Journal of
Animal Health and Behavioural Science

Hypothesis Open Access

Olfactory Mediation of Canine Gastrointestinal Neurobiology

Terry E Brady[1], Sarah K Abood[2], Rachel Tinker-Kulberg[1], Kristen Dellinger[1,3], Melinda KM Goddard[1], Lee Robertson[1] and Anthony Lee Dellinger[1,3*]

[1] Kepley BioSystems Incorporated, Greensboro, NC, USA
[2]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada
[3]Joint School of Nanoscience and Nanoengineering, Greensboro, NC, USA

Abstract

Dogs sniff the ground in advance of defecation, irrespective of sex, breed and location. This discussion proposes that canines are not casual "sniffers," but are rather evolutionarily predisposed to search for particular molecules that activate gastrointestinal neurobiology and physiology *via* olfaction. Given that canines possess an extremely discriminating olfactory system, it is further proposed that specific scent-stimuli prompt defecation. Such olfactory responses may have been imprinted genetically or always instinctive and manifested in behaviour, biology and physiology (including the vomeronasal organ). Specifically, the canine sphincter reflex and final peristalsis appear to be scent-mediated through synaptic neurobiology, triggered by a specific family of organic aromatic amines. However, as dogs have been making the transition from rural-to suburban-to urban settings, their quest for olfactory stimulation has become more challenging due to increasingly "sanitized" municipal environments. Indeed, while being welcomed into indoor cohabitation with busy and preoccupied human companions, erratic owners' schedules can compound these dynamics and lead to recurrent frustration with the dogs' apparent searching with respect to a normal excretion routine.

Keywords: Behaviour; Biogenic amines; Canine; Defecation; Gastrointestinal; Neurobiology; Olfaction; Vomeronasal organ

Abbreviations: HRD: Human Remains Detection; PPT: Parts-Per-Trillion; VNO: Vomeronasal Organ; RAIR: Recto-Anal Inhibitory Reflex

Introduction

Dog and human bond

The dog/family cohort is a powerful social and political group, as reflected in emerging trends. Dog status and social freedom are exemplified by the off-leash latitude in New York City public parks between 9:00 PM and 9:00 AM. Recently, the New York state legislature unanimously ratified a law also allowing dogs privileges in outdoor restaurant areas [1]. New Yorkers can now adopt the Parisian custom of inviting the city dog into many stores and sidewalk cafés. In fact, such liberal dog laws are emerging throughout many parts of the world, and services such as Bring Fido®, DogFriendly®, Rover®, and Yelp® provide owners with lists and maps of pet-friendly establishments. However, the domestic canine is evolutionarily complex, since dogs have acute "wilderness" olfactory capabilities, yet they typically live indoors in an out-of-context urban environment [2-4]. Dr. Alexandra Horowitz, a renowned dog olfaction and cognition expert, notably refers to the "yard dog" as if it were an extinct species [5]. Her work transcends traditional research-based scientific media, while reaching into the popular press with substantial readership and influence in both audiences [3,5-7]. In tandem, the growing interest in dog cognition has followed the urbanization of the "indoor" canine now integral to more than 60 million American households [8]. Despite tens of millions of such mutually adoring human and canine families worldwide, bonding failures all too often begin in the kennel upon adoption [9-12]. Overstimulated by indoor odors, sheltered dogs frequently become anxious, which can decrease both the chance of adoption and successful bonding thereafter. A lack of staffing or volunteers often results in dogs receiving less attention and exposure to the outdoor environment; in turn, many are forced to relieve themselves in their cages, eroding housebroken habits. Thus, dogs are routinely relinquished back to shelters for behavioural problems, and chief amongst these is accidental house soiling [13]. As shelters and philanthropic societies are increasingly adopting "no-kill" standards, every failure compounds the behavioural impact of further crowding, as well as the societal pressures to break the cycle of returns in order to maintain donor confidence and vital funding.

The scent search

Dogs have seemingly enjoyed almost all aspects of their transition indoors as companion animals. Two important, but less desirable, aspects of this shift nonetheless include overwhelming exposure to "sanitized" indoor spaces and being left alone while interminably having to hold the canine bowel and bladder in check [14]. While relevant publications infrequently mention dog defecation, feces and zoonosis, municipalities dutifully regulate dog waste collection with widespread public cooperation. Dog owners have cleverly adapted their hours and logistics to meet the needs of millions of dogs while complying with such requirements. However, dogs are often picky and potentially nervous creatures that require meandering and extended sniffing in order to achieve normal defecation. As a result, owners constrained by human schedules for work, children and life events can become quite frustrated with the amount of time associated with routine canine relief [15-18]. Many owners also realize that the sniffing process often appears to be equally frustrating for their pets. Cajoling a dog to stop dawdling may be tempered by considering that the dog evolved in the wilderness, habituated to open-air olfactory molecules. Intently searching for distinct particles, innumerable outdoor scents

***Corresponding author:** Anthony Lee Dellinger, Kepley BioSystems Incorporated, 2901 East Gate City Blvd, Suite 2400, Greensboro, NC, USA, E-mail: adellinger@kepleybiosystems.com

Received April 09, 2019; **Accepted** April 26, 2019; **Published** May 08, 2019

Citation: Brady TE, Abood SK, Kulberg RT, Dellinger K, Goddard MKM, et al. (2019) Olfactory Mediation of Canine Gastrointestinal Neurobiology. J Anim Health Behav Sci 3: 116. doi: 10.4172/2157-7536.1000116

(nearly absent indoors) often appear to erratically and repeatedly shift a dog's attention. Nevertheless, as an expert in molecular detection [19,20], the canine's frequently unrelenting search may be explained as a primal effort to locate specific decay molecules that were once abundant and plentiful. Contrary to popular assumptions, this behaviour is not associated with "marking" or otherwise seeking prior excrement of other dogs in the vicinity. Concrete jungles with sparse vegetation in "sanitized" metropolitan environments, coupled with diligent waste removal operations that effectively purge aromatic signalling molecules, can lead to longer searches that challenge dogs to accomplish defecation in a timely fashion [21,22].

Canine olfaction

Both the sensitivity and specificity of canine olfactory capabilities have been well-documented [23-28]. As such, canine scent detection has been proven in a myriad of applications; whereby, service dogs routinely recognize signature chemicals in explosives [23,29] and drugs [30]; track forensic evidence [31,32]; and assist in some disease diagnostics [25,33-37]. Identifying and locating particles at Parts-Per-Trillion [PPT; three orders of magnitude more sensitive than current instrumentation; [25,38], dogs would thus be capable of detecting a single droplet diluted in 20 Olympic-size pools [25]. Renowned feats include the work of cadaver dogs, which are capable of Human Remains Detection (HRD) long after death, despite burial, submersion in water, or attempted concealment [39-41]. Dogs characteristically detect volatile compounds produced by organisms and associated with molecular death, as evidenced by spontaneously rubbing their snouts at the sites of decayed animals, absent any otherwise discernible evidence. In the case of HRD, such identification is elicited by a set of volatile organic aromatic amines that linger for an indeterminate period [42].

Vomeronasal organ

The canine Vomeronasal Organ (VNO) was examined extensively [43-46] and determined to be a robust and highly vascularized anatomical adaptation of the dog's olfactory system; however, before and since, there has been a paucity of further VNO research. That said, the VNO in dogs is developed and prominent, yet it belies simple evolutionary explanation [43]. Located in the roof of the canine mouth, the VNO has been classified as a secondary olfactory organ capable of detecting chemical signals released from conspecifics and food flavors [47,48], perhaps best described as sensing a combination of taste and smell. The VNO in long-snouted dogs is especially prominent and proportional, representing a large olfactory, gustation and neurobiological center at the apex of external stimuli. While human gustation and taste have been well characterized, the canine experience has been somewhat left to speculation. Most dogs typically devour mouthfuls of food in a voracious manner, and little time is given to mastication or the ostensible savoring of taste sensations [49]. Aromatic signals obtained from food are nonetheless amplified by the VNO; yet in contrast to food consumption, prolonged bone chewing may provide a much greater source of canine gustatory stimulation.

Canine olfaction, neurobiology and gastrointestinal reflexes

The olfaction-synaptic gastric link was notably demonstrated by Pavlov as a conditioning response to ringing a bell in association with scent stimuli. However, the observed response was simple salivation, secondary to smelling/association with food for the dog [50]. More recently, many have described distinct, bidirectional signalling between the gut and brain [51-57]. This pathway is essential for wellbeing, providing on-going feedback with respect to digestive status and the recognition of hunger or satiety, as well as imbalances or pathogens impeding ingestion that may reach awareness of discomfort. Described as the "gut-brain axis," these pathways are hormonal, immunologic and neural (*via* the central and enteric nervous systems). Humans likewise experience these connections, as evidenced by the relationships between anxiety, stress, sudden bowel movements, and the ultimate awareness of dyspepsia. Whereas, the rectum and the colon serve as reservoirs for gastric luminal contents, the link between intestinal motility and the central nervous system has also been well established; for example, just as the ability to decipher when and where defecation is appropriate has proven to be critical for survival and social acceptability [58]. Gastrointestinal motility is modulated by a number of colonic reflexes [59], and the activity in one part of the gut can affect others. For instance, feeding has been previously reported to increase proximal duodenal tone in canines; thus, reducing the capacity and prompting receptive relaxation of the colon, indicative of a gastrocolonic reflex [60].

Linking olfaction to neurobiology and gastrointestinal processes

The human brain perceives and interprets odors differently from all other senses; such as, pleasant cooking aromas that stimulate hunger and familiar scents that awaken memories by association. Conversely, many volatile aromatic amines at high concentrations can induce human retching. Clearly, the olfactory transmission and information exchange in the brain stem appears to be linked to the physiology of the autonomic and somatic nervous systems [61]. While further dynamics of these links remain the subject of scientific debate, the scent-brain-stomach relationship is arguably self-evident. In turn, the somatic nervous system regulates body movement through control of skeletal muscles and orients the organism to its environment through the reception of external stimuli, such as vision, hearing, taste and smell [62]. The myenteric plexus is the major nerve to the gastrointestinal tract that communicates with the central nervous system in response to an array of digestive conditions; these include colon distention, which would alert the brain when the bowel needs relief. In turn, fibers in the somatic nerve (pudendal) of the pelvic floor and anus respond to specific triggers to initiate the final-phase defecation reflex. Given the array of inputs to the somatic nervous system, an olfactory stimulant is consistent with this neurobiological process and could likewise initiate the defecation reflex in a similar fashion. In the canine, the olfactory response appears remarkable in both final peristalsis (voluntarily releasing the bowel) and in prompting the defecation reflex. Sniffing the ground occurs universally and consistently appears to activate the somatic nervous system; it is thus biologically and conceptually plausible that an olfactory stimulus is linked to the somatic defecation reflex. Notably, dogs also typically appear to lose interest in olfactory stimulation immediately following excretion.

Evolutionary milieu

As a context for these observations, canine evidence suggests that the species has developed over the last 35 million years with consistently carnivorous characteristics [63]. Such traits have included eating spoiled meat throughout its evolution without becoming a carrion-dependent species, as decay (and consequent volatile emissions producing characteristic olfactory stimulants) would have occurred on the cellular level immediately following expiration of the hunted or scavenged organisms were evolving at the same time [42]. These distinctive "decay" molecules have been associated with a family of volatile aromatic amines that have been manifested in varying concentrations and combinations over time [64]. As such, organic particles generated by the decomposition of animal carcasses, dense forestry, and plants, unique to parasitic acceleration, all produce pungent aromas. Specific olfactory receptors have evolved for these molecules, which are recognized in milliseconds by the synaptic pathway of the canine brain. The brain/gut neurobiological signals are likewise instantaneous, but peristalsis requires time for sphincter relaxation and bowel relief. Other species, especially carrion-eating creatures (e.g. condors, hyenas, jackals, leopards, lions, raccoons, seagulls, wolves, etc.) that hunt to feed, have also demonstrated exceptional sensitivity to these aromatic amines emitted from decayed flesh, including arthropods and gastropods, albeit the latter in undersea environments [65-68]. In fact, the apparent attraction appears to be shared among all predators and scavengers with chemosensory capabilities to associate these particles with sustenance. Crustaceans, in particular, have provided a model for demonstrating carrion-scavenging specificity in the laboratory and field. Nocturnal-foraging crustaceans chemotactically locate potential food sources, consistently entering traps with bait containing/releasing these amines. Crabs, lobsters and crayfish (in the wild, captive and in aquaculture) readily consume calcium-based matrices infused with these volatile amines in the absence of flesh or other organic matter [69]. With respect to dogs, they are drawn to pungent odors, including human sweat, tires, and urine [70,71]. As noted, from hunting to eating to gnawing bones, volatile biogenic amines have played a central role in the canine experience: These molecules have also been evolutionarily ubiquitous when dogs have been seeking to achieve bowel relief for millennia. However, urban planning and waste management have gradually diminished the sources of aromatic amines from the environment where tens of millions of dogs share human dwellings and the "outdoor" spaces needed for routine elimination in municipalities, worldwide. Obviously, dogs vary, and some breeds more efficiently and reliably detect such molecules, indicating a range of canine responses to stimulating particles (parts per billion *vs*. trillion; [38]). However, the inexorable presence of such organic compounds, combined with the resolute determination of dogs "sniffing" as a precursor to bowel relief, suggest that these are in fact the biogenic amines that provide olfactory stimulation for final canine peristalsis.

Aromatic amines and bone gnawing

A strong association between aromatic amines and canine digestion plausibly evolved with bone gnawing; whereby, prey bones have provided marrow and calcium nutrients and no doubt fostered dental health while releasing aromatic amines sufficient to not only attract canines in the first instance, but also to characteristically bury, locate and later retrieve them. Chewing is inherently linked to gastrointestinal stimulation and ultimately neurobiological signalling for bowel relief. Neurotransmitters, in particular, also require calcium ions, possibly in abundance, to reach the brain/gut pathways that credibly imprinted these instinctive reflexes. As a predator, the canine jaw and teeth clearly adapted to gnawing with powerful mastication muscles that can produce over 1,600 newtons of force [72]. As gnawing gradually micronizes bones into particulates mixed with saliva, it provides a requisite bath for the canine VNO. While hunger is the primary motivation of hunting and scavenging animals, domestic dogs generally do not hunt to feed, and their consumption of meat is a distinct behaviour from bone chewing [73]. Indeed, mastication of bones (and other dense materials) has been a generally acknowledged canine trait consistent with the VNO function.

Recto-Anal Inhibitory Reflex (RAIR) *vs.* Olfactory stimulation

The retention and subsequent release of gut contents following universal "sniffing" in advance of defecation yields compelling evidence of linkage to the canine olfactory bulb. The defecation reflex triggers two main sphincters around the anal canal: the internal sphincter (which cannot be controlled voluntarily) and the external sphincter (a skeletal muscle which can be controlled) [74,75]. The reflex also controls when the internal sphincter relaxes and the external sphincter contracts [76]. However, the apparent ability to "sniff" for extended periods of time and seemingly ignore a myriad of scents while "searching" suggests a Recto-Anal Inhibitory Reflex (RAIR), in response to rectal distention, in the absence of specific olfactory stimulation. RAIR allows the animal to delay defecation by moving the stool backward slightly and reducing the urge to defecate [77]. Upon stimulation, the canine can then activate voluntary muscles to initiate movement of the stool forward and outside of the body. Such behaviour associating the canine's "sniff search" prior to relief [78], further suggests that particular aromatic amines induce these reflexes and facilitate elimination. Notably, dogs have been used as pudendal nerve test models in the search of human methods for neurobiological repair due to disease and injury [79,80]. Such controlled experiments have shown that this neuro-pathway, when disturbed, readily malfunctions in dogs [81,82]. The myenteric plexus (or signalling) of the colon and the pudendal nerve systems are separate neurobiological pathways, requiring bidirectional synaptic signalling. It may follow that the olfactory-stimulating prerequisites also require both systems to respond independently, (e.g. final peristalsis and anus/sphincter reflex from the nerve bundle) for canine defecation.

Testing the Hypothesis: Field Trials Using Synthetic Aromatic Amines

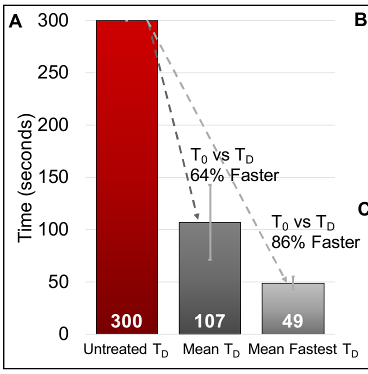
To examine this linkage, specific botanical and organic amines theorized to be sought by the "sniffing" canine in advance of defecation were identified and evaluated. The formulation was refined using an array of naturally occurring molecules. By placing a drop on the back of the hand, allowing to air dry, and washing vigorously with soap and water in the laboratory, the family dog's response without prompting upon returning home helped isolate key ingredients. Upon selection, an optimized (most consistent response) combination of aromatic biogenic amines was then placed on the dog's front paw following a routine digestion period (e.g. "time-to-let-the-dog-out" or "time-to-walk-the-dog") in a series of tests. As a note, the solution is odorless to humans but typically yields considerable canine sniffing and tracking. Such controlled aromatic amine exposure generally accelerated canine bowel relief following application just prior to giving access to "outdoor" locations. That is, further proof-of-concept trials engaged a sampling of volunteers and their dogs to see if the solution could expedite canine defecation in real world circumstances. Field test materials and tracking sheets were provided to participants with a simple protocol so as not to distract their dogs; all of which were responsive to the material – across a broad range of sizes and ages. Most achieved an improvement in the timeliness of the defecation reflex over seven daily applications (once, but occasionally twice, a day). In some instances, no discernible differences were noted by owners; however, the testing occurred during the winter months when some acknowledged they could not always observe the dogs due to darkness. In more controlled testing with daily use over a longer period (>80 tests) with a Labrador, the defecation times decreased to less than 2 minutes, from paw application to elimination; with a mean of 1:47 (± 36 seconds) (Figure 1). Average Defecation Times (T_D) without treatment were typically greater than 5 minutes (Figure 1). The three fastest observed T_D were 42, 52, and 53 seconds (average=49.0 seconds), and the three slowest observations (T_D) were 175, 181, and 186 seconds (average=180.7 seconds) (Figure 1). Overall, spanning 88

Citation: Brady TE, Abood SK, Kulberg RT, Dellinger K, Goddard MKM, et al. (2019) Olfactory Mediation of Canine Gastrointestinal Neurobiology. J Anim Health Behav Sci 3: 116. doi: 10.4172/2157-7536.1000116

tests, the stimulant solution reduced the T_D by 64% when compared to average untreated times (Figure 1). Comparable observations were made in a second set of controlled evaluations with smaller breeds, including a Yorkshire Terrier (4 years of age); Miniature Doberman (9 years); Jack Russel Terrier (1.5 years); and a Beagle (6 years), (Figure 2). Additional trials with a sampling of larger breeds yielded similar findings with a Golden Retriever (8 years of age); Weimaraner Pit Bull Mix (4 years); Rottweiler Australian Shepherd Mix (5.5 years); Labrador (4 years); and a Rhodesian Ridgeback (8 months). The field evaluator with a Labrador (4 years of age) reported typically needing to "walk the entire perimeter of the yard prior to elimination" requiring between 10 to 15 minutes prior to the testing. During the trial, the Labrador improved to an average time from drop administration to defecation of 4 minutes and 57 seconds (Figure 3). The research team and the volunteer dog owners found generally improved response times, i.e., consistent (T_0) *vs.* (T_D). Field evaluators perceived 75% of the daily tests to result in faster defecation times; while 21% were viewed as similar or unchanged; and 4% were perceived as slower (Figure 4). Notably, the slower responses were observed at the beginning of the trials, primarily occurring on days 1 and 2. In every test, the experiment was concluded when the animal defecated; and in nearly 90% of the evaluations, the dogs defecated and urinated. The trials were performed for at least seven days at the owners' (and presumably the dogs') preferred times of day. While some tests improved over time, the age, breed, sex, and location (i.e., rural, urban, city, backyard, or on leash) did not appear to affect the results of the evaluation. While one cannot ignore a conditioning aspect of the drop placement and the dogs' responses to their owners, the perceived confidence in overall defecation reflexes improved with daily use.

Next Steps: Real World Kennel Testing

The behavioural and environmental objective of developing this scent stimulant was to examine the potential for controlled exposure to "sniff-search" target molecules to accelerate routine canine defecation while improving dog waste management (e.g. owner management of when and where). A broader humane and societal goal would be to reduce the number of adoption failures and shelter returns due to indoor soiling. In effect, faster and more consistent "dog walking" to



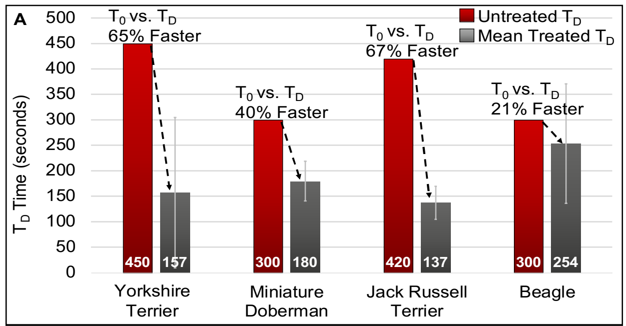
B

Breed	Labrador
Weight	65 lbs.
Sex	Male
Age	2.5 Years
Setting	Rural
Surface	Grass
Location	Fenced Backyard

C

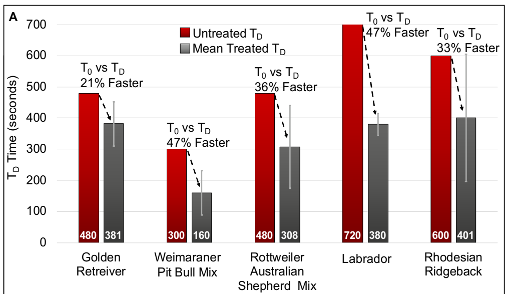
Untreated T_D [sec]	300
Average Treatment T_D [sec]	106.9 (±35.9)
Fastest Treatment T_D [sec]	49.0 (± 6.1)
Slowest Treatment T_D [sec]	180.7 (± 5.5)
Owner Perceived Faster	100%
Owner Perceived Slower	0%
Owner Perceived Same	0%

Figure 1: Field trial with a black Labrador (2.5 years of age); A) The average control 321 time (untreated) to defecation was 300-seconds. One drop of stimulant solution was placed on 322 the dog's front paw and recorded as T0, and the time required for defecation (TD) was recorded 323 for each test. The average TD across all observations (n=88) was 107 seconds (64% faster than 324 the untreated TD); the average of the three fastest TD was 49 seconds (86% faster than the 325 untreated TD); B) Indicates the breed, sex, weight, age, setting and preferred elimination 326 surface and location. C) Includes average untreated TD, treated TD and owner perceptions 327 from the field questionnaire.



B Dogs Less than 50 lbs.	Yorkshire Terrier	Miniature Doberman	Jack Russell Terrier	Beagle
Weight	6 lbs.	10 lbs.	17 lbs.	25 lbs.
Sex	Female	Male	Male	Male
Age	4 Years	9 Years	1.5 Years	6 Years
Setting	Suburb	Suburb	Suburb	Suburb
Surface	Grass	Grass	Grass	Grass
Location	Backyard	Leashed/Walk	Backyard	Backyard
Untreated (T_D) [sec]	450	300	420	300
Average Treated (T_D) [sec]	157 (±147.7)	180 (±38.7)	137 (±32.3)	254 (±117.5)
Average Fastest (T_D) [sec]	55 (±8.0)	150 (±30.0)	114 (±14.2)	160 (±34.6)
Average % Decrease in T_D	65%	40%	67%	15%
Optimal % Decrease in T_D	88%	50%	73%	47%

Figure 2: Field trials with four breeds weighing less than 50-lbs (Yorkshire Terrier, Miniature Doberman, Jack Russell Terrier, and Beagle); A) The average untreated elimination time (red) for each dog was established by the field evaluator; Each trial consisted of seven unique tests; One drop of stimulant solution was applied to the dog's front paw at T0, and the evaluator measured the time; Upon defecation, the time was recorded as TD; Gray bars represent the average TD across the seven-day trial; B) Indicates the animal information average untreated/treated elimination times, and percent change in TD *vs.* untreated tests (2018).



B Dogs Greater than 50 lbs.	Golden Retriever	Weimaraner Pit Bull Mix	Rottweiler Aust. Shepherd Mix	Labrador	Rhodesian Ridgeback
Weight	60 lbs.	60 lbs.	72.5 lbs.	75 lbs.	80 lbs.
Sex	Male	Male	Male	Male	Female
Age	8 Years	4 Years	5.5 Years	4 Years	8 Months
Setting	Suburb	City	Suburb	Rural	Suburb
Surface	Grass	Grass/Bush	Grass	Grass	Grass
Location	Backyard	Leashed/Walk	Backyard	Backyard	Backyard
Untreated (T_D) [sec]	480	300	480	720	600
Average Treated (T_D) [sec]	381 (±70.8)	160 (±72.0)	308 (±132.9)	380 (±34.6)	401 (±205.5)
Average Fastest (T_D) [sec]	320 (±31.2)	106 (±13.9)	195 (±19.9)	180 (±0.0)	238 (±82.5)
Average % Decrease in T_D	21%	47%	36%	47%	33%
Optimal % Decrease in T_D	33%	65%	47%	79%	60%

Figure 3: Field trials with five breeds weighing greater than 50-lbs (Golden Retriever, Weimaraner Pit Bull Mix, Rottweiler Australian Shepherd Mix, Black Labrador, and Rhodesian Ridgeback); A) The average untreated elimination time (red) for each dog was established by the field evaluator. Each trial consisted of seven unique tests. One drop of stimulant solution was applied to the dog's front paw at T0, and the evaluator measured the time. Upon defecation, the time was recorded as TD. Gray bars represent the average TD across the seven-day trial; B) Indicates the animal information, average untreated/treated elimination times, and percent change in TD *vs.* untreated tests. (2019).

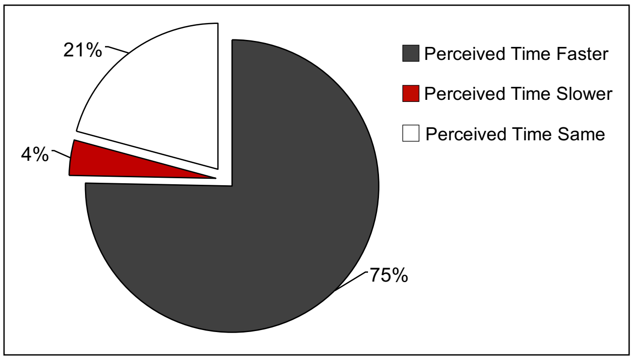


Figure 4: Owners' perceived efficacy of the stimulant solution; During each test of the trial, the volunteer evaluators were asked to rank the perceived efficacy of the solution in terms of time to defecation compared to untreated norms. Faster defecation times were observed 75% of the time (black); slower defecation was perceived 4% of the time (red); and no observed changes were perceived 21% of the time (white).

leverage limited shelter resources could facilitate the adjustment from "kennel life" to conditions in a new family environment [83]. That is, establishing pre-adoption and subsequent routines for more rapid and predictable dog defecation using the solution could play a critical role in prevention of indoor soiling accidents, decreasing the likelihood of adoption returns to the shelter. Over time, this technology could potentially prevent innumerable dogs from being relinquished or euthanized due to such incidents[46,84,85].

Discussion and Conclusion

Gastrointestinal health and defecation consistency are critical factors inextricably linked to the human:animal bond. This work to relate olfaction to canine gastrointestinal neurobiology is ongoing - and further studies are needed to examine the breadth of olfactory effects on canine behaviour, psychology and even disease states, including potential corollaries to pheromones and other volatile amines driving canine behaviour and socialization.

Authorship Statement

The canine "sniff-smell" target molecule hypothesis was conceived by TEB. ALD designed field research and data collection. TEB prepared the first draft. RTK and ALD performed literature review and reference documentation. All authors contributed to the new body of material and findings. All authors contributed to the manuscript and referenced material. MKMG refined and elucidated the thesis of this work. ALD, RTK, KD and LR performed early formulations and canine experimentation. SKA provided DVM and gastric expertise. ALD supervised the findings of this work.

Conflict of Interest Statement

TTEB and ALD are founder members of Kepley BioSystems, Inc. (kepleybiosystems.com). RTK, KD, MKMG and LR are also members of the Kepley BioSystems, Inc. team. SKA is Assistant Professor of Clinical Nutrition at the Ontario Veterinary College, University of Guelph and Owner of Sit, Stay, Speak Nutrition, LLC which specializes in nutritional coaching. The group has been working for several years to identify and select specific biogenic amines while developing synthetic delivery matrices for attracting crustaceans, which provided a framework for formulating them for dogs.

Ethics Statement

Any animal based included in this study were pet dogs voluntarily tested by their owners in their own homes.

Acknowledgements

The volunteer owners and their dogs contributed significantly with their time, patience and feedback in the preliminary field testing cited herein.

References

1. New York City Department of Health (2015) Notice of adoption of proposed amendments to title 24 of the rules of the city of New York.
2. Edensor T (2007) Sensing the ruin. The Senses and Society 2: 217-232.
3. Horowitz A (2010) Inside of a dog: what dogs see, smell, and know. Simon and Schuster
4. Henshaw V (2013) Urban smellscapes: understanding and designing city smell environments. Routledge. Taylor and Francis.
5. Horowitz A (2016a) Being a dog: following the dog into a world of smell. Simon and Schuster.
6. Horowitz A, Hecht J, Dedrick A (2013) Smelling more or less: investigating the olfactory experience of the domestic dog. Learning and Motivation 44: 207-217.
7. Horowitz A (2016b) Canis familiaris: companion and captive. In Gruen, L (Ed) (2014) The Ethics of Captivity. Oxford University Press, USA.
8. American Pet Products Association (APPA) (2018) 2017-2018 APPA national pet owners' survey statistics: pet ownership and annual expenses survey. American Pet Products Association Inc.
9. Kass PH, New Jr JC, Scarlett JM, Salman MD (2001) Understanding animal companion surplus in the United States: Relinquishment of nonadoptables to animal shelters for Euthanasia. J Appl Anim Welfare Sci 4: 237-248.
10. Marston LC, Bennett PC (2003) Reforging the bond-towards successful canine adoption. Appl Anim Behav Sci 83: 227-245.
11. Mondelli F, Previde PE, Verga M, Levi D, Magistrelli S, et al. (2004) The bond that never developed: adoption and relinquishment of dogs in a rescue shelter. J Appl Anim Welfare Sci 7: 253-266.
12. Herron ME, Lord LK, Hill LN, Reisner IR (2007) Effects of preadoption counseling for owners on house-training success among dogs acquired from shelters. J Am Vet Med Assoc 231: 558-562.
13. Segurson SA, Serpell JA, Hart BL (2005) Evaluation of a behavioral assessment questionnaire for use in the characterization of behavioral problems of dogs relinquished to animal shelters. J Am Vet Med Assoc 227: 1755-1761.
14. Tomlinson C (2016) Toileting troubles part 1: factors influencing house soiling in cats and dogs. Companion Animal 21: 351-357.
15. Hubrecht RC, Wickens S, Kirkwood J (1995) The welfare of dogs in human care. In The Domestic Dog. Cambridge University Press 180-198.
16. Dotson MJ, Hyatt EM (2008) Understanding dog-human companionship. J Business Res 61: 457-466.
17. Voith VL (2009) The impact of companion animal problems on society and the role of veterinarians. Veterinary Clinics of North America: Small Anim Prac 39: 327-345.
18. Gross M (2015) Natural waste: canine companions and the lure of inattentively pooping in public. Env Socio 1: 38-47.
19. Tripp AC, Walker JC (2003) The great chemical residue detection debate: dog versus machine. In Detection and Remediation Technologies for Mines and Minelike Targets VIII. International Society for Optics and Photonics 5089: 983-991.
20. Pickel D, Manucy GP, Walker DB, Hall SB, Walker JC (2004) Evidence for canine olfactory detection of melanoma. Appl Animal Behav Sci 89: 107-116.
21. Sussman D (2008) Since dogs do what they do, what can we do to improve the doo-doo situation at dog parks. Supplement to Context Alumni Magazine. Conway School of Landscape Design.
22. Gomez E (2013) Dog parks: benefits, conflicts, and suggestions. Journal of Park and Recreation Administration 31: 4.
23. Oxley JC, Waggoner LP (2009) Detection of explosives by dogs. in Aspects of Explosives Detection. 27-40.
24. Quignon P, Kirkness E, Cadieu E, Touleimat N, Guyon R, et al. (2003) Comparison of the canine and human olfactory receptor gene repertoires. Genome Bio 4: 80.
25. Angle C, Waggoner LP, Ferrando A, Haney P, Passler T (2016) Canine detection of the volatilome: A review of implications for pathogen and disease detection. Front Vet Sci 3: 47.

26. Marchal S, Bregeras O, Puaux D, Gervais R, Ferry B (2016) Rigorous training of dogs leads to high accuracy in human scent matching-to-sample performance. PLoS One 11: 2.

27. Duranton C, Horowitz A (2018) Let me sniff! nose work induces positive judgment bias in pet dogs. Appl Ani Behav Sci 2: 3-5.

28. Jenkins EK, DeChant MT, Perry EB (2018) When the nose doesn't know: canine olfactory function associated with health, management, and potential links to microbiota. Front Vet Sci 5: 56.

29. Lazarowski L, Foster ML, Gruen ME, Sherman BL, Fish RE, et al. (2015) Olfactory discrimination and generalization of ammonium nitrate and structurally related odorants in Labrador retrievers. Anim Cogn 18: 1255-1265.

30. Jezierski T, Adamkiewicz E, Walczak M, Sobczyńska M, Górecka-Bruzda A, et al. (2014) Efficacy of drug detection by fully-trained police dogs varies by breed, training level, type of drug and search environment. Forensic Sci Int 237: 112-118.

31. Furton KG, Caraballo NI, Cerreta MM, Holness HK (2015) Advances in the use of odor as forensic evidence through optimizing and standardizing instruments and canines. Philos Trans R Soc Lond B Biol Sci 370: 1674.

32. Cuzuel V, Cognon G, Rival, I, Sauleau C, Heulard F, et al. (2017) Origin, analytical characterization, and use of human odor in forensics. J Forensic Sci 62: 330-350.

33. Moser E, McCulloch M (2010) Canine scent detection of human cancers: a review of methods and accuracy. J Vet Behav 5: 145-152.

34. Cornu JN, Cancel TG, Ondet V, Girardet C, Cussenot O (2011) Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. Euro Urology 59: 197- 201.

35. Taverna G, Tidu L, Grizzi F, Torri V, Mandressi A, et al. (2015) Olfactory system of highly trained dogs detects prostate cancer in urine samples. J Urol 193: 1382-1387.

36. Pacik D, Plevova M, Urbanova L, Lackova Z, Strmiska V, et al. (2018) Identification of sarcosine as a target molecule for the canine olfactory detection of prostate carcinoma. Sci Rep 8: 4958.

37. Fischer TC, Johnen D, Nehls I, Becker R (2018) A proof of concept: are detection dogs a useful tool to verify potential biomarkers for lung cancer? Front Vet Sci 5: 52.

38. Walker DB, Walker JC, Cavnar PJ, Taylor JL, Pickel DH, et al. (2006) Naturalistic quantification of canine olfactory sensitivity. Appl Animal Behav Sci 97: 241-254.

39. Osterkamp T (2011) K9 Water searches: scent and scent transport considerations. J Forensic Sci 56: 907-912.

40. Papet LE, Ensminger J, Jezierski T (2016) Canine olfaction science and law: advances in forensic science, medicine, conservation, and environmental remediation. CRC press.

41. Swindells M (2016) The use of dogs in missing persons investigations. In: Morewitz S., Sturdy Colls C. (eds) Handbook of Missing Persons. Springer, Cham.

42. Stefanuto PH, Rosier E, Tytgat J, Focant JF, Cuypers E (2017) Profiling volatile organic compounds of decomposition. In Eline M. J. Schotsmans Nicholas Márquez‐Grant Shari L. Forbes (Eds.),Taphonomy of Human Remains:Forensic Analysis of the Dead and the Depositional Environment. Wiley Online Library.

43. Adams DR, Wiekamp MD (1984) The canine vomeronasal organ. J Anat 138: 771.

44. Halpern M (1987) The organization and function of the vomeronasal system. Ann Rev Neurosci 10: 325-362.

45. Keverne EB (1999) The vomeronasal organ. Science 286: 716-720.

46. Pageat P, Gaultier E (2003) Current research in canine and feline pheromones. Vet Clinics: Small Animal Prac 33: 187-211.

47. Halpern M, Martınez MA (2003) Structure and function of the vomeronasal system: an update. Progress in Neurobiology 70: 245-318.

48. Coli A, Stornelli MR, Giannessi E (2016) The dog vomeronasal organ: a review. Dog Behavior 2: 24-31.

49. Aldrich G, Koppel K (2015) Pet food palatability evaluation: a review of standard assay techniques and interpretation of results with a primary focus on limitations. Animals 5: 43-55.

50. Hare B, Woods V (2013) The genius of dogs. Oneworld Publications.

51. Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361: 512-519.

52. Lyte M, Li W, Opitz N, Gaykema RP, Goehler LE (2006) Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia citrobacter rodentium. Physio Behavior 89: 350-357.

53. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, et al. (2011) Ingestion of lactobacillus strain regulates emotional behavior and central gaba receptor expression in a mouse via the vagus nerve. Proceedings of the National Academy of Sciences.

54. Cryan JF, O'Mahony SM (2011) The microbiome‐gut‐brain axis: from bowel to behavior. Neurogastroenterol Motil 23: 187-192.

55. Foster JA, Neufeld KA (2013) Gut-brain axis: how the microbiome influences anxiety and depression. Trends in Neurosci 36: 305-312.

56. Quigley EM (2013) Gut bacteria in health and disease. Gastroenterol Hepatol 9: 560-569.

57. Carabotti M, Scirocco A, Maselli MA, Severi C (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol 28: 203-209.

58. Sarna SK (1991) Physiology and pathophysiology of colonic motor activity. Digestive Dis Sci 36: 827-862.

59. Bayliss WM, Starling EH (1899) The movements and innervation of the small intestine. The Jo Physio 24: 99-143.

60. Neri M, Phillips S, Fich A (1991) Measurement of tone in canine colon. J Physiol 260: 505-511.

61. Andrews PL (1992) Physiology of nausea and vomiting. Br J Anesth 69: 2S-19S.

62. Dorland WA (2011) Dorland's illustrated medical dictionary (32 Edn): Dorland's Illustrated Medical Dictionary. Elsevier Health Sciences.

63. Vilà C, Maldonado JE, Wayne RK (1999) Phylogenetic relationships, evolution, and genetic diversity of the domestic dog. J Heredity 90: 71-77.

64. Perrault KA, Stefanuto PH, Stuart BH, Rai T, Focant JF, et al. (2015) Detection of decomposition volatile organic compounds in soil following removal of remains from a surface deposition site. Forensic Sci Med Pathol 11: 376-387.

65. Preti G, Muetterties EL, Furman JM, Kennelly JJ, Johns BE (1976) Volatile constituents of dog (Canis familiaris) and coyote (Canis latrans) anal sacs. J Chem Ecol. 2: 177-186.

66. Su CY, Menuz K, Carlson JR (2009) Olfactory perception: receptors, cells, and circuits. Cell 139: 45-59.

67. Li Q, Liberles SD (2015) Aversion and attraction through olfaction. Current Bio 25: 120- 129.

68. Liberles SD (2015) Trace amine-associated receptors: ligands, neural circuits, and behaviors. Curr Opinion Neurobiol 34: 1-7.

69. Dellinger A, Plotkin J, Duncan B, Robertson L, Brady T, et al. (2016) A synthetic crustacean bait to stem forage fish depletion. Global Eco and Cons 7: 238-244.

70. Dunbar IF (1977) Olfactory preferences in dogs: the response of male and female beagles to conspecific odors. Behav Biol 20: 471-481.

71. Brian JL (1990) The filthiest show in town: sex and death. The Aus J Anthro 1: 180-191.

72. Ellis JL, Thomason J, Kebreab E, Zubair K, France J (2009) Cranial dimensions and forces of biting in the domestic dog. J Anatomy 214: 362-373.

73. Colard T, Delannoy Y, Naji S, Gosset D, Hartnett K, et al. (2015) Specific patterns of canine scavenging in indoor settings. J Forensic Sci 60: 495-500.

74. Karaus M, Sarna SK (1987) Giant migrating contractions during defecation in the dog colon. Gastroenterology 92: 925-933.

75. Matsufuji H, Yokoyama J (2003) Neural control of the internal anal sphincter motility. J Smooth Muscle Res 39: 11-20.

76. Bhadra N, Mortimer JT (2005). Selective block of external anal sphincter activation during electrical stimulation of the sacral anterior roots in a canine

model. Neurogastroenterol Motil 17: 721-726.

77. Chen JH, Sallam HS, Lin L, Chen JD (2010) Colorectal and rectocolonic reflexes in canines: involvement of tone, compliance and anal sphincter relaxation. Am J Physiol Regul Integr Comp Physiol 299: 953–959.

78. Hirabayashi T, Matsufuji H, Yokoyama J, Hagane K, Hoshino K, et al. (2003) Colorectal motility induction by sacral nerve electrostimulation in a canine model. Dis Colon Rectum 46: 809-817.

79. Ruggieri Sr MR, Braverman AS, Bernal RM, Lamarre NS, Brown JM, et al. (2011) Reinnervation of urethral and anal sphincters with femoral motor nerve to pudendal nerve transfer. Neurourol Urodyn. 30: 1695-1704.

80. Ju YH, Liao LM (2016) Electrical stimulation of dog pudendal nerve regulates the excitatory pudendal-to-bladder reflex. Neural Regen Res 11: 676-681.

81. Gomez-Amaya SM, Barbe MF, De Groat WC, Brown JM, Tuite GF, et al. (2015) Neural reconstruction methods of restoring bladderfunction. Nature Reviews Urology 12: 100-118.

82. Levine JM, Cohen ND, Fandel TM, Levine GJ, Mankin J, et al. (2017) Early blockade of matrix metalloproteinases in spinal-cord-injured dogs results in a long-term increase in bladder compliance. J Neurotrauma 34: 2656-2667.

83. Miller L, Zawistowski S (2012) Shelter medicine for veterinarians and staff. (2ndEdn.) John Wiley and Sons.

84. Christianson G (1981) Animal litter and method of preparation. US Patent No: 4,263,873. 28 Apr 1981.

85. Dodman NH (1995) Pheromone containing animal litter preparations. US Patent No: 5,415,131.16 May 1995.