Effect of a synthetic appeasing pheromone on behavioral, neuroendocrine, immune, and acute-phase perioperative stress responses in dogs

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Objective—To study the effects of a synthetic, dog-appeasing pheromone (sDAP) on the behavioral, neuroendocrine, immune, and acute-phase perioperative stress responses in dogs undergoing elective orchiectomy or ovariohysterectomy.

Design—Randomized, controlled clinical trial.

Animals—46 dogs housed in animal shelters and undergoing elective orchiectomy or ovariohysterectomy.

Procedures—Intensive care unit cages were sprayed with sDAP solution or sham treated with the carrier used in the solution 20 minutes prior to use. Dogs (n = 24 and 22 in the sDAP and sham treatment exposure groups, respectively) were placed in treated cages for 30 minutes before and after surgery. Indicators of stress (ie, alterations in behavioral, neuroendocrine, immune, and acute-phase responses) were evaluated perioperatively. Behavioral response variables, salivary cortisol concentration, WBC count, and serum concentrations of glucose, prolactin, haptoglobin, and C-reactive protein were analyzed.

Results—Behavioral response variables and serum prolactin concentration were influenced by sDAP exposure. Dogs exposed to sDAP were more likely to have alertness and visual exploration behaviors after surgery than were dogs exposed to sham treatment. Decreases in serum prolactin concentrations in response to perioperative stress were significantly smaller in dogs exposed to sDAP, compared with findings in dogs exposed to the sham treatment. Variables examined to evaluate the hypothalamic-pituitary-adrenal axis, immune system, and acute-phase responses were unaffected by treatment.

Conclusions and Clinical Relevance—sDAP appeared to affect behavioral and neuroendocrine perioperative stress responses by modification of lactotropic axis activity. Use of sDAP in a clinical setting may improve the recovery and welfare of dogs undergoing surgery. (*J Am Vet Med Assoc* 2010;237:673–681)

The perioperative stress response is a physiologic reaction to surgery and various associated conditions (eg, pain, analgesia- and anesthesia-induced dysphoria, human handling, and confinement to a hospital cage) that may be perceived as threatening by an animal.[1–6]

The activation of behavioral, neuroendocrine, immune, and acute-phase responses attributed to stress in dogs undergoing elective orchiectomy or ovariohysterectomy has been described in other reports.[1,2,6] A high incidence of communicative and explorative behaviors was detected prior to surgery, and incidence of the same behaviors was substantially decreased after surgery. Decreased physical activity and interactive behaviors, as well as changes in sleeping and waking patterns, were also observed during the immediate postoperative period after recovery from anesthesia. Salivary cortisol concentration proved to be a useful indicator for assessment of preoperative and postoperative stress, but WBC counts and serum concentrations of glucose, prolactin, and acute-phase proteins varied substantially after surgery. Stress-induced cortisol and glucose responses in those studies[1,2,6] suggested activation of the HPA axis, whereas detection of decreased serum prolactin concentrations indicated a potential dopamine suppression of the lactotropic axis. Increased neutrophil and monocyte counts in the blood and changes in circulating concentrations of proteins involved in the acute-phase response were reliable markers of inflammation,

Abbreviations

CRP	C-reactive protein
GPS	Glasgow pain scale
HPA	Hypothalamic-pituitary-adrenal
ICU	Intensive care unit
sDAP	Synthetic dog-appeasing pheromone

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but decreased lymphocyte and eosinophil counts were more sensitive indicators of early postoperative psychological stress and pain.

A synthetic pheromone (ie, sDAP[a]) is commercially marketed for the treatment of stress in dogs; it is a mixture of fatty acids that represent components of a natural pheromone identified in sebaceous gland secretions from the intermammary sulcus of lactating bitches. These secretions have been isolated during a period beginning 3 to 4 days after parturition and ending 2 to 5 days after weaning.[7] Although the mechanism of action of the natural pheromone is not well understood,[7] sDAP treatments have been described as having a calming effect on dogs in stressful situations, such as veterinary clinical consultation or kenneling, and as having a positive effect on behavioral problems, such as separation-related anxiety, fear associated with fireworks, and house soiling.[8–14]

The principal objective of the study reported here was to evaluate the effect of sDAP exposure on the perioperative stress response in dogs undergoing elective orchiectomy or ovariohysterectomy. Changes in behavior; changes in total WBC count, differential WBC count, and neutrophil-to-lymphocyte count ratio; and changes in concentrations of salivary cortisol, serum glucose, serum prolactin, serum haptoglobin, and serum CRP were analyzed in dogs exposed to sDAP and compared with findings in unexposed dogs. We hypothesized that sDAP would have an ameliorating effect on the multifactorial perioperative stress response.

Materials and Methods

Animals—Forty-six adult dogs (23 females and 23 males; mean ± SEM age, 29.1 ± 3.1 months; weight, 20.7 ± 1.2 kg [45.54 ± 2.64 lb]) underwent elective orchiectomy or ovariohysterectomy. Both purebred and mixed-breed dogs were included in the study; dogs < 6 months or > 8 years old were excluded. All dogs had been housed in a public animal shelter for ≥ 20 days, in a 6-m^2 pen (ie, the dogs' usual environment at the shelter) together with 1 or 2 other dogs, according to local bylaws.

Dogs were considered healthy on the basis of results of a physical examination, CBC, and serum biochemical analysis as well as seronegativity for antibodies against *Leishmania* spp (evaluated via a semiquantitative ELISA[b]). The reproductive status of each female was determined by use of an ELISA kit for progesterone,[c] and pregnant, lactating, or pseudopregnant females with serum progesterone values of 0 to 1 ng/mL were excluded from the study. Additionally, dogs that had stereotypical behavior (eg, pacing, circling, or tail chasing) or aggression toward humans were not included in the study. Procedures used in the study reported here were in compliance with the European Communities Council Directive of November 24, 1986 (Protection of animals used for experimental and scientific purposes; 86/609/EEC), and the study was approved by the Ethics Committee of the Autonomous University of Barcelona, Barcelona, Spain.

Experimental design—The study was performed between March and June, when the environmental temperature of the region was expected to be mostly constant (according to forecast databases). Mean ± SEM environmental temperature was 21.6 ± 0.4°C (70.9 ± 0.7°F) during the period in which the study was carried out.[15]

Dogs were randomly assigned to the sDAP or sham treatment exposure group. Fifty spray bottles were prepared for the study; 25 contained the sDAP (ie, a 2% solution of fatty acid methyl esters in ethanol),[a,d] and 25 contained the sham treatment (ie, the ethanol carrier solution only).[d] Each bottle was labeled with an identification code. A random code list was created by the manufacturer of the pheromone and sham treatments; the researchers and study supervisor were not provided with the code key until statistical analysis had been completed.

For each dog, 1 spray bottle was randomly selected and used to spray the 1.1 × 0.7 × 0.7-m cage in the ICU (ie, ICU cage) where the dog would be placed. Each bottle was only used for 1 dog. Prior to each use, the cage was thoroughly cleaned with a detergent containing a nonionic fraction[e] to control environmental contamination with natural pheromones. The cage floor was covered with an absorbent cotton towel, and its corners were sprayed according to the manufacturer's instructions (ie, with 10 pumps of the spray dispenser) 20 minutes prior to placing the dog in the cage before and after surgery. Thus, each dog was exposed to sDAP or the sham treatment for 30 minutes before and after surgery. No more than 2 dogs were confined in ICU cages at any time point to minimize the variability of environmental influences on the dogs' behavior.

Experiments began at the shelter between 10 AM and 11 AM on the day of surgery, when each dog's behavior was video recorded for 30 minutes to obtain behavioral category data in its usual environment; this was designated as the T0 time point for behavioral evaluation. After the video recording, blood and saliva samples were obtained from each dog in its usual environment; this was designated as the T0 time point for sample collection. Results for samples obtained at T0 were accepted as basal values.

Each dog was then walked to the ICU located in the same facility and placed in the prepared ICU cage, where behavior was video recorded for 30 minutes; then, a standardized dynamic interaction test for pain evaluation was performed and video recorded. After the interaction test, blood and saliva samples were obtained. The time points of behavioral recording and sample collection in the ICU prior to surgery were designated as T1. The dog was subsequently transferred to surgery.

After surgery, each dog was transferred to the prepared ICU cage, and the degree of sedation was assessed every 30 minutes. When the dog was able to stand in the ICU cage (ie, end of sedation), its behavior was video recorded for 30 minutes, after which a dynamic interaction test for pain evaluation was performed and video recorded. At the end of the pain evaluation test, blood and saliva samples were collected. The time points of behavioral recording and sample collection in the ICU after surgery were designated as T2.

Each dog was returned to its usual environment and behavior was video recorded for an additional 30 minutes at that location. At the end of the day, a saliva sample was obtained; to reduce the risk of complications after surgery, no blood was collected at this time. The time points of behavioral recording after the dog was returned to its usual environment and saliva collection at the end of the day were designated as T3. Additional blood samples were obtained at 24 hours (designated as T4), 48 hours (T5), and 8 days (T6) after surgery.

Surgical procedures—All surgeries were performed by 1 surgeon (MA) assisted by various veterinary undergraduates. Food was withheld from all dogs for ≥ 18 hours prior to surgery. A standard anesthetic and analgesic protocol was used for all surgical procedures. Each dog was premedicated with morphine (0.1 mg/kg [0.045 mg/lb], IM) and medetomidine (0.05 mg/kg [0.023 mg/lb], IM). Anesthesia was induced with thiobarbital (10 mg/kg [4.55 mg/lb], IV) and diazepam (0.5 mg/kg [0.23 mg/lb], IV) and maintained with 1% to 2% isofluorane vaporized in 0.5 to 1 L of 100% oxygen/min delivered via a semidisposable circle circuit. The vaporizer setting was adjusted to maintain a surgical plane of anesthesia, which was determined by evaluation of eye position, jaw tone, and lack of response to noxious stimuli. All dogs received a constant-rate infusion of lactated Ringer's solution (5 to 10 mL/kg/h [2.27 to 4.55 mL/lb/h], IV). Surgery was considered to end with extubation of the dog. After surgery, treatments with amoxicillin[f] (15 mg/kg [6.82 mg/lb], SC, q 48 h) and meloxicam[g] (0.2 mg/kg [0.09 mg/lb], SC, q 24 h) were initiated for 10 and 5 days, respectively.

Behavioral data collection—All behavioral data were recorded with a digital video camera.[h] All video recordings were viewed and analyzed on a 21-inch monitor[i] by 1 observer (CS).

Behavioral data were divided into categories and evaluated for frequency of behavioral events and duration of behavioral states (**Appendix**). Observations were recorded on prepared forms. Categories evaluated as events were logged by continuous recording, and the number of occurrences of each event during the observation period was analyzed. Behavioral events were recorded as having been detected once every 5 seconds if the behavior appeared to be continuous.[16] Categories evaluated as states were logged by instantaneous sampling at 2-minute intervals (ie, 15 instantaneous recording points in 30 minutes).[17] Although many behaviors were analyzed in the usual environment and in the ICU cage, it was not possible to perform all assessments in both locations, primarily because of environmental variability between the 2 settings that influenced the accuracy of video recording (eg, dogs housed individually or in groups, accessibility of hiding places, and distance of the video camera from the dogs).

Dynamic interaction test for pain evaluation—To minimize the effects of behavioral variability among dogs and eliminate problems with interobserver accuracy, 1 ethologist (CS) performed and recorded the results of all tests, as well as later analysis and assessment. This investigator was familiar with the behavior of the individual dogs included in the study.

The interaction test was performed as follows: the investigator knocked at the door of the ICU, opened it, and entered. The investigator reached for the cage, opened the door, and spoke to the dog with a gentle voice. If a dog was reluctant to approach the investigator and leave the cage, it was gently lifted out. Each dog was then gently stroked from the chest to the flank and up to the ventral surgery site.

Assessment of postoperative pain was based on the interactive behavior of dogs during the test (ie, response to handling by an investigator) and was analyzed by use of a GPS.[18,19] Investigators in our group had determined that the frequency of events observed among GPS behavioral categories was a most sensitive marker of low-intensity pain, compared with GPS score, in an earlier study.[6] The GPS behavioral categories determined for each dog enrolled in the present study were recorded. The frequency of observation for behaviors in each category before and after surgery and the corresponding total scores were analyzed qualitatively and quantitatively.

Saliva and blood sample collection—Saliva samples were collected by use of a commercially available saliva test kit[j] after stimulation of salivation via oral administration of 1 mL of a 3% citric acid solution in water.[20,21] Saliva sample collection always preceded blood sample collection; for each procedure, dogs were not handled for > 2 minutes to avoid any influence of handling on variables used to evaluate stress.[22] Swabs were used to obtain the saliva samples, and then placed in tubes provided with the kit. Tubes were kept at 4°C during transport to the laboratory. Saliva samples were then centrifuged at 2,000 × *g* for 15 minutes and stored at –80°C.

Blood samples (4 mL collected at each predetermined time point) were obtained via jugular venipuncture. One milliliter of the sample was stored in an EDTA-treated tube,[k] and 3 mL was transferred to a tube containing a coagulation activator.[l] The samples were kept at 4°C during transport to the laboratory. After clot formation, the tubes were centrifuged at 2,000 × *g* for 15 minutes. The serum obtained was transferred to 1.5-mL polypropylene tubes[m] and stored at –80°C.

Sample analysis—Saliva cortisol concentration was determined by use of a commercially available ELISA kit[n] for human salivary cortisol, which had been adapted in our laboratory to measure cortisol concentrations in canine saliva (data not shown).

The EDTA-treated blood samples were analyzed by use of a laser flow cytometer[o] within 6 hours after collection (at T0 to T2 and T4 to T6) to obtain total WBC and differential cell counts (neutrophils, monocytes, lymphocytes, and eosinophils).

Glucose concentrations in serum (obtained at T0 to T2) were detected via a standard hexokinase assay performed by use of an autoanalyzer[p] according to the manufacturer's instructions. Serum samples were additionally analyzed to measure prolactin (at T0 to T2), haptoglobin (at T0 and T4 to T6), and CRP concentrations (at T0 and T4 to T6). Serum prolactin concentrations were measured by use of a commercial ELISA kit,[q] haptoglobin concentrations were determined by use of an automated biochemical assay,[r] and CRP concentrations were measured by use of a canine CRP-specific, solid-phase sandwich immunoassay,[s] according to each manufacturer's instructions.

Immune and acute-phase response markers were evaluated on a long-term basis (ie, at 24 hours, 48 hours, and 8 days after surgery [T4, T5, and T6, respectively]) because long-term changes were expected in WBC counts and acute-phase protein concentrations on the basis of results of other studies.[6,23] Because changes in circulating glucose, cortisol, and prolactin concentrations in response to stress are known to be rapid,[24] evaluation of these markers was limited to the day of surgery to avoid influence of uncontrolled psychological stressors on these responses at 24 hours, 48 hours, and 8 days after surgery.

Data and statistical analysis—All results are reported as mean ± SEM. The normal distribution of data was determined with a Shapiro-Wilk test. Data were considered to be normally distributed when tests revealed a value of $P > 0.05$. Differences in sex of dogs assigned to sDAP and sham treatment exposure groups were analyzed by use of a χ^2 test, and differences in age and weight were analyzed by use of a Student *t* test. A Mann-Whitney *U* test was used to analyze differences in environmental temperature recorded on the days when video recordings and samples were obtained from dogs in either group, as well as differences in duration of surgery and duration of sedation (ie, time from the end of surgery until the dog was able to stand) between the 2 groups.

For behavioral data, intraobserver reliability was determined by analysis of the correlation between 2 different observations of the same video recording. Nine independent, 10-minute excerpts from recordings of 9 dogs (1 excerpt/dog) were used to calculate the Spearman rank correlation coefficient for the following randomly selected behavioral categories: nosing, lip licking, mouth opening, visual exploration, and alertness.[17] A commercially available statistical software package[t] was used for calculations.

Changes in behavioral categories that could be assessed in the usual environment and in the ICU cage were analyzed for each behavioral observation period (ie, from T0 to T3); for the other categories, comparisons were limited to the time points at which samples were collected in either environment (ie, T0 vs T3 for behaviors recorded only in the usual environment and T1 vs T2 for behaviors recorded only in the ICU cage). To determine whether sDAP influenced perioperative behavioral changes, the effect of sDAP exposure on the changes in behavioral categories over time, frequency of events for GPS behaviors, and GPS scores was analyzed by use of a generalized linear model for repeated measures.[u] A Bonferroni correction was applied to the level of significance for pairwise comparison of time points.

Hematologic and biochemical data that were not normally distributed were logarithmically transformed to achieve normality. The effect of sDAP exposure on the response of variables over time was analyzed via ANOVA for repeated measures.[v] For those variables significantly affected by the type of treatment, the interactions among treatment and sex, as well as differences between basal values and values obtained at later time points, were analyzed by post hoc pairwise comparisons. Univariate *F* statistics were corrected with Huynh-Feldt adjusted degrees of freedom when data sets deviated from the sphericity assumption. A Bonferroni correction was applied to the level of significance for pairwise comparison of the time points evaluated. Values of $P \leq 0.05$ were accepted as significant for all statistical tests.

Results

Twenty-four dogs were exposed to the sDAP treatment, and 22 were exposed to the sham treatment. The dogs in the sDAP and sham treatment exposure groups did not differ in regard to sex (12 males and 12 females, and 11 males and 11 females, respectively; $P = 1.000$), age (28.5 ± 4.1 months and 29.8 ± 4.9 months, respectively; $P = 0.968$), or weight (20.8 ± 1.4 kg [45.76 ± 3.08 lb] and 20.5 ± 2.1 kg [45.1 ± 4.62 lb], respectively; $P = 0.917$). The mean environmental temperature of days during which the sDAP exposure group was evaluated (21.6 ± 0.6°C [70.9 ± 1.1°F]) did not differ significantly ($P = 0.858$) from that of days when the sham treatment exposure group was evaluated (21.5 ± 0.5°C [70.7 ± 1.0°F]). Mean duration of surgery was 40.6 ± 2.7 minutes and 35.9 ± 2.4 minutes, and mean duration of sedation was 72.5 ± 8.4 minutes and 65.4 ± 6.1 minutes for dogs in the sDAP and sham treatment exposure groups, respectively. Duration of surgery and sedation did not differ significantly ($P = 0.307$ and 0.784, respectively) between the 2 groups.

Behavioral data—Intraobserver reliability, expressed by use of a Spearman rank correlation coefficient, was 0.95 for nosing, 1.00 for lip licking, 0.96 for mouth opening, 0.93 for visual exploration, and 1.00 for alertness ($P < 0.05$ for all categories). Among behavioral categories, changes in visual exploration behavior with time and changes in alertness were significantly ($P = 0.012$ and 0.020, respectively) influenced by exposure to sDAP. Dogs exposed to sDAP had a smaller decrease of these behaviors after surgery (at T2), compared with the decrease detected in dogs exposed to the sham treatment **(Figures 1 and 2)**. Males and females did not differ significantly ($P = 0.065$) for visual exploration or alertness ($P = 0.938$). The other behaviors evaluated were not significantly influenced by the type of treatment.

A significant ($P = 0.000$) effect of time (ie, between preoperative and postoperative results) was detected for GPS scores (overall score for dogs in both groups, 1.3 ± 0.2 at T1 vs 3.2 ± 0.5 at T2). Exposure to sDAP did not significantly ($P = 0.509$) affect the changes in GPS scores attributed to surgery (1.6 ± 0.2 at T1 vs 2.7 ± 0.3 at T2 for the sDAP exposure group; 1.0 ± 0.2 at T1 vs 3.6 ± 1.1 at T2 for the sham treatment exposure group). Similarly, exposure to sDAP had no significant ($P > 0.05$) influence on the perioperative changes of any of the GPS behavioral categories.

Hematologic and biochemical data—Logarithmically transformed data were recorded as geometric means and plotted on a semilogarithmic graph to clearly illustrate differences in the changes detected among time points between dogs exposed to sDAP and those exposed to the sham treatment. Exposure to sDAP did

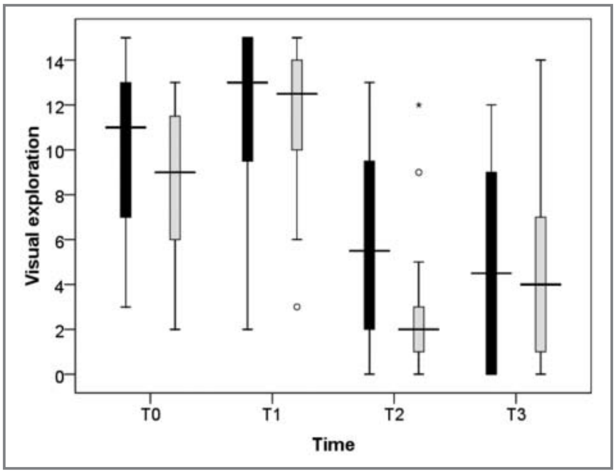

Figure 1—Box-and-whisker plots of the frequency of perioperative visual exploration behavior for healthy dogs housed in an animal shelter that were undergoing elective orchiectomy or ovariohysterectomy and that were (n = 24; black boxes) or were not (22; gray boxes) exposed to sDAP before and after surgery. Each dog's behavior was video recorded for 30-minute intervals at 4 time points in 2 locations: in the dog's usual environment prior to surgery (T0), after the dog was placed in an ICU cage that was sprayed with sDAP solution or sham treated with the ethanol carrier without sDAP prior to surgery (T1), in the sprayed ICU cage after recovery from surgery (T2), and after being returned to its usual environment (T3). The values on the y-axis represent the number of instantaneous samplings at 2-minute intervals per 30-minute evaluation period. For each box, the horizontal line represents the median value for the frequency of observed behaviors and the upper and lower boundaries represent the 75th and 25th percentiles, respectively. Whiskers represent the maximum and minimum data values. Extreme outliers (ie, values > 3 interquartile ranges [asterisk]) and outliers (ie, values between 1.5 and 3 interquartile ranges [white circles]) are indicated. The change in observed frequency of visual exploration behavior at T2, compared with values at T0, was significantly different ($P \leq 0.05$) between dogs exposed to sDAP and dogs exposed to the sham treatment.

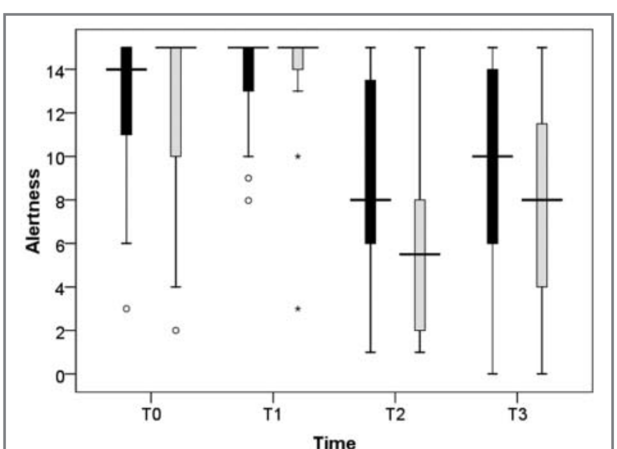

Figure 2—Box-and-whisker plots of the frequency of perioperative alertness behaviors for dogs in Figure 1 that were (n = 24; black boxes) or were not (22; gray boxes) exposed to sDAP before and after surgery. The change in alertness over time was significantly ($P \leq 0.05$) different between dogs exposed to sDAP and dogs exposed to the sham treatment. *See* Figure 1 for key.

not significantly ($P = 0.363$) affect the differences between preoperative and postoperative serum cortisol concentrations; changes in glucose also did not differ significantly ($P = 0.384$) with sDAP exposure. By contrast, the change in serum prolactin concentration with time was significantly ($P = 0.046$) affected by sDAP exposure **(Figure 3)**. Serum prolactin concentration decreased significantly ($P = 0.007$) after surgery, compared with the basal value at T0, for dogs in the sham treatment exposure group; however, the postoperative decrease was not significant for dogs in the sDAP exposure group. No significant ($P = 0.517$) difference in serum prolactin concentration was detected between male and female dogs after sDAP exposure. Total WBC, neutrophil, and lymphocyte counts and the neutrophil-to-lymphocyte count ratio were not significantly ($P = 0.762$, 0.603, 0.888, and 0.532, respectively) influenced by exposure to sDAP. Additionally, CRP and haptoglobin concentrations were not significantly ($P = 0.560$ and 0.760, respectively) affected by exposure to sDAP.

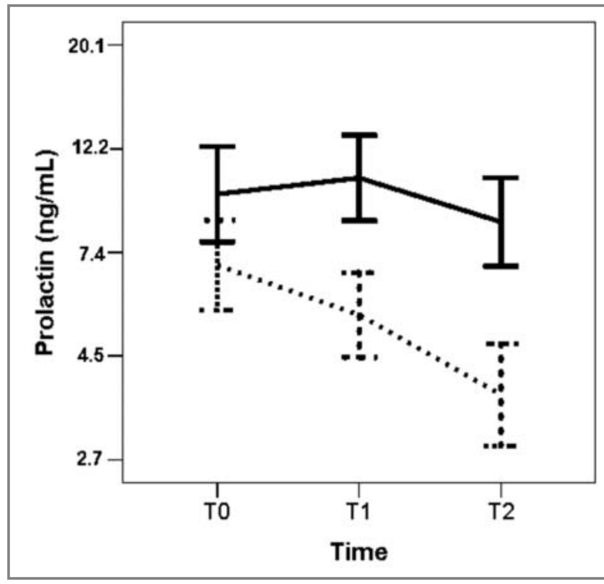

Figure 3—Geometric mean ± SEM perioperative serum prolactin concentrations in the dogs in Figures 1 and 2 that were (n = 24; solid line) or were not (22; dotted line) exposed to sDAP before and after surgery. Serum was obtained from blood samples collected in the dog's usual environment prior to surgery (T0), 30 minutes after the dog was placed in an ICU cage sprayed with sDAP solution or the sham treatment prior to surgery (T1), and after a dynamic interaction test for pain evaluation that was performed 30 minutes after the dog recovered from sedation in the sprayed ICU cage (T2). The change in prolactin concentration over time was significantly ($P \leq 0.05$) different between dogs exposed to sDAP and dogs exposed to the sham treatment.

Discussion

Results of the study reported here suggest that sDAP modifies the perioperative stress response in dogs undergoing elective surgery. Nevertheless, sensitivity to sDAP varied greatly among the evaluated components of the stress response. Alertness and visual exploration behaviors were positively influenced by exposure to sDAP. Among neuroendocrine, immune, and acute-phase stress responses in dogs, only the lactotropic axis, a component of the neuroendocrine response, was sensitive to the type of treatment. In many mammalian species, a relationship has been determined between pheromones and the neuroendocrine response,[25] but to our knowledge, results of the present study revealed the first evidence of an interaction between pheromones and the endocrine system in dogs.

Among behavioral responses, visual exploration and alertness were significantly affected by exposure to sDAP. Dogs exposed to the sham treatment had large postoperative (T2) decreases in the duration of these behaviors for both categories, compared with smaller decreases in duration detected for dogs exposed to sDAP. These results suggested that exposure to sDAP increased postoperative alertness and visual exploration behaviors in dogs. Investigators in other studies[2,6] determined that alertness and visual exploration (also called scanning) behaviors were among the most sensitive behavioral variables for detection of postoperative stress in dogs when pain was a major component. However, the interaction test for pain evaluation performed in the present study did not detect any influence of sDAP on pain perception, based on analysis of GPS scores and frequency of events observed among GPS behavioral categories. It is possible that the GPS was not sensitive enough to detect minor changes in behavior, compared with the sensitivity of visual exploration and alertness behavioral categories. The difference in sensitivity could be attributable to the observational method on which GPS scoring is based. A 1-or-0 recording method was used to collect GPS behavioral data according to the instructions provided with the scale,[18,19] and all other behavioral data evaluated in the present study were collected by continuous observation, which is more accurate than the 1-or-0 method.[16]

Exposure to sDAP did not have a significant effect on the differences between preoperative and postoperative serum cortisol concentrations in the present study; differences between preoperative and postoperative glucose concentrations were similarly unaffected. These findings could result from a possible lack of influence of sDAP on the HPA axis. The sensitivity of serum prolactin concentrations to sDAP exposure suggested that the synthetic pheromone modified the lactotropic response to perioperative stress. The involvement of the lactotropic axis in the stress response is a consistent observation because prolactin values have been used for stress assessment in many mammalian species,[24] but few studies have been published regarding the prolactin response to stress in dogs.

Prolactin is involved in the emotional responses of dogs, and circulating concentrations increase during positive interaction with humans.[26,27] Dogs with generalized anxiety have hyperprolactinemia, but dogs with phobias or mild anxiety do not.[7,28] Increased prolactin concentrations after surgery in humans have been reported[29]; in contrast, a perioperative decrease in serum prolactin values in dogs undergoing elective surgery has been revealed.[6] In the latter study,[6] a slight decrease in values prior to surgery was caused by psychological stressors (eg, handling and confinement), but a greater decrease after surgery was attributed to the major influence of postoperative pain and anesthesia-induced dysphoria.

In the present study, a similar decrease was observed in serum prolactin concentrations of dogs exposed to the sham treatment but a significantly smaller decrease was detected in prolactin concentrations for dogs exposed to sDAP. Decreases in prolactin concentrations of dogs exposed to the sham treatment were detectable as early as 30 minutes after moving a dog from its usual environment to the ICU cage (T1) and persisted after surgery (T2). In contrast, prolactin concentrations of dogs exposed to sDAP treatment increased slightly after confinement in the ICU cage before surgery and decreased after surgery. These variations indicated that exposure to sDAP primarily influenced the psychological stress response caused by unaccustomed handling and confinement because ameliorating effects were most prevalent at the end of the initial stay in the ICU cage prior to surgery. After surgery, when pain and dysphoria were added stressors, exposure to sDAP did not prevent a mild (although nonsignificant) decrease in prolactin concentrations.

Because the pharmacological mechanisms of natural and sDAPs are still unknown, the reason that the effects revealed in the present study were only associated with the lactotropic axis is not readily apparent. The secretion of prolactin by the pituitary gland is used as a marker for lactotropic axis activation; it is regulated by the suppressive effect of dopamine produced by the hypothalamus and stimulatory effects of thyrotropin-releasing hormone, neurophysin, neuropeptide substance P, and other factors.[24] Thus, sDAP exposure could have a direct effect on prolactin concentrations or could regulate prolactin secretion indirectly via suppression of the dopaminergic system.

Prolactin has a direct influence on oxytocin secretion, and both hormones have been reported[25,30,31] to modulate the neuroendocrine acute stress response that influences maternal behavior. An increase in circulating serum prolactin and oxytocin concentrations had an anxiolytic effect in pregnant and lactating rats.[30,31] In turn, increased oxytocin concentrations in CSF and serum after parturition in sheep appeared to be triggered by stimulation of pheromones from the lamb and the amniotic fluid.[25] Thus, the natural pheromones, together with prolactin and oxytocin, could be involved in controlling the acute stress response after parturition, although their interaction is not well understood. A similar mechanism could be responsible for the smaller decrease in serum prolactin concentrations detected in dogs in the sDAP exposure group, compared with dogs in the sham treatment exposure group; however, the lack of significant interaction between sex of the dogs and change in serum prolactin concentrations among time points indicated that the response was not limited to females.

Perioperative changes in immune function and variation of CRP and haptoglobin serum concentrations have been reported[6,23,32] in dogs. The immune response (assessed via analysis of total WBC, neutrophil, and lymphocyte counts[33] and the neutrophil-to-lymphocyte count ratio) did not appear to be influenced by exposure to sDAP in the study reported here. The expected postoperative increase in neutrophils was not likely to be affected by sDAP treatment because of the major influence of inflammation at the surgical site. Conversely, a decrease in lymphocytes is reportedly a better indicator of perioperative psychological stress[6] and may potentially have been sensitive to the effects of sDAP, but this hypothesis was rejected after analysis of the results of the present study. The lack of a detectable effect of sDAP exposure on lymphocyte count in dogs in the present study was in agreement with the lack of sDAP influence on serum cortisol concentrations because the HPA axis is the principal regulatory system involved in the stress response of lymphocytes.[24,34]

Circulating concentrations of acute-phase proteins may also be increased in association with physical and psychological stress in humans, cattle, rats, and mice.[23,29,32] An increase in the hepatic synthesis of acute-phase proteins in response to cytokine-mediated HPA axis activation, with a consequent release of cortisol, has been proposed as the mechanism involved in the acute-phase response to stress.[32] A possible role of cortisol in the secretion of acute-phase proteins in response to stress would support our finding of a lack of detectable effect of sDAP exposure on both cortisol and acute-phase proteins. Moreover, the major influence of postoperative inflammation on CRP and haptoglobin concentrations, together with the long-term activation of this response,[6,23] makes it more difficult to detect what may be minor changes in indicators of psychological stress caused by a short-acting treatment such as sDAP exposure.[7] Through analysis of immune and acute-phase responses, we determined that exposure to sDAP had no influence on the postoperative inflammatory response in healthy dogs.

Overall, indicators of the perioperative stress response revealed in the present study were in agreement with results obtained in a preliminary study[6] of stress in dogs undergoing elective surgery. The changes detected in evaluated variables described in that study were similar to those in the study reported here, with the exception of cortisol concentrations; in the preliminary study,[6] the mean cortisol value peaked immediately after surgery but cortisol concentrations decreased to the basal value as early as 30 minutes after returning a dog to its usual environment. The rapid return to the basal values was not detected in the results of the study reported here. These findings were further confirmed by the GPS score; the postoperative increase of cortisol in dogs is related to the degree of perceived pain,[1,6] and the GPS is a good tool for assessment of moderate or severe pain.[6,18,19] In the preliminary study,[6] there was no significant difference between preoperative and postoperative GPS scores, and in the present study, the mean GPS score was significantly increased after surgery. This significant postoperative pain may explain the absence of a rapid postoperative decrease of cortisol observed in this study. Various forms of pain management can have different effects on pain perception measured by neuroendocrine or behavioral variables.[28,35] Buprenorphine, the analgesic treatment used in the preliminary study,[6] was more efficient than morphine in controlling the postoperative stress response (in which pain is a major activating stimulus) because it has a greater duration of effect than morphine.[36]

Dogs in the study reported here had been housed in an animal shelter for ≥ 20 days. Inclusion of dogs that experienced chronic stress because of long-term confinement[17,37] could have influenced the response to acute stress.[24] For example, the prolactin response to acute stress in dogs can be influenced by the release of dopamine caused by underlying chronic stress.[6,38] To minimize the confounding effect of chronic confinement, dogs that had overt behavioral signs of chronic stress (eg, stereotypical behaviors) were not included in the study reported here. The selection of dogs housed in the shelter for ≥ 20 days was based on the results of other studies,[39,40] which indicated that serum and urine cortisol values in dogs peaked within the first 17 days after relinquishment to a shelter and then declined steadily.

Although many studies[8–14] have revealed benefits associated with the use of sDAP to reduce stress in dogs, it is difficult to compare the results of the present study with those reported by other investigators because of the different methods used for stress assessment. Qualitative methods, mainly questionnaires submitted to owners, have been used to assess the efficacy of sDAP.[9–14] In these studies,[9–14] realized under a different variety of conditions (home environment, shelters, and veterinary clinics), the sDAP was applied to the environment via an electric diffuser. To our knowledge, the study reported here is the first to use multiple quantitative variables to analyze the effects of exposure to sDAP on behavioral, neuroendocrine, immune, and acute-phase perioperative stress responses in dogs.

Evidence detected in the present study supported the hypothesis that sDAP influences the perioperative stress response in dogs undergoing elective orchiectomy or ovariohysterectomy. In dogs in the study reported here, exposure to sDAP increased postoperative alertness and visual exploration behaviors and decreased the magnitude of suppression of the lactotropic axis caused by perioperative stress. However, the HPA axis, the immune response, and the acute-phase response were not affected by exposure to sDAP. These findings suggested that sDAP could be effective in controlling the perioperative stress response in dogs undergoing elective orchiectomy and ovariohysterectomy. Additionally, perioperative exposure to sDAP of bitches undergoing cesarean section might contribute to the maintenance of lactation. Although it has been reported that cesarean section (with or without concurrent ovariohysterectomy) should not affect the bitch's milk production if prolactin maintains lactation,[41] it is also known that agalactia may occur after cesarean section.[42] Considering the results of the present study, we speculate that bitches undergoing this procedure could have decreased milk production related to insufficient serum prolactin concentration. Thus, exposure to sDAP might favor the lactation of bitches after cesarean section via maintenance of unchanged serum prolactin values. Further studies are needed to confirm this hypothesis.

a. Dog Appeasing Pheromone, CEVA Sante Animale, Libourne Cedex, France.
b. Ingezim Leishmania Vet, Inmunologia y Genetica Aplicada SA, Madrid, Spain.
c. Target Canine Ovulation, BioMetallics, Princeton, NJ.
d. Provided by Pherosynthese Laboratories, Saint Saturnin les Apt, France.
e. Extran MA 01 Detergent, Merck KGaA, Darmstadt, Germany.
f. Bivamox LA, Boehringer Ingelheim España, Barcelona, Spain.
g. Metacam, Boehringer Ingelheim España, Barcelona, Spain.

h. Sony Handycam DCR-HF40, Sony Corp, Tokyo, Japan.
i. Sony Trinitron KV-21FV300, Sony Corp, Tokyo, Japan.
j. Salivette system, Sarstedt Co Ltd, Numbrecht, Germany.
k. EDTA 3K 1-mL microtube, Tapval Aquisel SL, Barcelona, Spain.
l. Clot activator and gel separator 5-mL tubes, Tapval Aquisel SL, Barcelona, Spain.
m. Safe-Lock 1.5-mL Tubes, Eppendorf AG, Hamburg, Germany.
n. Cortisol Saliva, BLK Diagnostics, Barcelona, Spain.
o. ADVIA 120 haematology system, Bayer, Fernwald, Germany.
p. Olympus AU400, Olympus, Hamburg, Germany.
q. Milenia Canine Prolactin, Milenia Biotec, Bad Nauheim, Germany.
r. Tridelta phase range serum haptoglobin, Tridelta Development, Wicklow, Ireland.
s. Tridelta phase range canine CRP kit, Tridelta Development, Wicklow, Ireland.
t. SPSS, version 15.0, SPSS Inc, Chicago, Ill.
u. GEE module SPSS, version 15.0, SPSS Inc, Chicago, Ill.
v. GLM module SPSS, version 15.0, SPSS Inc, Chicago, Ill.

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Appendix

Behavioral categories used to evaluate healthy dogs housed in an animal shelter that were undergoing elective orchiectomy or ovariohysterectomy and were or were not exposed to sDAP before and after surgery.

Category	Description	Time and location of assessment: Usual environment T0 and T3	Time and location of assessment: ICU cage T1 to T2
State			
Panting	Increased frequency of inhalation and exhalation often in combination with the opening of the mouth.		X
Visual exploration	Visual scanning or observation of the environment through cage or enclosure door.	X	X
Alertness	Eyes kept open.	X	X
Resting or sleeping	Eyes closed; dog inactive.	X	X
Trembling	Body shaking with small, high-frequency movements.		X
Walking	Movement from 1 point to another, with no clear effort to explore.	X	X
Exploring	Moving slowly, sniffing, and investigating the environment.	X	X
Recumbent	Fully positioned, lying with 1 side in complete contact with the ground; positioned on side with body, but not the head, in complete contact with the ground; lying with the ventral aspect of the trunk and limbs in contact with ground; or lying with the dorsal aspect of the trunk in contact with the ground and feet up.	X	X
Sitting	Sitting on the ground with the pads of the front paws in contact with the floor and forelimbs straight.	X	X
Standing	Positioned with just 4 paws in contact with the ground or 2 in contact with the ground and 2 in contact with a wall.	X	X
Leaning (wall)	Positioned (standing, sitting, or recumbent) against the walls of the cage or enclosure, with the eyes opened or closed.	X	X
Leaning (door)	Positioned against the door of the cage or enclosure, with the eyes opened or closed.	X	X
Hiding	Attempts to be concealed; the entire body or the cranial portion of the dog are not visible to the camera.	X	
Changing states	Changing from one of the following states to another: walking, lying, sitting, or standing.	X	X
Event			
Barking	Abrupt, low-frequency vocalization, either soft or raucous.		X
Growling	Throaty rumbling vocalization, usually low in pitch, sometimes used aggressively or defensively.		X
Whining	Repeated, relatively brief, exhalation vocalizations of varying pitch.		X
Yelping	Loud, high-pitched vocalizations.		X
Mouth opening	Opening and closing the mouth with rapid movements without extending the tongue; possibly yawning.		X
Lip licking	Running the tongue over the lips.		X
Self grooming	Behaviors directed towards the dog's own body (eg, scratching, licking, and chewing the skin and coat or licking and chewing of wounds).	X	X
Tail chasing	Pursuit of the dog's own tail with continuous circling movements.	X	X
Circling	Walking in a circle.	X	X
Pacing	Continuous movement back and forth between the limits of the cage or enclosure.	X	X
Digging	Scratching the floor with the forepaws in a digging motion.	X	X
Barrier manipulation	Chewing, pawing, or licking the cage or enclosure.	X	X
Jumping	Springing into the air, either to make contact with an object or a person or for no apparent reason.	X	X
Nosing	Moving the nose along objects or exhibiting clear sniffing movements.		X
Paw lifting	Raising a forepaw into a position of approximately 45°.		X
Tail wagging	Repetitive back-and-forth motion of the tail.		X
Dog interaction	Agonistic or antagonistic interaction with another dog.	X	

X = Assessment performed.

Behaviors were video recorded before and after surgery and evaluated as states (logged by instantaneous sampling at 2-minute intervals) or events (logged by continuous recording and counting the number of occurrences). Forty-six dogs were randomly assigned to sDAP or sham treatment exposure groups. Each dog's behavior was video recorded for 30-minute intervals in the dog's usual environment prior to surgery (T0), after the dog was placed in an ICU cage sprayed with sDAP solution or with a sham treatment consisting of the ethanol carrier without sDAP prior to surgery (T1), in the sprayed ICU cage after recovery from surgery (T2), and after being returned to its usual environment (T3).

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