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ORIGINAL PAPER

# The use of an intermittent schedule of reinforcement to evaluate detection dogs' generalization from smokeless-powder

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**Abstract**

Odor generalization is essential for detection dogs. Regardless of its importance, limited research is available on detection dog odor generalization. The objectives of this study were (1) evaluate the use of an intermittent schedule of reinforcement to assess generalization in dogs and (2) evaluate olfactory generalization from a single exemplar of smokeless powder (SP). Dogs ($N = 5$) were trained to detect SP in an automated olfactometer under an intermittent schedule of reinforcement where only 60% of correct responses were reinforced. After training, eight non-reinforced probe trials were inserted within a session. A total of 15 testing odors were evaluated across 15 consecutive sessions (one odor/session). Six of the testing odors were control and the remaining testing odors were objects indirectly exposed to SP, objects that contained or were directly exposed to SP, single-base SP and diphenylamine (the main volatile present in the headspace of SP). Dogs' response rate to all testing odors except for the cotton gauzes and t-shirt cloths exposed to the headspace of SP, the simulated IED, and Getxent tubes exposed to direct contact with SP were statistically lower than their response rate of actual SP. The response rate to SP was not different across all 15 testing sessions suggesting that the intermittent schedule of reinforcement, maintained dog motivation and performance. Data show that the outlined method is a good approach to study generalization in detection dogs. These results also highlight dog generalization to SP varieties and associated odors.

**Keywords** Olfactory generalization · Detection dog · Reinforcement schedule · Explosive generalization

## Introduction

Dogs have a remarkable and highly developed olfactory system. Because of this, humans have used dogs as detectors of explosives (Aviles-Rosa et al. 2021b; Gazit et al. 2021; Harper et al. 2005; Lazarowski et al. 2015; Porritt et al. 2015) missing persons (Greatbatch et al. 2015), narcotics (Furton et al. 2002; Lorenzo et al. 2003), diseases (Cornu et al. 2011; Jendrny et al. 2020), and endangered species (Cristescu et al. 2015). To perform any detection task, dogs must discriminate the target odor from other environmental odors or distractors and respond to variations of the target that are operationally relevant (e.g. concentration differences, differences in production methods, or the presence of contaminants). A common practice in detection dog training is to train the dogs to detect and alert to specific targets with the assumption that dogs will also alert (generalize) to many additional variations or mixtures containing the target. Thus, it is essential to evaluate and confirm to what degree dogs generalize to odor similarities between the trained

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target odor and variations of the target odor (e.g., different manufacturer or different form) or mixtures containing the trained target odor.

Olfactory generalization is the psychological phenomenon where an animal responds to perceptually similar, but not identical odor stimuli (Moser et al. 2019). An example of olfactory generalization in detection dogs is when a dog alerts to a different source or to a mixture containing ammonium nitrate (AN) after being trained exclusively to the pure compound AN (Lazarowski et al. 2015).

The ability of a working dog to generalize from a trained substance to a novel configuration is one of upmost importance but, existing research shows that dogs tend to show limited odor generalization (Aviles-Rosa et al. 2021a; DeChant et al. 2021; Dorman et al. 2021; Lazarowski et al. 2015; Lazarowski and Dorman 2014). For instance, Lazarowski et al. (2014) found that 87% of the dogs tested failed to alert to an explosive mixture containing potassium chlorate (PC) after being trained exclusively with PC. DeGreeff et al. (2020) found greater generalization to odorants with similar carbon chain length independent of the functional group, and Dechant et al. (2021) found that dogs were not able to spontaneously generalize to isoamyl acetate when presented at a different concentration (at least a tenfold difference). Similarly, Aviles-Rosa et al. (2021b) found that dogs were not able to generalize to a 13 kg ammonium nitrate fuel oil explosive after training with a 30 g subsample of the material.

Even though smokeless powder (SP) is a commonly used target odorant in routine canine explosive detection training and certification procedures, little is known about dogs' olfactory generalization to different types of SP or to objects containing or exposed to SP. Furthermore, the available literature suggests that dogs' generalization to SP varieties could be poor. For instance, one of the few studies that evaluated this topic found that 52% of the dogs trained exclusively with one commercial brand of SP did not generalize to a different brand (Oxley and Waggoner 2009). This most likely was due to differences in the odor profile between different brands, as they might use different additives in their manufacturing process (Harper et al. 2005). Importantly, however, training to multiple exemplars of SP varieties did lead to improved generalization (Oxley and Waggoner 2009).

Research in olfactory generalization is essential to better understand how dogs perceive odors and to improve detection dog performance. However, evaluating detection dog generalization is a complex procedure because different factors can affect test results. Unintentional training to the testing odor or unintentional extinction of responses to the testing odor are the two main things that could affect or bias the results of a generalization test. The standard procedure to test detection dog generalization is to give dogs "probe trials" where the testing odor is presented within a session. Commonly, responses to the testing odor during a probe trial are not reinforced to prevent the dog from quickly learning the new odor (Lazarowski et al. 2015). However, this method limits the number of generalizations probes a dog can receive because after multiple probe trials the dog could learn to not alert to the testing odor, because alerts have not been reinforced (extinction of the response) or the lack of reinforcement could reduce dog motivation to search in subsequent trials. One alternative method to prevent extinction of responses is to reinforce all correct and incorrect responses non-differentially (e.g., responses to the testing odor or false responses will be reinforced on probe trials) (e.g. Hall and Wynne 2018). This method can be used to maintain motivation if many probe trials are to be inserted but would explicitly reinforce responses to the probe odor and false alerts to non-targets equally. An alternative method is to reinforce only responses to the generalization target odor in probe trials to prevent extinction of response or motivation decrement (Lazarowski et al. 2021). However, this method might not be suitable to study spontaneous generalization across many repeated trials because it may produce within-session learning. By reinforcing responses to the probe, the experimenter is indirectly training the dog to respond to the new odor. This unintended training could bias the results of subsequent probe trials because after reinforcement, the testing odor becomes a discriminative stimulus rather than a novel odor. Thus, if multiple reinforced trials of the testing odor are given to a dog, the only one that is measuring spontaneous generalization will be the first one. Because of the underlying limitations of these traditional methods, only one or two probe trials are given to a dog during a testing session. Thus, the amount of data that can be collected from each dog using this traditional approach is limited. This is an important limitation because it may prevent the study of individual factors associated with generalization, such as potential breed, motivation, and past training and experience variables that would be difficult to assess if generalization propensity can only be scored in a handful of trials.

Training dogs under an intermittent schedule of reinforcement could be a solution to prevent within-session learning or extinction. This procedure is commonly used in laboratory animals to assess stimulus generalization (e.g., Guttman and Kalish 1956; Kalish and Guttman 1959). Intermittent schedules of reinforcement are also used to train dogs under laboratory conditions (Sargisson and McLean 2010). As canine training and assessment techniques are becoming more automated and amenable to laboratory conditions (Aviles-Rosa et al. 2021a), the use of intermittent schedules of reinforcement could be ideal to conduct generalization procedures that otherwise would be inefficient using traditional methods. Under an intermittent schedule of reinforcement, dogs are trained such that not all correct responses are reinforced. This schedule of reinforcement could make dogs more resistant to extinction when responses to generalization probes are not reinforced (Hall 2017; Nevin 2012) while simultaneously preventing unintentional training to the test odor. This schedule of reinforcement could maintain dogs' motivation and responsiveness during testing, allowing researchers to increase the number of probe trials per dog. An intermittent schedule of reinforcement was successfully used by DeChant et al. (2021) to evaluate dogs' generalization to different concentrations of the same odorant and by Kranz et al. (2014), to evaluate generalization to different explosives pseudo-training aids. However, methods to assess generalization to different odors over multiple sessions still needs to be evaluated.

The aims of this research were (1) evaluate the use of an intermittent schedule of reinforcement to assess generalization in dogs and (2) evaluate the ability of dogs trained to detect a single source of double-base SP (Hodgdon H335®) to generalize to a different type of SP (single-base), different objects exposed or containing the same SP, and to the main volatile present in the headspace of SP using an automated olfactometer. Our purpose was to further evaluate the use of an intermittent schedule of reinforcement as a method to test dogs' spontaneous generalization and to have a better understanding of dogs' generalization to SP. Our goal was to evaluate a method to test generalization in detection dogs that could overcome some of the limitations of other methods and to advance the knowledge of detection dog generalization to SP.

## Materials and methods

### General

The study was conducted at the Texas Tech University Canine Olfaction Research and Education Lab (CORE). All procedures used were approved by the TTU Institutional Animal Care and Use committee (protocol # 19,093-10). The participants of this study were five mixed breed neutered male dogs (1–4 years). These dogs were the participants of a previous study where they were trained to detect double- base smokeless powder (Hodgdon H335®) in an automated olfactometer. Initially, dogs were selected from local shelters as part of our train to adopt program. Canines were housed in a climate-controlled room in indoor kennels (2.43 × 1.22 m) with free access to outdoor kennels (2.43 × 1.22 m). We used food as the primary reinforcer during training. Thus, to maintain food motivation, only 25% of the daily food ration was given in the morning (0800) and the remaining 75% in the afternoon once training was completed for the day (1600). Dogs had free access to water in their kennels and during training sessions.

## Apparatus

Dogs were tested using an automated 3-alternative line-up olfactometer. Detailed description of the apparatus and its validation as an automated odor delivery system can be found elsewhere (Aviles-Rosa et al. 2021a). The apparatus was made of 3 independent olfactometers. Each olfactometer was equipped with six valves that allowed the presentation of six different odorants (e.g., one target odorant and five distractors). After the activation of a solenoid valve, filtered air carried the odorant dilution (33% air dilution v/v) to the ports for the dogs to sample. The apparatus was equipped with infrared beam sensors that measured the amount of time a dog sniffed a port, the number of times a dog sampled a port, and dogs' response during a trial. Dogs' alert response during a trial consisted of a 4 s nose in one of the ports. An "all clear" response consisted of sampling all three ports and not alerting to any of them The apparatus recorded all this information for each trial and saved it in a csv. file at the end of the session.

### Training

Prior to the study, dogs were trained to detect and alert to the odor of 10 g of double- base smokeless powder (H335 rifle powder Hodgdon®). As part of the previous study, dogs received two 40-trials sessions daily for 4 months. Within a session, ninety percent of the trials contained a target odor, and the remaining ten percent of the trials (e.g., 4 of the 40 trials) were blank trials where the dogs had to search all three ports and not alert to any of the distractors (all clear). Cotton gauze (EquateTM), nitril gloves (Med Pride, MPR-50504), food grade mineral oil (Bluewater Chemgroup), limonene ($10^{-3}$ v/v dilution in mineral oil; CAS # 5989-54-8), and clean air were used as distractors because they are common laboratory components. All correct responses to the target odor were reinforced (e.g., with a treat), but correct "all clears", false alerts, misses, or "timeouts" were not reinforced. A correct all clear consisted of a dog sampling all the ports and not alerting to any of them when the target odor was not present (e.g., during a blank trial). A false alert consisted of the dog alerting to the wrong port during a blank or odor trial, and a miss consisted of a dog sampling all three ports and not alerting to the one containing the target odor during an odor trial (an all-clear response when odor was present). After the initiation of a trial the dog was allowed to search for 45 s. If the dog did not search all three ports within 45 s after the initiation of the trial, the trial was scored as a timed out and terminated. At the beginning of the current study all dogs had an accuracy > 85% correct responses when trained under these conditions.

For the first training phase of the study, we trained the dogs with only 100 mg of SP to train them to detect a lower quantity of the target odor. By not saturating the surface area of the vial we intended to reduce the odor availability and train dogs to alert to potential lower headspace concentrations of SP. This was to minimize the possibility that a potential lack of generalization was not due to changes in headspace concentration but rather to perceptual differences between SP and the testing odors (Aviles-Rosa et al. 2021b). After dogs' performance with 100 mg of SP was > 85% correct responses, we gradually trained them to an intermittent schedule of reinforcement where the probability of reinforcement for a correct response to SP was 60%. For initial training, dogs received two 40-trial sessions in a day where the odor prevalence and the reinforcement rate were set at 80%. For instance, 8 of the 40 trials were blank trials and only 26 of the 32 possible correct responses were preprogramed to be reinforced. Trials intended to be reinforced were completely randomized by the computer program at the beginning of the session. A correct response during a non-reinforced odor trial resulted in the termination of the trial without a reward. Correct all clears and incorrect responses (false alert, misses, timeouts) were also not reinforced during training or testing and just resulted in the termination of the trial. Dogs continued training under these parameters until their performance was > 80% correct responses in two consecutive training session. Once they reached training criterion, dogs moved to the next training phase where the reinforcement rate of correct responses to SP was reduced to 60% (e.g., Only 19 of the 32 possible correct responses were preprogramed to be reinforced). This training phase continued until dogs scored > 80% correct responses in two consecutive sessions. Testing did not start until dogs reached training criterion.

## Testing

A testing session was similar to the last training phase with the exception that the computer program was modified to introduce 8 non-reinforced probe trials randomly within the 40-trial session. During a probe trial, the testing odor was presented in one of the three ports while the other two ports contained a distractor odor. The same distractor odors used during training were used for testing. The computer program was modified to first randomize the trials to be reinforced (e.g., to select the 19 out of the 32 trials containing the target odor that were going to be reinforced if the dog alerted correctly). Probe trial selection was then randomized on top of this programmed rate. Given that probe trials were not reinforced, the actual reinforcement rate of correct responses to SP varied from 30 to 60% during a testing session depending on the randomization process. In addition to the probe trials, some correct responses to SP were also not reinforced during a testing session. This was to ensure that dogs had experience of non-reinforcement for correct responses to the SP target. Thus, non-reinforcement of an alert to a perceived target odor (such as on a probe) was not readily predictive of whether the odor was the actual target or a non-target (extinction stimulus). A testing session also contained 20% blank trials in which no port contained the target or testing odor (e.g., 8 out of the 40 trials). Blank trials were randomly distributed within blocks of 10 trials (e.g., on average there were 2 blank trials every 10 trials). If the dog alerted to a non-target odor, timed out, or missed the target odor during a trial intended to be reinforced, the trial was scored as incorrect and terminated without reward. Dogs received up to three testing sessions per day.

## Testing odorants

During each testing session we assessed dogs' response to one testing odor. As mentioned before, within a session a dog received eight 8 non-reinforced probe trials where the testing odor was presented. Overall, we tested dogs' spontaneous generalization to 15 different testing odors (15 testing sessions). Six of these testing odors were control odors with no previous exposure or direct relation to SP. These testing odors served as a negative control to ensure dogs were not alerting to random novel odors but rather to odor similarities between SP and the testing odor. The remaining nine testing odors were variations of SP (e.g., Single base), objects containing SP (bullets, simulated Improvise Explosive Device) or potentially contaminated with SP (fired gun), objects exposed to SP (cotton gauze, t-shirt), a commercially available training aid (Getxent tubes), or the most common volatile present in the headspace of double-base SP (diphenylamine). To facilitate the use of the olfactometer and to reduce the chance of odor contamination by continuously changing odor vials from the olfactometer, odors were tested in a particular order. This order was selected randomly but the same order was used for all dogs. Table 1 shows the testing order and a description of the testing odors.

The simulated Improvise Explosive device (simulated IED) was built by placing 10 g of SP (the same used during training) in a 1-inch diameter PVC pipe (9 cm length) and sealed with PVC caps. The simulated IED was placed in an odor vial (250 mL) with electronic cables, and solder wire and used for testing (Fig. 1A) by piercing Teflon tubing through the cap of the tube and connecting it to the olfactometer. The control simulated IED was identical but contained no SP.

An unloaded firearm (Smith & Wesson 9 mm M&P Shield) was cleaned with gun bore cleaner oil (Hoppe's No.

**Table 1** Testing odors description

| Testing session | Testing odor | Odor description | Testing purpose |
|---|---|---|---|
| 1 | Non-contact Gauze | Cotton Gauze (5 × 5 cm) exposed to the headspace of 10 g of SP for 48 h at 40 C | Evaluate if gauze can be impregnated with SP odor to be used as training aids |
| 2 | Pentanol | Pentanol ($10^{-4}$ v/v)* | Control odor |
| 3 | Single Based smokeless powder (SP[a]) | 10 g of Single-base Smokeless Powder | Evaluate generalization to different type of Smokeless Powder |
| 4 | Blank Gauze | Clean Gauze (5 × 5 cm) | Control test for session 1 |
| 5 | Diphenylamine (DPA) | 100 ppm solution[*] | Evaluate generalization to the most common volatile in SP headspace |
| 6 | Bullets[b] | 5 bullets were placed in an odor jar | Evaluate generalization to ammunitions |
| 7 | Blank Shirt | 100% cotton t-shirt cloth (5 × 5 cm) | Control test for session 8 |
| 8 | Non-contact t-shirt | 100% cotton t-shirt cloth (5 × 5 cm) exposed to the headspace of 10 g of SP for 48 h at 40 C | Evaluate if t-shirt cloth can be impregnated with SP odor and be used as a training aid |
| 9 | Benzaldehyde dimethyl | Benzaldehyde dimethyl ($10^{-4}$ v/v)[*] | Control odor |
| 10 | Control simulated improvise explosive device (IED) | A 9 cm PVC pipe together with electric cables | Control test for session 11 |
| 11 | Simulated improvise explosive device (IED) | 10 g of SP sealed in a 9 cm PVC pipe together with electric cables | Evaluate if dogs can detect a simulated IED after been trained with SP |
| 12 | Direct-contact Getxent tube[c] | Getxent tube exposed to direct contact with 10 g of SP for 48 h at 40 C | Evaluate generalization to a commercial training aid |
| 13 | Clean unloaded Gun[d] | The slide and chamber of the clean gun were wiped with cotton gauzes and the gauzes used for testing | Evaluate generalization to firearms and control test for session 14 |
| 14 | Fired Gun | The slide and chamber of the fired gun were wiped with cotton gauzes and the gauzes used for testing | Evaluate generalization to fired firearms |
| 15 | Non-contact Getxent tube[c] | Getxent tube exposed to the headspace of 10 g of SP for 48 h at 40 C | Evaluate generalization to a commercial training aid |

[a] Hodgdon H322®

[b] Blazer Brass ammunition, 115 mg

[c] Rue Georges-Auguste-Matile, 71 2000 Neuchâtel, Switzerland

[d] Gun was cleaned with Hoppe's No. 9 gun bore cleaner

[*] Odorants were diluted in Mineral oil

9) before testing. The slide and chamber of the cleaned gun were then wiped with three sterilized and previously pretreated cotton gauze (5 × 5 cm; Dukal Corporation). Prior work by authors has suggested that biologically sterile materials do not equate to analytically clean (Prada et al. 2011), hence all cotton gauzes were pretreated prior to test use. This laboratory pretreatment consisted of a direct spike with methanol solvent, followed by oven heating at 105 C° for 2 h to eliminate possible volatile odor contamination. After sampling, each gauze was placed in a 40 mL odor jar for testing (one gauze per olfactometer). Subsequently, the same gun was fired using 9-mm bullets (Blazer Brass ammunition, 115 mg; 75–100 fired shots) in an indoor shooting range. The fired gun was then wiped around the chamber and the slide area with 3 gauzes which were then utilized in the testing sessions.

Pretreated cotton gauzes (5 × 5 cm), pre-treated t-shirt cloths (5 × 5 cm 100% cotton shirt; Gildan Brands), and Getxent tubes were exposed to the headspace of 10 g of SP for 48 h at 40 C (Non-contact exposure). For odor impregnation 10 g of SP were placed in a 250 mL mason jar (Ball®). A perforated stainless-steel cap (6.35 cm) was placed on the top of the SP, and the gauze, the t-shirt, or the Getxent tube were placed on the top of the cap to prevent direct contact with the SP (Fig. 1B). A single Getxent tube was placed in the mason jar for odor impregnation. Five cotton gauzes or t-shirt cloths were placed together for odor impregnation in a mason jar (e.g., 3 jars with 5 cotton gauze or t-shirt cloths). Three gauzes and t-shirt cloths were removed from the impregnation jar and placed in a 40 ml odor jar for testing. Three fresh gauzes and t-shirt were used for each dog (one for each olfactometer per dog). Only three Getxent tubes were used to test all five dogs (one for each

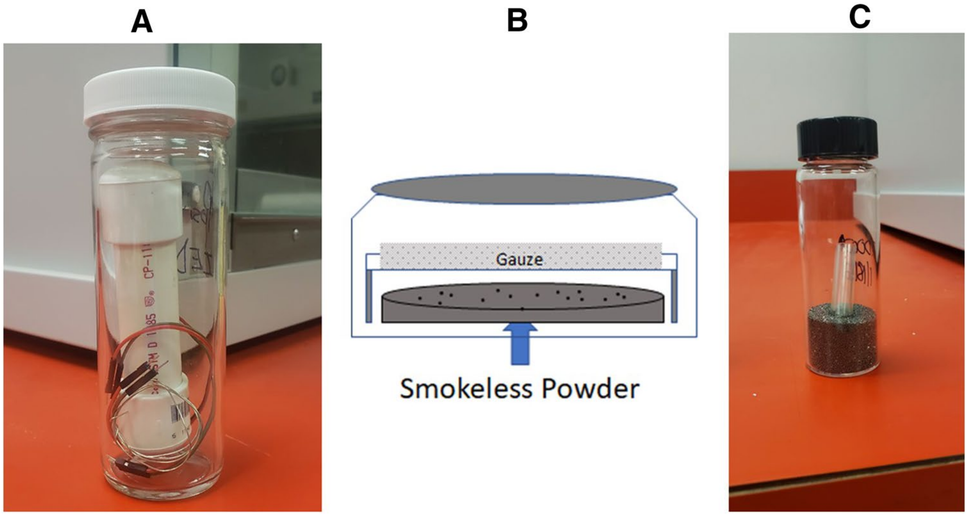

**Fig. 1** An illustration of some of the testing odors. **A** Picture of the simulated IED. Ten grams of SP were placed in a 9 cm PVC pipe and caped. The IED was placed together with electric cables and soldering wire in a 250 ml vial for testing. **B** An illustration of the method used for odor impregnation. Ten grams of SP were placed in a 250 ml mason jar. A perforated stainless-steel cap (6.35 cm) was placed on the top of the SP, and the gauze, the t-shirt, or the Getxent tube were placed on the top of the cap to prevent contact with the SP. **C** A picture of the direct exposure of a Getxent tube to SP. Ten grams of SP were placed in a 40 ml vial and the tubes was placed on the top

olfactometer) as the manufacturer indicates the tubes hold odor for up to 6 months. Control gauzes and t-shirt were also pre-treated and incubated in the oven for 48 h at 40 C° in a jar without SP. Additionally, different Getxent tubes were exposed to direct contact with 10 g of SP in a 40 mL vial and incubated in the oven for 48 h at 40 C° (Fig. 1C). This was to evaluate the best way to impregnate the odor in the tubes.

Diphenylamine (DPA) was found to be the most common additive in different commercially available SP samples (Joshi et al. 2009). Thus a 100-ppm solution of diphenylamine diluted in mineral oil was prepared to evaluate if dogs were able to generalize to a solution containing only this molecule. Ten milliliters of the 100-ppm solution were placed in a 40-ml jar for testing.

## Statistical analysis

For each dog we calculated the proportion of correct responses to SP (# of correct alerts / # of trials where SP was presented) and the proportion of responses to the testing odors (# of alerts to the testing odor / # trials where the testing odor was presented) within each session. Dogs' responses during blank trials were not included in the statistical analysis because we were exclusively interested in dogs' response to SP and the testing odors. In addition, we calculated the average amount of time a dog spent sniffing the port containing SP and the testing odors within a session. This was measured automatically by the IR sensors of the olfactometer. Residual plots from each model were used to evaluate normality and homoscedasticity. Parametric analyses were conducted if the residual plots did not show a significant violation of these assumptions and non-parametric analysis were conducted when these assumptions were violated.

A linear mix model was used to evaluate differences in the proportion of correct responses and sniff time between SP and the testing odors. The model included the fixed effect of odor and the random effect of dog. The Dunnett's multiple comparison test was used to evaluate differences in the sniff time and the proportion of responses between SP and the different testing odors.

When a linear mix model was used to evaluate the effect of session on dogs' response rate to SP, the residual plots showed significant heteroscedasticity. Thus, the non-parametric Friedman test was used to evaluate the effect of testing session on the proportion of correct responses to SP. This was to evaluate if dogs' motivation and performance remained stable after multiple testing sessions at a low reinforcement rate or if after multiple sessions dogs' performance declined. This model included the effect of dogs as block and the fixed effect of session (1 -15).

A binomial test was used to determine if the proportion of correct responses to the testing odors was above chance (Three alternative choice test: $H_o\ p = 0.33$). The Cochran test was used to evaluate the effect of probe trial (1 to 8) on the probability of response to a testing odor (Conover 1999). This model included dog as a block effect and a fixed effect of trial. This analysis was conducted by each testing odor independently. The aim of this statistical analysis was to evaluate if dogs' response rate to the testing odor changed after multiple unreinforced presentation of the testing odor. A significant effect of trial would suggest that either extinction of responses or within-session learning occurred during testing. A statistical significance was declared if $p<0.05$. All statistical analysis were conducted in SAS 9.4. (SAS version 9.4; SAS Inst., Inc., Cary, NC, USA). and data visualization in R and Rstudio (R Core Team; R Foundation for Statistical Computing: Vienna, Austria, 2017).

## Results

All five dogs met training criterion. After training with 10 g of SP all dogs were able to spontaneously generalize to 100 mg of SP. Thus, after two training sessions where the target odor was 100 mg of SP, we started training to reduce the reinforcement rate. None of the dogs showed a performance or motivation decrement as a result of the reduction in reinforcement rate. We were able to maintain performance and reduce the reinforcement rate from 100 to 60% in four training sessions. By the end of training at a 60% reinforcement rate, the proportion of correct responses for all dogs was > 0.95. All dogs had 100% correct responses except for Wishbone who had two timeouts during the session. None of the dogs showed any false alerts during the last training session and the false alert rate remained low during testing (see Figures in the supplementary materials).

The average reinforcement rate during testing was 44.67 ± 0.49 (SEM) % and it ranged from 34.37% to 53.00%. Even when the reinforcement rate was lower than 60% during testing, dogs' proportion of correct response to SP was ≥ 0.95 in 13 of the 15 testing sessions (Fig. 2). A slight performance decrement was observed during sessions 6 and 8 where the proportion of correct responses to SP decreased to 0.83 and 0.82, respectively. However, this difference did not reach statistical significance ($p=0.07$). The average proportion of correct responses to SP across all 15-testing session was 0. 95 ± 0.09 (SEM) (e.g., only 82 incorrect responses to SP in 1,800 trials where SP was presented).

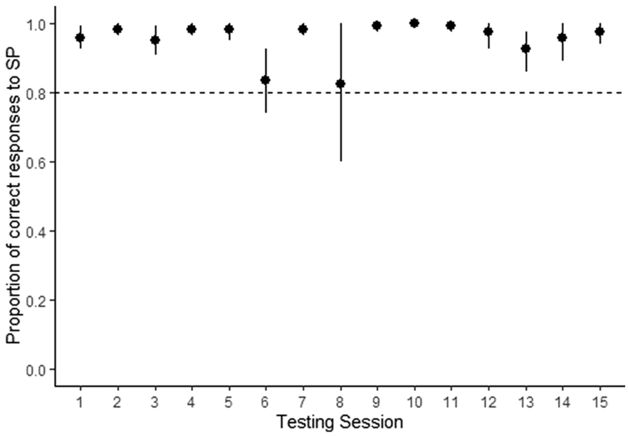

**Fig. 2** Dogs ($N=5$) mean ± 95% confidence interval of the proportion of correct responses to SP during each testing session. Dashed line indicates 0.80 proportion of correct responses, our training criterion. The effect of session on dogs' proportion of correct responses was not significant ($p=0.07$). The average detection performance of all dogs was > 0.90 for most of the sessions. During sessions 6 and 8 a slight reduction was observed, but performance was still above 0.80, which was our training criterion

Dogs responded only to the gauze and t-shirt exposed to the headspace of SP, Getxent tubes (direct and non-contact exposure), the simulated IED, and Single-base SP at a rate greater than chance (Binomial test $p<0.05$). The response rate to all other testing odors was significantly lower than expected by chance (Binomial test $p<0.05$; Fig. 3). The effect of testing odor on the proportion of responses was statistically significant ($p<0.0001$). Dogs alerted to all probe trials of the t-shirt and gauze exposed to the headspace of SP. Dogs' response rate to the Getxent tubes exposed to SP by direct (0.87 ± 0.05 SEM) and indirect contact (0.62 ± 0.20 SEM), and to the simulated IED (0.60 ± 0.18 SEM) were 8%, 33%, and 35% lower than their average response to SP, respectively. However, these differences did not reach statistical significance based on the Dunnett's test. The response rate to single-base SP, bullets, clean (unloaded) and fired gun, control gauze, control t-shirt, control simulated IED, pentanol, DPA, and benzaldehyde were all statistically different than the proportion of correct responses to SP (Fig. 3).

The effect of testing odor on the sniff time was also statistically significant ($p<0.001$). The evaluation of the difference in sniff time between testing odors showed similar results as the proportion of responses. The amount of time dogs spent sniffing bullets, DPA, clean and fired gun, control gauze, control t-shirt, control simulated IED, pentanol,

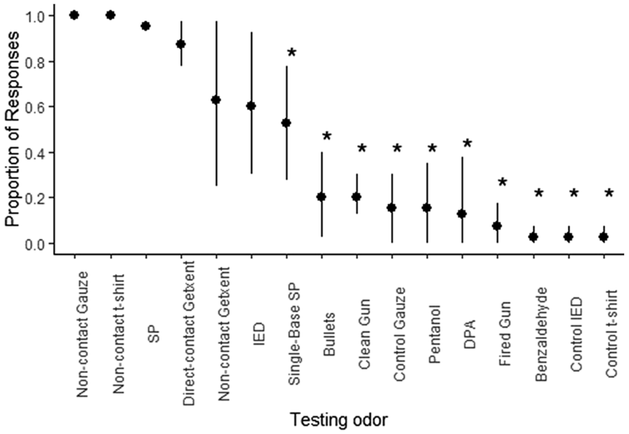

**Fig. 3** Dogs ($N=5$) mean ± 95% confidence interval of the proportion of responses to the testing odors. Values with * are statistically different from SP based on the Dunnett test. The proportion of correct responses to the non-contact gauze and t-shirt, Getxent tubes, and IED were not statistically different from SP

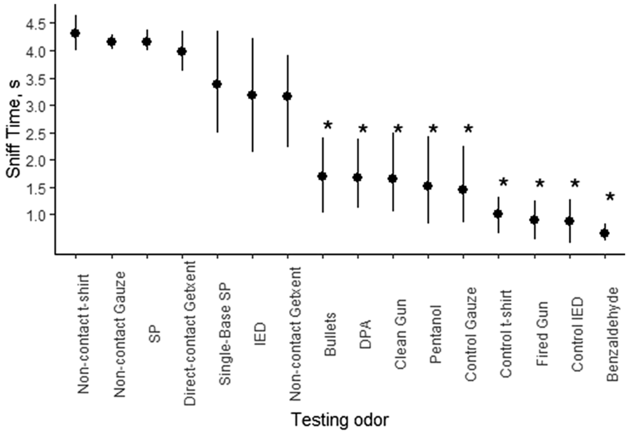

**Fig. 4** Mean ± 95% confidence interval of the amount of time (s) dogs ($N = 5$) sniffed the port containing the testing odor. The sniff time of odors with * are statistically different from the sniff time of SP. The sniff time for the non-contact gauze and t-shirt, Getxent tubes, single-based SP, and IED were not statistically different from SP. This suggest that these testing odors are more similar to SP that the others and induced more odor exploration (sniff)

and benzaldehyde was significantly lower than the time they spent sniffing SP (Fig. 4). No statistical differences were found on the time dogs spent sniffing the gauze and t-shirt exposed to SP, Getxent tubes (direct and non-contact exposure), simulated IED, or single-base SP relative to SP (Fig. 4). The effect of probe trial on the response rate to the testing odor was not statistically significant for any of the testing odors (Fig. 5). The lack of statistical significance indicates repetitive presentation of the testing odor within a session had no effect on dogs' response and further suggests that the method used prevented extinction of responses or within-session learning.

## Discussion

We evaluated the use of an intermittent schedule of reinforcement to assess generalization in detection dogs. The outlined method allowed us to test generalization to 15 different odorants without observing any performance or motivation decrement. Except for sessions 6 and 8, the proportion of correct responses to SP was consistently > 0.95 during testing. This indicates that the intermittent schedule of reinforcement did not affect dogs' performance across all 15 sessions and that dogs performed at their best despite the low reinforcement rate during testing. Eight probe trials were inserted in each of the 40-trials sessions. Dogs' responses to the different testing odors were not affected by trial number, indicating that the method used prevented extinction of responses or unintentional training to the probe trials, which are the two main limitations of traditional methods. Altogether, the data suggest that training dogs under an intermittent schedule of reinforcement seems to allow a repeatable test of spontaneous olfactory generalization in probe trials for detection dogs.

Five of the 15 odors tested (DPA, Getxent tubes direct and non-contact exposure, and cotton gauze and t-shirt exposed to the headspace) can be considered training aids (Simon et al., 2020). The goal of an explosive training aid is to safely provide a highly similar representation of the odor of the true material. Accordingly, a partial or complete generalization failure to a training aid would suggest perceptual differences between the training aid and the true material. For instance, perceptual differences could occur if the substrate of the training aid itself has a more salient odor than the target odor adsorbed onto it. Substrates or diluents with strong odors are not good training aids because the dog could learn to find the substrate and not the target odor itself. Based on Simon et al. (2020) training aid classification, t-shirt and gauze with non-contact exposure to SP and Getxent tubes exposed to direct or indirect contact to SP can be consider non-pseudo training aids. Dogs alerted to all the trials containing the gauze and t-shirt exposed to SP. This is in agreement with previous studies that have found that cotton gauzes absorbed SP volatiles after non-contact exposure (Sanchez et al., 2016). This suggests that the cotton gauze and t-shirt exposed to the headspace of SP were perceptually similar to SP and thus can represent a potential approach for SP training aid development. However, future studies are needed to confirm that dogs trained using cotton materials impregnated with SP will generalize to the actual explosive. Because all the participant dogs were previously trained to detect SP, this was not evaluated in this study.

All dogs alerted to the Getxent tube exposed to direct contact with SP at a rate greater than expected by chance, but even when the response rate was above chance, it was lower than the observed response to the gauzes and t-shirt exposed to the headspace of SP. Surprisingly, dogs' response rate to the Getxent tubes exposed only to the headspace of SP was 33% lower than their response to the true material. This difference in response rate between the Getxent tubes exposed to direct or indirect contact with SP could be because tubes exposed to direct contact with SP had some particle of SP incidentally attached. An alternative explanation can be that the tubes exposed only to the headspace of SP might not have captured all the relevant odor volatiles of SP or did not replicate the concentration of SP volatiles used during training. Getxent tubes are currently used as training aids for the detection of COVID-19 in different countries (Grandjean et al., 2021) however the data suggest that their use as explosive training aid must be further evaluated.

Diphenylamine (DPA) is a common additive used in different types and brands of smokeless powder (Harper et al. 2005; Joshi et al. 2009; Kranz et al. 2014). Because its

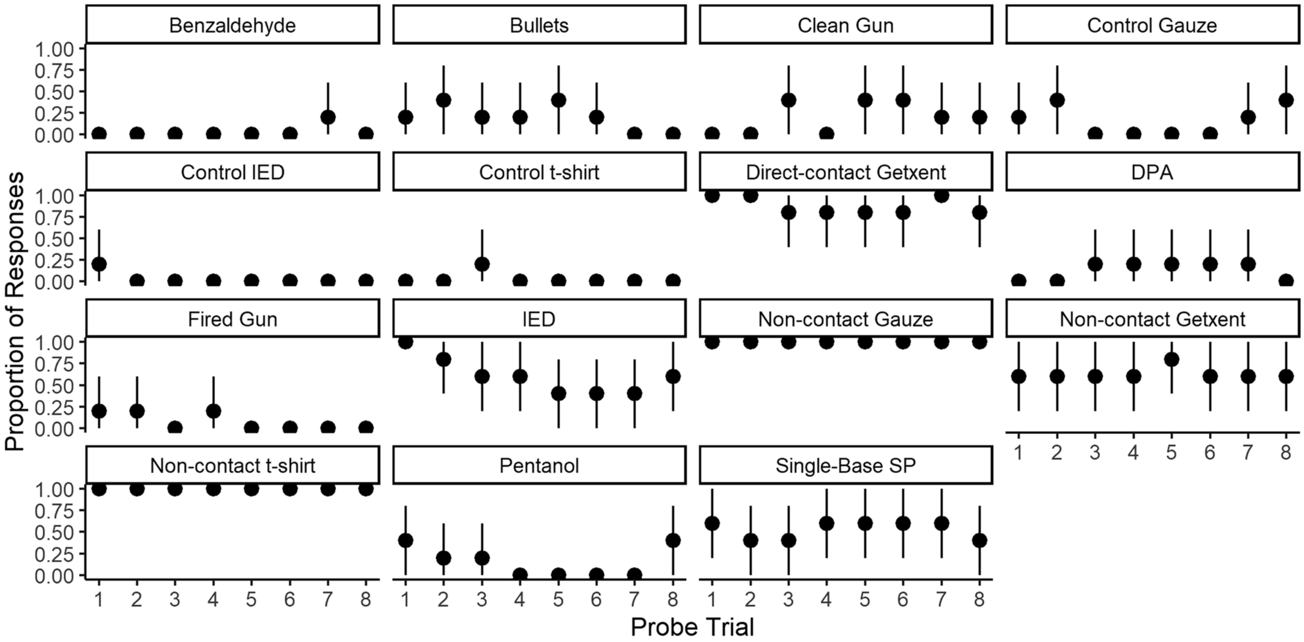

**Fig. 5** Mean ± 95% confidence interval of dogs' (*N* = 5) response to the testing odors during each probe trial. IED = simulated improvised explosive device. DPA = diphenylamine. Direct-contact Getxent = Getxent tube exposed to direct contact with 10 g of double-base smokeless powder (SP). Non-contact gauze, t-shirt, and Getxent tube were exposed to the headspace of 10 g of SP. The effect of trial was not statistically significant for any of the testing odorants. Suggesting that extinction of the response or within-session learning were prevented by the method

presence in the headspace of different types and brands of SP has been previously reported, in this study we evaluated if dogs were able to spontaneously generalize to a solution of DPA. Spontaneous generalization to DPA could suggest that DPA is the main volatile dogs utilized to detect SP and that a DPA solution could be used as an effective pseudo training aid (Simon et al. 2020). Dogs' response to DPA was lower than expected by chance and statistically lower than their response to SP. Furthermore, four of the five dogs tested did not alert to any of the eight probes. This agrees with previous studies where they found that none of the individual components of the headspace of SP elicited a response in dogs when tested individually and that dogs did not alert to pseudo-training aids containing DPA after training with the actual explosive (Harper et al. 2005; Kranz et al. 2014). Additionally, in this study we trained dogs to not alert to mineral oil (MO) as it was used as a distractor odor. Thus, MO could have been a discriminative stimulus for not responding due to the low set of distractors used in the study. By diluting DPA in MO we could have changed dogs' perception to DPA by mixing it with a distractor odor, or alternatively, the concentration used in the solution did not match the concertation found in the headspace of SP. Altogether, this and previous results suggest that, although DPA is one of the main volatiles present in the headspace of SP, dogs could be responding to the mixture of volatiles present in the headspace of SP and not to a single unique volatile. This highlights the importance of validating analytical findings with dog behavior, as both do not always correlate.

Smokeless powders (SP) are classified based on the number of energetic materials used in their manufacturing process (Joshi et al. 2009). Single-base SP contains only nitrocellulose, whereas double-base and triple-base contain nitroglycerine, and nitroguanidine in addition to nitrocellulose, as their energetic materials, respectively (Harper et al. 2005; Joshi et al. 2009). In addition, different manufactures add different additives and stabilizers to improve burning properties, shelf life, and performance (Harper et al. 2005). These differences in the number of energetic materials and manufacturing processes can lead to significant differences in the volatiles present in the headspace of different SP, creating perceptual differences between different types of SP and/or different brands or manufacturers of the same type (Harper et al., 2005). In this study we found that after exclusive training with double-base SP, dogs' overall response rate to single-base SP was statistically lower than their response rate to double-base SP. Only two of the five dogs tested alerted to the single-base SP at a rate greater than chance (See supplementary materials for individual dog response). Interestingly, however, we did not find a statistically significant difference in the amount of time dogs sniffed the double or single-base SP. The average sniff time for single-base SP was 3.36 s whereas for control odors and distractors it was less than 1 s. This suggest that dogs showed some odor recognition or more exploration to single-base SP than to the controls, but that the single-base SP was perceptually different enough for them to not show a full alert (4 s nose hold). Previous studies have also found that most dogs were not able to spontaneously generalize to different brands of SP (Oxley and Waggoner 2009) or to different sources of Ammonium nitrate (AN) after initial training with laboratory grade AN (DeGreeff and Peranich 2021). However, a recent study found that after training with laboratory-grade triacetone-triperoxide (TATP) dogs generalized to different clandestine TATP formulations (Lazarowski et al. 2021). This indicates that generalization failures after training with a single source of the target odor could be odor specific and that each target odor should be evaluated independently. Studies have found that generalization can be improved by training dogs with different sources/exemplars of the target odor or to different target odors (Oxley and Waggoner 2009; Waggoner et al. 2022). For instance, Oxley and Waggoner (2009) found that generalization to SP improved after training with 3 different types of SP, suggesting that one exemplar of SP may not lead to adequate generalization to SP varieties, but dogs will rapidly generalize after a few additional exemplars. In the context of the present study, this suggest that the decreased response to single based SP may be alleviated if dogs had prior training to additional SP varieties.

Improvised explosive devices (IEDs) are a significant threat to national security and were responsible for over 50,000 death or injuries of troops in Iraq and Afghanistan (Kang et al. 2012). IEDs can be made with inexpensive and commercially available materials and explosives (Kang et al. 2012; Östmark et al. 2012). Even when most of the IEDs are manufactured using ANFO, smokeless powders can represent a risk in IED manufacture because they are extremely dangerous when confined in a small container and smokeless powders with high nitroglycerin concentration can be induced to detonate. The capability of detection dogs to find IEDs relies on their ability to detect the trained explosive encapsulated within a casing and, frequently, with other materials (e.g., detonator, fuel, sugar, aluminum etc.). Thus, to evaluate in a laboratory setting dogs generalization to a simulated IED, we encapsulated 10 g of SP in a PVC pipe together with electronic materials to mimic what a dog could encounter in the field. The response rate to the simulated IED was 35% lower than their response to SP, however this difference did not reach statistical significance. All dogs alerted to the first probe of the simulated IED and their response to the subsequent probes varied between individuals. Two dogs alerted to all probes, and one dog alerted to only 1 probe. This variable response rate could be a result of intrinsic dog variation or of the encapsulation of SP in the PVC pipe. For instance, the encapsulation could have limited the amount of odor available or could have increased the time needed for odor regeneration in the vial between trials. Another possibility is that the presentation of SP together with electronic cables and PVC pipe was more perceptually different to some dogs than others.

The alert rate to bullets and to a fired gun swab were significantly lower than chance and dogs' alert rate to SP. The lack of generalization to bullets could have been due to a different type of SP used as propellant (Zeichner 2003), the small amount of SP within a bullet, or to the encapsulation of SP within the cartridge casing. The type of powder contained by the bullets tested was unknown as it was not specified by the manufacture label. Efforts were made to contact the manufacturer to address this question, but were not successful. The shooting process leaves residues on the fired gun. Some of these residues are components of SP but most of them are a result of the burning process that occurs during the shooting (Weyermann et al. 2009). By evaluating dogs' generalization to a fired gun swab, we wanted to evaluate if dogs were able to detect SP residues in a gun after shooting after dogs had been exclusively trained with SP. We found that dogs did not generalize to the fired gun swab. This lack of generalization most likely was a result of the burning process and possible human scent residues. For instance, even when Weyermann et al. (2009) found DPA in the muzzle of a fired gun, they also found other volatiles that were not present in the headspace of SP. Thus, this increment in volatiles could result in perceptual differences between SP and the fired gun. Recent work from the authors also have found that the headspace of guns (loaded and unloaded) and magazines are different from the headspace of SP (Nettles et al. 2022). Altogether the available literature suggests that the volatile profile of the headspace of guns (fired and clean) is different form the headspace of SP and thus these differences can explain the lack of generalization observed in this study.

Dogs alert rate and sniff time to all the control odors (blank gauze, blank t-shirt, pentanol, benzaldehyde, control IED, and clean gun) were statistically different from SP and statistically below chance. As none of the control odors share any relation or similarity with SP, we were expecting dogs to not show any response or increase sniff time to them. This shows that the dogs were alerting to olfactory similarities between the testing odor and SP and not to any novel random odor. The lack of response to the control odor shows that the method was effective in measuring generalization and odor similarities as dogs responded only to the testing odors that share some odor similarities with SP. The outlined method will be ideal to develop generalization gradients in the future as it shows to be effective at measuring perceptual similarities between the trained and testing odors.

It is important to note that the participants of the study spent over 4 months of training and testing with a single source of SP as part of a previous study. This could be a limitation of the current study as over training could reduce generalization (Moser et al. 2019). For instance, in mice, increasing the number of sessions where an odor was paired with a reward narrowed the generalization gradient (Cleland et al. 2009). The narrowing of the generalization gradient in Cleland et al. (2009) study was observed within six sessions. This study in rats indicates that by training our dogs for over 4 months with a single odor we could have increased their specificity to the target odor (Moser et al. 2019). Although Cleland et al. (2009) suggest that over training could occur rapidly in rats, to date, it is still unknown the time at which over training occurs in dogs and thus is difficult to correct for this possible extraneous factor. Another factor that can be perceived as a limitation is that the study was conducted in a laboratory setting with mixed breed dogs, and that this setting does not necessarily resemble operational dog breeds or operational work settings. Although possible genetic differences should be further evaluated, we do not foresee this as a real limitation as a previous study found that non-working dogs outperformed working dog breeds in an odor detection task (Hall et al. 2015).

## Conclusion

Intermittent schedules of reinforcement are known to increase resistance to extinction and maintain unreinforced responses. The use of an intermittent schedule of reinforcement showed to be a useful method to evaluate generalization in dogs. Testing generalization under a variable intermittent schedule of reinforcement did not induce any performance or motivation decrement during testing. This allowed us to give dogs multiple unreinforced trials of a testing odor within a session. All dogs showed high generalization to cotton gauzes and t-shirt cloths exposed to the headspace of SP and to the Getxent tubes exposed to direct contact. Although not statistically significant, dogs had a 30% decrement in their response rate to the simulated IED and to the Getxent tubes exposed only to the headspace of SP. Dogs' response rate to single-base SP was statistically different from their response rate to double-base SP. Similarly, dogs' response rate to a fired and clean gun were significantly lower than their response rate to SP. This highlights that a dog trained to detect SP will not generalize its training to firearms, hence suggesting that explicit training to firearms is needed. However, the sniff time data showed some odor recognition of single-base SP, even when dogs did not fully alert. Dogs' responses to the control odors (unrelated to SP) were significantly lower than their response to SP. Altogether, the data show that intermittent schedules of reinforcement are a good method to study generalization as it allowed researchers to give multiple unreinforced trials without reducing performance or motivation. Our findings have implications to detection dog teams as this highlights the importance of training dogs with variations of the target odor (e.g., different type and sources of SP) to prevent generalization failures.

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## Declarations

**Conflict of interest** All authors state that there is no real or perceived conflicts of interest.

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