B litter engaged in fewer occurrences of aggression than the cats receiving CON litter during the first six hours, both near and away from the litter box. In a small area such as a kennel, the odor likely dispersed throughout the environment and thereby minimized the effect of
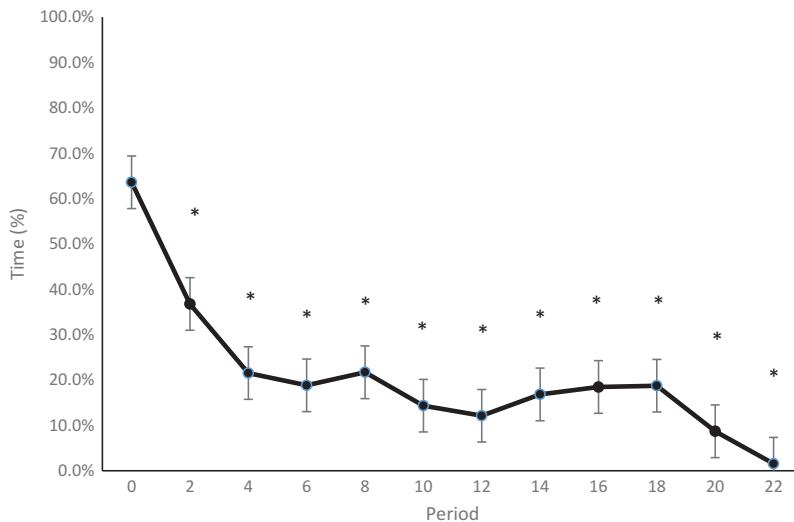
Figure 4. Average anxiety levels of cats after being introduced into a pair-housing situation. Each period represents two hours. $N = 16$ cats. SE = 0.06. *Denotes a significant difference in LS means compared with Period 0, $p < .05$.

distance from the litter box. These findings are consistent with the hypothesis that 2M2B reduces aggression after introduction of cat pairs.

Kessler and Turner (1997) found that stress in newly pair-housed cats declined significantly from Day 1 through Day 4. Approximately two thirds of the cats they studied reached stress levels of "weakly tense or lower" after the second week of pair housing, but the remaining third of the animals remained in a highly stressed state for a prolonged period. In the current study, there were no differences between the 2M2B and CON groups during the 24-hour trial, but anxiety in both groups decreased consistently throughout the study. In another study, when cats that had been socialized toward conspecifics were group-housed with a novel cat, stress scores were lower than for non-socialized cats (Kessler & Turner, 1999). Because these cats were accustomed to pair housing, it is possible that anxiety may have been due to social restructuring and may have facilitated this rapid adjustment to the novel situation.

Providing a single litter box in a multicat household may make a cat vulnerable to ambush; therefore, if a nonfamiliar cat is nearby, an anxious or nervous cat may either soil inappropriately or avoid elimination altogether (Neilson, 2008). In Study 2, litter box uses for both CON and 2M2B groups increased slightly during the 24-hour period, and no inappropriate elimination was observed in either group. In a confined space such as a kennel, cats may be more inclined to use the litter box to avoid soiling near resting sites or food (Olm & Houpt, 1988). In contrast to the hypothesis, cats in the CON group spent slightly more time using the litter box than did cats in the 2M2B group, which is consistent with the idea that cats adapt to their surroundings gradually and may become less anxious over time.

The delivery method utilized in this study was shown in a previous study to provide a consistent volatile molecule release over a 30-day period (May, Surowiec, & McGlone, 2015). Owners may perceive this application as more convenient than daily reapplication of a spray treatment. Additionally, by introducing the odor directly into the litter, initial aggression near the litter box can be reduced and decrease the potential for associated anxiety associated with the litter box in the future.

Suggestions to decrease stress, deter aggression, and facilitate adaptation when introducing a new cat into a household include adoption of a young kitten of the opposite sex (Landsberg et al., 1999), providing sufficient enrichment such as puzzle feeders and toys (Ellis, 2009), and ensuring sufficient

resources and space are available to accommodate the number of animals in the home (Dantas-Divers et al., 2011). The sensitive period of socialization in cats occurs from three to eight weeks of age; removal from the mother and littermates as well as negative experiences during this critical period can result in improper responses, such as aggression, to future stressors (Bateson & Young, 1981; Crowell-Davis & Wolfe, 1997; Overall et al., 2005). Proper management of litter boxes can help make litter boxes more appealing to cats and reduce inappropriate soiling. Proper management includes providing one litter box per cat plus an additional litter box, scooping waste from the box daily, washing the box and changing the litter weekly, and using the type of litter cats find preferable (Carney et al., 2014; Chew & Buffington, 2007; Herron, 2010; Neilson, 2004). Even with careful precautions taken to alleviate these problems, it may not always be possible to prevent them entirely.

This study suggests a preference for and potential social effects of 2M2B in newly introduced domestic cat pairs in a controlled laboratory setting. This type of environment is not a home environment in which factors such as human interaction or other resources such as hiding spots may impact the outcome of the study. Another limitation of this study was the short study time, which was used to minimize the short-term stressful nature of the study. This intervention method should be tested in a home environment for a longer period and should be compared to other methods of intervention.

Conclusions

Unfamiliar cats introduced into a pair-housed setting experienced varying levels of stress as evidenced by occurrences of aggression, displays of anxiety-like behaviors, and infrequent litter box use in the first hours after introduction. Aggression occurred less frequently in cats exposed to 2M2B in the litter box. There are many precautions that can be taken to reduce stress and aggression when introducing unfamiliar cats into a household. The results of this study suggest that 2M2B may act as an interomone in domestic cats, but it merits further study to refine its full potential to improve cat welfare.

Acknowledgments

Disclosure statement

Funding

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