Canine Research

Effects of gonadectomy on scent-marking behavior of shelter dogs

Betty McGuire*

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York

A B S T R A C T

In male domestic dogs, castration appears to reduce frequency of sexually dimorphic behaviors, such as mounting and roaming, but results for urine-marking, another highly dimorphic behavior, are less clear. Using two different experimental designs, I studied effects of gonadectomy on scent-marking behavior during leash walks of dogs at two animal shelters in NY (Cortland Community SPCA and Tompkins County SPCA). In the between-dog study, I compared intact males and females and gonadectomized males and females with respect to rate of urination (a highly sexually dimorphic behavior), likelihood of ground-scratching (a somewhat sexually dimorphic behavior), and likelihood of defecation (a sexually monomorphic behavior). In my within-dog study, I compared rate of urination before and after gonadectomy in a subset of males and females. For the between-dog study, I found that castrated males urinated at lower rates than intact males and that spayed females and intact females urinated at similar rates; this pattern characterized dogs at both shelters. Gonadectomy did not influence likelihood of ground-scratching by either males or females at either shelter. For males and females at the Tompkins shelter and females at the Cortland shelter, gonadectomy did not influence likelihood of defecation. However, intact males were more likely to defecate than castrated males at the Cortland shelter. For dogs observed both before and after gonadectomy in the within-dog study, overall rates of urination decreased after castration in males but did not significantly change after spaying in females (sample sizes sufficient for analysis at the Cortland shelter only). Individual differences characterized male behavioral response to castration: while one male showed a very slight increase in mean rate of urination after castration (4%), the remaining sixteen males differed in degree of response, with percentage decreases in mean rate of urination after castration ranging from 14% to 72%. The source of this individual variation remains to be identified. In summary, spaying did not influence urination, ground-scratching, or defecation by female dogs. For male dogs, I found the most robust effects of castration on urine-marking, the most sexually dimorphic of the three scent-marking behaviors studied. This study is the first research based on direct observations of individual dogs to find decreased urine-marking by males after castration.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Mammalian communication via scent-marking is mediated by hormones that influence both signalers and receivers. Androgens, for example, affect the chemical composition of scent marks (meadow voles, *Microtus pennsylvanicus*; Ferkin and Johnston, 1993) and in many species, intact males mark at higher rates than castrated males (rabbits, *Oryctolagus cuniculus*; Mykytowycz, 1965;

Mongolian gerbils, *Meriones unguiculatus*; Blum and Thiessen, 1971; golden hamsters, *Mesocricetus auratus*; Vandenbergh, 1971; mice, *Mus musculus*; Kimura and Hagiwara, 1985). Androgens also influence the investigatory behavior of receivers, such as when intact males spend more time than castrated males investigating scent marks of intact females (domestic dogs, *Canis lupus familiaris*; Lisberg and Snowdon, 2009).

Urine-marking is a common form of scent-marking in canids, and studies of free-living and captive packs suggest a role for gonadal hormones in regulating this behavior. In gray wolves (*Canis lupus*), urine-marking by high-ranking individuals increases during the breeding season (Asa et al., 1985; Peters and Mech, 1975), a time when testosterone levels also increase in high-ranking wolves (Asa et al., 1990). Urine-marking by high-ranking coyotes (*Canis latrans*)

* Address for reprint requests and correspondence: Betty McGuire, PhD, Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, NY 14853 USA. Tel: (+607) 279-6405; Fax: (+607) 255-8088.
E-mail address: bam65@cornell.edu.

also increases during the breeding season (Gese and Ruff, 1997). Finally, gonadal hormones influence the chemical composition of gray wolf urine (Raymer et al., 1986).

Domestic dogs differ from other canids in that levels of testosterone in males do not fluctuate seasonally and females display biannual, aseasonal estrus (Asa, 1997; Ortega-Pacheco et al., 2006). Dogs also are more accessible for observation and experimentation than wild canids, which has resulted in numerous studies on effects of gonadal hormones on canine behavior; these studies were either observational or based on interviews of dog owners. The general pattern to emerge from studies of male dogs is that castration reduces behaviors thought to be sexually dimorphic, such as mounting and roaming (Hopkins et al., 1976; reviewed by Hart and Eckstein, 1997; Houlihan, 2017). However, the effect of castration on urine-marking, another behavior thought to be highly dimorphic, is less clear. With respect to observational studies, Beach (1974) and Ranson and Beach (1985) found that development of the mature male pattern of urinary behavior (i.e., frequent marking of vertical targets using the raised-leg posture) requires testosterone around the time of birth. Based on finding no difference in frequency of urine-marking and urinary posture between seven intact males and six males castrated in adulthood, Beach (1974) concluded that although testosterone early in life was necessary for development of the adult male urinary pattern, it was not later necessary to maintain that pattern. Beach (1974) also reported that administration of gonadal hormones to males and females gonadectomized in adulthood resulted in increased frequency of urination. Hart (1974) found no difference in frequency of urine-marking by seven adult males, each observed many times before and after castration. By contrast, Maarschalkerweerd et al. (1997) interviewed 122 dog owners and reported that castration resulted in decreased urination inside the house in most dogs; however, most dogs showed no change in urination outside the house. Similar results were obtained by Hopkins et al. (1976) and Neilson et al. (data from inside the house only; 1997), who conducted phone interviews with owners whose dogs were castrated for behavior problems (sample sizes of 42 and 57 dogs, respectively). Given that pronounced individual differences characterize behavioral responses to castration in dogs (Hart and Eckstein, 1997), sample sizes in the observational studies by Beach (1974) and Hart (1974) might have been too small to detect effects of castration in adulthood on urine-marking by male dogs. With respect to studies based on owner interviews (Hopkins et al., 1976; Maarschalkerweerd et al., 1997), why castration would reduce urination in the house, but not outside the house, remains an open question. Spaying appears to have no effect on urinary behavior of female dogs (Balogh et al., 2018; Martins and Valle, 1948; O'Farrell and Peachey, 1990; Wirant and McGuire, 2004).

For this observational study, I tested the hypothesis that gonadal hormones influence sexually dimorphic scent-marking behaviors in male dogs, but not female dogs (intact females were not in either proestrus or estrus). Urine-marking is highly dimorphic, with male dogs marking more frequently than female dogs (Beach, 1974; Cafazzo et al., 2012; McGuire, 2016; McGuire and Bemis, 2017). I predicted that gonadectomy would decrease urine-marking in males but have no effect on urine-marking in females. Although there is a tendency for more male than female dogs to display ground-scratching, significant sex differences in this behavior have not been reported (Bekoff, 1979; McGuire, 2016), so one would expect little or no effect of gonadectomy on ground-scratching by either males or females. Defecation is not a sexually dimorphic behavior in dogs (McGuire, 2016; McGuire and Bemis, 2017; Sprague and Anisko, 1973), so no effect of gonadectomy was predicted for defecation by either males or females. The hypothesis was tested using two different experimental designs. First, using

large sample sizes from two different animal shelters, scent-marking behaviors of intact dogs were compared with those of gonadectomized dogs (between-dog design). Second, in a subset of dogs, urine-marking behavior was compared both before and after gonadectomy (within-dog design).

Materials and methods

Dogs and housing

Dogs were observed during walks at the Cortland Community SPCA (hereafter called the Cortland shelter) in Cortland, NY, and at the Tompkins County SPCA (hereafter called the Tompkins shelter) in Ithaca, NY. Dogs were at least 1 year of age and most were mixed breeds surrendered by owners; some dogs were either picked up as strays or transferred from other shelters. All dogs were available for adoption at the time of observation, having received veterinary treatment and undergone behavioral evaluation by shelter staff. At the Tompkins shelter, most dogs were gonadectomized before placement on the adoption floor (and all before going home with adopters). By contrast, at the Cortland shelter, intact dogs often were placed on the adoption floor (most were gonadectomized before going home with adopters; some were scheduled for spaying or castration at the next clinic and were placed in homes as foster to adopt). Accordingly, there was greater access to intact dogs at the Cortland shelter. Dogs with known medical issues and females in either proestrus or estrus (based on physical signs and behavioral changes) were excluded from the study. Additional details of veterinary care and behavioral testing are described in the study by McGuire (2016).

Most dogs at the Cortland shelter were housed individually in a room that contained seven chain-link cages from 2.3 m^2 to 4.5 m^2. Some dogs were housed in two other rooms, one of which contained a wall unit with ten stainless steel cages reserved for very small dogs. The other room contained four cages, divided by brick walls and accessed via chain-link doors, which housed small- to medium-sized dogs. At the Tompkins shelter, dogs were housed individually in 13 cubicles from 5.2 m^2 to 7.3 m^2. Dogs at both shelters had a raised bed, blanket, toys, and water bowl, and were fed twice daily by staff (between 08:00 and 09:00 h and between 15:00 and 16:00 h). At the Cortland shelter, dogs were rotated through outdoor enclosures and walked once or twice a day. At the Tompkins shelter, dogs were either walked or taken to an outdoor enclosure several times a day.

Experimental procedures

Data collection occurred between February 2013 and February 2018. All walks occurred between 11:00 and 17:00 h. Dog walking equipment provided by each shelter was used. For walks at the Cortland shelter, we connected either a 1.8 m cloth lead (Weiss Walkie, Dr. Emily Weiss, Wichita, KS) to a metal ring on the collar or a 1.8 m cloth lead to a harness (either a Yup! Mesh Non-Pull Harness, The Sporn Company, New York, NY, or a Kong Comfort Harness, The Kong Company, Golden, CO). Dogs were led to the lawn outside the shelter (0.3 ha; 42°34′55″ N, 76°13′01″ W), which was bordered by a gravel parking lot and road. At the Tompkins shelter, we typically connected a harness (either a PetSafe Easy Walk Harness, Radio Systems Corporation, Knoxville, TN, or a Zack and Zoey Nylon Pet Harness, Pet Any Way LLC, model US2395 14 99) to a cloth lead at least 1.8 m long; at the start of the study only, 5-m retractable leashes also were used (Flexi North America, LLC, Charlotte, NC). Each dog was walked from the shelter to a nearby field (16.6 ha; 42°28′20″ N, 76°26′22″ W), the substrate of which was mostly grass.

Given differences in the areas available for walking at the two shelters, each dog was observed for 20 min at the Tompkins shelter and 10 min at the Cortland shelter. Dogs were allowed to set the pace of walks and fully investigate the environment. Dogs were not kept in a "heel" position. Each urination, defecation, and occurrence of ground-scratching that immediately followed either urination or defecation was recorded by me or by one of my trained students in my presence. We typically collected behavioral data using a check sheet. When a student was occasionally unavailable, I verbally recorded observations using the voice memo app on an iPhone 5 (model ME306LL/A, Apple Inc., Cupertino, CA) and transferred these data to check sheets after the series of walks. We measured height at withers (in cm) and photographed each dog once the observation period had ended. On returning to the shelter, the following information was recorded for each dog from shelter records: name, identification number, intake date, age, body mass (in kg), and date of surgery if the dog was gonadectomized. Only dates for dogs in which surgeries were performed at the shelters were used; estimates provided by owners on surrender forms were not used. Surgery date was used to calculate number of days that had passed from day of surgery to day of walk (= time since surgery). At both shelters, some dogs were walked once and others multiple times, depending on time spent on the adoption floor (see in the following).

Statistical analyses

Between-dog study

We observed 245 dogs at the Cortland shelter and 427 dogs at the Tompkins shelter. Dogs from 1 year of age up to 8 years of age were considered adults; dogs 8 years or older were classified as seniors. Number of dogs observed by shelter, age class, sex, and reproductive status (intact versus castrated/spayed) is shown in Table 1. Data from each shelter were analyzed separately. For each shelter, I analyzed data from the first walk of each dog and data from all walks of each dog. At the Cortland shelter, number of walks per dog ranged from one to 63 (1-2 walks: 133 dogs; 3-4 walks: 53 dogs; 5-6 walks: 23 dogs; 7-8 walks: 12 dogs; 9-10 walks: 8 dogs; ≥11 walks: 16 dogs). At the Tompkins shelter, the number of walks per dog ranged from one to 13 (1-2 walks: 237 dogs; 3-4 walks: 119 dogs; 5-6 walks: 33 dogs; 7-8 walks: 24 dogs; 9-10 walks: 8 dogs; ≥11 walks: 6 dogs).

Generalized linear models were used to analyze rate of urination per minute for first walks. Logistic regression was used to determine significant predictors of defecating or ground-scratching during the first walk. The models for rate of urination per minute, likelihood of defecating at least once during the first walk, and likelihood of ground-scratching at least once during the first walk included the following fixed factors: sex, reproductive status, age class, and the interaction between sex and reproductive status.

When analyzing all walks, the three response variables (rate of urination, at least one occurrence of defecation, and at least one occurrence of ground-scratching) were calculated by determining the average for each dog that were then averaged across the dogs. A general linear model was used to analyze average rate of urination. Logistic regression was used to model average proportion of walks with either defecation or ground-scratching; the distribution was quasibinomial to account for overdispersion. The models for average rate of urination per minute, average proportion of walks with at least one defecation, and average proportion of walks with at least one occurrence of ground-scratching included the following fixed factors: sex, reproductive status, age class, and the interaction between sex and reproductive status.

Age class was included as a fixed factor when analyzing data from first walks and all walks to control for this variable in the models. However, the effects of age class on the scent-marking behavior of smaller samples of dogs from each shelter have been described previously (McGuire, 2016), so limited details concerning age effects are provided here.

Dogs gonadectomized at each shelter varied in the number of days that passed from day of surgery to day of observation during a

Table 1
Number of dogs walked by shelter, age class, sex, and reproductive status in the between-dog study

Shelter and age class	Male		Female	
	Intact	Castrated	Intact	Spayed
Cortland				
Adult	53	63	50	41
Senior	4	14	9	11
Tompkins				
Adult	29	170	29	122
Senior	3	32	3	39

Table 2
Dogs observed for the within-dog study at the Cortland shelter and the Tompkins shelter

Dog by shelter	Sex	Age (years)	Number of walks intact	Number of walks spayed or castrated	Days after spay or castration spanned by walks
Cortland shelter					
Rachel	Female	10	2	6	13-58
Peanut[a]	Female	9.5	3	5	129-199
Diamond	Female	7	3	9	6-50
Kimberly[b]	Female	5.5	9	4	8-18
Cassidy	Female	5	10	4	11-14
Sweetie	Female	3	9	5	1-18
Ruby	Female	2	2	13	1-55
Lady	Female	2	4	11	6-71
Elvis	Male	7	3	4	1-22
Elmer	Male	7	3	4	24-34
Pete[c]	Male	5	3	6	1, 124-204
Patches	Male	5.5	3	6	1-11
Gunner	Male	1.5	5	5	3-17
Rudy	Male	1.5	4	8	1-43
Grover[d]	Male	2	6	8	42-67
Buddy	Male	6	10	7	6-22
Lucius	Male	2.5	7	11	4-19
Barry	Male	5	2	16	4-32
Mars	Male	2.5	6	15	4-33
Lincoln	Male	10	5	16	1-55
Billy	Male	7	14	16	3-67
Oliver[e]	Male	2	15	25	1-38
Red	Male	6	8	35	1-187
Tompkins shelter					
Felicia	Female	10	3	5	5-11
Farrah	Female	2	2	6	1-8
Aphrodite	Female	1.5	5	7	2-20
Frost	Male	5	2	14	1-16
Rutherford	Male	2	2	18	1-34

These dogs were observed during at least two walks when intact and at least four walks when either spayed or castrated; walks when gonadectomized spanned more than the first week after surgery. Within each sex, dogs are ordered from least to most total walks.

[a] Peanut was adopted shortly after spaying and returned to the shelter several months later; all postspay walks occurred after her return.
[b] Kimberly was transferred from the Cortland shelter to the Tompkins shelter, where her last two 10-minute walks occurred.
[c] Pete was adopted shortly after castration and returned to the shelter several months later; five of the six postcastration walks occurred after his return.
[d] Grover was adopted shortly after castration and returned to the shelter about six weeks later; all postcastration walks occurred after his return.
[e] Oliver was fostered to adopt while intact; 10-minute walks five through 40 occurred at the foster home.

Table 3
Descriptive statistics (mean ± SD) for rate of urination by dogs at two shelters. Percentages of walks in which dogs defecated or ground-scratched also are shown

Behavior by shelter	Male		Female	
	Intact	Castrated	Intact	Spayed
Cortland				
Urination rate[a]	1.02 ± 0.50	0.62 ± 0.46	0.23 ± 0.24	0.24 ± 0.22
% Walks with defecation	66.3	46.2	44.3	44.1
% Walks with ground-scratching	32.4	38.0	26.0	15.8
Tompkins				
Urination rate[b]	0.59 ± 0.32	0.37 ± 0.26	0.14 ± 0.16	0.16 ± 0.12
% Walks with defecation	51.6	64.8	52.0	62.0
% Walks with ground-scratching	40.3	42.1	13.3	28.0

Data are from all walks of each dog in the between-dog study. All variables were calculated by determining the average for each dog and then averaging across the dogs.
[a] Total number of urinations/10 minutes.
[b] Total number of urinations/20 minutes.

walk. For example, days elapsed from surgery to observation during the first walk at the Cortland shelter (median, range) were as follows: males ($n = 35$), 7 days, 1-225 days; females ($n = 24$), 8 days, 1-65 days. At the Tompkins shelter, days elapsed from surgery to observation during the first walk were as follows: males ($n = 91$), 8 days, 1-662 days; females ($n = 67$), 8 days, 1-481 days. Using data from both the first walk and all walks, I looked at whether time since surgery influenced urination rate of dogs spayed and castrated at each shelter. For each shelter, two linear mixed effects models were run using data from first walks. The first modeled urination rate as a function of time since spay or castration. The second model additionally included sex and an interaction of sex with time since spay or castration. Similarly, for each shelter, two linear mixed effects models were run using data from all walks. The first modeled average rate of urination as a function of time since spay or castration with dog as a random effect. The second model additionally included sex and an interaction of sex with time since spay or castration. The results from these analyses indicated no relationship between time since gonadectomy and either urination rate (first walk) or average urination rate (all walks) at either shelter. In addition, none of the interaction effects was significant, indicating that sex did not moderate how time since gonadectomy was associated with either urination rate (first walk) or average urination rate (all walks) at either shelter. These results indicate that it is reasonable for me to consider spayed females as a group and castrated males as a group, despite differences in time since surgery among individual dogs within these groups. For males,

absence of an effect of time since surgery on urination rate is consistent with reports of rapid declines in testosterone levels within hours of castration, with testosterone undetectable in some dogs by 3 days after surgery and other dogs by 7 days (DePalatis et al., 1978; Taha et al., 1982). For females, my results are consistent with those of Balogh et al. (2018), who found no effect of time since spay on urinary behavior of Labrador retrievers.

Data were analyzed using either JMP Pro 12 (2015. SAS Institute, Cary, NC) or R, version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Specific R packages included Bates et al. (2015) for generalized linear models (lme4) and Length (2017) for estimated marginal means (emmeans).

Within-dog study

Based on the hormonal profiles of male dogs after castration (DePalatis et al., 1978; Taha et al., 1982) and taking into consideration the difficulty associated with collecting repeat observations on specific dogs on the adoption floors of shelters where continued access is never guaranteed, I selected for analysis dogs for which I had collected behavioral data on at least two walks before surgery and at least four walks after surgery, with postsurgery walks not restricted to the first week after surgery. This selection process resulted in sample sizes of eight females and 15 males from the Cortland shelter and two males and three females from the Tompkins shelter (Table 2).

Results from the between-dog study indicated robust sex by reproductive status interactions at both shelters for rate of urination (first walk) and average rate of urination (all walks) and the occasional significant or near-significant predictor for likelihood of defecation or ground-scratching at one shelter but not the other (see in the following). Therefore, the analysis focused on average rate of urination for the within-dog study. Number of days between observations varied between dogs and within a dog (e.g., some observations occurred on consecutive days whereas others occurred with several days or more between them), so the average rate of urination per minute before spaying or castration and the average rate of urination per minute after spaying or castration for each dog was calculated. For the Cortland data, a linear mixed model was used, which included sex, reproductive status, and the interaction between sex and reproductive status as fixed factors and dog as a random effect. A second model was run that included age as a continuous predictor. However, age was not a significant predictor ($P = 0.811$), so it was not included in the final model.

Given the small number of dogs that met the selection criteria from the Tompkins shelter (three females and two males), data from these dogs were not formally analyzed. Instead, descriptive statistics for average rate of urination per minute before and after

Table 4
Effects of sex, reproductive status, and age class on average rate of urination per minute by dogs during either 10-minute walks (Cortland shelter) or 20-minute walks (Tompkins shelter)

Parameter	Cortland				Tompkins			
	β	SE β	t	P	β	SE β	t	P
Intercept Female[a]	0.207	0.051	4.08	< 0.0001	0.136	0.038	3.58	0.0004
Male Intact[b]	0.804	0.071	11.31	< 0.0001	0.447	0.054	8.26	< 0.0001
Spayed/castrated Adult[c]	−0.003	0.073	−0.04	0.970	−0.001	0.042	−0.033	0.974
Senior	0.175	0.068	2.57	0.011	0.088	0.027	3.19	0.002
Male: castrated	−0.420	0.098	−4.26	< 0.0001	−0.222	0.059	−3.79	< 0.0002

Data are from the between-dog study using all walks of each dog.
[a] Female is the reference.
[b] Intact is the reference.
[c] Adult is the reference.

Table 5

Effects of sex, reproductive status, and age class on likelihood of defecation by dogs during either 10-minute walks (Cortland shelter) or 20-minute walks (Tompkins shelter)

Parameter	Cortland				Tompkins			
	β	SE β	z	P	β	SE β	z	P
Intercept Female[a]	−0.296	0.243	−1.22	0.225	0.049	0.328	0.15	0.882
Male Intact[b]	0.944	0.348	2.71	0.007	−0.016	0.467	−0.03	0.973
Spayed/castrated Adult[c]	−0.032	0.347	−0.09	0.928	0.363	0.362	1.00	0.316
Senior	0.430	0.328	1.31	0.192	0.331	0.252	1.31	0.191
Male: castrated	−0.845	0.476	−1.77	0.077	0.165	0.510	0.32	0.747

Data are from the between-dog study using all walks of each dog.
[a] Female is the reference.
[b] Intact is the reference.
[c] Adult is the reference.

gonadectomy for these dogs are included in the text and raw data for the two males in a figure.

Results

Between-dog study

The results using first walks of each dog were the same as those using all walks of each dog. Results from first walks are presented in Supplementary Tables 1 through 4. Results using all walks are presented here. Descriptive statistics for the three scent-marking behaviors are shown by shelter in Table 3. The statistics in Table 3 are meant to provide a general overview of the raw data collected over all walks of dogs.

At both shelters, the sex by reproductive status interaction was significant for average rate of urination per minute (parameter, male: castrated, Table 4). At the Cortland shelter and at the Tompkins shelter, the average rate of urination was higher for intact males than castrated males whereas the average rate of urination was similar for intact females and spayed females. At both shelters, rates of urination were higher for senior dogs than adult dogs (parameter, senior, Table 4), as has been previously documented (McGuire, 2016).

Sex predicted likelihood of defecation at the Cortland shelter (parameter, male, Table 5). Specifically, males were more likely to defecate than females, although this effect was largely because of intact males, as evidenced by the borderline significance of the sex by reproductive status interaction (parameter, male: castrated, Table 5). There were no significant predictors of defecation at the Tompkins shelter (Table 5). Likelihood of defecation during walks at the Tompkins shelter did not differ among intact males, castrated males, intact females, and spayed females.

The logistic regression analyses for the Cortland shelter revealed no significant predictors for ground-scratching (Table 6). Pairwise comparisons revealed that castrated males were more likely to ground-scratch than spayed females. At the Tompkins shelter, sex was a significant predictor of ground-scratching (parameter, male, Table 6). Specifically, intact males were more likely to ground-scratch than intact females, and castrated males were more likely to ground-scratch than spayed females.

Within-dog study

Descriptive statistics (mean ± SD) from the raw data for average rate of urination per minute at the Cortland shelter were as follows: males ($n = 15$), when intact, 0.92 ± 0.27, when castrated, 0.58 ± 0.27; females ($n = 8$), when intact, 0.30 ± 0.25, when spayed, 0.33 ± 0.16. These same statistics for average rate of urination per minute at the Tompkins shelter were as follows: males ($n = 2$), when intact, 1.03 ± 0.01, when castrated, 0.40 ± 0.15; females ($n = 3$), when intact, 0.10 ± 0.06, when spayed, 0.10 ± 0.06.

At the Cortland shelter, the sex by reproductive status interaction was significant for average rate of urination per minute (parameter, male: castrated, Table 7). Within males, the average rate of urination was higher when intact than when castrated, but within females, the average rate of urination did not differ when intact and when spayed. In addition, intact males at Cortland had a higher urination rate than intact females; this pattern continued after gonadectomy, but to a lesser magnitude.

To highlight individual differences in response to castration, raw data on rate of urination per minute for 14 of the 15 males from the Cortland shelter are shown across days after castration in Figure 1. I excluded Pete from Figure 1 due to the long period that elapsed from the first observation after castration to subsequent

Table 6

Effects of sex, reproductive status, and age class on likelihood of ground-scratching by dogs during either 10-minute walks (Cortland shelter) or 20-minute walks (Tompkins shelter)

Parameter	Cortland				Tompkins			
	β	SE β	z	P	β	SE β	z	P
Intercept Female[a]	−1.060	0.295	−3.59	<0.001	−1.890	0.507	−3.73	<0.001
Male Intact[b]	0.299	0.389	0.77	0.443	1.481	0.612	2.42	0.016
Spayed/castrated Adult[c]	−0.733	0.473	−1.55	0.122	0.903	0.534	1.69	0.092
Senior	0.344	0.362	0.95	0.344	0.176	0.255	0.69	0.491
Male: castrated	0.940	0.581	1.62	0.107	−0.821	0.649	−1.26	0.206

Data are from the between-dog study using all walks of each dog.
[a] Female is the reference.
[b] Intact is the reference.
[c] Adult is the reference.

Table 7
Effects of sex and reproductive status on average rate of urination per minute by dogs during 10-minute walks at the Cortland shelter; the model included a random effect of dog

Parameter	β	SE β	t	df	P
Intercept Female[a]	0.301	0.090	24.98	3.34	< 0.003
Male Intact[b]	0.619	0.112	24.98	5.54	< 0.00001
Spayed/Castrated	0.025	0.053	21.00	0.47	0.64
Male: Castrated	−0.361	0.066	21.00	−5.47	< 0.00001

Data are from adult and senior dogs in the within-dog study.
[a] Female is the reference.
[b] Intact is the reference.

observations because of his adoption and return to the Cortland shelter after several months (Table 2). Raw data for the two males from the Tompkins shelter are depicted across days after castration in Figure 2. Using mean rate of urination per minute before castration and mean rate of urination per minute after castration, I also calculated percentage decrease in mean urination rate for each dog (or in one case, Rudy, a percentage increase of 4%). Percentage decrease in mean rate of urination after castration ranged from 13.8% shown by Buddy at the Cortland shelter (Figure 1) to 71.6% shown by Frost at the Tompkins shelter (Figure 2). Considering the 13 males shown in Figure 1 whose mean rate of urination fell after castration (i.e., all except Rudy), Pete (percentage decrease of 30.1%; not included in Figure 1), and the two males shown in Figure 2, percentage decreases in mean rate of urination after castration were as follows: ≤ 25%, three dogs; 26%-50%, seven dogs; 51%-75%, six dogs.

Discussion

Consistent with the prediction that gonadectomy would decrease urine-marking in male dogs and have no effect on urine-marking in female dogs, I found that castrated males urinated at lower rates than intact males and that spayed females and intact females urinated at similar rates. This finding was robust as it characterized dogs at both the Cortland shelter and the Tompkins shelter, whether data from first walks of dogs or all walks of each dog were analyzed for the between-dog study. In addition, for the subset of dogs observed both before and after gonadectomy in the within-dog study, overall rates of urination decreased after castration in males but did not significantly change after spaying in females at the Cortland shelter, where sample sizes were sufficient for analysis. Finally, the five male and female dogs observed both before and after gonadectomy at the Tompkins shelter displayed behavior consistent with that found overall for males and females observed before and after gonadectomy at the Cortland shelter.

In contrast to previous observational studies, effects of castration on rate of urination by males in both the between-dog study and within-dog study were found. Beach (1974) observed adult beagles in an outdoor pen and found no difference in frequency of urination between seven intact males and six males castrated in adulthood. Hart (1974) observed seven adult mixed breed males in an outdoor pen during two series before castration and three series after castration (after castration days 20-32, 50-62, and 146-158) and found no significant difference in number of urine marks before and after castration. In the between-dog study, no relationship was found between time since castration and either rate of urination (first walk) or average rate of urination (all walks) in males castrated at each shelter. These findings, together with patterns of decrease in rate of urination shown by dogs in the within-dog study (Figures 1 and 2), indicate that decreases in frequency of urination

occur soon after castration, with frequencies typically remaining below precastration levels thereafter. Thus, it is unlikely that Hart's (1974) extensive observations of urinary behavior after castration occurred too early to detect effects of castration; time since surgery was not included for the castrated males in Beach's (1974) study. Although my between-dog study differed from that of Beach's (1974) and my within-dog study differed from that of Hart's (1974) in many ways (e.g., source and housing of dogs, and my observations were conducted during leash walks of dogs whereas their observations were of dogs in outdoor pens), the larger sample sizes in both studies reported here relative to sample sizes in their studies may be the most likely explanation for why significant effects of castration on rate of urination by males were found in this study. Given the individual variation that characterizes behavioral response to castration in dogs (Hart and Eckstein, 1997), sample size may be an especially important component of experimental design.

Based on their studies of development of urinary behavior in beagles, which included castration at various developmental stages, Beach (1974) and Ranson and Beach (1985) concluded that testosterone around the time of birth was necessary to organize the adult male pattern of frequent urine-marking of vertical targets using the raised-leg posture. Finding no differences between intact adult males and males castrated in adulthood in either frequency of urination or use of the raised-leg posture (Beach, 1974), they further concluded that once organized, adult male urinary behavior continues without regression in the absence of testosterone (Ranson and Beach, 1985). The results from the studies reported here indicate that although castrated adult males still urinate at higher rates than adult females, they urinate at lower rates than intact adult males. In addition, we previously reported that castration in adulthood was associated with decreased use of the raised-leg posture by shelter dogs: while 96% of intact males used the raised-leg posture as their predominant urinary posture during walks, 83% of castrated males did so (i.e., some males used the lean-forward posture, in which no hindlimb is raised; McGuire and Gough, 2017). Regardless of reproductive condition, most females used the squat posture, in which no hindlimb is raised (82% of intact females and 86% of spayed females; McGuire and Gough, 2017). Thus, our research with shelter dogs suggests that withdrawal of testosterone in adulthood causes declines in rate of urination and use of the raised-leg posture, at least in some dogs, although sexual dimorphism in urination rate and urinary posture persist.

There was variation in response to castration among individual males in the within-dog study, even though overall there was a significant decrease in rate of urination after castration. One male, Rudy, showed a very slight increase in mean rate of urination after castration (4%) and the remaining sixteen males differed in their degree of response, with percentage decreases ranging from about 14% to 72%. Such individual differences also were evident in the data collected through owner surveys. These studies reported that castration reduced urination in the house in some percentage of dogs, but not all dogs (50% of males; Hopkins et al., 1976; 69% of males, Maarschalkerweerd et al., 1997; improvement ≥ 50% in at least 60% of males and improvement ≥ 90% in 25%-40% of males, Neilson et al., 1997). Several factors seem unlikely to explain this individual variation in response to castration. For example, likelihood that castration will alter behaviors, such as urine-marking in the home, mounting, and roaming, is unrelated to age at castration in adulthood and length of time such behaviors were displayed (Hopkins et al., 1976; Neilson et al., 1997). Hart and Eckstein (1997) reviewed evidence that persistence in some males of unwanted behaviors after castration cannot be explained by either residual amounts of testosterone or compensatory secretion of androgens by the adrenal glands. Instead, these authors suggested two possibilities that might explain individual differences among males in
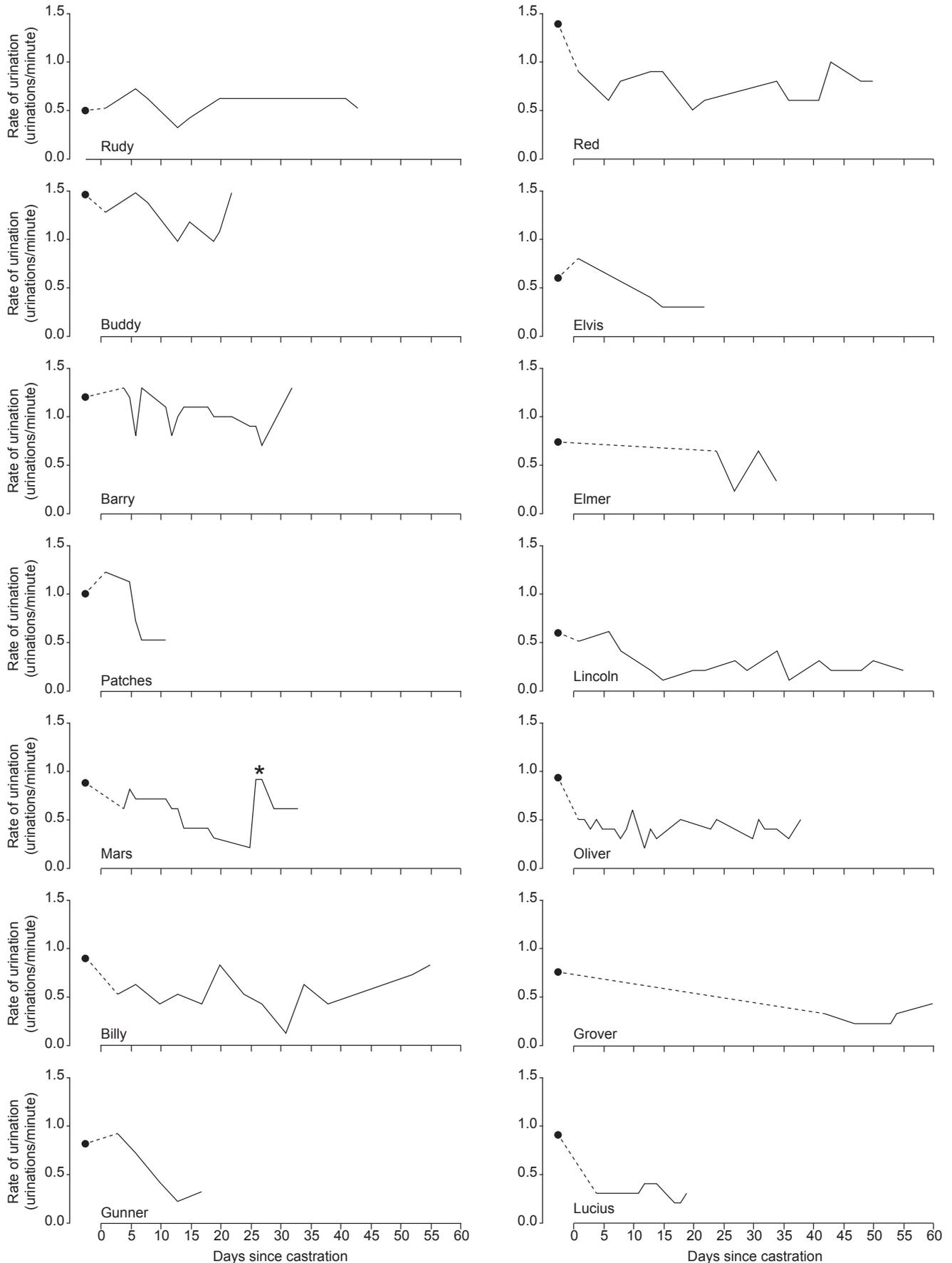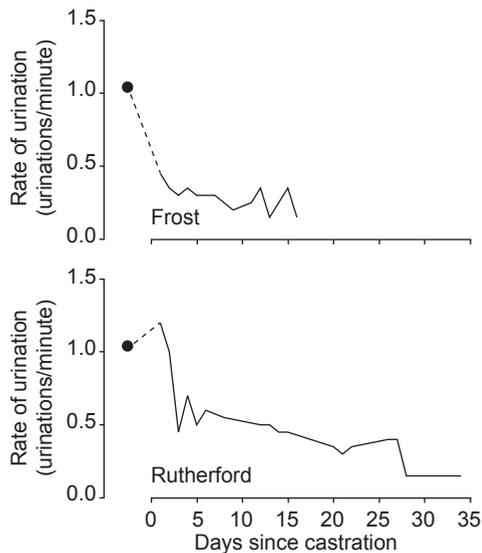
Figure 1. Changes in rate of urination exhibited by 14 male dogs at the Cortland shelter after castration. These data were collected during 10-minute leash walks of each dog. The filled circle represents mean rate of urination when each dog was intact, and the dashed line connects this value to the rate of urination on the first postcastration walk of the study. A long, dashed line, such as shown for Grover, reflects his adoption right after surgery and his return to the shelter several weeks later, when we conducted his first postcastration walk. The solid line shows raw data on rate of urination during postcastration walks. The asterisk for Mars indicates two walks that occurred when he shared the adoption floor with two females in heat.

Figure 2. Changes in rate of urination exhibited by two male dogs at the Tompkins shelter after castration. These data were collected during 20-minute leash walks of each dog. The filled circle represents mean rate of urination when each dog was intact, and the dashed line connects this value to the rate of urination on the first post-castration walk of the study. The solid line shows raw data on rate of urination during postcastration walks.

their behavioral responses to castration: 1) genetic differences among individual males in how the nervous system responds to decreased androgen levels and 2) individual differences in exposure to gonadal steroids *in utero* as a result of intrauterine position effects (Hart and Eckstein, 1997). To my knowledge, both possibilities remain untested for dogs and could explain individual variation in responses to castration seen here.

My finding of no effect of spaying on rate of urination by female dogs agrees with results from both observational studies and studies based on owner interviews (Martins and Valle, 1948; O'Farrell and Peachey, 1990; Wirant and McGuire, 2004; Balogh et al., 2018). However, my results do not indicate that gonadal hormones have no effects on urinary behavior of female dogs. I excluded from my studies intact females in proestrus and estrus, periods during the ovarian cycle when specific gonadal hormones increase (Beach et al., 1982). Free-ranging female dogs in estrus exhibited increased rates of urine-marking (Pal, 2003) and privately owned Jack Russell terriers directed more of their urinations at targets in the environment when in proestrus and estrus than when in anestrus (Wirant et al., 2007). Increased urination also characterized beagles spayed as adults and administered exogenous gonadal hormones (Beach, 1974). Thus, the intact females that I observed were not experiencing increased levels of gonadal hormones, which likely explains why their rates of urination were similar to those of spayed females.

Consistent with my prediction, gonadectomy did not influence likelihood of defecation by either males or females at the Tompkins shelter or females at the Cortland shelter. However, intact males were more likely to defecate than castrated males at the Cortland shelter. Effect of castration on likelihood of defecation by male dogs has not been examined previously, which makes it difficult to put my conflicting findings for males from the two shelters in context; this difficulty is further compounded by sample size differences between the shelters (number of intact males observed at the Cortland shelter was almost twice that at the Tompkins shelter). These findings of no difference between intact and spayed females with respect to likelihood of defecation at either shelter agree with

an earlier report for privately owned female Jack Russell terriers (Wirant and McGuire, 2004). Other studies of scent-marking in dogs (McGuire, 2016; McGuire and Bemis, 2017; Sprague and Anisko, 1973) and coyotes (Gese and Ruff, 1997) reported no sex differences in frequency of defecation, which is consistent with what I found at the Tompkins shelter. In feral dog packs, however, placement of feces on conspicuous objects was limited to adult males of high social status (Cafazzo et al., 2012).

As predicted, gonadectomy did not influence likelihood of ground-scratching by either males or females, and this characterized dogs at both Cortland and Tompkins shelters. My results for female shelter dogs agree with those for female Labrador Retrievers (Balogh et al., 2018) but disagree with findings for female Jack Russell terriers in which spayed females were more likely than intact females to ground-scratch after defecation (Wirant and McGuire, 2004). To date, no other study has examined potential effects of castration on ground-scratching by male dogs. At the Tompkins shelter, males were significantly more likely than females to ground-scratch. Similarly, for free-living coyotes, high-ranking males ground-scratch more frequently than high-ranking females (Bowen and Cowan, 1980; Gese and Ruff, 1997). Previous studies of dogs reported no significant sex difference in ground-scratching, although percentages of males that ground-scratched were always higher than those of females (Bekoff, 1979; McGuire, 2016).

Limitations of this study stem largely from challenges of collecting data on shelter dogs. Most importantly, their availability for observation is difficult to predict because it depends on length of stay at the shelter before adoption, which varies among dogs (McGuire, 2016). In the between-dog study, some dogs were walked once and others many times. Rather than establishing a set number of walks that each dog would need for inclusion in the between-dog study, I chose instead to look at both first walks and all walks of each dog to reach sufficient sample sizes, while at the same time making use of all data collected. Collecting data for the within-dog study was especially difficult because it required access to individual dogs both before and after gonadectomy, and for multiple postsurgery walks over a biologically meaningful span of time. These requirements resulted in much smaller sample sizes for the within-dog study than the between-dog study. In addition, some dogs in the within-dog study were adopted shortly after gonadectomy and subsequently returned, resulting in long periods either between postsurgery observations (e.g., Pete; Table 2) or between surgery and the first observation thereafter (e.g., Peanut and Grover; Table 2). Finally, one dog was transferred between shelters (Kimberly; Table 2) and another dog was fostered to adopt (Oliver; Table 2) during my study; both of these situations resulted in walks occurring at different locations. These challenges regarding experimental design and data collection make studying shelter dogs somewhat similar to field studies in which routine access to study animals cannot be guaranteed.

Conclusions

Canine scent-marking behaviors differ in degree of sexual dimorphism, with urine-marking highly dimorphic (Beach, 1974; Cafazzo et al., 2012; McGuire, 2016), ground-scratching somewhat dimorphic (Bekoff, 1979; McGuire, 2016), and defecation not dimorphic (McGuire, 2016; McGuire and Bemis, 2017; Sprague and Anisko, 1973). These results for urine-marking support the hypothesis that gonadal hormones influence highly sexually dimorphic behaviors in male dogs. This is the first observational study to measure decreased urine-marking by male dogs after castration. Previous studies based on owner interviews found postcastration reductions in urine-marking were limited to inside the house (Hopkins et al., 1976; Maarschalkerweerd et al., 1997; Neilson et al.,

1997). Data from the between-dog study and the within-dog study extend the effects of castration to urine-marking outdoors. Consistent with previous research, individual differences in degree of response to castration were evident in my within-dog study; the source of such variation remains to be identified. Finally, for the most part, gonadectomy did not influence ground-scratching and defecation, two scent-marking behaviors that are not strongly sexually dimorphic.

Acknowledgments

Authors' contributions: The idea for this article was conceived by Betty McGuire. The experiments were designed by Betty McGuire. The data were analyzed by Stephen Parry, Statistical Consultant, and Betty McGuire. The article was written by Betty McGuire.

Ethical considerations

All procedures were carried out under protocol 2012-0150, which was approved by Cornell University's Institutional Animal Care and Use Committee.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jveb.2018.11.002.

References

Asa, C.S., 1997. Hormonal and experiential factors in the expression of social and parental behavior in canids. In: Solomon, N.G., French, J.A. (Eds.), Cooperative Breeding in Mammals. Cambridge University Press, Cambridge, pp. 129—149.

Asa, C.S., Mech, L.D., Seal, U.S., 1985. The use of urine, faeces, and anal-gland secretions in scent-marking by a captive wolf (*Canis lupus*) pack. Anim. Behav. 33, 1034—1036.

Asa, C.S., Mech, L.D., Seal, U.S., Plotka, E.D., 1990. The influence of social and endocrine factors on urine-marking by captive wolves (*Canis lupus*). Horm. Behav. 24, 497—509.

Balogh, O., Borruat, N., Meier, A.A., Hartnack, S., Reichler, I.M., 2018. The influence of spaying and its timing relative to the onset of puberty on urinary and general behaviour in labrador retrievers. Reprod. Domest. Anim. 53, 1184—1190.

Bates, D., Maechler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Soft. 67, 1—48.

Beach, F.A., 1974. Effects of gonadal hormones on urinary behavior in dogs. Physiol. Behav. 12, 1005—1013.

Beach, F.A., Dunbar, I.F., Buehler, M.G., 1982. Sexual characteristics of female dogs during successive phases of the ovarian cycle. Horm. Behav. 16, 414—442.

Bekoff, M., 1979. Scent-marking by free-ranging domestic dogs - olfactory and visual components. Biol. Behav. 4, 123—139.

Blum, S.L., Thiessen, D.D., 1971. The effect of different amounts of androgen on scent marking in the male Mongolian gerbil. Horm. Behav. 2, 93—105.

Bowen, W.D., Cowan, I.M., 1980. Scent marking in coyotes. Can. J. Zool. 58, 473—480.

Cafazzo, S., Natoli, E., Valsecchi, P., 2012. Scent-marking behaviour in a pack of free-ranging domestic dogs. Ethology 118, 955—966.

DePalatis, L., Moore, J., Falvo, R.E., 1978. Plasma concentrations of testosterone and LH in the male dog. J. Reprod. Fertil. 52, 201—207.

Ferkin, M.H., Johnston, R.E., 1993. Roles of gonadal hormones in control of 5 sexually attractive odors of meadow voles (*Microtus pennsylvanicus*). Horm. Behav. 27, 523—538.

Gese, E.M., Ruff, R.L., 1997. Scent-marking by coyotes, *Canis latrans*: the influence of social and ecological factors. Anim. Behav. 54, 1155—1166.

Hart, B.L., 1974. Environmental and hormonal influences on urine marking behavior in the adult male dog. Behav. Biol. 11, 167—176.

Hart, B.L., Eckstein, R.A., 1997. The role of gonadal hormones in the occurrence of objectionable behaviours in dogs and cats. Appl. Anim. Behav. Sci. 52, 331—344.

Hopkins, S.G., Schubert, T.A., Hart, B.L., 1976. Castration of adult male dogs: Effects on roaming, aggression, urine marking, and mounting. J. Am. Vet. Med. Assoc. 168, 1108—1110.

Houlihan, K.E., 2017. A literature review on the welfare implications of gonadectomy of dogs. J. Am. Vet. Med. Assoc. 250, 1155—1166.

Kimura, T., Hagiwara, Y., 1985. Regulation of urine marking in male and female mice: effects of sex steroids. Horm. Behav. 19, 64—70.

Length, R., 2017. Emmeans: Estimated Marginal Means, Aka Least-squares Means. R package version 0.9.1. https://CRAN.R-project.org/package=emmeans.

Lisberg, A.E., Snowdon, C.T., 2009. The effects of sex, gonadectomy and status on investigation patterns of unfamiliar conspecific urine in domestic dogs, *Canis familiaris*. Anim. Behav. 77, 1147—1154.

Maarschalkerweerd, R.J., Endenburg, N., Kirpensteijn, J., Knol, B.W., 1997. Influence of orchiectomy on canine behaviour. Vet. Rec. 140, 617—619.

Martins, T., Valle, J.R., 1948. Hormonal regulation of the micturition behavior of the dog. J. Comp. Physiol. Psychol. 41, 301—311.

McGuire, B., 2016. Scent marking in shelter dogs: effects of sex and age. Appl. Anim. Behav. Sci. 182, 15—22.

McGuire, B., Bemis, K.E., 2017. Scent marking in shelter dogs: effects of body size. Appl. Anim. Behav. Sci. 186, 49—55.

McGuire, B., Gough, W.T., 2017. Body size influences urinary posture but not hindlimb laterality in shelter dogs. J. Vet. Behav.:Clin. Appl. Res. 21, 38—44.

Mykytowycz, R., 1965. Further observations on the territorial function and histology of the submandibular cutaneous (chin) glands in the rabbit, *Oryctolagus cuniculus* (L.). Anim. Behav. 13, 400—412.

Neilson, J.C., Eckstein, R.A., Hart, B.L., 1997. Effects of castration on problem behaviors in male dogs with reference to age and duration of behavior. J. Am. Vet. Med. Assoc. 211, 180—182.

O'Farrell, V., Peachey, E., 1990. Behavioural effects of ovariohysterectomy on bitches. J. Small Anim. Pract. 31, 595—598.

Ortega-Pacheco, A., Bolio-González, M.E., Colin-Flores, R.F., Sauri-Arceo, C.H., Gutiérrez-Blanco, E., Jiménez-Coello, M., Linde Forsberg, C., 2006. Evaluation of a burdizzo castrator for neutering of dogs. Reprod. Domest. Anim. 41, 227—232.

Pal, S.K., 2003. Urine marking by free-ranging dogs (*Canis familiaris*) in relation to sex, season, place and posture. Appl. Anim. Behav. Sci. 80, 45—59.

Peters, R.P., Mech, L.D., 1975. Scent-marking in wolves. Am. Sci. 63, 628—637.

Ranson, E., Beach, F.A., 1985. Effects of testosterone on ontogeny of urinary behavior in male and female dogs. Horm. Behav. 19, 36—51.

Raymer, J., Wiesler, D., Novotny, M., Asa, C., Seal, U.S., Mech, L.D., 1986. Chemical scent constituents in urine of wolf (*Canis lupus*) and their dependence on reproductive hormones. J. Chem. Ecol. 12, 297—314.

Sprague, R.H., Anisko, J.J., 1973. Elimination patterns in the laboratory beagle. Behaviour 47, 257—267.

Taha, M.B., Noakes, D.E., Allen, W.E., 1982. Hemicastration and castration in the beagle dog; the effects on libido, peripheral plasma testosterone concentrations, seminal characteristics and testicular function. J. Small Anim. Pract. 23, 279—285.

Vandenbergh, J.G., 1971. The effects of gonadal hormones on the aggressive behaviour of adult golden hamsters (*Mesocricetus auratus*). Anim. Behav. 19, 589—594.

Wirant, S.C., McGuire, B., 2004. Urinary behavior of female domestic dogs (*Canis familiaris*): influence of reproductive status, location, and age. Appl. Anim. Behav. Sci. 85, 335—348.

Wirant, S.C., Halvorsen, K., McGuire, B., 2007. Preliminary observations on the urinary behaviour of female Jack Russell Terriers in relation to stage of the oestrous cycle, location, and age. Appl. Anim. Behav. Sci. 106, 161—166.