Canine Threshold to Double Base Smokeless Powder

By

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A Thesis

In

Animal Science

Submitted to the Graduate Faculty

of Texas Tech University in

Partial Fulfillment of

the Requirements for

the Degree of

MASTER OF SCIENCE

Approved

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August, 2021

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ACKNOWLEDGEMENTS

First and foremost, I want to thank my advisor, Dr. Hall, for accepting me as a graduate student two years ago. Not only have we survived two years of learning and growth, but we survived a worldwide pandemic. I have learned so many new skills and topics, from epoxying floors to maximizing humidity in hot temperatures to a dogs' detection capabilities, or lack thereof in some cases. I will cherish all I have learned and carry it with me in my next chapter. I would also like to thank my committee members, Dr. Katy Schroeder, and Dr. Paola Tiedemann, as you both have guided me through different projects over the last two years.

Additionally, I want to thank all the undergraduates, graduate students, and the post-doc for your kindness and guidance throughout my time at the Canine Olfaction Lab. You have each made an impact on my educational career, and I look forward to seeing how each of you thrive! I also want to thank the dogs who survived months of rigorous training and testing to help me complete my studies, especially the six German Shorthaired Pointers and one Labrador Retriever.

Most importantly, I want to thank my family, my boyfriend, and my two dogs. Without your support, I probably would have lost my sanity. Your love, willingness, and dedication allowed me to be standing where I am today.

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ABSTRACT

Canines have been employed to thoroughly search susceptible areas such as schools, sporting events, concerts, and more for explosives and other weapons. Unfortunately, while dogs are so readily utilized, little is known about dogs' detection limits to essential odorants. This study aimed to determine eleven dogs' thresholds to double-base smokeless powder utilizing an air dilution olfactometer to evaluate individual differences. Using a 2-up, 1-down adaptive descending staircase procedure, dogs completed two threshold assessments which were required to meet a specific repeatability criterion. More assessments were required if dogs did not meet our repeatability criterion. Using dog as a fixed effect in our linear model, we found that there were important differences in detection limits between individuals, with some dogs showing consistently lower thresholds and an overall variation of 1,100-fold difference between the best and worst threshold. The results from this study validated the use of an air dilution olfactometer with a correlation measure of 0.86. highlighted individual differences in detection dogs. In addition, the data highlighted individual differences in detection dogs, suggesting that some dogs are inherently better at detecting smokeless powder.

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CHAPTER I

INTRODUCTION

With an increased concern for mass shootings and bombings, canines have been employed to search susceptible areas such as schools, sporting events, concerts, and more for explosives, weapons, and other contraband. Although dogs are readily utilized, little is known about dogs' thresholds (or limits of detection) to key odorants for which they are employed to detect. Therefore, there is an important need for more research on odorants related to explosives and narcotics which specifically pertain to national homeland security applications (Waggoner et al., 1997). This thesis will report on eleven dogs' thresholds (detection limits) for double-base smokeless powder.

Working Dogs and Their Noses

Dogs appear to rely on olfaction more so than any other sense (Gazit & Terkel, 2003), and is the primary sensory system relied upon for detection dogs. A dog's sense of smell has been leveraged for a variety of detection tasks such as cancer (Cornu et al., 2011; McCullock et al., 2006; Roizen, 2006; Willis et al., 2004;), landmines (Sargisson, McLean, Brown, & Bach, 2012), explosives (Furton, 2001; Gazit & Terkel, 2003), narcotics (Marks, 2002; Riva et al., 2012), human scent (Brisbin & Austad, 1993; Greatbatch et al., 2015), and many species' scat (Cablk & Heaton, 2006; Cristescu et al., 2015), among many others. A dog's keen sense of smell is a result of many anatomical and physiological adaptations (Quignon et al., 2011).

One physiological adaptation presumed to enhance the detection capabilities of a dog are the inhalation airflow patterns (Craven et al., 2007). When a dog inhales, odors rush through the alar fold which directs the air flow to olfactory or respiratory purposes (Evans et al., 2013). Odor delivered for olfactory purposes passes through the dorsal meatus, where it then is delivered to an area known as the olfactory recess, located in the back of the olfactory path. The olfactory recess is made up of a structure known as the ethmoturbinates, a scroll-like structure, which increases olfactory epithelia surface area (Evans et al., 2013). Within the epithelium are olfactory sensory neurons which protrude cilia. These cilia contain olfactory receptors which bind to the odor and are critical in deciphering the odor (Mainland & Sobel, 2005; Uemura, 2019). Because the olfactory recess is in the rear of the olfactory path, when a dog expires air, the odorant is not expired with the other air due to unidirectional flow, allowing for additional sampling before a new set of odorants are delivered (Craven et al., 2007). Additionally, when the dog exhales, air escapes through lateral slits on the side of the nostrils (Horowitz, 2017). This expired air is pushed down and to the side which disrupts odorants and serves to entrain odorants from further distances allowing for more potential odor sampling (Staymates et al., 2016).

If an odor in the air catches the attention of a dog, they switch to “active sniffing” which causes a dramatic increase in the inhale and exhale rate (Craven et al., 2009). Craven et al. (2009) measured active sniffing in bouts, which were described as shorter sniffing periods followed by a larger inhalation of air. The bouts lasted anywhere between half a second up to several seconds. During active sniffing, a dog’s sniffing

frequency ranged from 4-7 Hz (Craven et al., 2009), which is similar to that of the rat at 6-9 Hz (Kepecs et al., 2007), but less than a mouse at 9-12 Hz (Wesson et al., 2008) during exploration of an odorant. According to Spencer et al. (2021) sniffing frequency appears to decrease as body size increase. In the study, sniffing frequency was measured for a rat (average 8 Hz), dog (average 5 Hz), giraffe (2 Hz), and elephant (2 Hz). It appears that the faster the sniff, the faster the relay of data; however, this comes at a price of sacrificing in-depth information about the odor (Spencer et al., 2021).

For humans, the sniffing frequency lies around 0.3-0.7 Hz (Craven et al., 2009). Humans' slower sniffing frequency can cause what Sela and Sobel (2010) coined as "change anosmia". In other words, humans sniffing frequency is slow enough to get in the way of detecting small changes of an odorant. Therefore, a higher sniffing frequency would appear to be linked to a higher sensitivity when it comes to detecting odorants. Based on these assumptions, it could be expected for dogs, mice, and rats to have better olfactory capabilities than a human.

Canine Threshold Assessments

There are a variety of methods to complete threshold assessments; however, several adaptive procedures and the method of constant stimuli are most utilized in current practices. Commonly, the method of constant stimuli is viewed as the "gold-standard" of threshold assessments (Wise et al., 2008). In olfactory threshold assessments, the method of constant stimuli presents a wide range of concentrations set by researchers prior to the study. These concentrations range below, at, and above the perceived threshold. Each concentration is presented multiple times and randomly

throughout a test. The individuals' performance is then plotted on a psychometric curve. Threshold is determined by extracting from the entire sample. Because of the increased number of concentrations and presentations, it provides a more accurate threshold measurement. However, the increased number of concentrations increases the time necessary to complete an assessment, which hinders the ability to use this method (Leek, 2001). In contrast, in adaptive procedures the concentration presented is based on the performance of the last trial. This produces a faster threshold measurement, with the caveat being less data points (Wise et al., 2008). However, despite their dissimilarities, both assessments have shown similar threshold measurements in humans (Wise et al., 2008; Lotsch et al., 2004). Unfortunately, there is currently no literature for these threshold assessment tools in canines.

Olfactory capabilities are often characterized by odor recognition, odor discrimination, and odor threshold tests (Hall et al., 2014; Lubow et al., 1973). While odor recognition and odor discrimination tests are necessary, odor threshold tests aim to determine the lowest concentration of an odorant which can be reliably detected. Doty, 2019). Knowing odor thresholds allows inferences to be made, such as the minimum concentration necessary for detection to occur. These inferences are extremely necessary in the working dog field where dogs are required to detect important odorants, such as explosives and drugs, for human safety at airports, in the military, and as police dogs.

Interestingly, relatively little known about canine thresholds in comparison to other species (McGann, 2017), but across the last five decades, there have been a few studies to evaluate canine detection thresholds. Some of the earliest work in North

America was conducted by Moulton et al. (1960) where they assessed two dogs' threshold to nine n-aliphatic acids from formic acid to iso-butyric acid. Threshold tests were completed by diluting the target acid with distilled water. The odor/water mixture was placed in a metal crucible and hid in one of four metal pots in a corner of the room. The additional three empty crucibles and pots were filled with distilled water. The dogs were trained to sit when they identified the target acid and ignore the pots that contained distilled water.

One dog tended to detect acids with higher carbon counts more easily than those with less carbon chains. The second dog's threshold did not statistically correlate with the compounds' carbon chain length (See Table 1 for threshold measurements). Fluctuations in thresholds from acid to acid may have, in part, been due to the varying water solubilities of the acids (Moulton et al., 1960). Additionally, the results of the first dog in this study align with many other published studies for non-human primates (Laska & Seibt, 2002; Laska et al., 2000) as well as rats and mice (Johnson et al., 1999; Laska, et al., 2006; Moulton, 1960)

A few years later, Moulton and Marshall (1976; see in review Passe & Walker, 1985) used four German Shepherds in a three-choice behavioral threshold assessment. A descending staircase procedure was utilized, and the α- ionone/air mixture started at 10^{-3} vapor dilution up to 10^{-9} vapor dilution. Dilution steps were $10^{-1.5}$. An olfactometer delivered the odorant, α- ionone, and air to ports available to the dog in a "bay." One bay contained odor, while the other two had blank air. A correct answer was measured when the dog remained at the odor bay for 5 seconds. After a correct response, the dog received

access to water for seven seconds. Incorrect responses were not rewarded or punished. Threshold results ranged between 1 x $10^{-9.5}$ to 1 x $10^{-7.5}$ of vapor saturation ($10^{-17.68}$- $10^{-15.68}$ M: see Table 1; note threshold from the best performing dog was determined using extrapolation based on performance from the session), with about a 50-100-fold difference between their best and worst performing dog. Moulton and Marshall (1976) noted that the large dilution steps utilized in this study could have been cause for worse threshold measurement. Leek (2001) also recognizes this potential issue; however, it is unknown what effect dilution step sizes have on the overall threshold measurements for an individual.

Using an air dilution olfactometer, Krestel et al. (1984) evaluated and compared human and dog thresholds to amyl acetate. Six beagles and ten humans were evaluated during the study. The equipment was identical for both dogs and humans. In study one, the dogs were initially trained to press a lever to gain access to water. Next, dogs were trained to discriminate odor (amyl acetate) versus no odor through conditioned suppression (Krestel et al., 1976; Henton, 1969; Pierson, 1974). Whenever odor was presented, it was followed by a shock. When testing began, dogs would press the lever for water continuously. However, when odor was presented, lever pressing ceased. The research group then compared the number of pre-odor lever presses, the number of lever presses while odor was present, and the number of lever presses when odor was no longer present.

In the second study, Krestel et al. (1984) asked human participants to place their nose into the breathing chamber and indicate the presence or absence of odor. The results

revealed dogs to be more sensitive to amyl acetate. Humans were able to detect, on average 6.92×10^{-9} M while dogs were able to detect $1 \times 10^{-9.43}$ M (Krestel et al., 1984; see in review Passe & Walker, 1985; Thresholds are also listed in Table 1). In humans, there was a 3-fold difference between the best and worst threshold. In dogs, there was a 450-fold difference in threshold concentrations from the best to the worst dog.

Walker et al. (2006) utilized permeation tubes to tests dogs' threshold to amyl acetate. The permeation tubes were used to dilute the amyl acetate at a steady, constant rate. Dogs received odor from a Teflon box with a small door for odor sampling. On the top of the Teflon box, the permeation tube was housed in a small glass container. Air entered through the top of the glass container, blew across the permeation tube, and escaped through a small exhaust in the bottom of the Teflon box. Electronic mass flow controllers and compressed air allowed for proper dilution of amyl acetate from the permeation tube to provide a wide range of odorant concentrations. Dog A was tested with concentrations ranging between 2.6×10^{-13} M to 8.5×10^{-15} M while Dog B was tested between 6.7×10^{-13} M to 2.6×10^{-14} M, correlating to a 2-fold difference between the lowest concentrations from Dog A and Dog B and a 3-fold difference between the larger threshold concentrations from Dog A and Dog B

Dogs were trained to sit if they detected the odorant. If a dog responded incorrectly, the handler said "no" and would quickly lift the dog out of a sit. The two dogs were able to detect 4.74×10^{-14} M of amyl acetate (See also in Table 1). This was approximately 30 to 20,000-fold lower than Krestel et al.'s study (Walker et al., 2006). Walker et al. (2006) spent 6 months training the dogs involved, as well as an additional 7

months in testing, which in turn may have increased the dogs' overall sensitivity to the odorant.

With different testing equipment, Concha et al. (2019) utilized a scent carousel to determine dogs' threshold to amyl acetate. The amyl acetate was diluted in mineral oil, which is a frequently used solvent for olfactory work. Ten dogs were trained for 30 weeks and then completed 12 threshold assessments. Dilutions were initially yoked to the dogs' previous performance. In a second part of the study, concentrations were randomly probed. Individual dogs' thresholds ranged from $1.66x10^{-9}$ M to $5.24x10^{-14}$ M, a 31,000-fold difference between the best and worst threshold concentrations. This is a great example of substantial subject variability. Concha et al. (2019) attributed this variation to genetics, as well as an array of individual characteristics such as ability to focus and motivation.

While the previous studies focus on n-aliphatic acids and amyl acetate, it is useful to analyze dogs' thresholds to important chemicals that working dogs are frequently trained to detect. Waggoner et al. (1997) analyzed dogs' threshold to methyl benzoate (an odorant associated with illicit cocaine). Illicit cocaine was also tested. A total of thirteen dogs were tested in experimental chambers. An air dilution olfactometer presented the odor stimuli (either illicit cocaine or methyl benzoate) into a glass chamber, which was mounted to an aluminum interface on the wall. A right, middle, and left lever were located on the aluminum interface- the right lever meant the target odorant was present, the left lever meant no odor was present, and the middle lever was pressed if a non-target odor was presented. Non-target odors included amyl-butyrate, allyl sulfide, amyl

butyrate, dimethyl thiazole, and alpha-ionone (Waggoner et al., 1997). If dogs responded correctly, they received kibble as a reward. If a dog responded incorrectly, the lights went out for 15 seconds. The range of threshold concentrations was from 1.1×10^{-9} M to 2.1×10^{-10} M, which converts to a 5-fold difference. The average threshold for methyl benzoate was 6.66×10^{-10} M (See also in Table 1).

It is important to consider the actual levels of methyl benzoate within the illicit cocaine sample utilized during this study. It was determined there was approximately 4.16×10^{-12} M of methyl benzoate in illicit cocaine. Therefore, the amount of methyl benzoate in the cocaine was lower than the dogs' average threshold to methyl benzoate. Because of this, Waggoner et al. (1997) points out the concern for developing training samples based on the compounds within, specifically methyl benzoate when discussing cocaine. If cocaine training samples (illicit or pseudo) are mostly comprised of methyl benzoate, dogs may not generalize to smaller levels of methyl benzoate in an illicit cocaine sample.

Threshold Assessments for Other Animals

When discussing thresholds, it is important to consider species other than dogs. Analyzing other papers allows for deeper reflection on the equipment and study design. In addition, it provides a deeper understanding of just how profound dogs' noses are, or are not, in relation to other species. Laska et al. (2000) trained three squirrel monkeys to detect n-aliphatic acids, from n-propionic acid to n-heptanoic acid, as well as 1-pentanol, 1,8-cineole, 1:1Mio n-heptanal (Mio means diluted in mineral oil), and 1:30Mio amyl acetate. The threshold tests assessed the monkeys' ability to discriminate varying

concentrations of the target odor versus diethyl phthalate, which was the solvent. Odorant thresholds began at 1:100 and increased 10-fold in a descending staircase procedure. Each dilution step was tested a total of four times, until the monkey was no longer significantly discriminating between the target odor and solvent.

The odorant or solvent was placed on the lid of a flip top cup. Odor (S+) signaled a peanut reward inside, while diethyl phthalate (S-) signaled no peanut reward. Eighteen cups (nine with the target odor and nine with diethyl phthalate) were placed horizontally on a climbing wall in the monkey's enclosure. Each monkey had one minute to "forage" for cups. Out of the four sessions for each dilution, the best two sessions were analyzed. The researchers calculated the percentage of correct choices. Incorrectly selected cups and cups that were not selected which contained odor were also calculated into the percentage of correct choices. Other than n-hexanoic acid and 1,8- cineole, the three monkeys scored very similarly to each other. In addition, just as Moulton et al. (1960) observed, there was a clear change in threshold as related to carbon chain length. Thresholds improved (concentration decreased) as carbon chain length increased (see Table 1).

Williams and Dewan (2020) used eight mice in a go-no go design to test olfactory sensitivity for five aliphatic alcohols, from 1-propanol to 1-heptanol (see Table 1). Each of the alcohols were diluted and carried in nanopure water. The mice received water as a reward for correct responses and were punished for incorrect responses with a longer intertrial interval. To avoid contamination and desensitization, only one dilution was tested every day, and every dilution was tested five times total. When diluting the

odorant, a 10-fold liquid dilution step was utilized. The maximum liquid dilutions tested for each odorant are as follows: 1-butanol: 2.17×10^{-5} M; 1-pentanol: 2.81×10^{-5} M; 1-propanol: 1.79×10^{-6} M; 1-hexanol: 2.64×10^{-7} M; 1-heptanol: 3.19×10^{-7} M. Just as Moulton et al. (1960) and Laska et al. (2000) found, Williams and Dewan (2020) noted higher sensitivity was correlated with shorter carbon chain length. Additionally, threshold concentration similarity varied depending on the odorant. The best and worst threshold for 1-propanol, 1-pentanol, and 1-heptanol was within 3-fold. 1-hexanol was a 145-fold difference between the best and worst threshold concentrations. 1-butanol was within 300-fold.

Threshold Assessments for Humans

There are a plethora of published studies discussing odor thresholds in humans, such as: autism (e.g., Muratori et al., 2017), Parkinson's disease (e.g., Quinn et al., 1987), women during menstruation (e.g., Navarrete-Palacios et al., 2003), and individuals with multiple sclerosis (e.g., Lutterotti et al., 2011). Human research varies from that of animal threshold testing due to the communication limits with animals. Therefore, more research exists with humans than other species.

Attempting to develop an accurate sensitivity test for humans, Walker et al. (2003) tested twelve participants on amyl acetate using an air dilution olfactometer. Each participant had 30 trials for each session and were tested multiple times over a period of 20+ weeks. Of the 30 trials, 15 consisted of clean air and 15 trials presented the odorant in a randomized order of varying concentrations ($10^{-4.4}$, $10^{-4.1}$, $10^{-3.8}$, $10^{-3.5}$ vapor saturation). Amyl acetate thresholds ranged between 6.97×10^{-9} M to 2.96×10^{-10} M. This

range of concentrations equates to a 23-fold difference between the best and worst threshold (Walker et al., 2003) (See in Table 1). Therefore, both dogs and monkeys appear to have a higher sensitivity to amyl acetate than humans (See in Table 1).

Behavioral data seems to suggest that using neuroanatomical features to predict how a species will perform in an olfactory test is insufficient (Laska et al., 2000). The terms "microsmatic" and "macrosmatic" are used to label different species based on the size of the olfactory bulb and overall surface area of the olfactory epithelium (Smith et al., 2004). However, with each of the studies presented in this paper, it is evident that detection capabilities vary greatly depending on the odorant, as well as the individual. In Moulton and Marshall (1976), they saw a 50-100-fold difference when testing dogs' thresholds to α-ionone. Krestel et al. (1984) saw a 3-fold difference in humans between the best and worst concentration to amyl acetate, while the worst and best dog had a 450-fold difference in thresholds to amyl acetate. Walker et al. (2006) recorded a 2 to 3-fold difference in dogs for amyl acetate. Waggoner et al. (1997) noted a 5-fold difference for methyl benzoate detection thresholds for dogs. Williams and Dewan (2020) saw a 3-fold for 1-propanol, 1-pentanol, and 1-heptanol in mice. They also saw a 145-fold difference between the worst and best concentrations to 1-hexanol. Lastly, a 300-fold difference was seen for 1-butanol in mice. Walker et al. (2003) saw a 23-fold difference for amyl acetate threshold concentrations in humans. Laska et al. (2000), who tested monkeys, did not provide individual thresholds for odorants, and therefore was excluded from this calculation. In Concha et al., (2019), a 31,000-fold difference was observed between the worst detection dog and the best detection dog. This was the largest magnitude of

variability within a study; however, it was also one of the larger sample sizes which could contribute to the increased variability. Additionally, these variabilities can be described by a variety of factors such as genetics, learning, and prior experiences (Concha et al., 2019; Smith et al., 2004). Based on the large range of concentrations, it can be expected to see large variabilities in thresholds to odorants; however, further research is necessary to determine what factors most contribute to the variability.

Furthermore, there appears to be a wide range of threshold measurements from odorant to odorant. For example, there was a 1,400-fold difference between the monkeys' performance on butanoic acid and 1,8-cineole (Laska et al., 2000; See in Table 1). Dogs were best able to detect α- ionone, while they struggled to detect formic acid the most, creating over a 204-billion-fold difference in threshold concentrations. Due to the limited number of threshold assessments for dogs, it is difficult to conduct species comparisons regarding odorants tested; however, dogs did outperform humans, monkey, and mice on amyl acetate threshold assessments (See in Table 1). All these comparisons again may be related to learning, length of training, genetics, or prior experiences (Concha et al., 2019; Smith et al., 2004).

Another important consideration is instrumentation and the study design used to complete the threshold assessment. Concha et al. (2019) was the only study to utilize a scent carousel to conduct canine threshold. Not only was it the only time this instrumentation was used, but this study also produced the largest inter-subject variability compared to all other studies presented in this paper. Olfactometers are also frequently utilized to allow for precise and accurate dilution steps when testing; however, with the

wide array of olfactometer types, it is hard to accurately assume their efficacy. Unfortunately, no data exists regarding equipment reliability as applied to threshold assessments.

In addition, to the equipment utilized for testing, the overall procedure for testing thresholds can vary. A descending or ascending staircase procedure (Moulton & Marshall, 1976; Krestel et al., 1984; Laska et al., 2000; Williams & Dewan, 2020) and method of constant stimuli like in Walker et al. (2003; Walker et al., 2006; Waggoner et al., 1997; Concha et al, 2019; Moulton et al., 1960) are commonly used. Test-retest reliability has been measured in human olfactory tests. For example, Doty et al. (1995) found that a staircase procedure was much more reliable than tests that utilize an ascending presentation of dilutions for an odorant. In addition, reliability of the test was correlated with session length. Then, Doty et al. (2003) found that a descending procedure produced more reliable measures when dealing with lower concentrations of an odorant. In addition, the number of choices presented to the participant also play a role in the efficacy of the threshold assessment. It appears that in human research, a 3-AFC task is more reliable than a 2-AFC (Leek, 2001).

Further studies are necessary to determine what learning and background factors play a role in threshold measurements. It is also necessary to determine what type of testing provides the most accurate and reproducible threshold measurements. Threshold research could allow handlers to know the minimum amount of odor necessary for their dog to detect an odorant. Once this information is determined, handlers could strategically alter their training plan to help lower their dogs' overall threshold (DeChant

& Hall, 2021). It would also allow handlers to focus more time on detecting small, hidden odors, rather than just large quantities. Threshold assessments for important compounds such as ethanol (arson detection), ANFO (bomb detection), and smokeless powder (gun and bomb detection), among many others, will help strengthen training procedures which will in turn provide greater security for civilians.

CHAPTER II

EVALUATION OF CANINE THRESHOLD TO SMOKELESS POWDER

Introduction

Based on the relatively small collection of canine threshold assessments, it would be beneficial to conduct more threshold tests with dogs. Not only could more species comparisons be analyzed, but training methodology for working dogs could be re-evaluated if necessary.

Currently, published studies on canine thresholds use a variety of testing methods, not only for equipment (scent carousel, olfactometer, behavioral measures) but also for assessment measurements. Some research uses adaptive procedures while others probe varying concentrations of the odorant (method of constant stimuli). Adaptive procedures including a descending/ascending staircase are commonly utilized in human research since they require less time commitment from participants (e.g., Besser et al., 2019; Cometto-Muñiz & Cain, 1990; Kleinbeck et al., 2011; Lehrner et al., 1999); however, with animals, probing varying concentrations allows for the collection of more data points and an overall deeper understanding of threshold for that specific odorant. Unfortunately, there is no current data suggesting the test-retest reliability of threshold assessment standards for canines (i.e., method of constant stimuli or adaptive procedures). A large majority of animal studies require months of training, especially for behavioral studies (Moulton & Marshall, 1976; Concha et al., 2019). If a shorter threshold assessment proves to be reliable, this could help decrease training time.

In the present study, we tested dogs' threshold to double base smokeless powder. Smokeless powder is a propellent that was developed to reduce the amount of residue left in guns after being shot (Heramb & McCord, 2002). There are three types of smokeless powder: single base, double base, and triple base. The differences lie within the compounds which make up the smokeless powder. Single base smokeless powder is made of nitrocellulose (Heramb & McCord, 2002). Double base smokeless powder contains nitrocellulose and nitroglycerin (Heramb & McCord, 2002). Lastly, triple base smokeless powder, the least common of the three, contains nitrocellulose, nitroglycerin, and nitroguanidine (Heramb & McCord, 2002). Smokeless powder is one component of bullets used in guns. Smokeless powder is also a common ingredient in improvised explosive devices (IED's). It is an easily accessible component that when mixed with other ingredients (ammonium nitrate or fuel oil) can cause an explosion. When confined to a small container, pressure builds causing expansion, which eventually causes the explosion (Heramb & McCord, 2002). Because of the high likelihood for pipe bombs (which are commonly made using smokeless powder), canines have been trained to detect smokeless powder.

It is important to assess threshold to smokeless powder so working dog handlers can see the minimum concentration that dogs can detect of the compound. Moreover, showing the variability between dogs will also help demonstrate how temperament, training, and learning may play a large role when selecting a working dog. If a handler recognizes the minimum amount of odorant necessary, this can open a window into new

training methodology, such as training with limited odorant available to the dog (smokeless powder hidden in a plastic container inside a backpack).

Previously, descending staircase procedures were utilized to determine dogs' threshold to α- ionone (Moulton & Marshall, 1976) and amyl acetate (Krestel et al., 1984). With the frequency of use (Laska et al., 2000; Williams & Dewan, 2020), as well as the minimum amount of time to complete a threshold assessment, this method was chosen for the present study. Additionally, an air dilution olfactometer was selected to complete the dilution series. Because the odorant, smokeless powder, was not a liquid, the air dilution olfactometer was able to systematically decrease the odorant by half-log steps.

The last goal of this study was to determine test-retest reliability utilizing the descending staircase procedure when testing with an air dilution olfactometer. Due to the low concentrations that this study would utilize, a descending staircase procedure was chosen based on previous research (Doty et al., 1995). Additionally, a 3-AFC task was selected to maximize the efficiency of the threshold assessment (Leek, 2001). If this method proved accurate, it would be a step towards developing a more standardized method of canine threshold testing.

Before beginning this study, three hypotheses were created. First, the adaptive descending staircase procedure can be completed repeatedly without concern for learning and adaptation effects. For the second hypothesis, we assumed that individual differences would appear between the eleven dogs. Lastly, the third hypothesis tested whether the

olfactometer and descending staircase procedure would produce a high test-retest correlation.

Methods

Subjects

Eleven dogs were recruited from local shelters in Lubbock, Texas and brought to the Canine Olfaction Lab in New Deal, Texas. The Canine Olfaction Lab works with shelter dogs to complete a variety of olfactory and behavioral tests. They are housed in indoor/outdoor kennel runs and receive two daily walks and/or play sessions. In addition, the dogs receive daily training and social enrichment with people and other dogs. Dogs were selected based on their food motivation and willingness to work. Breeds were mixed breed between nine months and seven years of age and including four spayed females and seven neutered males. The procedures for this study were reviewed and approved by the Texas Tech University Institutional Animal Care and Use Committee (IACUC #20027-04).

Materials and Methods

The testing environment was a climate-controlled room approximately 3.6m x 3.6m. The olfactometer ports were placed in the corner of the room, on a wall opposite of the HVAC and heating systems. Temperature was set to 21°C ± 2°C and humidity was 60% RH ± 10%.

Testing was completed using an air dilution olfactometer, which allowed us to achieve the proper dilutions of the smokeless powder. The olfactometer delivered diluted

smokeless powder pseudo randomly to one of three odor ports and clean Praxair UN1002 Air Compressed "zero" air to the other two ports. A total of 3 stainless steel ports were mounted flush with a polypropylene plastic sheet which was $34\frac{1}{8}$ inches wide x $11\frac{1}{8}$ inches tall. Each port was nine inches from the other. To trigger a response for the olfactometer, IR beams were placed approximately four inches above and below each port. The IR beams would measure the time each dog spent with their nose in the odor port. This air dilution olfactometer used nine AliCat® (Tucson, AZ, USA) mass air flow controllers (Figure 1). "Zero" air was pulled in from a compressed air tank and then mixed with the headspace of the smokeless powder to dilute the odor from 1% air dilution to 0.00000001% air dilution. Air and odor were delivered to the odor ports at a final flow rate of 10 L/min.

Odorant

Prior to participation in this study, the eleven dogs were trained and tested on smokeless powder for approximately 3-4 months. Hodgdon H335® Double Base Smokeless Powder was obtained from Hodgdon® and received on August 4th, 2020. Ten grams of smokeless powder were measured and placed in an amber glass vial with a sealed PTFE lid. This vial was then attached to the air dilution olfactometer. The vial was kept at ambient temperatures to best represent real life situations (15.5ºC ± 5 ºC and 35% RH ± 5%)

Procedure

Pre-Training: Prior to the experiment, dogs were trained on a 3-choice olfactometer to detect the target odor (i.e. 10g Hogdon® Smokeless Powder) at a 33% air dilution. The dogs were trained to hold their nose in a Teflon port for a duration of 4 consecutive seconds. The IR beams registered a four second nose hold and provided a tone for a correct response and a different tone for an incorrect response. Dogs demonstrated accuracy >90% on their previous training prior to the start of training on the air dilution olfactometer for this study.

Transfer to Air Dilution Olfactometer: Dogs were introduced to the air dilution olfactometer at the highest concentration produced by the olfactometer (1% dilution of vapor saturation) in 20 trial sessions, until achieving 90% accuracy or higher (18+ of 20 trials correct).

Threshold Assessments: The assessments began at 1% dilution of vapor saturation. A two-down, one-up descending staircase procedure was utilized in which dogs were required to make two consecutive correct responses at a concentration before decreasing to the next concentration by a half-log step (DeChant & Hall, 2021). If a dog made an incorrect response, the dilution increased by a half-log step. If dogs did not make a response on a trial within 60 seconds, it was considered an incorrect response and the concentration increased by a half-log. The threshold assessment continued with the two-down, one-up procedure until eight reversals were met. A reversal was met whenever the concentration hit a turning point (e.g., the dog answered incorrectly, and the concentration increased (one reversal)). When the concentration increased, the dog then was required to get two correct

responses to meet the criterion for a second reversal, in which the concentration decreased again by a half-log). This pattern continued until there were eight reversal points. A repeatability criterion was set and required dogs to have two consecutive threshold sessions within 10-fold of each other. If this criterion was not met, the dogs continued testing. The cutoff point was set at six threshold assessments, at which point the dog's threshold would not be considered repeatable. This did not occur.

Statistical Analysis

The concentration of the last six reversal points were extracted from each session and log transformed. To calculate the overall threshold from a session, we calculated the mean of the log transformed concentration of the six reversal points (Figure 2 pictures each dogs' sessions and individual reversal points). For eight of the eleven dogs, the thresholds obtained for the first two sessions were within 10-fold and met our repeatability criterion. The ninth dog (Dale) met the repeatability criterion within three session, the tenth dog (Sasha) within five sessions and the last dog (Raven) was unavailable for more than the first two sessions. For subsequent analyses, we used the last two sessions from each dog that met our repeatability criterion.

To assess whether there was a difference in performance overall between the last session and the one immediately preceding (hypothesis one), we fit a linear mixed effect model. The dependent variable was the log transformed threshold, which was predicted by the session and a random effect of the dog (intercept model). An ANOVA with a Satterthwaite's correction for degrees of freedom was used to evaluate the effect of session using the lmerTest package (Kuznetsova et al. 2017).

To analyze differences between individual dogs (hypothesis two), highlighting individual performance capabilities, we compared the six reversals points from the two sessions that met our repeatability criterion across dogs. We used the reversal points instead of the mean threshold from each session to maximize individual data and variability for analysis. A linear model was used to predict the log concentration of reversals by the individual dog. An ANOVA with Satterthwaite's correction for degrees of freedom was used to analyze the effect of dog. Additionally, a compact letter display (Figure 4) based on Tukey adjusted post-hoc comparisons was created to visually compare reversal measurements for between individual dog. The boot-strap estimated 95% confidence intervals were used to compare dog performance.

Lastly, test-retest correlation was analyzed (hypothesis three; Figure 5). The threshold concentrations from the two threshold assessments that met our repeatability criterion were casted utilizing the reshape2 package (Wickham, 2007). This data set was then plotted with the first threshold assessment was on the x-axis, while the second session was on the y-axis. Both axes were log transformed. Next, Pearson's correlation was utilized to determine the correlation between the threshold concentration for assessments one and two. Two correlations were completed. The first included Raven, whose two threshold assessments did not meet our repeatability criteria. She was unable to complete further testing; however, we felt it necessary to provide the correlation with her included. The second correlation measure excluded Raven leaving ten dogs whose two sessions did meet our repeatability criterion**.**

Results

To assess whether there were systematic differences between the last session and the one prior to it (indicative of learning or improvement effects), we utilized a linear mixed effect model in which session predicted the threshold obtained. There was no statistical difference between the last threshold and the preceding threshold ($F_{1,10}= 0.24$, $P >0.05$; See Figure 3), indicating that performance did not get systematically better across these consecutive tests (Figure 3).

Dog-Dog Comparisons

Next, we evaluated whether there were systematic differences in thresholds between dogs, highlighting individual variability. The last six reversal points from both threshold assessments were analyzed. According to the linear model ANOVA, there was a statistical difference between individual dogs ($F_{10,121}= 12.21$, $P <0.0001$.) The compact letter display (Figure 4) visually displayed apparent groups within the eleven dogs- above average, average, and below average threshold concentrations. The largest difference fell between Bullseye (the best threshold- $8.25x10^{-8}$ air dilution) and Dale (the worst threshold at $9.08x10^{-5}$ air dilution), which converts to a 1,100-fold difference.

Test-Retest Reliability

Part of the importance of this study was to develop a reliable, accurate, and repeatable measure for dog threshold assessments utilizing an air dilution olfactometer. Pearson's correlation was conducted twice- the first correlation including Raven and the second correlation excluding Raven. With Raven, there was a weak positive correlation

was $r(10)=0.61$ $p= 0.04$. Without Raven, there was a strong positive correlation increased to $r(9)=0.86$, $p<0.01$. Figure 5 displays the correlation between session one and session two for each dog. A strong, positive correlation is visible, except for Raven who was a clear outlier. Therefore, the repeatability measure of threshold session concentrations being 10-fold appears to increase test-retest reliability substantially.

Discussion

From this study, no apparent session effect was evident. This demonstrates that no learning effects were apparent between sessions the final two sessions. Additionally, adaptation, which is a concern in threshold tests, appears to not develop, at least within the limits of this study. This can become an issue in studies where tests are completed in the same day (Williams & Dewan, 2020); however, some human threshold methodologies have shown no adaptation effects, no matter then length of the study or frequency of tests (Albrecht et al., 2008). Therefore, this issue could be a study in and of itself to determine if adaptation is an imperative topic to focus on in canine threshold assessments.

It was determined that there was a 1,100-fold difference between the best dog (Bullseye) and the worst dog (Dale). However, when the worst dog, Dale, is removed from the calculation, the best to worst threshold difference is 74-fold. This suggests that most dogs perform within a similar range when detecting smokeless powder, except for our one outlier. Within this paper, threshold literature ranges between a 2-fold difference to a 450-fold difference. However, Concha et al. (2019), saw a 31,000-fold difference between the best and worst performing dogs. This huge leap in threshold differences can

be attributed to sample size, testing design, equipment used, and the odorant utilized. For example, Concha et al. (2019) tested ten dogs, while Moulton et al. (1960) and Walker et al. (2006) only tested two dogs. On the other hand, Waggoner et al. (1997) tested thirteen dogs and saw a 5-fold difference between the best and worst thresholds. Therefore, more research is required to determine what exactly may contribute to the wide range of threshold concentrations. However, within the limits of this paper, our 1,100-fold difference, and even 74-fold difference without Dale, does suggest that some dogs are inherently better at detecting smokeless powder than others which may influence the choice of selecting one dog over another for detection work.

This study was also able to highlight a potentially consistent apparatus and testing method when assessing canine threshold capabilities. Threshold assessments were fast to complete if dogs have previously been trained to detect the odor, while also providing accurate and repeatable measures, as shown by our test-retest correlation of 0.86. For the test-retest reliability to be as accurate as possible, it was necessary that two consecutive threshold assessments must be within a 10-fold range of one another. Therefore, two of our dogs- Dale and Sasha- were required to complete more than two assessments. Raven's data also showed that she should have continued testing; however, she was unable to return for more testing. While setting a repeatability criterion allowed for a higher test-retest reliability, this forced repeatability could have skewed our overall correlation in our favor. Future tests could complete research to evaluate the positives and negatives when utilizing a repeatability criterion.

There were limitations to the present study. A laboratory study can be hard to replicate in the field. While the dogs tested in this study had an 1,100-fold variability in their threshold measurements, testing thresholds outside of a lab setting may increase variability. This extenuated variability could be related to amount of target odor, quality of the odor, temperature of the odor, location of testing, environmental distractions, and the ambient temperature/humidity.

In addition, this study utilized an adaptive descending staircase procedure. With no research for canine threshold testing structures, this method could potentially prove not to be the most efficient or reliable, as compared to an ascending staircase or method of constant stimuli designs. For example, the introduction of this paper noted five researchers who utilized a method of constant stimuli design (Walker et al., 2003; Walker et al., 2006; Waggoner et al., 1997; Concha et al, 2019; Moulton et al., 1960), which are being directly compared to this study. Direct comparisons may provide an inaccurate representation of threshold concentrations; therefore, it is imperative that canine threshold assessment designs be directly compared and analyzed.

Many of the previously published papers utilizing dogs had extremely long training times (30 weeks in Concha et al., 2019; 6 months in Walker et al., 2006). In the present study, dogs had previously been working with smokeless powder for approximately 3-4 months. Training time could be an important consideration for dog threshold assessments, as there are no studies to properly evaluate the effect of training time on thresholds. Therefore, this study proved the air dilution olfactometer, as well as a

descending staircase procedure, beneficial as they require less testing time and appear to provide an accurate and reliable measure of threshold, without the sacrifice of time.

Further studies in this field could focus on the application of these threshold measurements to real life situations. For example, in this study, Bullseye was the best at detecting smokeless powder, while Dale was the worst. In the training completed before this study, all dogs were achieving >90% accuracy in their detection tasks; however, the odor was kept at a constant 33% air dilution. If we asked Bullseye and Dale to search a stadium for a concealed and enclosed IED, would Bullseye be the only one to find it because of his lower threshold detection? Similarly, it has been shown that it is necessary dogs are trained to detect these trace amounts of odorants. DeChant and Hall (2021) found that dogs who were not strategically trained with decreasing samples had worse thresholds than dogs who were trained with varying solutions. Therefore, a dogs' prior training history will most likely impact the dogs' threshold performance. A dog who has only trained on high concentrations may appear to have a worse threshold to an odorant than a dog who has spent more time training with low concentrations. For working dog handlers, they could have their dog complete a series of threshold assessments shortly after beginning training. Then, they could strategically train to lower concentrations over a set period of time. Lastly, the dog could complete another set of threshold assessments. This would provide a before and after threshold to determine if their training is successful.

In conclusion, this study highlights the inter-dog variability when it comes to smokeless powder threshold detection. This suggests that while dogs are capable of being

trained to detect specific quantities of smokeless powder, that some dogs are inherently better. In addition, this provides a solid foundation for utilizing an air dilution olfactometer with an adaptive descending staircase procedure for determining dogs' thresholds.

Table 1: Previous Studies Threshold Assessments

The reported thresholds from each study are listed in the table. All thresholds were converted to vapor Molarity for proper comparison. Some studies used a range of threshold measurements from the highest threshold to the lowest threshold rather than creating an overall average measurement. Dilution method refers to the presentation and determination of dilution of odorant. If a diluent was present, it was calculated into the overall threshold. Estimated vapor concentrations by multiplying molar fraction by vapor pressure.

Study	Species	Odor	Dilution Method	Diluent	Threshold (M)
Walker et al., 2006	Dog	Amyl Acetate	Vapor	N/A	4.74×10^{-14}
Concha et al., 2019	Dog	Amyl Acetate	Liquid	Mineral Oil	1.77×10^{-11} to 5.30×10^{-13}
Waggoner et al., 1997	Dog	Methyl Benzoate	Vapor	N/A	6.66×10^{-10}
Moulton & Marshall, 1976	Dog	Alpha Ionone	Vapor	N/A	$1 \times 10^{-15.68}$ to $1 \times 10^{-17.68}$
Krestel et al., 1984	Dog	Amyl Acetate	Vapor	N/A	$1 \times 10^{-9.43}$
Moulton, Ashton, & Eayrs, 1960	Dog	Formic Acid	Liquid	N/A	$1 \times 10^{-6.37}$
Moulton, Ashton, & Eayrs, 1960	Dog	Acetic Acid	Liquid	N/A	$1 \times 10^{-8.02}$
Moulton, Ashton, & Eayrs, 1960	Dog	Propionic Acid	Liquid	N/A	$1 \times 10^{-9.78}$
Moulton, Ashton, & Eayrs, 1960	Dog	Butyric Acid	Liquid	N/A	$1 \times 10^{-11.25}$
Moulton, Ashton, & Eayrs, 1960	Dog	Valeric Acid	Liquid	N/A	$1 \times 10^{-9.82}$
Moulton, Ashton, & Eayrs, 1960	Dog	Caproic Acid	Liquid	N/A	$1 \times 10^{-10.57}$
Moulton, Ashton, & Eayrs, 1960	Dog	Heptylic Acid	Liquid	N/A	$1 \times 10^{-11.37}$

Table 1 Continued

Moulton, Ashton, & Eayrs, 1960	Dog	Caprylic Acid	Liquid	N/A	$1 \times 10^{-12.11}$
Moulton, Ashton, & Eayrs, 1960	Dog	Iso-Butyric Acid	Liquid	N/A	$1 \times 10^{-11.20}$
Laska, Seibt, Weber, 2000	Monkey	Propionic Acid	Liquid	Diethyl Phthalate	4.8×10^{-8} to 1.6×10^{-8}
Laska, Seibt, Weber, 2000	Monkey	Butanoic Acid	Liquid	Diethyl Phthalate	7.0×10^{-9} to 1×10^{-9}
Laska, Seibt, Weber, 2000	Monkey	Pentatonic Acid	Liquid	Diethyl Phthalate	3.3×10^{-9} to 9.8×10^{-10}
Laska, Seibt, Weber, 2000	Monkey	Hexanoic Acid	Liquid	Diethyl Phthalate	5.1×10^{-10} to 1.7×10^{-11}
Laska, Seibt, Weber, 2000	Monkey	Heptanoic Acid	Liquid	Diethyl Phthalate	2.5×10^{-11} to 8.2×10^{-12}
Laska, Seibt, Weber, 2000	Monkey	Pentanol	Liquid	Diethyl Phthalate	1.7×10^{-8} to 5.0×10^{-9}
Laska, Seibt, Weber, 2000	Monkey	Heptanal	Liquid	Diethyl Phthalate	3.1×10^{-10} to 1×10^{-10}
Laska, Seibt, Weber, 2000	Monkey	Amyl Acetate	Liquid	Diethyl Phthalate	1.6×10^{-11} to 4.9×10^{-12}
Laska, Seibt, Weber, 2000	Monkey	Cineole 1,8	Liquid	Diethyl Phthalate	4.7×10^{-10} to 4.7×10^{-11}
Williams & Dewan, 2020	Mice	Propanol	Vapor	N/A	1.3×10^{-10}
Williams & Dewan, 2020	Mice	Butanol	Vapor	N/A	4.5×10^{-9}
Williams & Dewan, 2020	Mice	Pentanol	Vapor	N/A	2.3×10^{-10}
Williams & Dewan, 2020	Mice	Hexanol	Vapor	N/A	3.0×10^{-11}
Williams & Dewan, 2020	Mice	Heptanol	Vapor	N/A	1.2×10^{-10}
Walker et al., 2003	Human	Amyl Acetate	Vapor	N/A	2.96×10^{-10}
Krestel et al., 1984	Human	Amyl Acetate	Vapor	N/A	6.92×10^{-9}

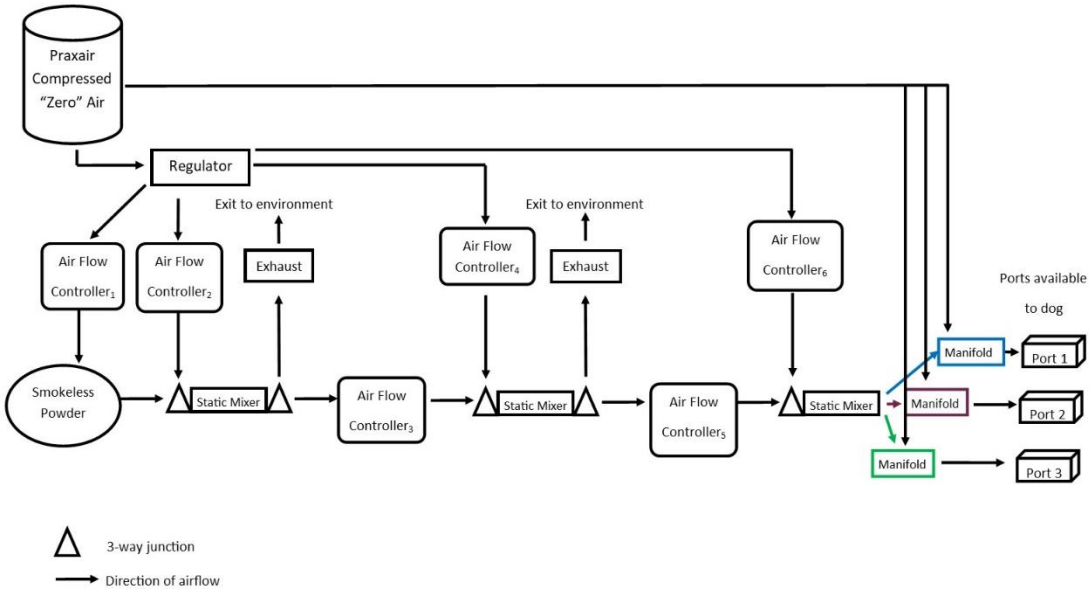

Figure 1: Air Dilution Olfactometer Schematic

Above, the air dilution olfactometer has been broken down into its simplest form. The headspace from the smokeless powder jar is pulled and air is pushed through a series of mass flow controllers, as well as static mixers. The final dilution of the odor and air mixture is presented in one of three odor ports pseudo randomly and "zero" air is delivered to the additional two ports.

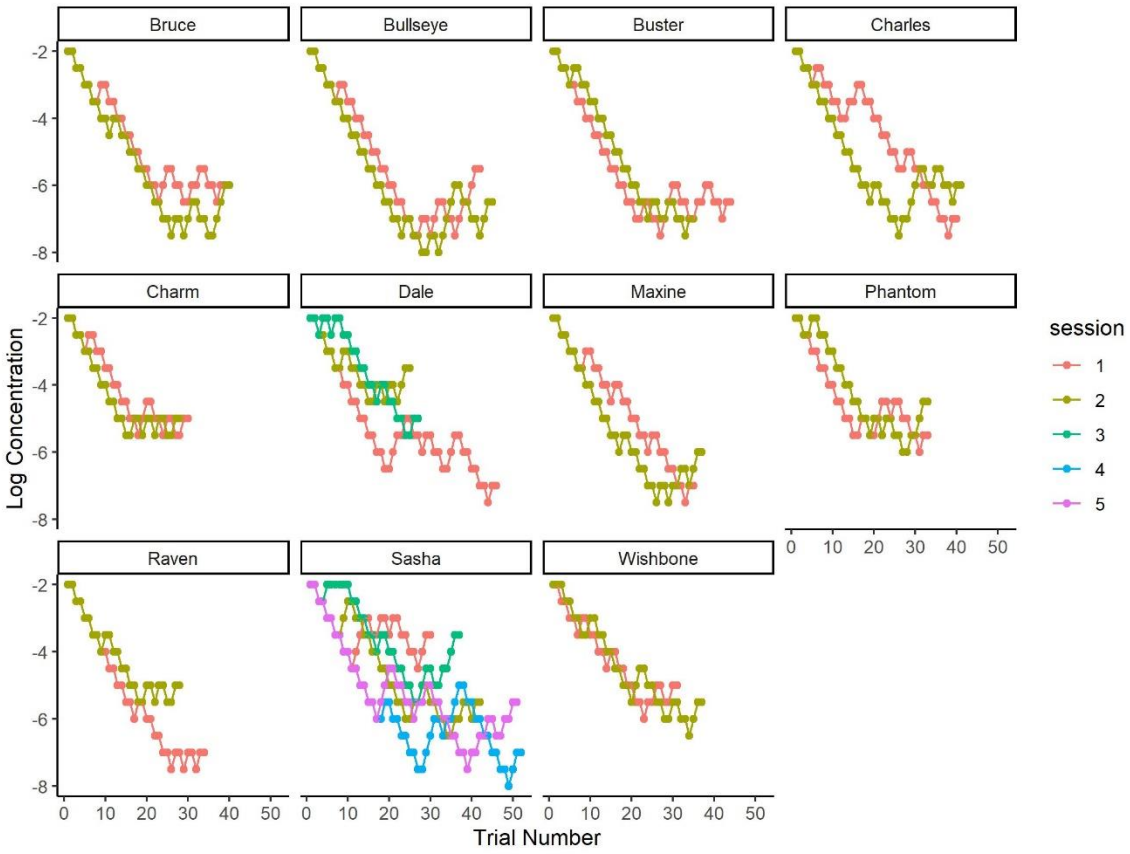

Figure 2: Threshold Assessments and Reversals

Threshold assessments are pictured above. This graph includes every trial the dogs had during each assessment. Dale and Sasha had multiple sessions to increase the test-retest reliability. This allows for easy comparison between each session and between each dog.

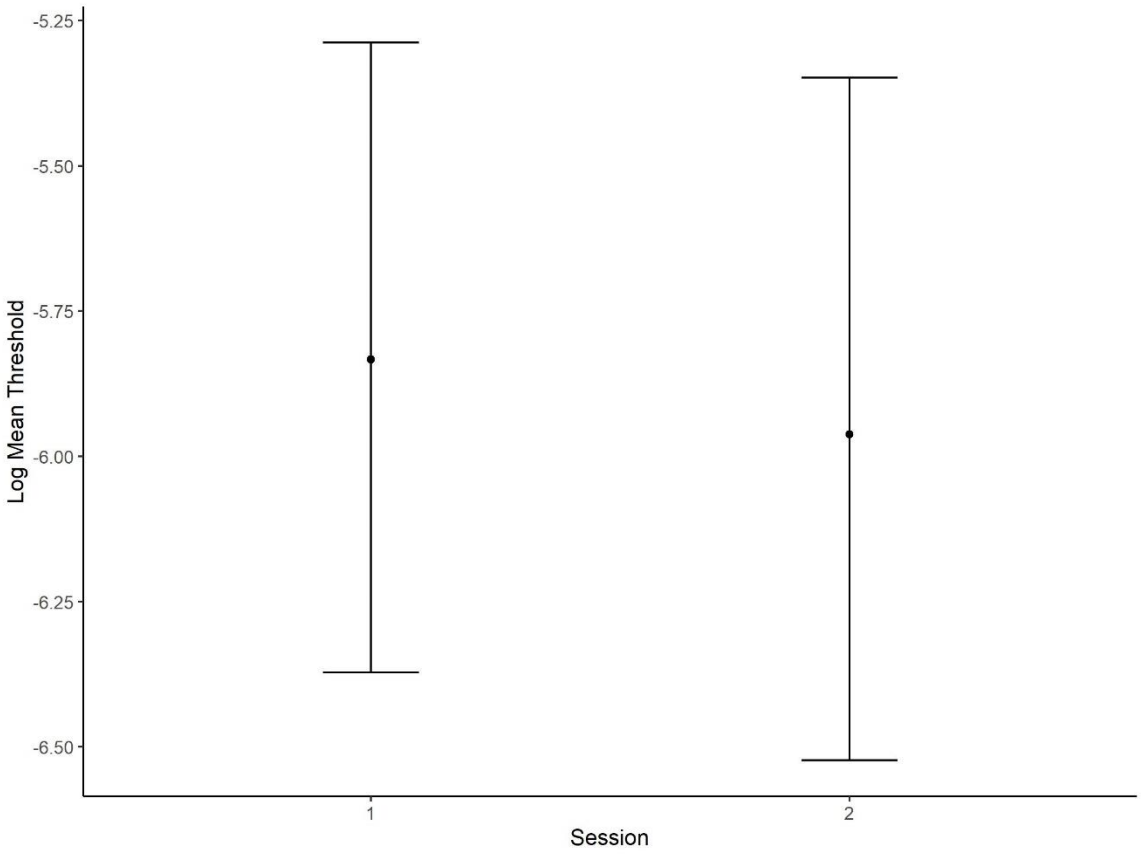

Figure 3: Individual Session Comparison

Threshold concentrations were not statistically significant ($F_{1,10}= 0.24$, $P > 0.05$). 95% confidence interval error bars are shown above. This graph, along with our p-value, shows there was not a substantial difference between the two threshold assessments. Again, the two threshold assessments used were required to meet our repeatability criterion (two consecutive sessions within 10-fold).

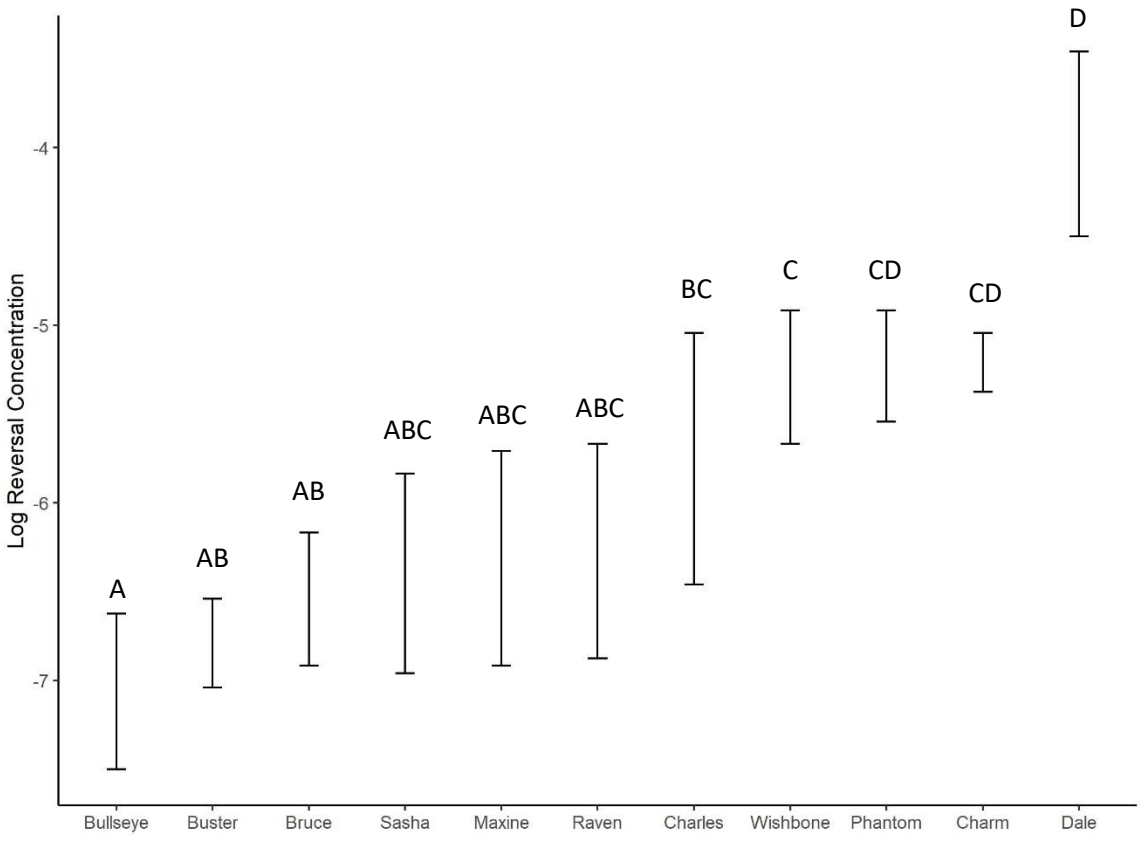

Figure 4: Compact Letter Display

A display of each dogs' threshold is pictured above. Error bars show the 95% confidence intervals (bootstrap estimated) of each dogs' threshold. Two sessions which met the repeatability criterion were used for this graph. Each dog with a letter in common means they were not statistically different. Therefore, three main groups emerged- Bullseye, Buster, Bruce, Sasha, Maxine, and Raven proved to be above average. Charles and Wishbone's performance was mediocre, while Phantom, Charm, and Dale scored below average.

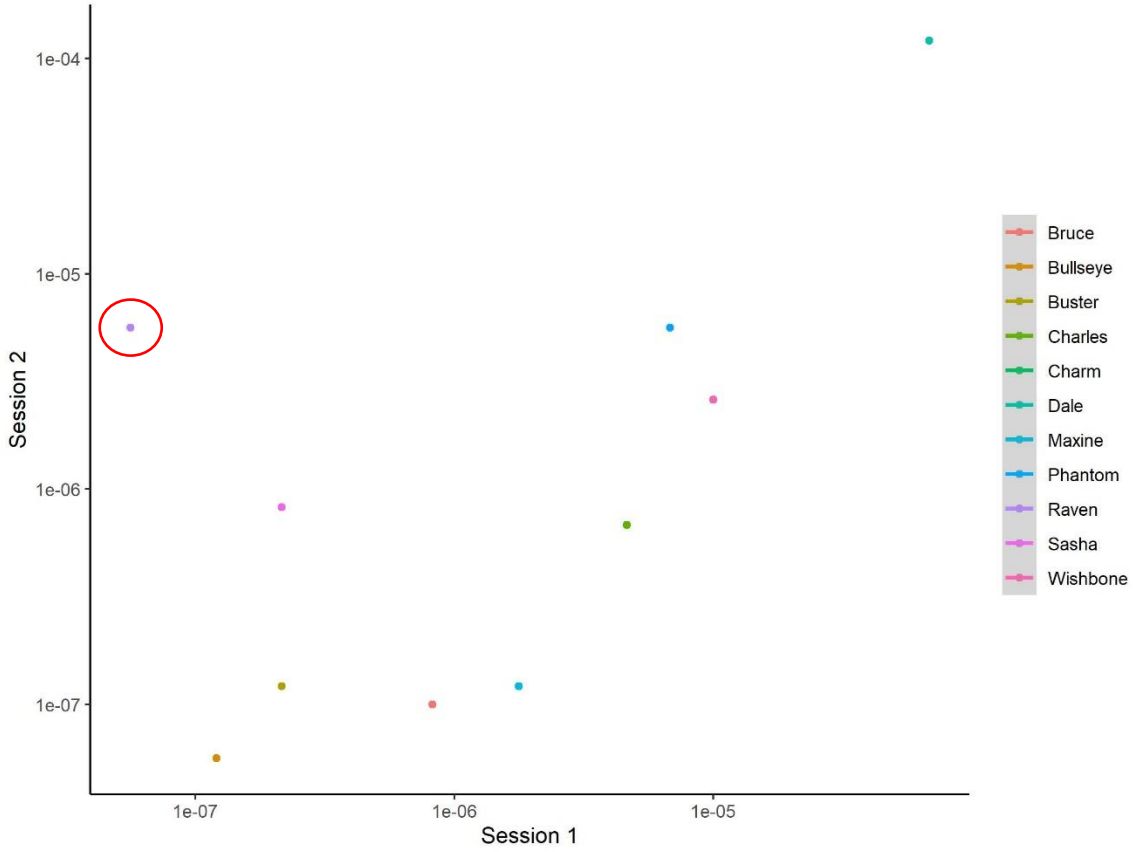

Figure 5: Correlation Between Threshold Measurements

This graph pictures a positive correlation between the two threshold assessments for each dog. Raven's correlation is circled in red, as she is a clear outlier among the other ten dogs tested. She was unable to complete further testing, and therefore, her two sessions did not meet our repeatability criterion. Without Raven, our test-retest correlation was a 0.85.

CHAPTER III

CONCLUSION

The working dog community continues to grow in the United States, as dogs are trained to detect more compounds. With the use of working dogs in security and safety positions, research is required to answer imperative questions, such as this study analyzing smokeless powder thresholds. This research can be applied in a variety of contexts, as these threshold sessions prove to be repeatable session to session (hypothesis one), inter-dog variability was shown to be substantial (hypothesis two), and test-retest reliability (hypothesis three) was demonstrated for an olfactometer adaptive threshold assessment for dogs.

Threshold analyses may prove beneficial in the working dog field. For example, if threshold truly correlates to in-field performance (i.e., Bullseye could detect a well-contained IED, whereas Dale could not), threshold assessments may become a normal standard. This would prove beneficial as the working dog and research community continue to delve into what characteristics and temperament measures may predict a good working dog (Brady et al., 2018). Not only would it provide fast and repeatable results, it also provides quantitative data for detection capabilities. Currently, qualitative analyses are utilized to determine a dogs' potential for work (Brady et al., 2018).

The inter-dog variability should be considered in the working dog community. Many dogs are selected and trained to one concentration of odorant (DeChant & Hall, 2021; Aviles-Rosa et al., 2021). If every dog varies with their detection capabilities, standards for working dog training should evolve to best capture the individual dogs'

performance. It appears that over time, training to an odorant can help decrease the overall threshold; therefore, a dog like Dale may require excess time on lower concentrations to ensure detection and overall generalization to lower quantities (DeChant et al., 2021).

Lastly, our procedure utilizing an adaptive descending staircase procedure with an olfactometer produced repeatable measures necessary for accurate threshold testing. However, there is no research directly comparing adaptive procedures to method of constant stimuli for canines. This research is necessary if the working dog community wants to embrace threshold testing as a standard for working dog selection processes.

In addition, these threshold tests can be standardized to test an array of different hypothesis. For example, little is known about how the environment may assist or hinder canine olfaction (e.g., Greatbatch et al., 2015; Jinn et al., 2020; Mendel et al., 2018; Schneider & Slotta-Bachmayr, 2009; Sargisson et al., 2012). Utilizing this threshold test, researchers could determine how the environment may impact a dogs' overall threshold concentration, which could directly affect those in the military or search and rescue dogs who are deployed.

Overall, this research brings to light an important, yet overlooked topic of canine thresholds. As this field grows, more research analyzing thresholds to different odorants, its application in the field, and best methods for threshold assessments is necessary. From this study, it appears that the adaptive descending staircase was a repeatable test, especially coupled with the air dilution olfactometer. In addition, there appears to be a

high inter-dog variability, suggesting that every dog will have a different detection capability.

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