



Olfactory Mediation of Canine Gastrointestinal Neurobiology

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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

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(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 (\pm 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

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

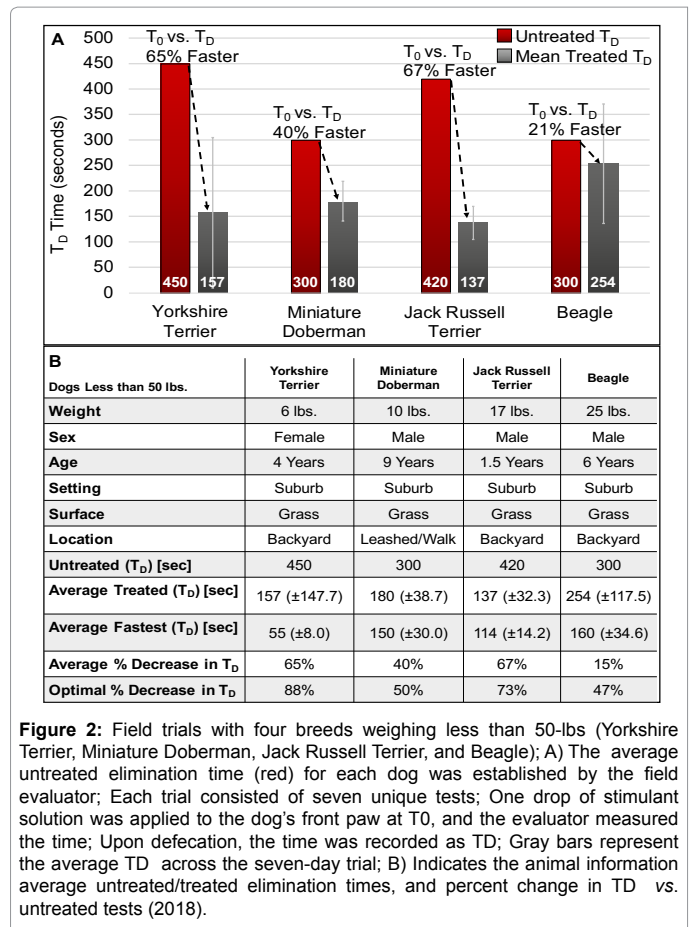


Figure 2: Field trials with four breeds weighing less than 50-lbs (Yorkshire Terrier, Miniature Doberman, Jack Russell Terrier, and Beagle); A) The average untreated elimination time (red) for each dog was established by the field evaluator; Each trial consisted of seven unique tests; One drop of stimulant solution was applied to the dog’s front paw at T_0 , and the evaluator measured the time; Upon defecation, the time was recorded as T_D ; Gray bars represent the average T_D across the seven-day trial; B) Indicates the animal information, average untreated/treated elimination times, and percent change in T_D vs. untreated tests (2018).

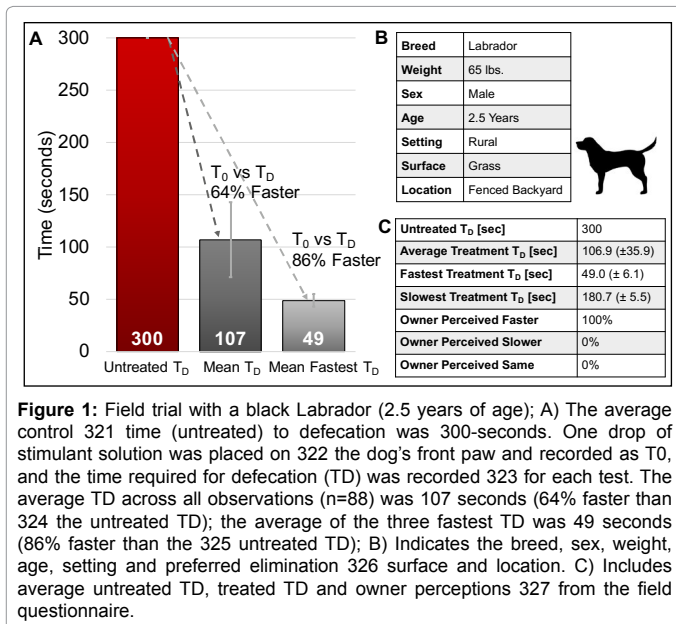


Figure 1: Field trial with a black Labrador (2.5 years of age); A) The average control 321 time (untreated) to defecation was 300-seconds. One drop of stimulant solution was placed on 322 the dog’s front paw and recorded as T_0 , and the time required for defecation (T_D) was recorded 323 for each test. The average T_D across all observations (n=88) was 107 seconds (64% faster than 324 the untreated T_D); the average of the three fastest T_D was 49 seconds (86% faster than the 325 untreated T_D); B) Indicates the breed, sex, weight, age, setting and preferred elimination 326 surface and location. C) Includes average untreated T_D , treated T_D and owner perceptions 327 from the field questionnaire.

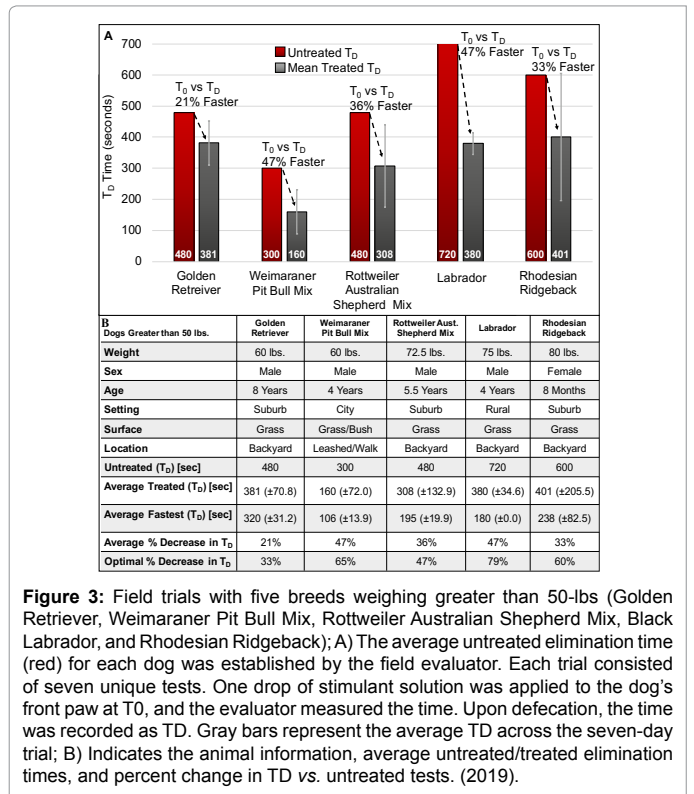
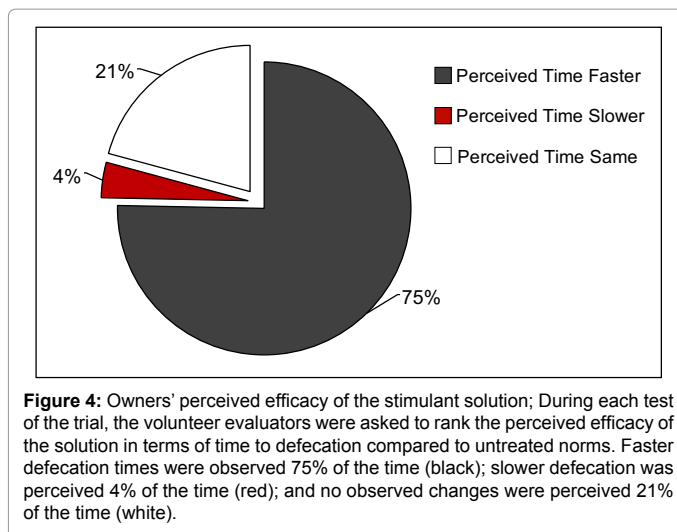


Figure 3: Field trials with five breeds weighing greater than 50-lbs (Golden Retriever, Weimaraner Pit Bull Mix, Rottweiler Australian Shepherd Mix, Black Labrador, and Rhodesian Ridgeback); A) The average untreated elimination time (red) for each dog was established by the field evaluator. Each trial consisted of seven unique tests. One drop of stimulant solution was applied to the dog’s front paw at T_0 , and the evaluator measured the time. Upon defecation, the time was recorded as T_D . Gray bars represent the average T_D across the seven-day trial; B) Indicates the animal information, average untreated/treated elimination times, and percent change in T_D vs. untreated tests. (2019).



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.

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