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Generalization and Discrimination of Molecularly Similar Odorants in Detection Canines and the Influence of Training

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ABSTRACT

Operationally-deployed canine detectors are often trained on one or a limited number of materials representing a single target odor, and training frequently occurs using materials of a high purity grade in controlled scenarios with minimal other background odors. Conversely, in the field, canine detectors are expected to generalize and identify variations of the target odor, while discriminating from similar extraneous or background odors. This exemplifies the balance between generalization and discrimination required for effective canine detectors.

This research explored the tendency for detection canines to generalize or discriminate between similar odorants. Two groups of related odorants were used in two separate studies; (1) odorants of similar functional groups with differing carbon chains, and (2) odorants of similar carbon chain length but differing functional groups. Within each odorant set, the effect of training was addressed by incrementally increasing the number of odorants each canine was trained to detect.

Initially, discrimination increased with increasing molecular dissimilarity in both odorant groups. After further training on additional related odorants, generalization increased across the set of odorants of the same carbon chain length, but there were no significant changes in either generalization or discrimination across the set of odorants of the same functional group. The results suggest that the canines in this study were more likely to generalize across compounds of the same chain length with differing functional group than across compounds of the same functional group, but differing chain lengths. Furthermore, some variation in performance between individual canines indicated that the tendency to generalize differed with experience, breed, and other factors affecting olfaction.

1. Introduction

Olfactory generalization occurs when an animal responds to similar odors in the same way that they would to the original target (Moser et al., 2019). Small differences between similar stimuli are over-looked either intentionally through training or inadvertently, allowing an animal to place similar odors into a common category with a single meaning, and thus give the same response as the trained odor. Conversely, olfactory discrimination follows when those small differences between similar stimuli are instead accentuated and made meaningful, placing closely related odors into separate categories resulting in dissimilar responses (Mishra et al., 2010). Detection canines must constantly generalize throughout the learning process, as an initial learned odor will never be encountered in exactly the same way a second time. Early research regarded generalization as a passive process, but discrimination as an active one (Stokes and Baer, 1977). In other words, generalization was thought to occur due to a random variation in stimuli with repetition, and the seeming absence of generalization was due to tightly controlled stimuli (removing all variation), as opposed to a purposeful act. Discrimination, on the other hand, was thought to be an active process that could be improved through training and practice, making generalization simply a failure to discriminate (Stokes and Baer, 1977). However, for a canine detector to be an effective field detection tool it must be trained in such a way as to optimize discrimination between target odorants and similar non-target or background odors, minimizing false positives as well as generalization

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across similar target odorants varying only by origin, variant, or purity, minimizing false negatives. This process can be compared to what is termed as "selectivity" in analytical instrumentation, and is largely considered to be superior in the canine olfactory system as compared to instrumental detectors (Furton and Myers, 2001).

As an example, canine detectors are often used to locate obscured or hidden narcotics, such as cocaine. The main odorant of cocaine, as perceived by canine detectors, is methyl benzoate, an odorant that is also present in the odor of snapdragon flowers. Research has shown that trained canines discriminate between the two methyl benzoate-containing odorant mixtures, in that narcotics detection canines trained on cocaine do not show interest in snapdragons (Cerreta and Furton, 2015). While discrimination in such instances is important, there is also the occasion where it is necessary for canines to generalize across like odorants. To extend the example further, for a canine to be a proficient cocaine detector, the canine should generalize across cocaine samples regardless of small differences in the odor due to packaging variations or the presence of cutting agents. In another example, Wright et al. (2017) explored the ability of canines to correctly categorize accelerant odors versus non-accelerant odor. It was shown that, through training, detection canines could learn to categorize odor stimuli for the presence or absence of an accelerant, and further showed that the canines generalized to novel accelerant odors. This paradigm of simultaneously optimizing generalization and discrimination has been referred to as the generalization-discrimination balance by Mishra et al. (2010) and is akin to optimizing selectivity in instrumental detection. This is particularly important when novel odors are presented, where over-generalization can lead to false positives and over-discrimination will increase false negatives (Oldenburg et al., 2016).

There is limited peer-reviewed research available concerning how the generalization-discrimination balance affects operational canine detection. Cerna et al. (2011) revealed that when explosive detection canines previously trained to a single TNT (2,4,6-trinitrotoluene) source were expected to detect TNT of a different origin, the canines did not readily detect the alternative sources of the TNT odor indicating difficulty of the canines to generalize. A similar trend was also found in the detection of varying ammonium nitrate samples (Lazarowski et al., 2015). Although further research did indicate that, while overall generalization across ammonium nitrate variations was low, some individual canines showed a greater tendency to generalize across ammonium nitrate variations than others when given the same detection tasks (DeGreeff et al., 2018). These studies underscore the importance of maintaining a proper generalization-discrimination balance in detector canines and suggest that further research is necessary to ensure proper training practices are used for canines to achieve maximum detection capabilities.

Mishra et al. (2010) demonstrated that it was possible to alter the perception of similar odorants through associative learning, illustrating adaptive shifting of the generalization-discrimination balance in larval *Drosophila*. Using the odorant pair of 1-octen-3-ol and 3-octanol, the researchers separately assessed both generalization across the similar compounds, as well as discrimination. It was found that if the larvae were explicitly trained to make a discrimination between the two similar odorants, they were capable of doing so; however, if discrimination was not specifically rewarded, generalization occurred instead. This implied that there was indeed a small, but perceptible difference between the odorant pairs that could be ignored (i.e. generalization) or exploited through training (i.e. discrimination).

Discrimination of the innumerable possible odorants, trained and untrained, that a canine might experience throughout their lifetime is accomplished through the activation of an array of olfactory receptors (ORs) activated in a unique pattern for each odorant (Buck and Axel, 1991). Studies suggest that while mammals possess an array of structurally dissimilar ORs, the number does not account for the discriminatory capacity of the olfactory system. Instead, research has revealed that odorants are perceived based on an olfactory code, of sorts, made up by combinations of ORs (Buck, 2005). ORs can have a high specificity for a particular molecular feature, but a high tolerance for other regions of the same molecule, such as receptors that bind a specific carbon chain length independent of varying functional groups (Firestein, 2001; Araneda et al., 2004). In turn, a single OR can recognize multiple odorants, and a single odorant may be recognized by multiple receptors, thus creating the olfactory code capable of discrimination between an immense number of odorants (Malnic et al., 1999).

OR coding research tends to focus on how the olfactory system is able to discriminate between a huge array of seemingly similar compounds, but says little about generalization across like odorants. Input from the olfactory receptors is initially processed in the glomeruli in the olfactory bulb (OB) (Purves et al., 2001), and researchers have mapped glomeruli activation in the OBs of rodents and other animals to better understand odor perception and the basis for some odorant pairs being perceived as similar and others dissimilar. Uchida et al. (2000) mapped glomeruli activation in the rat OB across a panel of more than 200 odorants that varied in carbon chain length, configuration, and functional groups. Compounds with similar functional groups mapped closely together, with particular functional groups being segregated into separate areas in the dorsal OB. Furthermore, within these separate areas, a gradual shift in the position of the activated glomeruli was correlated to systematic changes in the carbon chain and configuration.

The pattern of neural responses in the OB can also be used to predict odorant perception. Linster and Hasselmo (1999) showed that overlapping neural responses were predictive of overlapping perception using a homologous series of aldehydes. The research indicated that generalization across like odorants was related to the overlap in neural response, though the generalization-discrimination balance could be driven in the other direction through training, allowing rats to discriminate between the similar aldehydes.

To better understand how related odorants are perceived by canines, we can turn to behavioral research. Olfaction studies on rats (Cleland et al., 2002; Yoder et al., 2014; Braun and Marcus, 1969), primates (Laska and Freyer, 1997), humans (Laska and Teubner, 1999), elephants (Rizvanovic et al., 2013), and insects (Laska et al., 1999; Daly et al., 2001) have used homologous series of odorants differing in carbon chain length, which have been frequently chosen by researchers as they provide a simple manner to study generalization among a linear progression of dissimilar odors (Cleland et al., 2009). These studies have shown that generalization is more likely between odorants with greater molecular similarity specifically in the case of homologous series. For example, Hall et al. (2016) studied the discrimination between a homologous series of aliphatic alcohols using six companion dogs not previously trained for olfactory detection. Similar to the studies using other animals, a loss of discrimination ability (shift towards apparent generalization) did occur from the trained odorant (1-pentanol) to the related compounds (series from ethanol to octanol), though discrimination increased with greater difference in carbon chain length in relation to the pentanol (Hall et al., 2016). This same tendency has been demonstrated across a variety of functional groups tested in various species (Cleland et al., 2002; Laska and Freyer, 1997; Laska et al., 1999; Laska and Teubner, 1999; Rizvanovic et al., 2013; Yoder et al., 2014, Daly et al., 2001).

Though homologous series have been frequently studied, generalization or discrimination between functional groups is equally interesting. Functional groups are specific substituents or collections of atoms attached to the carbon backbone of a molecule, such as an alcohol or ester, and are responsible for that molecule's behavior in chemical reactions as well as its chemical properties. Regardless of the length of the carbon chain, compounds with a common functional group behave the same or similarly in a given chemical reaction. As such, the functional groups on molecules are thought to play an important role biologically in the activity of ligands and receptors. Similar to homologous series, research has also shown that generalization

Table 1
Study 1 (Generalization across carbon chain lengths) – Training and testing odorants for Sessions 1-3.

	Session 1	Session 2		Session 3
Training Odorant(s)	Pentanoic acid (T)	**Group T + 2**: T and T + 2	**Group T-Branched**: T and T-branched	T, T + 2, and T-branched
Testing Odorant 1	3-Methylbutanoic acid (T-branched)	**Group T + 2**: T-branched	**Group T-Branched:** T + 2	T-1
Testing Odorant 2	Heptanoic acid (T + 2)	T-1		T-1 branched
Testing Odorant 3	Butanoic acid (T-1)	T-1-branched		T + 1
Testing Odorant 4	2-Methylpropanoic acid (T-1-branched)	T + 1		n/a
Testing Odorant 5	Hexanoic acid (T + 1)	n/a		n/a

occurs across similar compounds with differing functional groups (Carcaud et al., 2018; Daly et al., 2001). For example, Daly et al. (2001) demonstrated that, moths (*Manduca sexta*) conditioned to the alcohol 1-hexanol, readily generalized to ketones of equivalent chain length, and that generalization across like odorants was a function of molecular length, carbon chain configuration, and functional group.

The balance between generalization and discrimination in odor recognition is affected by neurobiology and behavioral perception through both the molecular structure of the target odorant as well as the influence of training. The current study was designed to address this fundamental paradigm in selectivity by exploring how previously trained detection canines generalize to similar compounds, and the effect of training and conditioning on the generalization-discrimination balance. Furthermore, prior to conducting any type of odor-based research with working detection canines, it is imperative that all odorants provided to the canines for experimentation be delivered at known, quantifiable, and reproducible levels, though this is often overlooked (Simon et al., 2019).

The research described herein uses a controlled method of odor delivery and two separate sets of molecularly-similar compounds to explore the concepts of molecular structure and training as they relate to canine odor perception in previously-trained detection canines. To ensure constant, controllable, and reproducible delivery of the odorants during field-testing, controlled odor mimic permeation systems (COMPS) were used, providing consistent permeation rates across all odorants. COMPS were used to deliver the analyte odorants at similar concentrations (i.e. similar odor availability). The first odorant set included aliphatic carboxylic acids of varying carbon chain length and confirmation (Study 1), and the second set was comprised of compounds of the same chain length, but varying functional groups (Study 2). Both studies included canines previously trained for odor detection. No less than 14 canines were used in each session, with a different set of subjects used for Study 1 and Study 2, allowing for statistical comparison of canine olfactory performance. The research was carried out as a series of three sequential detection sessions testing the canines' tendency to generalize from one compound to other molecularly similar compounds, and the effects of training.

2. Methods

This research examining generalization across molecularly-similar compounds was carried out as two independent studies using separate groups of canines. Study 1 considered generalization from pentanoic acid, a five-carbon, straight chain, carboxylic acid, to other aliphatic, carboxylic acids of varying chain length and confirmation, while Study 2 investigated generalization, again, from pentanoic acid to other five-carbon, aliphatic compounds with varying functional groups. In the first session of each study, following a period of off-site training, canines were tested on their ability to detect pentanoic acid, the trained odorant, as well as the molecularly-similar compounds (testing odorants).

Following the first session, canines continued to train for an additional period of time on pentanoic acid in addition to another odorant from the group of similar testing odorants. After this additional training time, the testing was repeated for a second session assessing the canines for increased generalization or discrimination to the remaining testing odorants. Finally, this process was repeated a third time, where the canines were trained on three related odorants, followed by a final testing session. Details of the studies, odor delivery, training, and testing design are defined below.

It should be noted that olfactory generalization may be confused with a failure to discriminate between two similar odorants, and in this, as in many of the above-referenced studies, it is easy to conflate the two. Furthermore, it is difficult to design a testing scenario that excludes the possibility of such perceptual errors; however, should generalization be shown to increase with training, this would tend to indicate that the canines are indeed categorizing (i.e. generalizing) and not simply failing to discriminate.

2.1. Study 1 odorants: Generalization across varying chain length and confirmation

In Study 1, the canines' inclination to generalize from pentanoic acid to other aliphatic carboxylic acids with varying chain lengths and confirmations was measured (Table 1). The odorants in this study each possessed a hydrocarbon chain, straight or branched with 4, 5, or 6 carbons terminating in a carboxylic acid group. All canines were initially trained on the 5-carbon, straight chain acid, pentanoic acid (training odorant = T). They were then tested on other related acids (testing odorants), as listed in Table 1. After the first session of Study 1, the canines were assigned randomly to one of two groups for second training odorant assignments. Group T + 2 was trained on heptanoic acid, the 7-carbon, straight chain acid in addition to pentanoic acid. Group T-Branched was also trained on pentanoic acid, as well as 3-methylbutanoic acid, a 5-carbon, branched chain acid. Finally, for the third session, all canines were trained on both heptanoic and 3-methylbutanoic acids, as well as pentanoic acid (Table 1).

2.2. Study 2 odorants: Generalization across varying functional groups

In Study 2, the canines' tendency to generalize from one five-carbon compound (pentanoic acid) to others that differ only by functional group was tested (Table 2). Like pentanoic acid, all testing compounds possessed a five-carbon straight chain, but differed in the terminating group to include ketones (R-CO-R'), aldehyde (R-COH), alcohol (R-OH), and ester (R-COO-R'). Like Study 1, canines were initially trained with pentanoic acid (training odorant = T), then evaluated with like compounds containing other functional groups (testing odorants). Again, after the first session, the canines were randomly assigned to one of two groups for second training odorant assignments. Group 2-Ketone was trained to 2-pentanone, and Group Alcohol to pentanol. For the third session, all canines were trained on both 2-pentanone and pentanol, as well as pentanoic acid.

Table 2
Study 2 (Generalization across functional groups) – Training and testing odorants for Sessions 1-3.

	Session 1	Session 2		Session 3
Training Odorant(s)	Pentanoic acid (T)	**Group Alcohol**: T and Alcohol	**Group 2-Ketone**: T and 2-Ketone	T, 2-Ketone, and Alcohol
Testing Odorant 1	2-Pentanone (2-Ketone)	**Group Alcohol**: 2-Ketone	**Group 2-Ketone**: Alcohol	3-Ketone
Testing Odorant 2	Pentanol (Alcohol)	3-Ketone		Methyl Ester
Testing Odorant 3	3-Pentanone (3-Ketone)	Methyl Ester		Aldehyde
Testing Odorant 4	Methylpentanoate (Methyl Ester)	Aldehyde		n/a
Testing Odorant 5	Pentanal (Aldehyde)	n/a		n/a

2.3. Vapor delivery for testing and training

COMPS were comprised of 5 μL of neat compound (all chemicals in Tables 1 and were purchased neat from Sigma-Aldrich) spiked onto a gauze pad (sterile 2"x2", 12-ply, cotton gauze, Dukal Corporation) which was subsequently placed into a COMPS device (Furton and Harper, 2007). The vapor from the compound, while in the COMPS, dissipates at a controlled and measurable rate allowing for reproducible odorant delivery over a period of hours. Dissipation rate is dictated by the compound vapor pressure, the intermolecular interactions between the compound and the gauze material, and the thickness of the permeable bag. Permeable bags (2"x3" industrial poly bags, Uline) of varying thicknesses (1 to 6 MIL) were utilized to control the permeation rate with thicker bags slowing the dissipation of higher volatility compounds and allowing the matching of permeation rates between analytes of varying vapor pressures. The odorant concentration from each COMPS was measured to confirm approximate equivalence prior to canine testing (although it should be noted that controlling for available vapor concentration does not necessarily control for perceived intensity). The COMPS method and quantitative testing of the odor delivery is described in greater detail in Simon et al. (2019). For this research all COMPS were created prior to each session, and were never reused.

To prevent cross-contamination during transport and storage, COMPS were individually contained in smaller (3.4" x 4.5") Mylar bags (ESP Packaging LLC), and then grouped by odorant in a secondary outer container, either a jar (16 oz. glass canning jars with lids) or a larger (7.5" x 11.5" x 3.5") Mylar bag (ESP Packaging LLC). Blank materials consisting of a clean gauze pad in a permeable bag were prepared and handled in a similar fashion. For testing, new COMPS were prepared for each session. For training, handlers were instructed to dispose of the COMPS after a maximum of 6 hours of use or upon contamination. The procedure for handling and usage of the COMPS was determined by previous research (Simon et al., 2019).

2.4. Canine subjects

Canine participants were obtained from the National Association of Canine Scent Work®, LLC (NACSW™), a.k.a. K9 Nose Work®. K9 Nose Work is a sporting group for domestic (pet) canines offering classes and competitions in scent detection using essential oils (birch, anise, and clove). The group trains and tests in scenarios that mimic search and testing scenarios used by operational detection canines. All canine participants were previously shown to be competent at odor detection through K9 Nose Work competitions and training.

The K9 Nose Work sporting group includes canines from a range of breeds, ages, experience level, and behavior. The subjects were selected for this study subjectively by outside K9 Nose Work trainers based on their perceived proficiency in the detection sport. A total of 37 canines, both male and female, participated across both studies. The mean age was 6.0 years and the mean years of experience was 2.9. Breeds included more traditional detector dog breeds, such as Labradors, German shepherds, Belgian malinois, and beagles, as well as less traditional breeds to include Australian shepherd, Lagotto Romagnolo, German wirehair and shorthair pointers, Wheaton terrier, black mouth cur, sheltie, Doberman, Welsh corgi, golden retriever, husky, King Charles cavalier, and various mixed breeds. Additional information on the age, breed, and experience of individual canine participants can be found in the Supplemental Data.

2.5. Canine training and testing

All testing protocols were reviewed and approved by the Florida International University Institutional Animal Care and Use Committee as well as the Navy Bureau of Medicine and Surgery prior to conducting any animal testing.

2.5.1. Canine training

Those compounds listed as "Training Odorants" in Tables 1 and 2 were provided to canine handlers for off-site training prior to each session. All participating canine handlers received multiple freshly made materials (odorants and blanks) four to six weeks prior to the first session and as needed through the duration of the study.

Upon receipt of the training materials, handlers were instructed to train at home with the provided training odorant(s) "as usual," meaning that they were to continue training in the same manner in which they train with K9 Nose Work odors. Additional details of recommended training practices are included in Supplemental Materials, though each participant trained off-site and the exact method and duration of training varied.

2.5.2. Canine testing

All testing was done in a double-blind fashion, meaning neither the handler nor the impartial assessors knew the locations or identity of the odorants, distractors, or blanks in the testing. Impartial assessors of the testing recorded canine responses as an alert, interest, or no interest. In order to maintain the double-blind, the impartial assessor completed recording canine response data prior to any verbal comment or physical action by any other experimenters in the room that could indicate the position or identity of the target. Compounds listed as "Testing Odorants" in Tables 1 and 2 were presented to canines only one time during each test in the manner described below. Odorant recognition tests (ORTs) provided a uniform method of determining a canine's ability to locate and identify a target odorant. Each session consisted of six or seven ORTs, dependent on the number of odorants being tested for the specific session. Each ORT was comprised of a line of five 8" x 6" x 4" cardboard boxes (Uline) consisting of one target, one distractor, and three blanks. Additional negative ORTs containing one distractor and four blanks were included. Finally, one or more ORTs containing the trained odorants with no distractor were used to validate the canines' recognition and were treated as positive controls. Distractor odorants were selected at random from the following list: limonene, cinnamaldehyde, α-amyl-cinnamaldehyde, citral, cuminaldehyde, pinene, eucalyptol, phenol, linalool, carvone, β-caryophyllene, isoamyl acetate, pinene, nerolidol, 3-carene, furanmethanol, 2-pentylfuran, or farnesene (all purchased neat from Sigma-Aldrich). Canines did not see

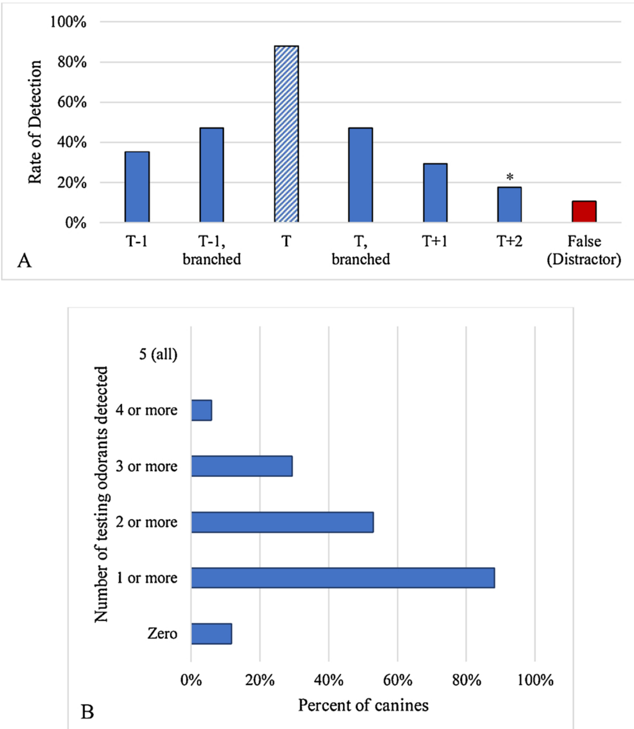

Fig. 1. Results from Study 1 (*Generalization across carbon chain lengths*), Session 1 – (A) percent of canines that detected each compound in the ORT (T = Trained odorant; Testing odorants are labelled as T + or – number of carbons or branched); (B) Percent of canines that detected 0 to 5 odorants. All testing odorants were detected at rates significantly greater than chance, and significantly less than the trained odorant, pentanoic acid (T) unless otherwise noted by. *Statistically the same as chance (χ^2, $p < 0.05$).

a single distractor odorant more than one time in a single session.

The order of the ORTs was randomized for each testing day using a random number generator. Additionally, within each ORT, the locations of the target, testing, and distractor odorants were assigned by a random number generator for individual canines. While within-session learning of novel (testing) odorants could not be completely eliminated, it was minimized by presenting each testing odorant to a canine no more than once per session (i.e. each canine saw each testing odorant only one time per session) with a minimum of four weeks (and no more than 6 weeks) between sessions. Preventing or minimizing within-session learning of novel odorants was imperative to distinguish between generalization and simply learning to recognize two different target odorants.

Like in K9 Nose Work sessions and training, the type of reward was chosen by the handler based on knowledge of how best to motivate their canine. A majority of the canines that participated in the study were rewarded with food and a few with play. The canine participants had not previously been trained on a variable reward schedule, and thus to prevent deterioration in motivation during the study, canines were rewarded for a positive response either to their trained odorant or related testing odorants. For this purpose, an experimenter, an additional person with knowledge of the location of the target, was present during testing. This person was placed in such a manner to not disturb or influence the canine/handler, assessors, or recording of data in any way, allowing the study to remain double-blind. When the canine indicated to an odorant, the handler was instructed to call an "alert", to which the experimenter would reply simply with a yes or no, allowing the handler to reward the canine as appropriate. The type and manner of alert was determined by the handler and communicated to the assessor prior to testing.

Additionally, to validate that the canines were indeed capable of locating their trained odors, a minimum of four positive control searches was incorporated into the testing session. There were two types of searches containing the canines' trained odors. First, room searches incorporated some combination of indoor container searches, room / furniture searches, or external vehicle searches, and contained one or two target odorant(s), depending on the size of the search area, as well as blank COMPS. Second was ORT searches, as described above. Results from only the ORT's were tallied in the data as only these could be directly compared to the testing data. The room searches were not included in the data and were only used to assess the canines' ability to recognize the trained odorants; however, the data from any canine that did not detect the trained odorants in at least three of the four total control tests in a given session were not included.

2.6. Data collection and analysis

Correct and false alert rates were subsequently calculated as a percent of responses for all canines included in each session. Two false alert rates were calculated: (1) false alert rate to distractor odorants (calculated as number of false alerts to distractor odorants out of total number of distractor odorants presented in the session), and (2) total possible false alert rate to include blanks and distractors (calculated as total number of false alerts by a canine out of total possible). This distinction was made because excessive false alerts to distractor odorants could be indicative of a canine simply locating a novel odorant instead of finding a recognized odorant. Any canine that did not successfully locate 75% of the trained odorants or that had excessive false alerts was excluded from the data. Excessive false alerts were defined as more than two false alerts on distractor odorants or more than six total false alerts in a single session. Detailed pooled data for all canines included in each session can be found in the Supplemental Materials.

Chi-square statistical analyses of the data collected were used to compare canine responses to training and testing odorants, as well as responses between canine testing groups. All canines were exposed to each testing odorant only once during each session to minimize learning of the odorants, and for this reason confidence intervals could not be calculated. However, the testing did include a higher number of subjects than most detector canine research allowing use of the chi-square test of independence.

3. Results

3.1. Study 1 – Generalization across differing carbon chain lengths

A summary of results from Study 1, Session 1 are given in Fig. 1 (all session data can be found in the Supplemental Materials). Of the canines, 88% located their trained odorant, pentanoic acid (T), with a false alert rate of 11% to distractors, and an overall false alert rate of 8% (including distractors and blanks). Four canines were excluded from the data for excessive false alerts or non-recognition of the trained odorant. The alert rate to the testing odorants was statistically lower than the trained odorant (χ^2 [1, N = 17] > 6.58, $p < 0.01$), ranging from 18-47% detection. Although, also significantly greater than the false alert rate ; (χ^2 [1, N = 17] > 4.24, $p < 0.05$), with the exception of heptanoic acid (T + 2) detection rates (χ^2 [1, N = 17] = 0.68, $p = 0.41$) (Fig. 1A) indicating some minimal generalization to these odorants. Similar to previous research, canines were more likely to detect the compounds that differed from the target odorant by only one or no carbons (i.e. 47% - 2-methylpropanoic [T-1-branched], 47% - 3-methylbutanoic [T-branched], and 35% - butanoic acids [T-1]). The compound with the lowest alert rate (18% - heptanoic acid [T + 2]) was the molecule most different from the target odorant, followed by hexanoic acid (T + 1) (29%). Interestingly, the branched acids yielded the highest alert rates, compared to the straight chain configurations. Fig. 1B compares the canines' overall propensity for generalization to this series of compounds. Nearly all canines (88%) identified at least

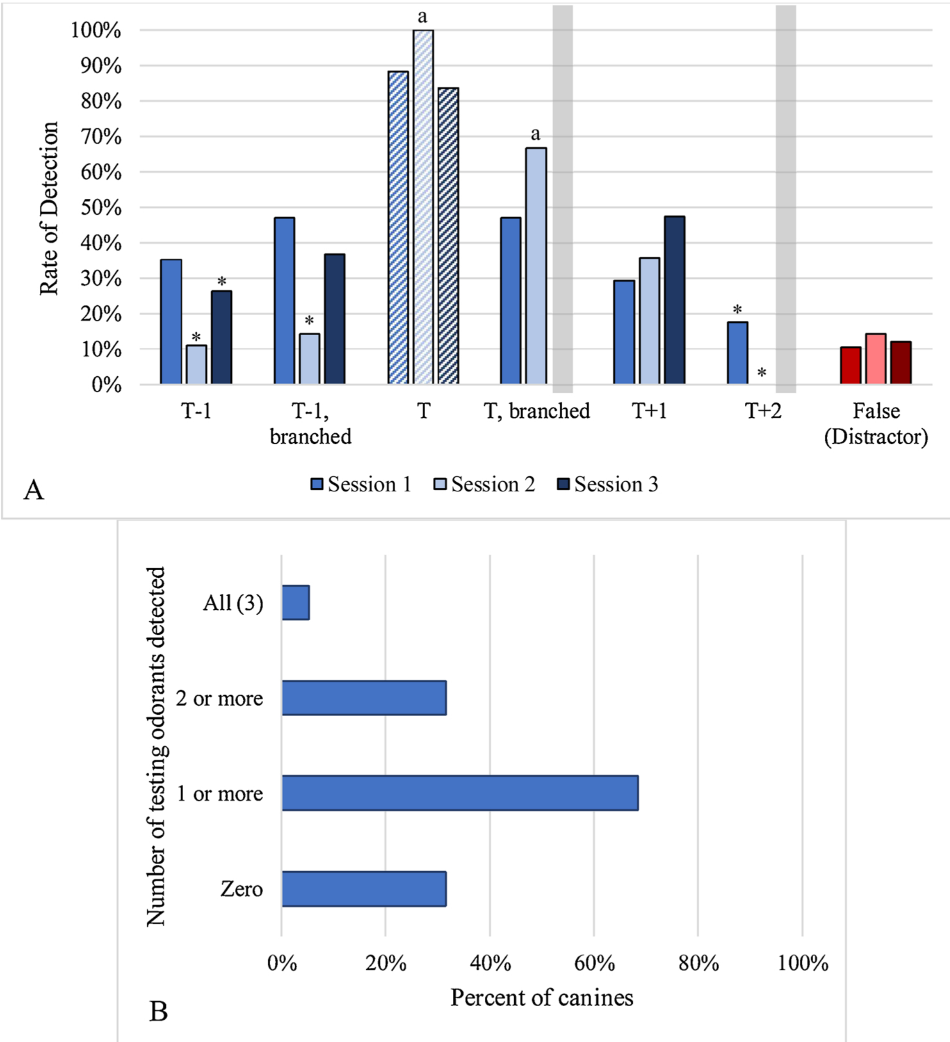

Fig. 2. Results from Study 1 (*Generalization across carbon chain lengths*), (A) comparison of Sessions 1-3, given as percent of canines that detected each compound in the ORT (T = Trained odorant; Testing odorants are labelled as T + or – number of carbons or branched). In Session 3, no canines were presented with 3-methylbutanoic (T, branched) or heptanoic acids (T + 2) as testing odorants, and thus the absence of data is depicted as gray bars; (B) Percent of canines that detected 0 to 3 odorants in Session 3. All testing odorants were detected at rates significantly greater than chance, and significantly less than the trained odorant, pentanoic acid (T) unless otherwise noted. [a]Statistically the same as pentanoic acid (χ^2, $p < 0.05$); *statistically the same as chance (χ^2, $p < 0.05$).

one of the testing odorants, though no canines identified all testing odorants and only one located four, showing that most canines had some propensity to generalize, but not necessarily to the same compound and not to the same degree.

After Session 1, Sessions 2 and 3 further explored the generalization-discrimination balance by addressing the effect of training on multiple compounds from the series. Data from Sessions 1 through 3 are compiled in Fig. 2 (complete data from the sessions are included in the Supplemental Materials). The data from five canines in Session 2 and three canines in Session 3 were excluded for excessive false alerts or non-recognition of the trained odorants. Pentanoic acid (T) was detected by 100% of canines in Session 2, but dropped to 84% in Session 3, and the false alert rates to distractors increased in Session 2 to 14%, and 12% in Session 3 (total false alert rates were 7% for both sessions). Throughout the three sessions, canines detected some testing odorants at a rates greater than chance (χ^2 [1] > 4.24, $p < 0.05$) (Fig. 2A); however, there was no indication of improved generalization with more than the two thirds of the canines (68%) detecting one or none of the training odorants, and thus indicating no additional generalization through learning (Fig. 2B). Furthermore, canines that located three or more of the five testing odorants in the first session, located no more than two of the three possible testing odorants in Session 3, again indicating that increased training time potentially increased discrimination and not generalization for these compounds.

In Session 2, canines were split into two groups and trained on two different compounds in the set, in addition to the pentanoic acid. Group T + 2 was trained on heptanoic acid (T + 2) and Group T-Branched was trained on 3-methylbutanoic acid (T-branched) (Fig. 3). As might be hypothesized, Group T + 2 did have a higher alert rate to hexanoic acid (T + 1) compared to Group T-Branched after being trained on pentanoic acid (T) and heptanoic acid (T + 2), although this difference was not statistically significant (χ^2 [1, N = 11] = 0.75, p = 0.39; ϕ = 0.26) due to the few number of canines from Group T + 2. Furthermore, canines in Group T-Branched were the only canines to detect 2-methylpropanoic acid (T-1-branched) in Session 2 after being trained on 3-methylbutanoic (T-branched) and pentanoic acids (T).

3.2. Study 2 – Generalization across differing functional groups

A separate group of canines, not previously trained to pentanoic acid or similar compounds were used in Study 2. This group was initially trained to pentanoic acid (T) and tested on molecularly similar compounds of the same chain length (five carbon) with differing oxygenated functional groups (Table 2). Results of the first session are given in Fig. 4 (additional data for this and further sessions are included in the Supplemental Material). All canines located pentanoic acid (T), the trained odorant (100%), and only one canine was excluded from the data for non-detection of the trained odor. The alert rates to the testing odorants varied drastically from 7% for the 2-ketone and the alcohol, statistically the same as the false alert rate (8%; (χ^2 [1, N = 30] = 0.023, p = 0.88), to 87% for the methyl ester, statistically the same as pentanoic acid (T) (χ^2 [1, N = 30] = 0.52, p = 0.047)

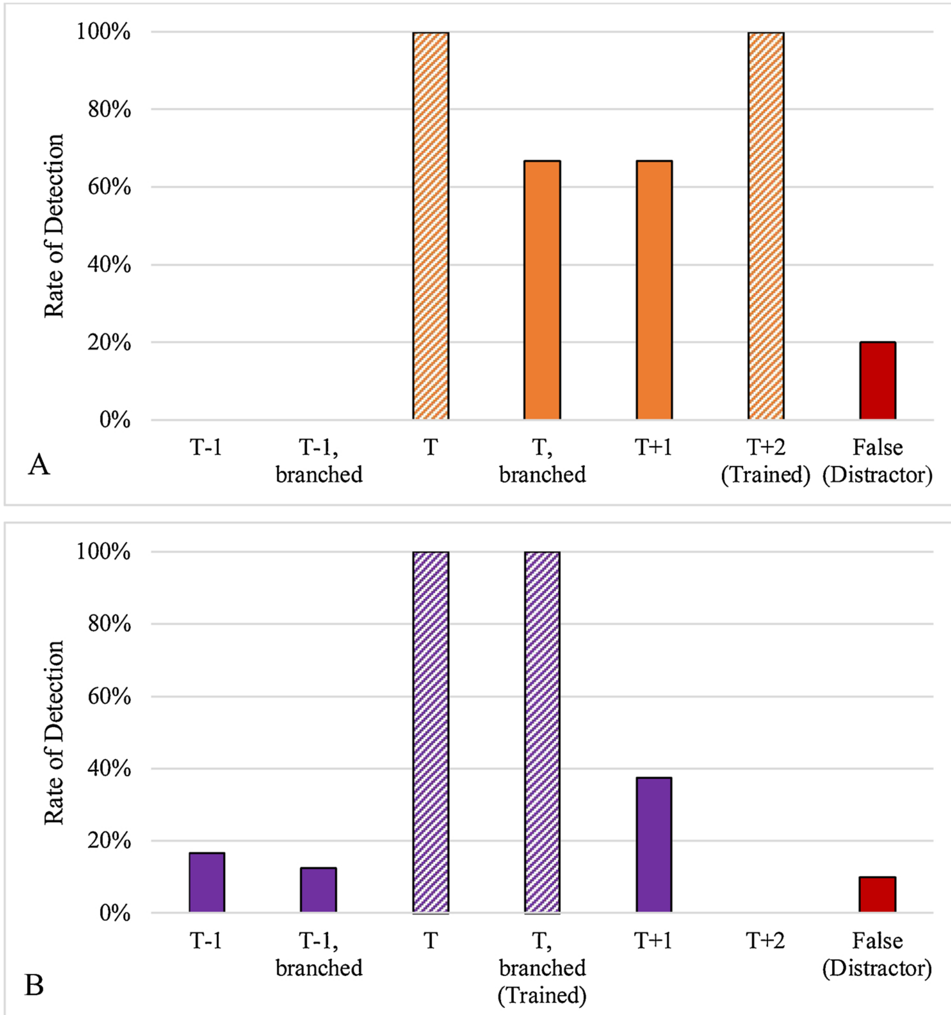

Fig. 3. Percentage of canines that detected each compound in Study 1 (*Generalization across carbon chain lengths*), Session 2, comparing (A) Group T + 2 (trained to pentanoic [T] and heptanoic acids [T + 2]) to (B) Group T Branched (trained to pentanoic [T] and 3-methylbutanoic acids [T, branched]). T = pentanoic acid; Other odorants are labelled as T + or – number of carbons or branched. Striped fill indicates a trained odorant and solid fill indicates a testing odorant.

(Fig. 4A), implying the that methyl ester of pentanoic acid (methylpentanoate) was statistically similar to the pentanoic acid training odorant (T). The detection rate to the aldehyde was also greater than chance (χ^2 [1, N = 30] = 3.28, p = 0.070), indicating that a significant number of the canines generalized to the five-carbon aldehyde, as well. The alcohol, on the other hand, was the only compound lacking a carboxyl group, and, along with the 2-ketone, elicited the lowest alert rates (7%). Only one canine alerted to the alcohol and one other canine alerted to the 2-ketone. Both of these canines showed a relatively high degree of generalization across this session, alerting to at least two additional testing odorants. All canines showed some tendency to generalize, with all alerting to at least one testing odorant, though no single canine alerted to all of the testing odorants (Fig. 4B).

Fig. 5 compares results of all three sessions. The rate of detection of the trained odorants remained high for Sessions 2 and 3, 86% and 93%, respectively, while the rate of false alerts on distractor odorants were below 10% for all sessions. No canine data were removed from either Session 2 or 3 for excessive false alerts or non-recognition of trained odorants. Detection of the methyl ester also remained very high, with all dogs (100%) locating the odorant in Session 3. Additionally the rate of detection of the aldehyde and 2-ketone increased with each session reaching 93% and 100% in Session 3, respectively. The chance of canines detecting the 3-ketone and alcohol increased by the third session, as well (Fig. 5A). By Session 3, generalization by all canines increased, as can be seen in Fig. 5B, where 100% of canines detected at least two of the three testing odorants, and 64% detected all three. In Study 2, there was notable initial variance between tendency to generalize in Session 1, as can be noted by Fig. 4B, but then all canines either increased generalization or remained the same (Fig. 5B).

Prior to the second session, in addition to pentanoic acid (T), Group Alcohol was given pentanol, the five-carbon alcohol for training, while Group 2-Ketone was given 2-pentanone. The addition of the pentanol training odorant yielded 100% detection of the 2-ketone for Group Alcohol. However, this trend was not reversed, and Group 2-Ketone did not see a significant increase in the detection of the alcohol after training on 2-pentanone (Fig. 6). These results suggest that the training odorant does affect the compound to which a canine will generalize.

3.3. Comparison of canine performance

There was a notably greater propensity for the canines in Study 2 (generalization across functional groups) to generalize from pentanoic acid to the testing odorants, compared to Study 1 (generalization across carbon chain lengths). Table 3 compares the percentage of positive alerts to the testing odorants for Sessions 1 and 3 in each study. Initially, in the first sessions, canines had approximately the same tendency to generalize from pentanoic acid to testing odorants in either set (35% and 37%, respectively); however, in Study 1 canine alerts were

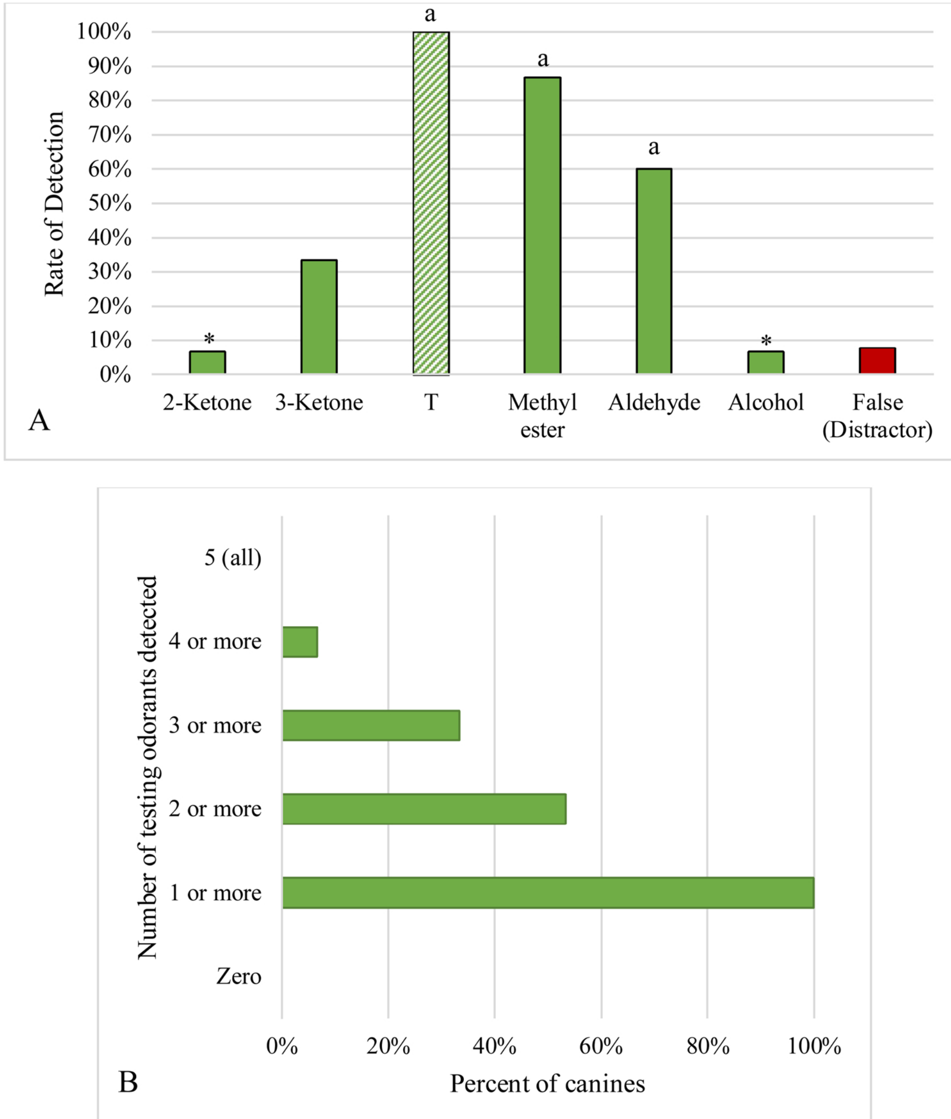

Fig. 4. Results from Study 2 (*Generalization across functional groups*), Session 1 – (A) Percent of canines that detected each compound in the ORT (T = trained odorant and all others are the related 5-carbon compound); (B) Percent of canines that detected 0 to 5 odorants. All testing odorants were detected at rates significantly greater than chance, and significantly less than the trained odorant, pentanoic acid (T) unless otherwise noted. [a]Statistically the same as pentanoic acid (χ^2, $p < 0.05$); *statistically the same as chance (χ^2, $p < 0.05$).

spread out more broadly across the possible testing odorants (Fig. 1) as compared to Study 2, where alerts were concentrated on methylpentanoate and pentanal (Fig. 4). By the third session in Study 1, there was no notable change in alert rate to the testing odorants (i.e. no increased generalization), as opposed to Study 2 where the percentage of positive canine alerts went from 37% to 88% on the testing odorants indicating canines more readily generalized to those testing odorants.

As mentioned previously, it was noted that some canines had a greater propensity to generalize than other canines. Similar trends were seen in previous generalization studies with ammonium nitrate variants (DeGreeff et al., 2018), and in another study where human subjects were asked to discriminate between a homologous series of aliphatic alcohols (Laska and Teubner, 1999). As would be expected, discrimination was negatively correlated to increasing chain length, but there were also notable differences in individual's ability to discriminate ranging from an error rate of 8% to 41%.

To better understand differences between individual canines, the data was examined further. First, comparing canine performance based on detection proficiency to the trained odorants. Of the canines that missed a trained odorant in the ORT, the average number of testing odorants detected in Session 3 was 0.78 (± 1.09) of three possible compounds to detect, while the average number detected by those canines that did not miss a training odorant was 2.17 odorants (± 0.76), again out of three possible compounds that could be detected. This difference illustrates that greater proficiency for detecting the training odorant or the detection task significantly increased the canine's ability to locate the testing odorants ($t[32] = 4.18$, $p = 0.0002$), and thus appears to have enhanced generalization.

No statistical differences in the data were observed based on canine age or gender; however, there was some distinction based on experience level. Canines were assigned to the "novice" or "expert" groups based on years of odor detection experience. A majority of the canines that participated in this session had one or more years of experience in odor detection. As such, the novice group included canines with less than 2.5 years of experience in odor detection and the expert group included those with more than three years of experience. A comparison of novice and expert groups is given in Fig. 7 and suggests that more experienced canines in odor detection tended to generalize more than

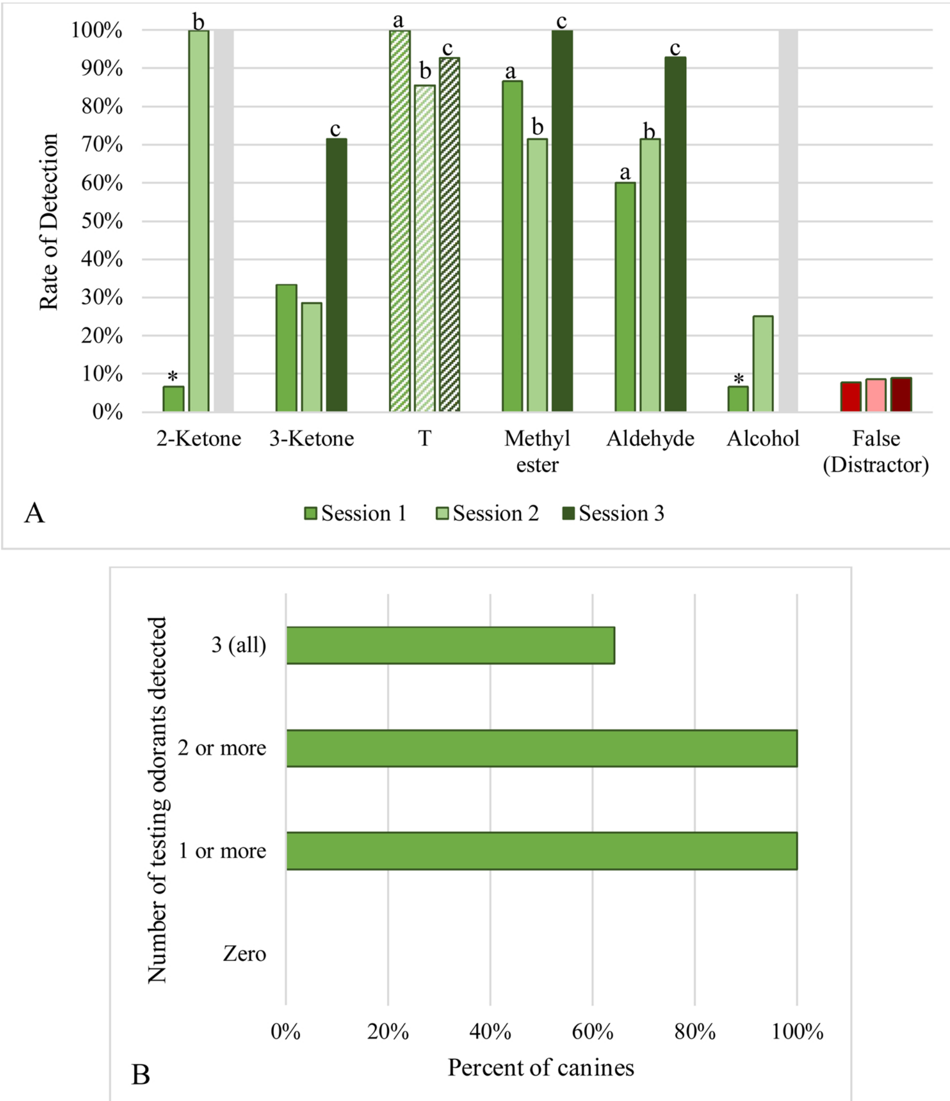

Fig. 5. (A) Result from Study 2 (*Generalization across functional groups*) comparison of Sessions 1-3, given as percent of canines that detected each compound in the ORT. T = trained odorant (pentanoic acid) and all others are the related 5-carbon compound. In Session 3, canines were not presented with the 2-ketone or alcohol as testing odorants, and thus the absence of data is depicted as gray bars; (B) Percent of canines that detected 0, 1, 2, or 3 odorants in Session 3. All testing odorants were detected at rates significantly greater than chance, and significantly less than the trained odorant, pentanoic acid unless otherwise noted. [a,b,c]Statistically the same as pentanoic acid (T) (χ^2, $p < 0.05$); *statistically the same as chance (χ^2, $p < 0.05$).

novice canines, regardless of the odorant set, though the difference between the groups was not statistically significant (χ^2 [1, N = 145] = 3.42, $p = 0.064$, $\phi = 0.15$). Repeating the experiments with a group of canines with less experience (i.e. more canines with less than 1 year of experience) could yield more meaningful results.

There is often a great deal of interest in differences between canine breed as it relates to detection capabilities. This study allowed for data to be recorded on the performance of a variety of breeds, although also noting that all of the canines used in this research were chosen by the owner to participate in Nose Work because of their odor detection ability. Though there was not enough of any single breed to make comparisons, canines results could be reviewed as a comparison of traditional detector dog breeds versus non-traditional detector dog breeds (Note. The breeds included as “traditional” and “non-traditional” detector dog breeds were chosen by the authors). The traditional breeds group included Labradors, German shepherds, Spaniels (English springers and fields), Belgian malinois, and beagles (the authors acknowledge that there are other breeds that could fall into this category, however no other “traditional breeds” participated in this research). The non-traditional breeds group encompassed all others in the study. Several studies comparing canine breed performance in olfactory detection tasks have shown some enhanced capabilities in certain breeds over others. Polgar et al. (2016) compared previously untrained scenting, non-scenting, and short-nosed breeds in their ability to locate food by olfaction and found that, indeed, the scenting group performed better than the other groups at the task. Similarly, Jerierski et al. (2014) compared detection capabilities of trained law enforcement canines of several breeds and showed that German shepherds gave significantly more correct indications than terriers in the given tasks. Fig. 8 gives the percent of correct alerts to the testing odorants in Session 1 of Studies 1 and 2 for both breed groups. While canines of traditional breeds did appear more likely to generalize to the testing odorants, the difference between groups was not significant (χ^2 [1, N = 165] = 2.36, $p = 0.12$, $\phi = 0.12$) for either study. Again, a greater number of canine participants might improve statistical confidence. In both this and the previous comparisons, it should be noted that a greater degree of generalization by a canine does not necessarily imply a better detection canine. As discussed previously, both discrimination and generalization is necessary in the field to make a successful detection canine.

4. Discussion

4.1. Study 1 – Generalization across differing carbon chain lengths

Study 1 explored canine generalization to similar compounds of differing carbon chain lengths and degree of branching. In Session 1, the testing odorants with the highest alert rates were those most similar to the trained odorant in chain length, i.e. 2-methylpropanoic (T-1-

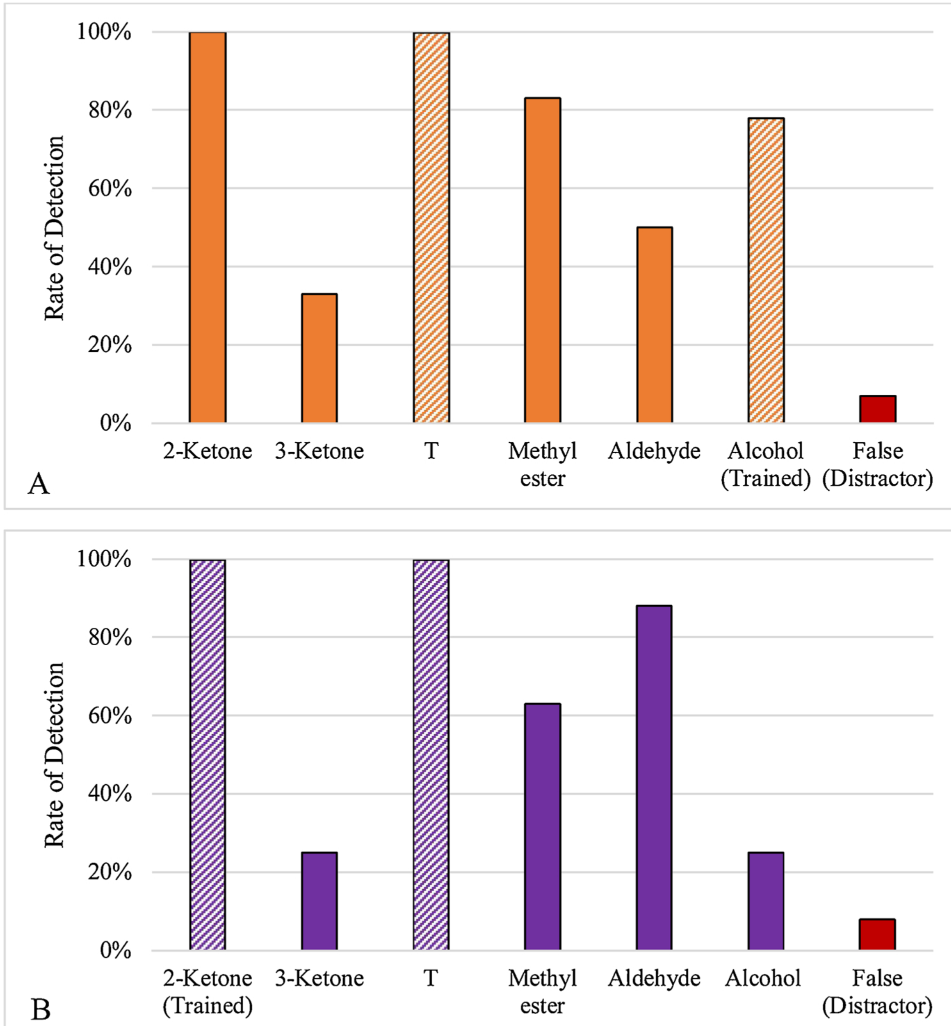

Fig. 6. Percentage of canines that detected each compound in Study 2 (*Generalization across functional groups*), Session 2, comparing (A) Group Alcohol (trained to pentanoic acid [T] and pentanol [alcohol]) to (B) Group 2-Ketone (trained to pentanoic acid [T] and 2-pentanone [2-ketone]). T = trained odorant and all others are the related 5-carbon compound. Striped fill indicates a trained odorant and solid fill indicates a testing odorant.

Table 3
Percentage of positive canine alerts to testing odorant for Sessions 1 and 3 in each study.

	Session 1	Session 3
Study 1	35%	39%
Study 2	37%	88%

branched), 3-methylbutanoic (T-branched), and butanoic acids (T-1), which differed in length from pentanoic acid (T) by either one carbon or only by chain confirmation. The compound with the lowest alert rate (heptanoic acid [T + 2]) was the most different testing odorant compared to the training odorant, with two additional carbons. This initial data agrees with previous studies that found that animals do have more difficulty in discriminating (i.e. some tendency to generalize) between molecularly similar compounds (Cleland et al., 2002; Hall et al., 2016; Kaluza and Breer, 2000; Laska and Freyer, 1997; Laska and Teubner, 1999; Laska et al., 1999; Rizvanovic et al., 2013; Yoder et al., 2014; Carcaud et al., 2018), though in the previous studies this was measured as an inability to discriminate between pairs of more similar

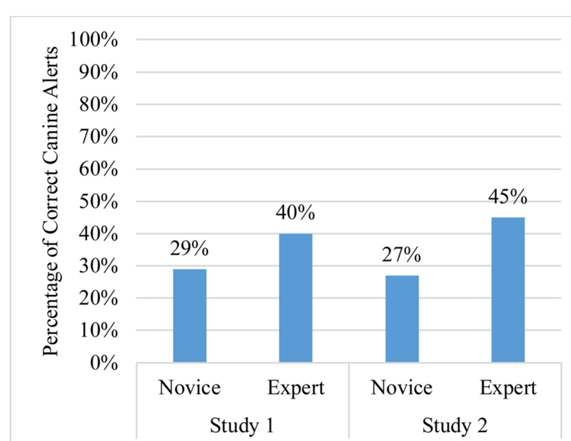

Fig. 7. Number of testing odorants collectively detected by canines in each study (Session 1) comparing previous detection experience. Canines with no more than 2.5 years of experience in odor detection are considered novice, while canines with at least three years of experience are considered experts.

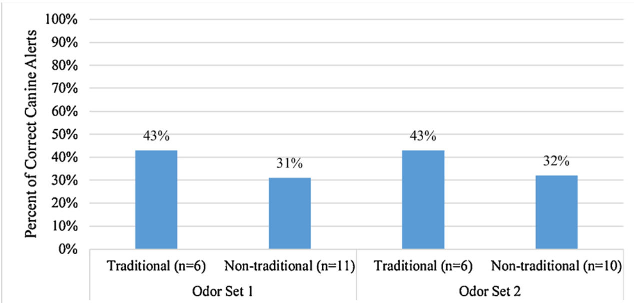

Fig. 8. Percent of correct alerts to testing odorants by traditional (Labrador, German shepherd, Spaniel, Belgian manilois, and beagle) and non-traditional detector dog breeds in both Studies 1 and 2 (n = number of canines).

compounds. This is an important distinction as the study herein was designed to study generalization. Interestingly, of the three acids, 2-methylpropanoic (T-1-branched), 3-methylbutanoic (T-branched), and butanoic (T-1), those to which individual canines generalized seemed to be based on individual preferences. In agreement with this study, previous research showed that some individual canines seem to display a higher tendency to generalize than others, and the odorants to which canines generalize was variable between individuals (Lazarowski et al., 2015; DeGreeff et al., 2018; Laska and Teubner, 1999), where some canines located most or all of the testing odorants, and others found none.

The effect of training on an increasing number of molecularly similar odorants was considered. From the beginning, in Session 1, some generalization did occur, but additional training (Sessions 2 and 3) did not increase generalization for this set of odorants. In fact, the number of canines that did not detect any testing odorant increased from 12% in Session 1 to 32% in Session 3, perhaps suggesting that the additional training time enhanced discrimination, as has been seen in previous studies (Cleland et al., 2009, for example).

Research on the neural activity patterns in the glomerular layer of the olfactory bulb has clearly shown that even though odorants with similar functional groups have similar activity pattern, accounting for generalization behavior, there is enough differentiation to account for an animal's ability to discriminate between such odorants (Linster and Hasselmo, 1999; Uchida et al., 2000; Johnson and Leon, 2000). Johnson and Leon (2000) compared the neural activity in the glomerular layer of the olfactory bulbs for a series of acids, to include pentanoic, 3-methylbutanoic, and hexanoic acids. The activity maps between the isomers pentanoic and 3-methylbutanoic acids were notably more similar than between pentanoic and hexanoic acids, in agreement with data from this study. It has also been noted that there exist positional differences between individuals' activity patterns for the same odorant, perhaps accounting for differences in individual tendency to generalize or discriminate like odorants (Oka et al., 2006; Belluscio and Katz, 2001).

4.2. *Study 2 – Generalization across differing functional groups*

Study 2 was designed to demonstrate generalization-discrimination between related compounds of differing functional groups. Canine responses were related to compound structure, specifically the presence and location of a carbonyl group on the molecule. Canines were more likely to generalize from the carboxylic acid to other carbonyl-containing compounds compared to the alcohol. Methylpentanoate (Methyl Ester) was the most structurally similar testing odorant, and consistently had the highest alert rate after Session 1. Pentanal (Aldehyde) had the second highest alert rate, and was the only other testing odorant with the carbonyl group on C-5. Generalization greatly increased from Session 1 to Session 3, and, in Session 3, the alert rates were well above chance for all testing compounds (χ^2 [1, N = 45] < 3.50, $p < 0.61$) with no statistical difference compared to the training odorants (χ^2 [1, N = 70] > 25.98, $p < 0.00001$). These results indicate that, for the selected set of odorants, generalization across functional groups was common. Several research groups did measure generalization/discrimination across odorants of varying functional groups in both the honeybee (Carcaud et al., 2018) and the moth (Daly et al., 2001). In agreement with this research, both groups demonstrated a reluctance to discriminate between closely related compounds with differing functional groups. Moreover, in this research, training on target odorants with a variety of functional groups did enhance generalization across the odorant set, and, unlike in Study 1, the generalization-discrimination balance was indeed shifted through training.

Mapping neural activity in the glomerular layer of the olfactory bulb has shown that compounds of some functional groups have similar activity maps that can be differentiated from groups of compounds with other functional groups. Uchida et al. (2000) measured responses of the rat olfactory bulb to groups of carboxylic acids, aldehydes, alcohols, esters, and ketones of varying chain lengths and confirmations. Their results showed that the acids, aldehydes, and some esters activated glomeruli in a similar area of the OB, while alcohols, ketones and other esters activated the glomeruli in another area (Uchida et al., 2000). These data align closely with the results of Study 2 of this research, where canines readily generalized from pentanoic acid to the related aldehyde and ester, but not the alcohol or ketones.

Likewise, structurally-related odorants, such as those used in this study, are recognized by an overlapping, though not identical, combination of ORs creating olfactory codes utilized for discrimination (Buck, 2005). Firestein (2001) suggests that based on this combinatorial approach to odorant identification, the theoretical number of odorants that could be discriminated could be in the billions, though none or few animals harness the full scope of this capability. It was suggested that increased training, such as that which canine detectors undergo, enhances the animal's ability to discriminate between similar odors. Such research exploring OR "coding" in the brain has been used to explain the olfactory system's great capacity for discrimination between a vast number of odorants, though few have considered how this relates to generalization. It has been shown that single ORs can bind odorants of several consecutive chain lengths (i.e. C7-C9 or C5-C7) and, though no single OR can bind all functional groups, many can bind more than a single compound class (Malnic et al., 1999). As molecularly similar odorants do indeed have common ORs, it could be hypothesized that this similarity in OR code as well as the neural activity patterns allow for the generalization behavior described herein and by others, and the degree of similarity could be related to the ease in shifting the generalization-discrimination balance in the direction of the generalization side.

5. Conclusion

This research explored the concepts of olfactory selectivity and odor perception in canine detection to determine how the molecular structure of the odorant and training influence the generalization-discrimination balance using groups of approximately 15 canines trained previously for odor detection. Unlike many other similar research studies, the available odor concentration for each odorant was controlled so that canines experienced similar odorant availability for each compound, and thus differences in vapor pressure minimally influenced the results, allowing for better evaluation of perceptional differences. Generalization across groups of compounds with dissimilar alkane chains or dissimilar functional groups was assessed separately. Results showed significantly more generalization across compounds with varying functional groups but the same chain length (Study 2). Furthermore, the hypothesis that generalization would increase with increased training was true for the odorant set in Study 2, while increase in generalization was insignificant in Study 1, where chain length and confirmation was varied but the functional group was held constant. Individual ability or tendency to generalize or discriminate between like compounds was noted, with some correlation to previous experience in detection exercises and knowledge of the trained odorant, as well as canine breed. Such research has important implications for trained detection canines, and additional research should be done to supplement these results and related topics in order to optimize the canine generalization-discrimination balance, improve training efficiency, and minimize false positives and negatives in the field.

CRediT authorship contribution statement

Lauryn E. DeGreeff: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Alison G. Simon:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing. **Kimberly Peranich:** Conceptualization, Methodology, Investigation, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Howard K. Holness:** Investigation, Resources, Writing - review & editing, Supervision. **Kelvin Frank:** Investigation, Writing - review & editing. **Kenneth G. Furton:** Writing - review & editing, Project administration.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.beproc.2020.104148.

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