Systematic review of the use of pheromones for treatment of undesirable behavior in cats and dogs

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Objective—To systematically review the scientific literature to identify, assess the quality of, and determine outcomes of studies conducted to evaluate the use of pheromones for treatment of undesirable behavior in cats and dogs.

Design—Systematic review.

Study Population—Reports of prospective studies published from January 1998 through December 2008.

Procedures—The MEDLINE and CAB Abstracts databases were searched with the following key terms: dog OR dogs OR canine OR cat OR cats OR feline AND pheromone OR synthetic pheromone OR facial pheromone OR appeasing pheromone. A date limit was set from 1998 through 2008. Identified reports for dogs (n = 7) and cats (7) were systematically reviewed.

Results—Studies provided insufficient evidence of the effectiveness of feline facial pheromone for management of idiopathic cystitis or calming cats during catheterization and lack of support for reducing stress in hospitalized cats. Only 1 study yielded sufficient evidence that dog-appeasing pheromone reduces fear or anxiety in dogs during training. Six studies yielded insufficient evidence of the effectiveness of dog-appeasing pheromone for treatment of noise phobia (2 reports), travel-related problems, fear or anxiety in the veterinary clinic, and stress- and fear-related behavior in shelter dogs as well as vocalizing and house soiling in recently adopted puppies.

Conclusions and Clinical Relevance—11 of the 14 reports reviewed provided insufficient evidence and 1 provided lack of support for effectiveness of pheromones for the treatment of undesirable behavior in cats and dogs. (*J Am Vet Med Assoc* 2010;236:1308–1316)

Pheromones are enigmatic bioactive compounds; only a few mammalian pheromones have been identified. Such compounds are classically defined as chemical cues emitted and detected by animals of the same species that influence social and reproductive behavior. Pheromone communication is a 2-component system: signaling pheromones and receiving sensory neurons. Despite the importance of these chemical cues in regulating essential animal behaviors, the nature of these ligands remains largely unknown.

A growing body of evidence indicates that the structural and functional characteristics of pheromones may be far more diverse than revealed by classic experiments.[1] Results of bioassays have suggested that pheromones are nonvolatile, activate vomeronasal sensory neurons, and regulate innate social behavior and neuroendocrine release.[1] More recent studies[2,3] have revealed that pheromones may be nonvolatile or ephemeral, activate vomeronasal organ neurons or main olfactory epithelium neurons, and have their effects altered by context. Results of experiments in swine indicate that some pheromone-mediated behaviors are generated by main olfactory epithelium neurons.[4] Mechanistic studies of the perception of environmental odorants in vertebrates (mostly mice) including putative pheromones have been facilitated by the identification of genes for receptors of main olfactory epithelium neurons[5] and the sequencing of genes for receptors of vomeronasal organ neurons.[6]

Additional experiments have shown that mouse vomeronasal organ neurons can be stimulated by odorants not emitted from other animals, such as floral and woody-smelling compounds. One study[7] revealed that in mammals and insects, odorous compounds released from plants or other animal species may act as so-called semiochemicals or signaling molecules that elicit stereotyped behaviors. Results of that study also suggested that the vomeronasal organ is highly sensitive to low concentrations of both pheromones and odorants.

A synthetic analogue of the facial pheromone found in cats (FFP)[a] is marketed as an effective way to control and manage unwanted feline behaviors such as urine

Abbreviations

DAP	Dog-appeasing pheromone
FFP	Feline facial pheromone
GABA	γ-Aminobutyric acid
ITT	Intention-to-treat
RCT	Randomized, controlled clinical trial

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marking and scratching of furniture. Other claims for this product on the manufacturer's website or in marketing materials include calming cats in unknown and stressful situations or environments such as transport in a cage or in a car and boarding or moving to a new house. According to the patent, the composition of the synthetic facial pheromone is an emulsion comprising a mixture of fatty acids or derivatives thereof and a compound of vegetal origin (an extract of *Valeriana officinalis*) that has an attractant effect on cats.[8]

A structural analogue reproducing the soothing properties of the appeasing maternal pheromone in dogs (DAP)[b] is available to help alleviate anxiety associated with travel, new environments (eg, moving to a new home or adoption), introduction of visitors or strangers, veterinary visits, and crate confinement or boarding in kennels as well as other situations in which a dog may feel uncomfortable. Information from the manufacturer also indicates that this pheromone can be used as a treatment for stress- or fear-related behaviors in puppies by helping dogs to cope with loud noises or to stay alone and for reducing signs of arousal in puppy classes. In adult dogs, this treatment can be used to help dogs cope with staying alone, to avoid separation problems, or to manage fear of noises.

To the authors' knowledge, there has been no systematic review of the literature to analyze the efficacy of these products. The purpose of the review reported here was to examine and critically appraise the evidence of efficacy of commercially available pheromones used as a treatment for canine or feline behavioral problems. Whenever possible, conclusions regarding recommendations for or against their use were sought.

Materials and Methods

Search methods—To retrieve all reports of clinical trials involving dogs or cats treated with commercially available pheromones, a wide electronic search was carried out by use of the MEDLINE database. A broad query was conducted with the following terms: dog OR dogs OR canine OR cat OR cats OR feline AND pheromone OR synthetic pheromone OR facial pheromone OR appeasing pheromone, with a limit set from 1998 through 2008. These dates were chosen because commercial pheromone products were developed in the early 1990s, and no independent study of efficacy was published prior to 1998. A second focused search was made with the same string limited to the treatment category of the clinical queries by use of methodology filters. To increase the retrieval of additional veterinary medical citations, the CAB Abstracts database was queried with the aforementioned terms. Bibliographies of all relevant reports and book chapters covering pheromone treatment of undesirable behaviors in cats and dogs were examined for additional relevant citations.

Selection of clinical studies—This systematic review was limited to prospective studies on the use of pheromones in dogs or cats for the treatment of behavioral problems that were published in peer-reviewed veterinary medical journals from 1998 through 2008. Two reviewers did the initial screening of the abstracts. There was no limitation based on language of publication. Two canine studies[9,10] in which the owner of the company that produces the commercially available canine and feline pheromones was a coauthor were excluded. A short retrospective communication[11] published 10 months later on long-term follow-up of the effect of pheromone treatment on feline urine-spraying behavior was also excluded. Selection based on specific behavioral diagnosis was not possible because of the low number of published reports that satisfied our inclusion criteria.

Data extraction—Clinical trials that met the inclusion criteria were reviewed independently by all authors. Studies were assessed on quality of design, participant characteristics, objective information about the behavioral problem being treated, treatment intervention, and outcome measures. Data were summarized in tabular form. Results of individual reviews were compared, and when differences were found, they were discussed and amended. There was little discordance among the 3 reviewers, but when it occurred, it was resolved by consensus.

Assessment of methodological quality—The same approach as described by Olivry and Mueller[12] was used for this review. Three parameters were addressed to determine risk for biased estimates of treatment effect in the selected studies: randomization (method of generation[13] and concealment of allocation[14] to treatment groups), masking (blinding of researchers and clients), and loss to follow-up. These 3 parameters were graded as adequate, unclear, inadequate, or not applicable. When trials were not randomized, the term not applicable was given to the randomization parameter. Randomization was rated as adequate when the method was adequate for both generation of allocation sequences and allocation concealment as described in the report. Randomization for both the generation of allocation sequences and the allocation concealment was rated as unclear when the methods were not described in the report. Randomization was rated inadequate when the methods were not performed correctly. Masking was rated as adequate only when both client or owner and researcher or clinician were blinded. Intention-to-treat was characterized as adequate when dropouts were included in the statistical analysis (outcome), unclear when the reviewers were unable to determine whether dropouts had been included in statistical analysis, not applicable when there were no dropouts, and not done when statistical analysis did not factor in dropouts. All reports included statistical analyses. Statistical methods were evaluated to determine whether they were sufficiently explained, and problems with the analyses, if any, were recorded.

Grading of evidence quality—An overall grade of evidence quality was assigned as described elsewhere,[12] with 2 modifications. The evidence pyramid illustrated in the guide to research methods of the SUNY Downstate Medical Center[15] was used as reference for the 2 changes made. Grades were assigned on the basis of the aforementioned parameters as follows: A = blinded RCT (control treatment for studies in this review consisted of placebo); B = controlled trial lacking blinding or clear randomization; C = cohort study (first change)[15];

and D = case-control analytic study, case series (second change),[15] descriptive study, or case report. A placebo-controlled, randomized trial in which randomization (allocation generation and allocation concealment) was not clearly described was graded B. A number was also assigned on the basis of the number of study subjects as follows: 1 = > 50 subjects/group; 2 = 20 to 50 subjects/group; 3 = 10 to 19 subjects/group; and 4 = < 10 subjects/group. For example, an RCT with 40 enrolled subjects (20 in each group) was graded as A2, whereas a cohort study with 15 dogs was graded as C3. This grading system could at times be misleading. For example, one should not assume that a study graded A1 but with poorly defined outcome measures yields evidence superior to that of a well-designed smaller study graded B2.

Assessment of subject enrollment quality—Selection of enrolled subjects was reviewed to assess whether a behavioral diagnosis was required along with inclusion and exclusion criteria for a given study. The quality of subject enrollment was rated as well-defined (eg, sufficient details on the methods for diagnosis, including clinical signs, diagnostic rule-outs, and concomitant behavioral disorders), fairly defined (intermediate situations), or poorly defined (vague selection criteria or insufficient details on behaviors or absence of medical work-up). All animals were client-owned except in 1 study.[16]

Assessment of outcome measures—Outcome measures were assessed by considering clinical efficacy (percentage of dogs or cats with complete or nearly complete remission), percentage of dogs or cats that had partial clinical remission, and relapse rate (percentage of animals with a relapse after treatment was stopped, within a given time frame). Statistical significance of the relationships presented in each report was assessed, and for each variable, a simple ratio was calculated between mean or median values for treated versus nontreated or control animals. This ratio was calculated post hoc and dealt with quantitative measurements of treatment effects between groups within a given study. This ratio can be considered an estimate of effect size. However, it was not possible to obtain confidence intervals for this type of ratio given that in most reports, information on relevant interindividual variation was not available. To examine the extent of the bias attributable to loss to follow-up, reports were examined to determine whether ITT analyses had been performed.

Reporting of qualitative results—The same grading system as described by Olivry and Mueller[12] was used. When > 1 report, including at least 1 well-designed RCT, yielded sufficient outcome details to support high efficacy of the pheromone treatment, then this was considered good evidence for recommending use of the treatment. When at least 1 well-designed RCT revealed medium to high efficacy of the pheromone treatment, then this was considered fair evidence for recommending use of this product. When well-designed RCTs were not available or when multiple studies yielded controversial evidence of a treatment effect, then this was considered insufficient evidence for recommending use of the treatment. When ≥ 1 well-designed study or several less detailed studies revealed lack of efficacy of the pheromone treatment, then this was considered only fair evidence against recommendation of the treatment. When > 1 study, including at least 1 well-designed RCT, revealed lack of efficacy of the pheromone treatment, then this was considered good evidence against recommending use of the treatment.

Results

Fourteen English reports fulfilled our selection criteria. Seven involved cats, and 7 involved dogs.

Synthetic FFP—Of the 7 feline reports identified, 4 involved the effect of pheromone treatment on urine spraying or urine marking (defined as urine projected on vertical surfaces),[17–20] 1 concerned pheromone treatment of idiopathic cystitis,[21] 1 involved the effect of FFP on cat behavior prior to and during venous catheterization procedures,[22] and 1 described the effect of FFP on behavior and food intake in healthy and ill cats during hospitalization (**Table 1**).[23]

The 7 reports provided information on 8 studies. Reports included an RCT involving a placebo,[22] 3 blinded placebo-controlled trials that lacked clear randomization[19,21,23] (1 of which dealt with urine spraying,[19] and another of which also included a case series within the same report[23]), and 3 case series[17,18,20] (all of which dealt with urine spraying). The total number of cats involved in 6 of these reports was 243 (range per study, 12 to 77 cats). In 1 report,[18] it was unclear how many cats actually participated. Therefore, the total number of cats treated with synthetic FFP could not be definitively determined. In 5 reports,[17,19–21,23] 87 cats were treated. In the other 2 studies, either the number of cats treated was not mentioned[18] or allocation was described as 8 to 12 cats/group, with 2 groups treated with FFP and 2 groups serving as controls.[22] The synthetic FFP was applied in the environment by spraying the product either manually (spray bottles in 6 reports[17,18,20–23]) or with a plug-in diffuser (2 bottles for the diffuser in 1 report[19]). Amounts of synthetic pheromone sprayed were variable (3 reports), but spraying was done in accordance with the manufacturer's recommendation (1 to 3 sprays, 1 to 3 times daily on all urine marks in the home of urine-spraying cats).[17,18,20] In 1 report,[22] the amount of synthetic pheromone sprayed was standardized ("each act of spraying consisted of a complete actuation of the aerosol pump for 1 to 2 seconds with the head of the aerosol held vertically and approximately 10 cm from the cage paper"), whereas in another report,[23] amounts sprayed on the towels in the cages were not specified.

The length of pheromone treatment varied from 25 minutes to 8 weeks. An additional pharmacological intervention (acepromazine [0.04 mg/kg {0.018 mg/lb}, SC]) was used concurrently with the pheromone in 1 study.[22] Feline facial pheromone was specified as the only treatment used in 3 of the publications,[17,19,20] and no additional product was mentioned in the other reports.[18,21,23] No behavioral advice was given or environmental modifications performed in 6 studies.[17–22] In 1 study,[23] a plastic cat carrier was used for half of the participants.

Statistical methods described were adequate in all studies but one. That study[18] involved multiple tests at

Table 1—Characteristics of studies reported from 1998 through 2008 in which the efficacy of FFP for treatment of undesirable behaviors in cats was evaluated.

Characteristic	Frank et al[17]	Hunthausen[18]	Mills and Mills[19]	Ogata and Takeuchi[20]	Gunn-Moore and Cameron[21]	Kronen et al[22]	Griffith et al[23]*
Quality of evidence†	D2	D2	B3	D2	B4	A4	B2/D2 (2 studies)
Randomization (allocation generation)	NA	NA	Adequate	NA	Unclear	Adequate	Inadequate/NA
Randomization (allocation concealment)	NA	NA	Unclear	NA	Unclear	Adequate	Inadequate/NA
Masking of outcome assessor (clinicians and owners)	NA	NA	Adequate	NA	Adequate	Adequate	Unclear/NA
ITT analysis	Not done	Unclear	Not done	Not done	Not done	NA	NA
Inclusion criteria for study subjects	Well-defined	Fairly defined	Well-defined	Well-defined	Well-defined	Well-defined	Fairly defined/poorly defined
Selection based on diagnoses/behavior	Yes	Yes	Yes	Yes	Yes	No	No
No. of cats enrolled	34	Unclear	25	55	12	77	20
No. of dropouts	12	Unclear	3	19	3	0	0
No. of treated	22	Unclear	10	36	9	8 (12)	10 of 20
Mode of pheromone exposure	Spray	Spray	Diffuser	Spray	Spray	Spray	Spray
Treatment of controls	NA	NA	None	NA	None	Yes	None/NA
Amount of pheromone used	1–3 times/d	2 times/d	2 bottles	1 time/d (2–3 times/d in multiple-cat households)	Daily (1–2 cats); twice daily (3 cats or more)	3 spots/cage	NR
Duration of treatment	4 wk	4 wk	4 wk	4 wk	8 wk	25 min	125 min/24 h
Duration of follow-up after treatment withdrawal	1 mo	0	0	1 mo	0	0	0
Pharmacological intervention	None	NR	None	None	NR	Acepromazine	NR
Additional intervention	None	None	None	None	None	None	None/E
Proportion of participants with complete or nearly complete clinical remission	2/19 households	33% of cats	NR	37% of cats	NR	NR	NR
Proportion of participants with partial clinical remission	14/19 households	2/3 households	8 cats (7/12 cats)	40% of cats	NR	NR	NR
Proportion of participants with treatment failure	3/19 households	9.3%	1 cat	22.9%	NSD	Unknown‡	NSD/NR
Relapse rate	None	NR	NR	None	NR	NR	NR
Statistical method described	Adequate	Inadequate	Adequate	Adequate	Adequate	Adequate	Adequate

Results in parentheses are for the control (placebo-treated) groups.

*This report included 2 studies: a case series and a blinded placebo-controlled trial that lacked clear randomization; characteristics are presented for each, respectively. †Grades were assigned as follows: A = RCT (blinded, with control treatment consisting of placebo); B = controlled trial lacking blinding or clear randomization; C = cohort study; and D = case-control analytic study, case series, descriptive study, or case report. A placebo-controlled, randomized trial in which randomization (allocation generation and allocation concealment) was not clearly described was graded B. A number was also assigned on the basis of the number of study subjects as follows: 1 = > 50 subjects/group; 2 = 20 to 50 subjects/group; 3 = 10 to 19 subjects/group; and 4 = < 10 subjects/group. ‡There may have been interaction between the pheromone and acepromazine administered to half the cats.

E = Environmental modification. NA = Not applicable. NR = Not reported. NSD = No significant difference between treated and control subjects.

various times, without statistical correction for those multiple tests. Inclusion criteria were judged as well-defined in 4 studies,[17,19–22] fairly defined in 2 studies,[18,23] and poorly defined in 1 case series study.[23] All 4 reports[17–20] regarding treatment of urine-spraying cats included some data for outcome. The median number of urine marks decreased 2.7- to 6-fold with respect to the pretreatment number in 3 studies,[17,18,20] and in 1 study,[19]

the mean number of spraying incidents decreased 2.3-fold in treated cats versus control cats. Complete or nearly complete remission was evident in between 10% and 37.1%[17,18,20] of cats and was not applicable[22,23] or was not reported[19,21] for 4 studies. Partial clinical remission was variable, ranging from 36% to 74% of participants. In the placebo-controlled trial,[19] the frequency of urine spraying by week 4 of treatment had decreased from the pretreatment frequency in 58% of placebo-treated cats and 80% of FFP-treated cats. Treatment failure occurred in 9.3% to 22.9% of participants.[17–20] Relapse rate was not evaluated in 2 of the 4 studies[17,20] and was not reported for the other 2 studies.[18,19] Attrition rates were 35% (12/34),[17] 12% (3/25),[19] and 35% (19/55)[20] in 3 studies on FFP treatment for urine spraying in cats and were unreported in 1 study.[18] Follow-up was performed in 2 of 4 studies[17,20] for 1 month after cessation of treatment. Follow-up was not applicable[22,23] or was not performed prospectively[18,19] in the other 4 studies. Adverse effects were reported for 1 study[20] only, in which 4 (11%) owners indicated their cats were more aggressive or unfriendly than before treatment.

In the study[21] of pheromone treatment of idiopathic cystitis, no significant effect was detected, but effect sizes ranged from 0.43 to 0.83 in the predicted direction for a reduction in negative behavior (aggression, fear, or hiding) and fewer episodes and recurrences of feline interstitial cystitis. Effect size for appetite in the treatment group was improved by a factor of 5.31, compared with the effect with a placebo.[21] No information was available on additional recommendations for management of the cystitis that may have been given to owners other than the pheromone treatment. In 1 study,[23] no difference was identified in mean food intake in hospitalized cats exposed to synthetic FFP, compared with intake in those exposed to the vehicle (placebo); although initially listed as an objective of the study, the most common behaviors in hospitals seen in both groups of cats were not reported. In the second study within that same report,[23] the 24-hour food intake was significantly greater (increased by a factor of 2.9) in hospitalized cats exposed to a cat carrier and FFP within the hospital cage, compared with intake in cats exposed to the pheromone alone without a cat carrier in the cage. One report[22] included a description of changes in behavior associated with synthetic FFP; although the effect of treatment was nonsignificant, treatment did yield an effect in the predicted direction for calmness. In that study, there may have been interaction of the pheromone with the acepromazine administered.

Although all cats in the aforementioned studies were client owned, none of the studies included an evaluation of owner compliance with treatment protocols. Assessment of treatment efficacy in 5 of the 8 studies[17–21] relied entirely or heavily on subjective assessment by the owner.

In 5 of 8 studies included in the review, ITT analysis was not conducted[17,19–21] or was not described or unclear.[18] In the other 3 studies,[22,23] it was not applicable.

DAP—Of the 7 canine reports, 2 dealt with noise phobia treatment (fireworks)[24,25] and the other 5 dealt with stress-, fear-, or anxiety-related behaviors and the response to pheromone treatment (**Table 2**).[16,26–29] Four reports[16,27–29] were of placebo-controlled studies (grade B studies) that lacked a clear description of the treatment allocation concealment process. Two of these studies[27,29] had inadequate random allocation generation; the remaining 3 were case series.[24–26]

All studies considered, the total number of dogs reported as participating was 320. The total number of dogs treated with DAP was estimated at 195 because in 1 report,[25] the number of dogs actually treated was unclear. In 2 studies,[26,29] the treatment was applied by means of a collar impregnated with DAP, and in another study,[24] the pheromone was dispensed in the environment by a plug-in diffuser (1 bottle for the diffuser). For 4 studies,[16,25,27,28] the method of pheromone dispensation was not specified. A diffuser was most likely used because collars were initially not available. The amount of pheromones used in those 4 studies was either not reported or unclear.

Length of pheromone treatment varied from 7 minutes to 8 weeks[16,24,26–29] and was not reported for 1 study.[25] Follow-up (1 month, 3 to 5 months, or up to 12 months) was performed in only 3 studies.[26,28,29] Additional pharmacological intervention (anxiolytic or sedative treatment) was permitted in 1 grade D study[24] (fear of fireworks), on an as-needed basis. Dog-appeasing pheromone was the only product used for 3 trials,[16,25,28] and no other product was mentioned for the other 3 studies.[26,27,29] Behavioral advice was given in 4 studies,[24–26,29] 3 of which were cases series, and environmental modification (cage) was provided in 1 placebo-controlled experiment.[28]

Statistical methods described were adequate in most studies. In 1 report[16] of a placebo-controlled trial, results were not always directly compared among the treatment groups. In 2 studies,[26,29] no attempt was made to statistically correct comparisons for the large number of statistical tests.

Inclusion criteria for study subjects were questionable in 4 of the 7 studies. In those studies, behaviors or inclusion and exclusion criteria were poorly defined or characterized. Two reports[25,28] provided clearer details about specific behaviors and inclusion and exclusion criteria. One study[29] did not involve selection of subjects on the basis of behaviors.

Six publications[16,24,25,27–29] did not include data on treatment outcome (complete or nearly complete clinical remission). One report[26] was unclear with respect to outcome. Another study[24] revealed partial clinical remission in 22 of 30 (73%) dogs treated with DAP, but the number of dogs that in fact also received an as-needed pharmacological intervention is unknown. Additionally, no data about possible influence of behavioral modification versus DAP on dog responses were available for that study. However, significant reduction was detected in measures of frequency of 9 of the 14 most common behavioral signs of fear that the dogs displayed when exposed to fireworks, even when there were variations in the responses of individual dogs. The median frequency of certain behaviors decreased 2- to 6-fold in DAP-treated dogs, compared with pretreatment levels with many significant effects in the predicted direction, but these effects could not be at-

Table 2—Characteristics of studies reported from 1998 through 2008 in which the efficacy of DAP for treatment of undesirable behaviors in dogs was evaluated.

Characteristic	Sheppard and Mills[24]	Tod et al[16]	Gandia Estellés and Mills[26]	Mills et al[27]	Levine et al[25]	Taylor and Mills[28]	Denenberg and Landsberg[29]
Quality of evidence*	D2	B2	D1	B4	D2	B2	B2
Randomization (allocation generation)	NA	Unclear	NA	Inadequate	NA	Inadequate	Inadequate
Randomization (allocation concealment)	NA	Unclear	NA	Unclear	NA	Unclear	Unclear
Masking of outcome assessor (clinicians and owners)	NA	Adequate	NA	Adequate	NA	Adequate	Adequate
ITT analysis	Unclear	NA	Not done	NA	Unclear	NA	Not done
Inclusion criteria for study subjects	Poorly defined	Fairly defined	Poorly defined	Poorly defined	Fairly defined	Well-defined	Well-defined
Selection based on diagnoses/behavior	Yes	No	No	Yes	Yes	No	No
No. of dogs enrolled	30	54	62	15	54	60	48
No. of dropouts	NR	0	3	0	Unclear	0	3
No. of treated	30	37	59	15	Unclear	30	24
Mode of pheromone exposure	Diffuser	Diffuser	Collar	Diffuser	Diffuser	Diffuser	Collar
Treatment of controls	NA	None	NA	None	NA	None	None
Amount of pheromone used	1 bottle	NR	2 collars	NR	NR	NR	2 collars
Duration of treatment	3–5 wk	1 wk	6 wk	7 min	NR	4 wk	8 wk
Duration of follow-up after treatment withdrawal	0	0	3–5 mo	0	0	1 mo	12 mo
Pharmacological intervention	Yes	None	NR	NR	None	None	NR
Additional intervention	B	None	B	None	B	E	B
Proportion of participants with complete or nearly complete clinical remission	NR	NR	Unclear	NR	NR	NR	NR
Proportion of participants with partial clinical remission	22/30 dogs if no dropouts	NR	Unclear	NR	NR	NR	NR
Proportion of participants with treatment failure	NR	Unclear	Unclear	Unclear	NR	Unclear	Unclear
Relapse rate	NR	NR	Unclear	NR	NR	NR	NR
Statistical method described	Adequate	Adequate	Inadequate	Adequate	Adequate	Adequate	Inadequate

*Grades were assigned as follows: A = RCT (blinded, with control treatment consisting of placebo); B = controlled trial lacking blinding or clear randomization; C = cohort study; and D = case-control analytic study, case series, descriptive study, or case report. A placebo-controlled, randomized trial in which randomization (allocation generation and allocation concealment) was not clearly described was graded B. A number was also assigned on the basis of the number of study subjects as follows: 1 = > 50 subjects/group; 2 = 20 to 50 subjects/group; 3 = 10 to 19 subjects/group; and 4 = < 10 subjects/group.

For additional intervention, B = Behavioral modification program.

See Table 1 for remainder of key.

tributed solely to the pheromones. No results in terms of treatment failure or relapse rate were reported.

Another study[25] involved use of a combination of DAP with 1 of 2 compact discs commercially available as a noise-desensitizing program. No effort was made to separate effects of the behavior modification program from that of the pheromone treatment. Effect size was not applicable. Results in terms of percentage clinical remission (complete, nearly complete, or partial), treatment failure, and relapse rate were not reported.

One placebo-controlled trial (grade B study)[27] revealed that treatment in the waiting room increased the relaxed emotional state of dogs by a factor of 3 and decreased the anxious state by a factor of 1.2, compared with placebo. No effect was observed in the waiting room with regard to specific categories of recorded behaviors. In the consultation room, treatment decreased the anxious state duration by a factor of 1.8 and increased visitation of room edges by a factor of 1.2, with no other significant effect. The other placebo-controlled

trial (grade B study)[16] showed that during a walking test, treatment significantly reduced barking noises by a factor of 1.3, compared with placebo. During the recovery test, treatment significantly reduced barking noises by a factor of 1.6. No significant effect of treatment was evident for a neutral-stranger test. During a friendly stranger test, treatment significantly decreased barking frequency by a factor of 4, compared with pretreatment levels, although it is not clear what happened in the control group. Many behavioral categories usually associated with stress in dogs were unchanged in response to DAP during the neutral- and friendly stranger tests. In 1 study[26] in which significant improvements for many behavioral categories were observed, we were unable to calculate effect sizes.

Treatment significantly reduced the number of disturbed nights (nights when puppies primarily vocalized or scratched at the door) by a factor of 3, compared with placebo, for 1 breed of dog only.[28] In the same study, for recently adopted puppies with a tendency to cry, treatment reduced the number of disturbed nights by a factor of 2.3. Sleeping with another dog at night reduced a puppy's tendency to disturb owners at night. Puppies that were not sleeping with another dog and had a tendency to disturb at night during the first 3 nights in a new home tended not to continue the behavior in the presence of DAP. No significant effect of pheromone treatment was evident for house soiling.

For the study[29] on fear or anxiety of puppies during training, on the final day of assessment, excitability scores and degree of fear in the treated group were smaller by a factor of 2, compared with those in the control group. Higher scores in the control group indicated more excitability or more fear. In addition, socialization scores were higher by a factor 1.5 in the treated group, compared with those in the control group, 12 months after the first assessment (higher scores indicated better socialization). Relapse rate was not reported or was unclear in all reports.

All dogs used in the aforementioned 7 studies were client[24–29] or shelter[16] owned. None of the studies with client-owned dogs specifically included evaluation of owner compliance with treatment protocols. Assessment of treatment efficacy in 5 of the studies[24–26,28,29] relied entirely or heavily on owner's subjective assessment.

In 4 of 7 studies included in our review, ITT analysis was not conducted[26,29] or was not described or unclear.[24,25] In the other 3 studies,[16,27,28] it was not applicable.

Discussion

Fifteen studies (14 reports) in which the effects of 2 pheromones (FFP and DAP) on canine and feline behavior were reviewed systematically. Before recommendations regarding pheromone use can be made, it is important to evaluate the results regarding clinical efficacy for internal and external validity. Several types of biases may affect the internal validity of the studies in the analysis reported here. Selection bias results from inappropriate randomization schemes, whereas detection bias is the result of a lack of a blinded design. Selection bias can also be related to the inclusion and exclusion criteria for study subjects.

The process of randomization is aimed at the generation of treatment groups comparable with regard to known or unknown confounding factors.[30] The effectiveness of randomization depends upon the generation of allocation sequences by truly random methods[13] and from the adequate concealment of treatment sequences.[14,31] Within the present systematic review, only 1 report[22] included a description of the allocation concealment implementation process. All others omitted this information and were therefore ranked as B-level evidence studies. Therefore, there was 1 true RCT[22] and 7 blinded, controlled studies[16,19,21,23,27–29] that were lacking clear randomization. The remaining 7 studies were case series,[17,18,20,23–26] which are typically of minimal value to assess treatment effects. Performance bias (eg, 1 group of dogs treated preferentially with medications in addition to that being evaluated) was detected in some of the assessed trials. Attrition bias can arise from deviations from study protocols or loss to follow-up. Possible reasons for deviation from protocols include violation of eligibility criteria and nonadherence to treatment. In most studies assessed in the present review, deviation from protocols and nonadherence to treatment usually were not addressed comprehensively. Detection bias also likely existed in that 7 of the 15 studies[17,18,20,23–26] evaluated here were unmasked (ie, the 7 case-series studies).

Loss to follow-up refers to the unavailability of study subjects because of refusal to continue participating (eg, dropouts), loss of contact with researchers, or development of adverse drug effects or medical issues that justify participant withdrawal for health concerns. None of the studies included an evaluation of owner compliance with the study protocol. Intention-to-treat analysis was not conducted, not specifically reported, or not applicable. Attrition of enrolled feline subjects totaled 37, with 34 belonging to 3 studies[17,19,20] of urine spraying. Therefore, we believe attrition was a likely source of bias in this review of pheromone treatment of urine marking in cats. Attrition of enrolled canine subjects totaled 3 in 2 studies,[26,29] was not reported or unclear in 2 studies,[24,25] and was null in 3 studies.[16,27,28] Therefore, we believe that attrition was an unlikely source of bias in the review of pheromone treatment in dogs.

External validity relates to subject selection, nature and duration of administered treatments, and assessed modalities of outcome. Several studies[23,24,26,27] (1 feline case series and 3 canine studies) discussed in this review involved enrollment of subjects with poorly defined or vague inclusion criteria. Duration of treatment was variable, dosage varied between studies and within the same study, and in some situations, treatment effect was confounded by other elements such as environmental changes between groups, additional drugs, or behavior modification recommendations. Assessment of treatment efficacy in many studies relied entirely or heavily on an owner's subjective assessment of their pet's behavior (5 feline and 5 canine studies).

All animals were client owned with 1 exception (shelter dogs),[16] which may have impacted the ability of the researchers to standardize collection of outcome data. Several factors, such as vague inclusion criteria,

short treatment duration, and limited outcome evaluation, may have collectively weakened the external validity of the studies in this review.

In systematic reviews themselves, selection bias can occur because of the nature of studies selected and the language in which they are reported; publication bias can result because of the variable quality of result reporting. In the present review, we limited ourselves to reports published in peer-reviewed journals. We also excluded 2 reports published by the manufacturer of the pheromone products as well as abstracts or lecture notes from conference proceedings. Trials in which an intervention is found to be ineffective are likely not to be published; therefore, the results reported here may overestimate the true treatment effect.

On the other hand, review of abstracts does not always yield sufficient information on study design and treatment outcomes (benefit vs harm). All published reports that met our inclusion criteria were in English. Although some studies reported in other languages may have been missed, we believe that is unlikely, given our search of medical and veterinary databases as well as bibliographies of publications and book chapters. Additional searches were performed to locate any potential relevant studies reported in French, English, and Italian by contacting distributors of the pheromone products. Reporting of some studies was not of high quality with respect to information on study design, enrollment of subjects, and attrition rate, and outcome measures were insufficiently described. Therefore, there may have been some bias (selection and publication) involved in our review; but we believe that if such bias was present, then it was weak and should not prevent treatment decisions based on interpretation of this review's results.

The chemical structures of FFP and DAP have yet to be reported. Fourteen chemical compounds from diverse biological sources elicit a unique pattern of behavior in most felids.[8] Actinidine is purportedly the chemical basis for the attraction of some cats to valerian roots.[8] The pharmacological effects of *V officinalis* extract and valerenic acid appear to be mediated through modulation of $GABA_A$ receptor function.[32] Experiments have shown that *V officinalis* extract, principally valerenic acid, increases the availability of GABA by inhibiting its reuptake by as much as 50%, decreasing its degradation and increasing its release by induction of Ca^{2+} channels.[33] The chief inhibitory neurotransmitter in the mammalian CNS, GABA, plays an important role in regulating neuronal excitability throughout the nervous system. It is also directly responsible for the regulation of muscle tone. We speculate that the treatment effects (attractant and anxiolytic) associated with synthetic FFP are, in fact, a consequence of the *V officinalis* extract.

Whereas it is widely recognized that blinded, placebo-controlled RCTs provide the best available evidence, such trials are often demanding in terms of cost, manpower, and subject management.[34] In addition, they can raise ethical dilemmas, depending on the clinical problem and treatments evaluated. In situations of ethical dilemma, a cohort study with minimal bias can yield convincing results, even though such studies are not as reliable as RCTs because there is theoretically more bias associated with non-RCT studies.[15] Because of financial constraints in veterinary medicine and difficulties associated with RCTs, other trial designs are often used to address clinical questions. As Keene[34] said, "We cannot afford to exclude or ignore evidence from easier to perform, nonrandomized (historically controlled) or even uncontrolled (case-series) trials. However, we can also not afford to uncritically embrace the results of such studies." To this end, additional studies on the efficacy of pheromones in the treatment of behavioral problems in cats and dogs are warranted.

From the information obtained through this systematic review, several points can be made regarding the efficacy of FFP for treatment of undesirable behavior in cats. Success rates for treatment of urine-spraying cats with FFP may have been overestimated because dropouts were not included in the statistical analyses and may have been a consequence of treatment failure. Interestingly, 1 distributor of FFP advertises a 95% success rate for treatment of urine spraying in cats when used as directed.[35] It should also be considered that complete cessation of urine spraying occurs in fewer cats than the publicized success rates because those rates include both decrease and cessation of urine spraying.

With regard to efficacy in the treatment of idiopathic cystitis in cats, the 1 placebo-controlled study[21] (grade B with unclear allocation generation and concealment of allocation sequences) yielded insufficient evidence to support use of FFP to manage feline idiopathic cystitis. In the report[23] of 2 studies in cats (1 grade B study with inadequate allocation generation and concealment of allocation sequences and 1 case series), there was lack of support of efficacy of FFP and therefore fair evidence against the use of this treatment for hospitalized cats. Regarding efficacy in facilitating catheterization procedures, the 1 RCT[22] on the subject yielded insufficient evidence that FFP helps to calm cats in unfamiliar surroundings; however, there may have been interaction of the pheromone with the acepromazine used for sedation in half of the cats.

Two case series[24,25] provided insufficient evidence that DAP is effective for treatment of fear of fireworks in dogs. In 1 study,[25] effects of behavioral modification and pheromones could not be distinguished. In the other,[24] the effects of medication, behavioral modification, and pheromones could not be distinguished from each other.

One placebo-controlled study[27] with inadequate generation of allocation of sequences, unclear allocation concealment, and poorly defined inclusion criteria for dogs yielded insufficient evidence that DAP is effective for reducing signs of anxiety in dogs at the veterinary clinic. The 1 placebo-controlled study[16] (with unclear generation of allocation and concealment of allocation sequences) provided insufficient evidence that DAP is effective for reducing stress- and fear-related behavior in shelter dogs. During the friendly stranger test, treatment with DAP reportedly significantly decreased barking frequency, compared with the pretreatment frequency but it is not clear what happened in the control group. Many behavioral categories typically associated with stress in dogs were unchanged in response to DAP during the neutral- and friendly stranger tests.

With regard to efficacy of DAP in alleviating disturbance (primarily vocalizing and scratching at the door) and house soiling during the night of recently adopted puppies, 1 placebo-controlled study[28] (grade B study with inadequate generation of allocation and unclear concealment of allocation sequences) did yield evidence against use of DAP for soiling, as no significant effect of treatment was demonstrated for soiling. Sleeping with another dog at night reduced the puppy's tendency to disturb at night. Thus, the study provided insufficient evidence for the use of DAP for treatment of disturbance during the night of recently adopted puppies.

One case series[26] with poorly defined inclusion criteria provided insufficient evidence for the use of DAP for travel-related problems in dogs. It did not differentiate between behavioral modification effect and pheromone effect. However, 1 placebo-controlled study[29] (with inadequate allocation generation and unclear concealment of allocation sequences) yielded some evidence that DAP reduces fear or anxiety of puppies during training, resulting in better socialization.

a. Feliway, CEVA Santé Animale, Libourne, France.
b. DAP, CEVA Santé Animale, Libourne, France.

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