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ORIGINAL PAPER

Olfactory discrimination and generalization of ammonium nitrate and structurally related odorants in Labrador retrievers

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Abstract A critical aspect of canine explosive detection involves the animal's ability respond to novel, untrained odors based on prior experience with training odors. In the current study, adult Labrador retrievers ($N = 15$) were initially trained to discriminate between a rewarded odor (vanillin) and an unrewarded odor (ethanol) by manipulating scented objects with their nose in order to receive a food reward using a canine-adapted discrimination training apparatus. All dogs successfully learned this olfactory discrimination task ($\geq$80 % correct in a mean of 296 trials). Next, dogs were trained on an ammonium nitrate (AN, NH_4NO_3) olfactory discrimination task [acquired in 60–240 trials, with a mean ($\pm$SEM) number of trials to criterion of 120.0 $\pm$ 15.6] and then tested for their ability to respond to untrained ammonium- and/or nitrate-containing chemicals as well as variants of AN compounds. Dogs did not respond to sodium nitrate or ammonium sulfate compounds at rates significantly higher than chance (58.8 $\pm$ 4.5 and 57.7 $\pm$ 3.3 % correct, respectively). Transfer performance to fertilizer-grade AN, AN mixed in Iraqi soil, and AN and flaked aluminum was significantly higher than chance (66.7 $\pm$ 3.2, 73.3 $\pm$ 4.0, 68.9 $\pm$ 4.0 % correct, respectively); however, substantial individual differences were observed. Only 53, 60, and 64 % of dogs had a correct response rate with fertilizer-grade AN, AN and Iraqi soil, and AN and flaked aluminum, respectively, that were greater than chance. Our results suggest that dogs do not readily generalize from AN to similar AN-based odorants at reliable levels desired for explosive detection dogs and that performance varies significantly within Labrador retrievers selected for an explosive detection program.

Keywords Dog · Canine · Cognition · Olfactory discrimination

Introduction

The highly developed olfactory system of the domesticated dog (*Canis familiaris*) has been exploited for canine scent detection of cancers, insect pests, narcotics, and endangered animal species amongst other scents (reviewed in Johnen et al. 2013). One of the most important applications of scent detection is explosives and land mine detection (Gazit and Terkel 2003). For example, adult Labrador retrievers have been used by the US Marine Corps in combat zones as improvised explosive device (IED) detector dogs (Lamothe 2010). Explosive detecting dogs play a crucial role because IEDs and related undetonated munitions are a significant risk to civilian and military populations (Furton and Myers 2001; Gazit and Terkel 2003; Harper et al. 2005; Jones 2011). Indeed, IEDs were the main cause of bilateral lower limb amputation in British troops deployed to Afghanistan (Penn-Barwell et al. 2014). Scent detection canines are one of the most effective

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explosive detection technologies and are thought to meet or exceed the capabilities of many instrumental detection technologies (Harper et al. 2005).

Training dogs for IED and other homemade explosive detection presents several challenges. The chemical composition of IEDs and homemade explosives vary by region, local availability of materials, and quantities of explosives, fuels, gelling agents, distractors, and other materials used (Eisler 2012; Kopp 2008). Consequently, most target (explosive) odors encountered by dogs under field conditions are comprised of a combination of many different substances (Harper et al. 2005) that may differ from those used in training. Little is known about how scent detector dogs apply their learned recognition of training substances to operational variants. Another challenge is the paucity of training substances that can be handled safely and can provide the representative odor profile of an explosive of interest (Lazarowski and Dorman 2014). Finally, evaluating the scent detection capability of dogs is technically challenging. For example, inadvertent handler cueing can confound interpretation of field tests (Johnen et al. 2013; Lit et al. 2011).

Many canine explosive scent-training programs rely on the behavioral process of stimulus generalization (Herstik and Smith 2006). In this context, generalization refers to an individual's tendency to respond to novel, untrained stimuli based on prior experience with training stimuli that share physical similarities (Ghirlanda and Enquist 2003; Oxley and Waggoner 2009). Given the likelihood that explosives encountered in an operational work environment will vary in exact measurement, concentration, components, or other aspects compared to training materials, it is critical for a dog to be able to detect variations in explosives that were not explicitly trained. Oxley and Waggoner (2009) reported that dogs trained on one type of smokeless pistol gunpowder (Bullsye®) successfully detected an untrained powder (IMR 4064) from a different manufacturer only 52 % of the time. Recently, we demonstrated that the majority of Labrador retrievers that were trained to detect the scent of chemically pure potassium chlorate, an explosive used in IED manufacture, were unable to generalize this behavior to potassium chlorate-based explosive mixtures that contained a novel component (Lazarowski and Dorman 2014). Olfactory generalization of other explosive compounds of military interest in dogs remains largely underexplored (Johnston 1999). Factors that contribute to or inhibit generalization in canine explosive detection warrant further investigation.

Completion of this study's first specific aim established the dogs' ability to discriminate between vanillin and ethanol using an olfactory-based discrimination task adapted from a visual discrimination training paradigm for canines (Lazarowski et al. 2014). Since vanillin is a widely used odorant (Frasnelli et al. 2011), completion of this aim provides a useful olfactory performance benchmark for the cohort of dogs used in the experiment. Subsequent phases of the study evaluated the following specific aims: assessment of dogs' ability to discriminate chemically pure ammonium nitrate (AN, NH_4NO_3), assessment of generalization to fertilizer-grade AN and other chemicals containing the ammonium or nitrate chemical moiety, and examination of whether dogs trained to respond to AN would recognize a soil sample that contained 5 % AN—this phase served as a surrogate for assessing the ability of dogs to detect AN under certain field conditions. Our research interest in AN was driven by reports that AN-based chemicals are commonly used inorganic oxidizers in IEDs (Hess 2013; Kopp 2008). These aims were addressed in four separate phases, described below.

Materials and methods

Animals and housing

The subjects used for this study were drawn from a stock of candidate IED detector dogs that were procured by a military contractor that trains military working dogs (K2 Solutions, Inc. Southern Pines, NC, US). In order to be considered for procurement, dogs had to meet age, retriever training, and other entry requirements for the USMC IED detector dog program (Sherman et al. 2015). Procured dogs were collected throughout the USA and then transported by truck to south-central North Carolina, USA. After a 14-day quarantine at an off-site commercial boarding kennel, the dogs were transported to the K2 training facility. A K2 veterinarian performed a comprehensive physical evaluation and placed a subcutaneous microchip for subsequent identification. Procured dogs were held at the K2 facility and received varying amounts of retriever and other training before entry into this research project.

The dogs used in our study were 15 black- and yellow-coated Labrador retrievers between 2 and 4 years of age including eight intact males, four intact females, and three spayed females. Because the group size for spayed versus intact female dogs was small, spay status was not included in the analysis. Dogs were transported to an indoor canine facility at the North Carolina State University College of Veterinary Medicine (NCSU-CVM) Laboratory Animal Resources unit where they underwent a period of adjustment and health monitoring for several weeks before experimental testing began. Details on housing and husbandry procedures have been previously described (Lazarowski et al. 2014). Briefly, dogs were individually housed in temperature and humidity controlled indoor kennels (1.5 m × 2.4 m), fed a canine dry ration twice daily (Iams Mini Chunks, P & G Pet Care, Cincinnati, OH), provided with water ad libitum, and received enrichment in the form of outdoor walks and off-leash exercise, social interactions with people, and toys in their kennels.

All experimental protocols were reviewed and approved by the NCSU Institutional Animal Care and Use Committee and the US Army Medical Research and Material Command Animal Care and Use Review Office. This study was performed in an accredited animal facility (Association for the Assessment and Accreditation of Laboratory Animal Care International). All research was performed at NCSU-CVM by NCSU researchers. At the conclusion of research studies at NCSU-CVM, all dogs were returned to K2 and subsequently adopted to private homes as pets since their training during the experiment prevented their use as IED detector dogs.

Apparatus

Testing utilized the Toronto General Testing Apparatus (TGTA) which was originally developed by Milgram et al. (1994) for visual discrimination training with beagles (CanCog Technologies, Toronto, Canada). The system was modified to allow visual discrimination testing of larger breed dogs including Labrador retrievers and has been previously described (Lazarowski et al. 2014). Briefly, the apparatus was divided into two sections by stainless steel bars, which separated the portion in which the dog remained during testing from the stimulus presentation area. The experimenter sat in front of the testing chamber, separated from the dog by the front panel of the chamber which contained a one-way mirror on the inside to obscure the dog's view of the person. The lower portion of the front wall of the chamber contained a hinged door measuring 20 cm high that opened to allow the insertion of the sliding Plexiglas stimulus presentation tray. All dogs were successfully acclimated to the TGTA system prior to the present study and trained on a visual discrimination task using plastic, three-dimensional colored objects (Lazarowski et al. 2014).

Odorants

The odorants utilized in this study are presented in Table 1. Liquid odorants included solid vanillin ($\geq$98 % pure, Sigma-Aldrich Chemical Co., Milwaukee, WI) dissolved in pure ethanol (AAPER Alcohol and Chemical Co., Shelbyville, KY) to create a 1-M vanillin solution and pure ($\geq$99.8 %), ethanol solvent, and amyl acetate (Sigma-Aldrich Chemical Co., Milwaukee, WI). Solid odorants included chemically pure (>99 %) AN (Sigma-Aldrich Chemical Co., Milwaukee, WI), soil collected at Camp Victory, Iraq (Dorman et al. 2012), commercial grade (34-0-0, i.e., 34 % nitrogen) fertilizer-grade AN (Weaver Fertilizer, Winston-Salem, NC), ammonium sulfate [$(NH_4)_2SO_4$] and sodium nitrate ($NaNO_3$) purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI), and flaked aluminum (Indian Head Division Naval Surface Warfare Center, Indian Head, MD).

Chemists at the US Naval Energetics Test and Evaluation Division at the Naval Surface Warfare Center analyzed the fertilizer-grade AN sample using Fourier transform infrared spectroscopy (FTIR) and X-ray fluorescence (XRF). Sample analysis was consistent with a granular (i.e., prilled) material having a composition of >97 % AN with negligible levels of either limestone (calcium carbonate) or silica (silicon dioxide).

Stimulus presentation

Plastic egg-shaped containers measuring 10 cm $\times$ 5 cm with two 3 mm perforations on each end were used to contain and present odorants. Liquid odorants were dispensed onto standard weighing paper (2.5 $\times$ 2.5 cm) and placed inside the containers via syringe by an experimenter wearing gloves. Solid odorants, with the exception of AN and flaked aluminum, were deposited inside a nylon bag and then placed into the perforated plastic egg-shaped containers. Because of the inherent explosiveness of an AN/aluminum mixture, these chemical odorants were not physically mixed. Based on our previous work with potassium chlorate-based explosives (Lazarowski and Dorman 2014), we developed a plastic container that presented the odors to the dog while keeping the two explosive components separated. Hollow plastic blocks measuring 6.35 $\times$ 6.35 $\times$ 6.35 cm with a diagonal partition and a removable perforated lid were used for this phase. Two separate plastic perforated vials containing 5 g of AN and 0.4 g of flaked aluminum were placed on either side of the partition inside the cube. The comparison stimulus was an identical container that held a plastic vial containing 0.4 g of flaked aluminum and an empty vial on either side of the partition. Containers were mounted horizontally with hook and loop tape onto plastic coasters (10 cm diameter) and positioned over the left and right wells of the plastic rolling tray. Stimuli were wiped with a paper towel in between trials, and coasters were rotated every 3–4 trials so that the same coaster did not consistently hold the same odorant. The chamber and stimuli were wiped with a damp cleaning towel in between subjects and then thoroughly cleaned with a disinfectant solution (Virkon® S, E.I. duPont de Nemours Co., Wilmington, DE) after all subjects completed testing for the day.

Discrimination training

Table 1 provides the overall experimental design. All dogs were given a single daily 20-trial session, typically

Table 1 Experimental design

Phase	Step	Task	Odorant pair	
			S+	S−
1	1	Initial olfactory discrimination	Vanillin[a]	Ethanol[a]
2	1	Ammonium nitrate (AN) olfactory discrimination	Pure AN[b]	Nylon bag
	2	First negative stimulus transfer	Pure AN[b]	Iraqi soil[b]
	3	Second negative stimulus transfer	Pure AN[b]	Amyl acetate[a]
3	1	Generalization to structurally related odorants	Fertilizer-grade AN[c]	Menthol
	2		Ammonium sulfate[d]	Ascorbic acid
	3		Sodium nitrate[d]	Calcium chloride
	4		AN and flaked aluminum[e]	Flaked aluminum
4	1	Detection of AN in Middle East soil	Pure AN in Iraqi soil[f]	Iraqi soil

[a] Liquid odorant (10 ml)

[b] Solid odorant (5 g)

[c] Solid odorant (5 g)

[d] Solid odorant (equimolar quantity − equivalent to 5 g of AN)

[e] Solid odorant (5 g of AN and 0.4 g flaked aluminum)

[f] Solid odorant (5 g of AN in 95 g Iraqi soil)

conducted Monday–Friday. A tone signaled the beginning of a trial as the hinged door was opened and the experimenter inserted the stimulus presentation tray into the presentation portion of the chamber for 3 s. The tray was then fully inserted, and the dog was allowed 30 s to make a response. A response (defined as any movement of the object with the snout) to the rewarded (S+) container uncovered a 2-cm piece of a food reward (Pup-Peroni® Original bacon flavor treats, Del Monte Foods, San Francisco, CA) in the well beneath. Responses to the unrewarded (S−) container revealed an empty well. An inaccessible food reward was fixed to the bottom of the S− coaster to prevent dogs from using food odors to select the correct stimulus. If no response was made within 30 s, the tray was withdrawn, a nonresponse was recorded, and the next trial began. For sessions 1–10, dogs were allowed to respond to either stimulus until a correct choice was made and the reward consumed. After the tenth session, trials ended after the first response, regardless if correct or incorrect.

Data were collected using DogCog™ software (CanCog Technologies, Toronto, Canada) on a computer running a Windows 7 interface. The software randomized stimulus and reward positions, controlled trial timing, and recorded responses. Responses were recorded by the software as indicated by a keystroke from the experimenter, which initiated a 30-s intertrial interval (ITI) before the next trial began. Stimulus position throughout the session was balanced with odors presented on the left and right sides an equal number of times and no more than three consecutive trials on the same side.

Phase 1: vanillin–ethanol olfactory discrimination

Phase 1 assessed each dog's ability to discriminate between vanillin and ethanol. Vanillin has been characterized as a purely olfactory odorant; that is, the olfactory system but not the trigeminal system is stimulated by this chemical (Stephenson and Halpern 2009). Dogs were trained to discriminate between 10 ml of vanillin (S+) and 10 ml of ethanol (S−) using the procedures described above. A two-stage criterion adopted from previous studies utilizing the TGTA system was used (Tapp et al. 2003). In order to satisfy the first criterion stage, a score of 16/20 or better was required. The second stage of criterion required a total score of 28/40 over two consecutive sessions. Thus, a minimum of three sessions was required before criterion could be met.

Phase 2: ammonium nitrate olfactory discriminations

In the next phase, dogs were trained to discriminate between AN and various other odors. In the initial discrimination, AN (S+) was presented with a "blank" container as the S− (i.e., an empty nylon bag). The criterion used for this discrimination was the same as in phase I. Once criterion was met with this pair of odors, the blank stimulus was replaced with 5 g of Camp Victory soil as the S− for two sessions, followed by amyl acetate as the final S−. If performance was above 80 % on the first two sessions, dogs were advanced to the next phase; if performance fell below 80 %, dogs were required to meet the original 3-day criterion. This was done in order to expedite training, while ensuring stable performance with the odorants that would become the baseline pair in the next phase. Testing occurred continuously with no breaks in between sessions involving stimulus replacement.

Phase 3: generalization to structurally related odorants

Once dogs reached criterion with the AN and amyl acetate stimulus pair, next followed a series of transfer tests followed to assess the dogs' behavioral generalization from pure AN to fertilizer-grade AN, AN and flaked aluminum (a commonly used homemade explosive of military interest), and inorganic chemicals containing either an ammonium or nitrate moiety [i.e., ammonium, sulfate, $(NH_4)_2SO_4$, and sodium nitrate, $NaNO_3$]. Equimolar quantities of ammonium sulfate (8.3 g) and sodium nitrate (5.3 g) were each paired with a compound chemically unrelated to ammonium or nitrate as the S− (see Table 1 for stimulus pairs).

Transfer trials in which dogs were presented with the novel odors were presented a total of 20 times per odor. Each individual novel odor was presented over the course of six consecutive sessions, with 2–5 presentations per session. Only one novel odor appeared during a given transfer session, but dogs received transfer test sessions intermixed and in random order. Transfer sessions were conducted in a similar manner to the previous phase. AN (S+) and amyl acetate (S−) served as the baseline discrimination pair, with novel stimulus trials interspersed throughout the session. Transfer trials consisted of pairs of one AN-related compound and one unrelated compound (Table 1). Transfer trials were never rewarded in order to eliminate the possibility of rapid within-session learning (Wynne et al. 2008).

Phase 4: detection of masked ammonium nitrate (AN)

The final phase, which began on the session immediately following the completion of the previous phase, evaluated whether dogs would detect AN that was mixed with desert soil from the Middle East. A surface sand sample that was collected at Camp Victory, a US Army base situated on the grounds of the Baghdad international airport, was used for this experiment. Characterization of this sand sample has been previously reported (Dorman et al. 2012). Sessions were conducted in the same manner as the previous test sessions, with one container holding 5 g of pure AN mixed in 95 g of Iraqi soil and a second identical container holding 100 g of soil. Because this phase was concerned with dogs' ability to detect the previously trained AN when masked with soil and was not testing for generalization to novel odors, this phase was conducted in a single 20-trial session in which all trials were rewarded.

Data analysis

Response accuracy is a powerful indicator of learning and memory performance in dogs tested using the TGTA system (Callahan et al. 2000; Milgram et al. 1994). Thus, error scores were calculated by summing the total number of errors committed up to and including the last criterion day. Error rates were calculated as total errors committed divided by total trials to criterion (acquisition) where a nonresponse trial was not included in the analysis (i.e., the trial was discounted rather than counted as an incorrect response). Nonresponses generally occurred early in training and during sessions when dogs became distracted or demonstrated decreased motivation. However, due to the inability to repeat transfer trials which would result in unequal exposure to the novel odors, nonresponses during transfer tests were treated as incorrect responses.

The data were compared by tests for homogeneity of variance (Levene's test), and a multivariate analysis of variance (ANOVA) that examined the effect of coat color and/or sex as group factors on a test parameter. When a factor was identified as not significant, the data were pooled appropriately. If the Levene's test was significant, then a Welch's ANOVA test for unequal variances was used. A comparison of group performance versus chance was performed using a one-sample test for the mean in which the sample standard deviation was used to perform a two-tailed t test. To measure individual dog performance, the percentage of correct decisions per session was calculated for each individual and each session. For example, in the initial learning tasks, the criterion was set at 80 % hits, which corresponds to one complete session of 16/20 correct responses (corresponding to $P < 0.01$ two-tailed binomial test vs chance). Statistical analyses were performed using either SAS (JMP 9.0, Cary, NC) or Graphpad (Prism 6, La Jolla, CA) statistical software. A threshold of $\alpha = 0.05$ was used as the critical level of significance for all statistical tests. Unless otherwise noted, data presented represent mean (±SEM) values.

Results

Phases 1 and 2: vanillin–ethanol and AN-blank olfactory discrimination tests

Table 2 provides individual dog performance and overall performance on these tests (see also Fig. 1). All dogs successfully completed the vanillin–ethanol and AN-blank discrimination tasks; however, performance (acquisition and error rates) varied between dogs (Table 2). Learning the initial vanillin olfactory discrimination test typically occurred in two stages. The first included a period of

Table 2 Individual dog and overall (mean ± SEM) performance during initial acquisition of the vanillin and ammonium nitrate (AN) olfactory discrimination procedures

Name	Sex	Coat color	Vanillin olfactory discrimination (vs ethanol)			AN olfactory discrimination (vs blank)		
			Errors	Trials to criterion	Error rate %	Errors	Trials to criterion	Error rate %
Ace	M	B	143	340	42.1	10	60	16.7
Annie	F	Y	60	180	33.3	21	80	26.3
Baxter	M	B	145	359	40.4	87	240	36.3
Bullet	M	Y	95	240	39.6	38	140	27.1
Dakota	F	B	140	380	36.8	8	60	13.3
Honey	F	Y	84	240	35.0	21	100	21.0
Hunter	M	B	100	300	33.3	14	80	17.5
Jimmy	SF	B	101	280	36.1	40	140	28.6
Macks	M	B	55	160	34.4	48	200	24.0
Mercy	SF	B	176	440	40.0	8	60	13.3
Reno	M	Y	166	380	43.7	57	160	35.6
Rip	M	B	64	180	35.6	19	100	19.0
Ruby	SF	Y	141	380	37.1	21	80	26.3
Valentine	F	B	151	400	37.8	20	80	25.0
Wizard	M	B	62	180	34.4	80	220	36.4
Overall			112.2 ± 10.8	295.9 ± 24.3	37.3 ± 0.8	32.8 ± 6.5	120.0 ± 15.6	24.4 ± 2.0

M male, *F* female, *SF* spayed female, *B* black, *Y* yellow

random responding, in which correct responses did not differ from chance. In the second stage, the dogs showed progressive improvement. There were no significant effects of sex or coat color on either the number of trials required to reach criterion or the observed error rates during the vanillin olfactory discrimination test (Table 2). Male dogs required more trials (150.0 ± 23.6) to reach criterion on the AN-blank olfactory discrimination task when compared with female dogs (85.7 ± 10.4 trials) ($F_{9.6} = 6.2$; $P = 0.033$, Welch ANOVA). There were no significant effects of coat color on either the number of trials required to reach criterion or the observed error rates on the AN olfactory discrimination test (Table 2). Significant correlations were not observed between either the number of trials required to reach criterion or error rates associated with acquisition of the vanillin and initial AN olfactory discrimination tasks (data not shown).

The total number of trials required to acquire the AN olfactory discrimination task (mean = 120) was significantly lower ($F_{23.9} = 37.1$; $P < 0.0001$, Welch ANOVA) when compared with the vanillin olfactory discrimination task (mean = 296). Likewise, the overall error rate associated with the AN olfactory discrimination task (mean = 24.1 %) was significantly lower ($F_{18.7} = 35.6$; $P < 0.0001$, Welch ANOVA) when compared with the vanillin olfactory discrimination task (mean = 37.3 %).

Table 3 shows the results of replacing the original S− (nylon bag) with Iraqi soil and then amyl acetate. The dogs' overall performance on the AN (S+) and Iraqi soil

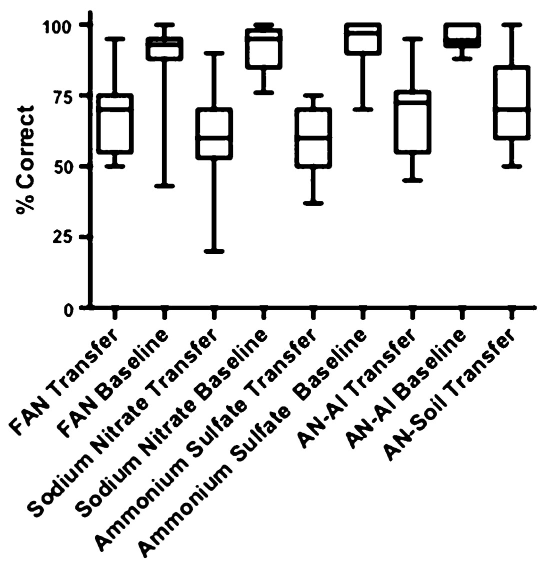

Fig. 1 Overall percent correct transfer and baseline performance for each of the odors presented during ammonium nitrate generalization transfer tests: fertilizer-grade ammonium nitrate (FAN), sodium nitrate, ammonium sulfate, AN and flaked aluminum (AN–Al), and an ammonium nitrate and soil mixture (AN–soil). Means and standard deviations are also shown

(S−) stimulus pair was significantly higher when compared with chance ($t_{14} = 13.0$; $P < 0.0001$, *t* test). The dogs' overall performance on the AN (S+) and amyl acetate (S−) pair was also significantly higher when compared with chance ($t_{14} = 23.5$; $P < 0.0001$, *t* test). Application of Friedman's test shows that there were some statistically

Table 3 Individual dog and overall (mean ± SEM) performance during discrimination tasks

Name	Sex	Coat color	AN versus Iraqi soil			AN versus amyl acetate		
			Errors	Trials completed	Error rate %	Errors	Trials completed	Error rate %
Ace	M	B	3	40	7.50	4	40	10.0
Annie	F	Y	4	40	10.00	4	40	10.0
Baxter	M	B	13	40	32.50	18	80	22.5
Bullet	M	Y	11	40	27.50	7	40	17.5
Dakota	F	B	7	40	17.50	22	100	22.0
Honey	F	Y	6	40	15.00	4	40	10.0
Hunter	M	B	10	40	25.00	4	40	10.0
Jimmy	SF	B	5	40	12.50	7	40	17.5
Macks	M	B	15	40	37.50	8	40	20.0
Mercy	SF	B	5	40	12.50	7	40	17.5
Reno	M	Y	4	40	10.00	10	40	25.0
Rip	M	B	8	40	20.00	3	40	7.5
Ruby	SF	Y	6	40	15.00	4	20	20.0
Valentine	F	B	7	40	17.50	6	40	15.0
Wizard	M	B	12	40	30.00	8	40	20.0
Overall			7.7 ± 0.9	40	19.3 ± 2.4	7.7 ± 1.4	45.3 ± 5.0	16.3 ± 1.4

M male, *F* female, *SF* spayed female, *B* black, *Y* yellow

significant changes in the distribution of dog performance over the three conditions, $\chi^2 = 7.356$, $df = 2$, $P = 0.025$.

Phase 3: generalization to structurally related inorganic salts

Table 4 presents the percentage of correct responses during generalization tests that used fertilizer-grade AN, sodium nitrate, AN, and a training aid with AN and flaked aluminum.

On an individual basis, 8/15, 4/15, and 8/14 dogs had a correct response rate with fertilizer-grade AN, sodium nitrate, and AN and flaked aluminum, respectively, that were greater than chance (≥70 %; $P < 0.05$, two-tailed binomial test). Percentage of dogs that responded correctly on the first trial of each test are shown in Table 4. Application of Friedman's test showed an absence of statistically significant changes in the distribution of dog performance over the three conditions, $\chi^2 = 0.667$, $df = 2$, $P = 0.667$. There were no significant effects of either coat color or sex on the response rate accuracy with either fertilizer-grade AN, sodium nitrate, or AN and flaked aluminum (Table 5).

We found that there was no significant effect of sex on the response rate accuracy with ammonium sulfate; however, black dogs performed significantly better than dogs with a yellow hair coat (Table 5). Analysis of black dogs' performance with ammonium sulfate was significantly higher than chance ($t_9 = 3.53$; $P = 0.0064$, t test). Overall, the yellow dogs' mean performance with ammonium sulfate was not significantly higher than chance. On an individual basis, four black-coated dogs had a correct response rate of ≥70 %, which exceeded chance ($P < 0.05$; two-tailed binomial test). None of the yellow-coated dogs had a correct response rate of ≥70 %, which exceeded chance ($P < 0.05$; two-tailed binomial test).

Phase 4: detection of AN in Middle East soil

The final experimental phase examined whether dogs trained to recognize AN alone would also detect AN when mixed in Iraqi soil (Table 4). Performance on this test was affected by sex and coat color. Mean (±SEM) correct response rate in female (85.7 ± 3.5 %) dogs was higher ($F_{14} = 19.79$; $P = 0.0007$, ANOVA) than that in male dogs (62.5 ± 3.8 %). Overall, both the male ($t_7 = 3.31$; $P = 0.013$, t test) and female ($t_6 = 10.13$; $P < 0.0001$, t test) dogs' performance with AN in Iraqi soil was significantly higher than chance. On an individual basis, 4/10 black-coated dogs and 4/5 yellow-coated dogs met or exceeded a 70 % correct response rate, which exceeded chance ($P < 0.05$; two-tailed binomial test).

Individual differences

A Kolmogorov–Smirnov test was used to determine whether the distribution of trials correct across dogs differed significantly from a binomial distribution with the

Table 4 Percentage of correct responses for dogs trained to detect ammonium nitrate (AN) when presented with structurally related materials including fertilizer-grade ammonium nitrate (FAN), sodium nitrate ($NaNO_3$), ammonium sulfate ($(NH_4)_2SO_4$), and ammonium nitrate with flaked aluminum (AN–Al). Response rates are also shown for ammonium nitrate mixed with Iraqi soil (AN/soil). Group means (±SEM) and first-trial performance (%) are also shown

Name	Sex	Coat color	FAN	$NaNO_3$	$(NH_4)_2SO_4$	AN–Al	AN/soil
Ace	M	B	70	73	53	80	60
Annie	F	Y	55	10	60	55	90
Baxter	M	B	80	55	60	nd	70
Bullet	M	Y	55	60	40	70	85
Dakota	F	B	70	65	75	95	80
Honey	F	Y	75	53	37	75	100
Hunter	M	B	70	50	60	65	55
Jimmy	SF	B	70	55	55	45	90
Macks	M	B	60	45	40	75	60
Mercy	SF	B	95	70	75	90	85
Reno	M	Y	55	90	50	55	60
Rip	M	B	60	55	75	45	50
Ruby	SF	Y	55	65	50	75	85
Valentine	F	B	80	70	70	65	70
Wizard	M	B	50	66	65	75	60
Overall			66.7 ± 3.2*	58.8 ± 4.5	57.7 ± 3.3	68.9 ± 4.0*	73.3 ± 4.0*
First trial (%)			33	67	67	71	75

M male, *F* female, *SF* spayed female, *B* black, *Y* yellow, *nd* not determined

* Overall dogs' mean (±SEM) performance is significantly higher when compared with chance (two-tailed binomial test)

Table 5 Summary statistics showing mean (±SEM) correct responses (%) in dogs trained to detect ammonium nitrate (AN) when presented with structurally related materials including fertilizer-grade ammonium nitrate (FAN), sodium nitrate ($NaNO_3$), ammonium sulfate ($(NH_4)_2SO_4$), and ammonium nitrate with flaked aluminum (AN–Al). Detection rates are also shown for ammonium nitrate mixed with Iraqi soil (AN/soil)

Name	% Correct				
	FAN	$NaNO_3$	$(NH_4)_2SO_4$	AN–Al	AN/soil
Female	71.4 ± 5.3	55.5 ± 8.0	60.2 ± 5.4	71.4 ± 6.8	85.7 ± 3.5*
Male	62.5 ± 3.5	61.9 ± 5.1	55.4 ± 4.3	66.4 ± 4.7	62.5 ± 3.8
Black	70.5 ± 4.0	60.5 ± 3.1	62.8 ± 3.6*	70.6 ± 5.9	68.0 ± 4.2
Yellow	59.0 ± 4.0	55.7 ± 13.0	47.3 ± 4.1	66.6 ± 4.6	84.0 ± 6.6

* $P < 0.05$

probability of success equal to the mean performance. Although the variations around mean performance did not deviate significantly from a chance distribution (Kolmogorov–Smirnov tests, $P > 0.10$ except for the AN vs soil test where $P = 0.086$), they were large enough to have practical importance. We observed that only 8/15, 9/15, and 9/14 dogs performed above chance (≥70 %) when presented with fertilizer-grade AN, AN and Iraqi soil, or AN and flaked aluminum, respectively (Table 4). We found that only three dogs (Dakota, Honey, and Mercy) had individual performances above chance during all three of these transfer sessions. Likewise, two dogs (Reno and Rip) failed to generalize to any of these three materials. The remaining 10 dogs reliably signaled the presence of either one (4 dogs) or two (5 dogs) of the three AN scents of possible military importance.

Discussion

The present study used the TGTA system to assess olfactory discrimination learning and generalization in a cohort of dogs selected for explosive detection. The initial discrimination used vanillin, a chemical frequently used in studies of olfaction in humans, dogs, and other species, and thus represents a useful odorant for future comparisons across species or dog breeds. Studies in animals and people show that vanillin preferentially activates the olfactory cortex (Frasnelli et al. 2011; Savic 2002). In addition to vanillin's "pure" olfactory stimulus (Frasnelli et al. 2011), we also chose vanillin because we anticipated that it would represent a novel odor for the dogs used in our experiments. When comparing speed of acquisition on the vanillin olfactory discrimination task with results from previous tests of nonhuman animals trained on other 2-choice olfactory discrimination procedures, it appears that dogs in the present study required fewer trials to reach criterion than old- and new-world monkeys (Hubener and Laska 2001; Laska et al. 2003), fur seals (Laska et al. 2008), or minipigs (Søndergaard et al. 2010) but more trials than rodents (Bodyak and Slotnick 1999; Slotnick et al. 1991) elephants (Arvidsson et al. 2012), and dogs in a previous study (Lubow et al. 1973). However, differences in training methods, procedures, odorants, and criteria used complicate direct comparisons across species and experiments (Laska et al. 2003). Moreover, the dogs used in our study were previously trained on a visual discrimination task (Lazarowski et al. 2014), requiring the dogs to shift from a visual-based strategy to one that focused on the use of olfactory cues. It is unknown whether prior training on the visual discrimination task affected acquisition of the olfactory discrimination task.

We next evaluated the olfactory discrimination performance of dogs using an AN and "blank" odor pair. Overall group performance on the AN olfactory discrimination task was significantly improved (i.e., fewer trials to reach criterion and lower error rate) when compared with the results of the vanillin olfactory discrimination task. Overall performance on the two olfactory discrimination tests (vanillin/ethanol and AN/blank) did not correlate. Once dogs learned to discriminate the odor of AN from the blank netting material used to hold the solid AN, we then explored the ability of dogs to maintain the ability to respond to AN when the S− was replaced with Middle Eastern soil (collected at Camp Victory, Iraq) and another commonly used odorant, amyl acetate (banana oil). These phases served to establish the robustness in which dogs would continue to respond to the trained odorant (AN) when the odorants in the paradigm were changed prior to introducing transfer tests.

The physicochemical features of an odorant are a key determinant of processing scents (Joussain et al. 2011), and thus, we also aimed to explore the physicochemical basis for the detection of AN. Most target odors are composed of many different vapor compounds and dogs can detect many compounds in such mixtures (e.g., Johnston 1999; Williams and Johnston 2002). In its relatively pure chemical form, the AN molecule is extremely simple, being composed of equimolar quantities of the ammonium cation (NH_4^+) and nitrate anion (NO_3^-). One question that we sought to answer was whether detection of AN was based upon recognition of either the ammonium or nitrate chemical moiety. To this end, we evaluated whether dogs trained to AN would respond to different ammonium- or nitrate-based salts. In our studies, the ability of Labrador retrievers to detect these other chemical salts was not statistically greater than chance (i.e., >50 % correct response rate), suggesting that the odor signature for AN differs from that associated with other ammonium- or nitrate-containing salts (Table 4). Important future directions in the study of canine explosive detection should include an investigation of which elements of a trained target odor are important for their detection by dogs.

Another goal of our study was to evaluate generalization of AN detection using novel odorants that mimic material that a dog may encounter during combat situations. In these phases, dogs were presented with fertilizer-grade AN, AN mixed with soil from the Middle East, and an odor source comprised of AN and flaked aluminum (components found in some IEDs used in the Middle East and elsewhere). We found that after training to respond to pure AN, dogs responded to these novel odorants at a success rate greater than chance. Our results are consistent with previous findings that demonstrated weak generalization to similar odors from different manufacturers (Oxley and Waggoner 2009). The fact that dogs did not robustly generalize from pure AN to a sample containing pure AN with flaked aluminum are also consistent with our previous findings in which dogs failed to generalize from pure potassium chlorate to samples that combined potassium chlorate with accelerants and fuels (Lazarowski and Dorman 2014). We also found that the ability of dogs to generalize to these more operationally relevant samples varied among individual dogs. This difference in performance may be due to differences in functional olfactory capability, cognitive ability, motivation, distractibility, and other factors. Future studies should further investigate such factors that may lead to these differences, and ways to increase rates of generalization with AN-based explosives. This is especially important since detection standards for working dogs in operational combat settings should be considerably higher than chance, since a more stringent criterion would be more desirable to prevent combat casualties.

Our study has several important limitations. One such limitation relates to the odor pairs used. For example, we were unable to match odor intensity between the odor pairs. We also did not evaluate whether the order in which the initial olfactory discrimination tests could have altered our results. Thus, we are unable to determine whether the improvement in performance seen between the initial training with vanillin followed by AN was a result of prior learning with vanillin. Further, because all dogs began training with vanillin as the S+ and ethanol as the S−, it is unknown whether characteristics of these particular odorants related to speed of initial learning. Additionally, it is unclear whether training on a visual discrimination task prior to the present study may have affected acquisition of the olfactory discrimination task. Finally, the use of Iraqi soil as an S− in earlier training phases may have affected later performance in which AN was mixed with the soil.

However, performance on the AN/Iraqi soil phase was greater than other phases, suggesting that previous training to avoid the Iraqi soil stimulus did not greatly affect detection of AN in the soil. Further, this paradigm may serve as a surrogate in which explosive detection dogs must distinguish traces of explosive odors amongst common background odors that they have previously encountered. Despite these potential limitations, our study demonstrates that the ability of dogs to generalize from AN to more operationally relevant samples was limited, suggesting that additional investigation into training aid development and factors that hinder or facilitate generalization is needed.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research complies with the current laws of the USA and was reviewed and approved by the NCSU Institutional Animal Care and Use Committee (IACUC) and the DoD US Army Medical Research and Materiel Command (USAMRMC) Animal Care and Use Review Office (ACURO). NCSU research animal facilities are inspected semiannually by the NCSU IACUC, and the CVM is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC, International).

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