Applied Animal Behaviour Science xxx (2016) xxx-xxx



Contents lists available at ScienceDirect

### **Applied Animal Behaviour Science**



journal homepage: www.elsevier.com/locate/applanim

# Effect of odorant pre-exposure on domestic dogs' sensitivity on an odorant detection task

### Nathaniel J. Hall<sup>a,\*</sup>, David W. Smith<sup>b</sup>, Clive D.L. Wynne<sup>a</sup>

<sup>a</sup> Department of Psychology, Arizona State University, United States

<sup>b</sup> Department of Psychology, University of Florida, United States

### ARTICLE INFO

Article history: Received 9 July 2015 Received in revised form 31 December 2015 Accepted 13 February 2016 Available online xxx

Keywords: Olfaction Odor sensitivity Pre-exposure Pavlovian conditioning Dogs

### ABSTRACT

Although dogs are widely trained and deployed for odor detection work, relatively little research has investigated procedures that may more efficiently train or increase detection performance. Prior research in rodents and humans suggests that odorant exposure may enhance sensitivity to that odorant; however, other research has suggested that exposure may have the opposite effect. Our aim was to assess whether exposure to odorants influences dogs' sensitivity to those odorants on a subsequent operant task. We specifically tested whether simply being non-contingently exposed to an odorant or being exposed to an odorant in an appetitive Pavlovian conditioning paradigm influenced dogs' sensitivity to that odorant. In a pre- post-test design we assessed changes in dogs' sensitivity to two odorants. In the first phase, dogs' sensitivity to both odorants was assessed using a descending series of half (binary) dilutions presented using a liquid-dilution olfactometer. Then half the dogs were non-contingently exposed or Pavlovian conditioned to one odorant while the second odorant remained an unexposed control. Sensitivity to both odorants was then re-assessed using the same procedures as during baseline. Dogs showed a significant increase in sensitivity to the Pavlovian conditioned odorant compared to both the control odorant (p < 0.01) and compared to the non-contingently exposed odorant (p < 0.01). These results suggest that Pavlovian conditioning may be a simple procedure to enhance olfactory sensitivity to a target odorant. © 2016 Elsevier B.V. All rights reserved.

### 1. Introduction

Dogs are deployed worldwide for a variety of chemical detection tasks such as the detection of explosives (Furton and Myers, 2001; Goldblatt et al., 2009), narcotics (Dean, 1972), wildlife (e.g. Cablk and Heaton, 2006) and more (e.g. Moser and McCulloch, 2010). Despite their importance as chemical detectors, relatively little research has investigated the efficiency and effectiveness of various training procedures that might enhance dog performance.

One such procedure might be repeated exposure to an odorant to facilitate sensitivity to that odorant. Behavioral research in rodents, humans, and electrophysiological study suggests that repeated exposure to an odorant can enhance overall sensitivity to that odorant (Dalton et al., 2002; Wang et al., 1993; Wysocki et al., 1989; Yee and Wysocki, 2001). If simply being exposed to an odorant enhanced dogs' sensitivity to that odorant, this would

http://dx.doi.org/10.1016/j.applanim.2016.02.003 0168-1591/© 2016 Elsevier B.V. All rights reserved. suggest a simple intervention that might enhance the performance of detection dogs at little cost.

In one study, Yee and Wysocki (2001) showed that following repeated exposure to an odorant, adult mice showed an increase in sensitivity to that odorant. In this study, Yee and Wysocki first tested mice's sensitivity to amyl acetate or androstenone using a descending series of binary (halved) dilutions by presenting up to four dilutions per day until performance dropped below a termination criterion. Next, they exposed the mice to the target odorant for 10 days. Following exposure, the mice were able to reach a lower odorant dilution than they did during baseline.

Unfortunately, enhanced sensitivity following exposure is not a universal finding. Researchers have found no effect of repeated odor exposure when tested in rodents that had been exposed to the odor starting from a young age (Cunzeman and Slotnick, 1984; Laing and Panhuber, 1980). Furthermore, research also suggests that adaptation may occur, leading to reduced sensitivity following prolonged odorant exposure in humans (for a review see Dalton, 2000; Dalton and Wysocki, 1996; Wysocki et al., 1997). Thus, the possibility that exposure could be useful for detection dog training needs further evaluation.

<sup>\*</sup> Corresponding author at: Department of Psychology, Arizona State University, Tempe, AZ 85295, United States.

E-mail addresses: njhall1@gmail.com, njhall1@ufl.edu (N.J. Hall).

#### N.J. Hall et al. / Applied Animal Behaviour Science xxx (2016) xxx-xxx

Instead of simply being exposed to an odorant repeatedly, an alternative simple intervention is Pavlovian conditioning. In an appetitive odorant Pavlovian conditioning intervention, brief exposures of an odorant are correlated with the presentation of an unconditioned reinforcer such as food. Prior laboratory research in rodents has demonstrated that Pavlovian conditioning can reduce training time of an operant discrimination (Bower and Grusec, 1964). Furthermore, our recent research in dogs has indicated that Pavlovian conditioning can enhance the acquisition of, and resistance to disruption in, an olfactory discrimination (Hall et al., 2014, 2015). In contrast, non-contingent exposure or exposure uncorrelated with food had no effect. However, prior laboratory research has yet to test whether Pavlovian conditioning to an odorant leads to enhanced sensitivity for that odorant in dogs.

The aim of the present experiment was to evaluate whether odorant exposure, either as non-contingent exposure or as Pavlovian conditioning, would influence dogs' sensitivity to that odorant. To do this, we compared the effects of non-contingent exposure, Pavlovian conditioning or no exposure on changes in dogs' sensitivity to an odorant. We hypothesized that both noncontingent exposure and Pavlovian conditioning would lead to greater increases in sensitivity compared to no exposure.

### 2. Methods

### 2.1. Subjects

Ten pet dogs of varying ages (1–5.5 years) and breed were recruited for the present study by soliciting owners who had registered their dogs in an online database for research studies, word of mouth, and handing out flyers at dog parks (see Table 1 for dog information). Five dogs had been previously trained on an odor detection task in prior studies; however, all dogs were naïve to the experimental apparatus and experimental odorants used in the present study. All dogs were reported by owners to be in good health and testing sessions took place in a quiet area in the owner's homes in the presence of the experimenter. All testing sessions took place at least 2 h after the dog's last meal and were scheduled around the owner's availability.

### 2.2. Ethical approval

All procedures in this study were conducted with the approval from the University of Florida Institutional Animal Care and Use Committee.

### 2.3. Materials

We assessed dogs' sensitivity to two odorants, 2phenylethanol (Sigma-Aldrich, CAS# 60-12-8) and isoamyl acetate (Sigma-Aldrich, CAS# 123-92-2), using a custom-built liquid-dilution olfactometer. These odorants were selected because they are common in olfactory research and to humans have a characteristic odor of banana for isoamyl acetate and of rose for 2-phenylethanol. The general design principles for the olfactometer in this study follow a similar principle to that of standard liquid-dilution olfactometers used for rodents (e.g. Slotnick and Restrepo, 2001). Fig. 1 shows the design of our olfactometer. A diaphragm air pump was used to generate an airflow that first passed through an activated charcoal filter. The air stream was then split three ways. One path passed a flow meter and needle valve that regulated airflow to 1.9 l/min (4.0 SCFH). This path provided a continuous diluting airflow to a final mixing manifold immediately before the odor port. The second path passed a different flow meter and needle valve regulating airflow to 0.42 l/min (0.9 SCFH). This path led to a manifold and series of solenoids. The solenoids

controlled which saturation jar that air would pass through. Each saturation jar held 10 ml of either odorant or diluent. After passing the saturation jar, the airflow then moved to a manifold where it was mixed with the dilution airflow to produce a  $\sim$ 30% air dilution of the odorant before moving to the odor port. The remaining path was an unregulated path that was normally closed with a solenoid. This path was only opened to clear the mixing manifold and odor port of residual odorant. All components that contacted the odorant (e.g. tubing, jars, and nose port) were comprised of Teflon (PTFE), glass, or Stainless Steel, except for the check valves. Those were composed of Kynar and Viton, but were replaced for each odor and odor dilution to prevent odor cross-contamination.

The olfactometer had six channels, four of which were used for dilutions of the S+ odorant and two for the diluent. Odor presentation was controlled by a custom written Python program on a laptop that interfaced with a digital I/O controller (Arduino Uno<sup>TM</sup>, Turin, Italy) that activated the solenoids. The odor port was continuously exhausted via an attached exhaust fan that emptied into another room or simply away from the olfactometer when that was not possible. The odor port also contained an infrared beam pair that permitted the detection of nose entry. In addition, to the left of the odor port there was a 2 cm × 2 cm response pad. The response pad was a force sensitive resistor (4.45 cm × 3.8 cm) for seven dogs, but was switched to a more robust micro switch with a plastic response pad on top of the switch, making the response area the same size as the force sensor, after one dog repeatedly destroyed the force sensitive resistor with its paw.

### 2.4. Initial training

Dogs were first trained to the go/no go olfactory discrimination procedure with 1-pentanol (1% v/v dilution in mineral oil, CAS# 71-41-0). In the go/no go procedure, dogs were required to indicate the presence of Pentanol by touching the response pad to the left of the odor port (a 'hit') using any part of their body, but most dogs used their nose or paw. If only the diluent was present (mineral oil), the dog was required to not touch the response pad (a 'correct rejection'). Fig. 2 shows the procedure for a go/no go trial and how dogs were trained on the go no/go task.

To train this behavior, the dogs were first trained to insert their nose into the odor port, breaking the infrared beam (Fig. 2A, left). This triggered the computer to make a "beep" which signaled to a handler standing by to give the dog a small treat. Once dogs readily poked their nose in the odor port, they were next required to touch the response pad following a nose poke before the experimenter delivered food. Once a dog readily placed its nose in the odor port and touched the response pad, they were transitioned to discrimination training.

During discrimination training (Fig. 2A; right), each trial started with a brief tone from the computer. If the dog did not approach and sniff at the odor port within approximately 10 s of the tone, the experimenter prompted the dog by saying, "go" and pointing to the odor port. Odorant presentation began once a dog entered its nose into the odor port as detected by a beam break. The odorant was presented for a maximum of 5 s. If the odorant was 1-pentanol (S+ odorant), the dog was required to touch the response pad within the 5s odor presentation (a 'hit'). If the dog made a 'hit' the computer made a tone indicating to the experimenter to deliver a treat to reinforce the behavior. If the odorant was the diluent, mineral oil (S-), the dog was required to not touch the response pad (a 'correct rejection'). There were no programed consequences for correct rejections. If the dog failed to respond when an S+ odorant was present, a 'miss' was scored and was not associated with any programmed consequences. Touching the response pad in the presence of the S- (a 'false alert') led to a 15 s time-out. Fig. 2B shows the beginning of a trial (Image 1), the dog entering its nose

Please cite this article in press as: Hall, N.J., et al., Effect of odorant pre-exposure on domestic dogs' sensitivity on an odorant detection task. Appl. Anim. Behav. Sci. (2016), http://dx.doi.org/10.1016/j.applanim.2016.02.003

2

### N.J. Hall et al. / Applied Animal Behaviour Science xxx (2016) xxx-xxx

### Table 1

Characteristics (breed, age, sex, sex status, and training history) of dogs and treatment assignment. M indicates intact male, NM indicate neutered male, F indicates intact female, SF indicates spayed female.

Subject	Group	Experimental odor	Breed	Age (in years)	Sex	Prior training
Paco	Exposure	2-Phenylethanol	Toy Yorkie	3	М	Yes
Bessa	Exposure	2-Phenylethanol	Lab mix	5	SF	Yes
Sonya	Exposure	2-Phenylethanol	Bull Terrier	5	SF	Yes
Zollie	Exposure	Isoamyl acetate	Greyhound	4.5	SF	No
Teddy	Exposure	Isoamyl acetate	Chihuahua	5.5	NM	No
Rex	Pavlovian	2-Phenylethanol	Pit mix	4	NM	Yes
Ben	Pavlovian	2-Phenylethanol	Rhodesian Ridgeback lab mix	4	NM	No
Gnarlie	Pavlovian	2-Phenylethanol	Pit mix	1.5	NM	Yes
Atlas	Pavlovian	Isoamyl acetate	Poodle	1	Μ	No
Cooper	Pavlovian	Isoamyl acetate	Australian Shepherd	2	NM	No



**Fig. 1.** Sketch of olfactometer. Air flow starts at air pump and passes through an activated carbon filter. Air flow is then split into two regulated lines: an odor line and continuous line. The continuous line provides a constant diluting flow to the odor port. When a solenoid is activated the air flows thorough a check valve then a saturation jar containing an odorant or diluent. The air passes another check valve and a final mixing manifold before being delivered to the odor port. ..... indicates repetition of the above solenoid, check valve, saturation jar, check valve system for a total of 6 lines (4 for S+ odorants and 2 for diluent S–). A clearing line was opened between trials to allow the full pump air flow to clear residual odorant.

in the odor port (Image 2), and the dog responding to the touch pad (Image 3).

The dogs were trained using between two and four sessions of 30 trials a day until reaching an 85% ((hits + correct rejections)/total number of trials) proportion correct criterion for two consecutive sessions. Once dogs reached this criterion, 1-pentanol was no longer used, and the target odorants were changed to a 1% v/v dilution (in mineral oil) of isoamyl acetate or a 1% v/v dilution of 2-phenylethanol. All dogs were trained on both odorants until reaching an 85% accuracy criterion. Only one odorant was presented per day, but the two odorants were alternated across days. Once dogs met an 85% accuracy criterion for both isoamyl acetate and phenylethanol, they entered sensitivity testing.

### 2.5. Sensitivity testing

Sensitivity testing was similar to baseline training, except that dogs were presented with a decreasing series of half (binary) dilutions of the odorant until their performance dropped below a 70% accuracy criterion. Contingencies for hits, correct rejections, misses and false alerts were the same as in training. As in training, the experimenter only provided treats to the dog when indicated by the computer that the dog made a correct 'hit'. The experimenter would also prompt the dog to start a trial by saying 'go' and pointing at the odor port if more than 10 s elapsed since the computer produced the trial starting 'beep' to indicate that start of a trial.

#### N.J. Hall et al. / Applied Animal Behaviour Science xxx (2016) xxx-xxx

4

Α.



Β.



**Fig. 2.** Training and discrimination trial outline. (A) Initial training. Indicates the steps in training from nose poke training to discrimination training. Discrimination go/no go trials. Outlines the procedure for go no/go trials. (B) Dog interacting with the apparatus. Going left to right, the first image shows the apparatus at the start of a trial when the laptop 'beeps'. The next image shows the dog sniffing at the odor port. The final image shows the dog touching the touch pad to the left of the odor port.

General procedures for sensitivity testing were adapted from Yee and Wysocki (2001). Each day dogs were tested with four binary dilutions of one of the odorants. Binary dilution 1 corresponded to a 1% v/v (liquid) dilution of the odorant. All sessions started with five un-scored 'warm-up' trials with the most concentrated dilution. Following the 'warm-up,' dogs were given 10 trials with go (S+) and no/go (S-) trials intermixed with the S+ at the most concentrated dilution. The trial order was pseudo-randomly determined so that the half of the trials were go trials and half no/go, and runs of the same trial type did not exceed three in a row. If the dog made at least seven correct responses, the next dilution (lower concentration) was presented. If dogs made six or fewer correct responses, another 10 trials were conducted at the same dilution. If, after repeating that dilution, the dog then achieved seven correct, the next dilution (lower concentration) was presented, otherwise, the dog was considered to have met the termination criterion. Each session consisted of a maximum of 40 trials, and dogs were tested in two identical sessions per day (a total of 80 trials). Odorants (isoamyl acetate and phenylethanol) alternated across days. After

#### N.J. Hall et al. / Applied Animal Behaviour Science xxx (2016) xxx-xxx

the first testing day, all days started with one dilution less concentrated than the dilution reached in the previous testing day.

Testing was discontinued once dogs met the termination criterion for an odorant (two blocks of 10 trials with 6 or fewer correct responses). If a dog, however, 'passed' a dilution during the first session of the day, but met the termination criterion at that dilution during the second session the dog was considered to have 'passed' the dilution and was moved to next dilution less concentrated.

### 2.6. Exposure

Dogs' sensitivity to the two target odorants was assessed twice, once before and once after an extended exposure phase to one of the odorants in a pre- post-test design (see Table 2). After the initial sensitivity test, half of the dogs were randomly assigned to receive daily non-contingent exposure to one odorant, or Pavlovian conditioning to one odorant for 7 days (described in detail below). For all dogs, one odorant always remained an unexposed control and provided a measure of test-retest reliability. For three dogs in each group, phenylethanol was the exposed/Pavlovian conditioned odor and isoamyl acetate was the control odorant. For the remaining two dogs in each group, isoamyl acetate was the experimental odorant and phenylethanol was the control.

### 2.6.1. Pavlovian conditioning

Odor Pavlovian conditioning was conducted using delay conditioning in which food was presented 10s into a 15s odor presentation while the dog rested in a confined area using the dog's normal crate or a baby gate. During the session the experimenter sat quietly  $\sim 1$  m away. Each Pavlovian conditioning session comprised six conditioning trials, with an inter-trial interval of 5 min. Odor delivery and food delivery were controlled via a computer. At the start of the session, an air pump delivered an odorant stream from a saturation jar containing only mineral oil. After 5 min, the odorant was changed to the conditioning odorant (phenylethanol or isoamyl acetate), and was pumped toward the dog for 10 s. Then, a SuperFeeder<sup>TM</sup> (Super-Feed Enterprises, Dallas, TX) connected to the computer delivered food to the dog. After the 15s odorant delivery, the odorant was discontinued, and the airflow from the jar containing mineral oil resumed. This was repeated until six trials were completed. Thus, all components of the exposure were automated so the experimenter's only duty was to start the program.

#### 2.6.2. Non-contingent exposure

In this condition, dogs were given longer periods of exposure which is more similar to the exposure procedures in prior studies (e.g. Yee and Wysocki, 2001). Similarly to the Pavlovian conditioning, the session was comprised of six trials. For non-contingent exposure, however, the dogs were exposed to the odorant for the entire 5 min period, but after every 5 min, there was a 15 s presentation of the mineral oil alone. No food was presented. This was repeated six times to produce an identical trial length to the Pavlovian conditioning group.

### 2.7. Controls

Several measures were taken to ensure dogs were utilizing the odorant cue and not potential unintentional cues from the experimenter or sounds from the olfactometer itself. First, all trials were conducted blindly. The experimenter never knew whether a trial was a go trial or no/go trial. During sensitivity testing, after every five trials there was a control trial. During these trials, prior to the dog initiating the trial, the solenoid for a go trial was activated, to produce the typical sound of a go trial, but was then closed immediately to prevent any significant amount of odorant from being delivered. The trial then began 3 s later (to allow any potential residual odorant to clear) and thus assessed whether dogs treated these trials as a go trial (sound from olfactometer) or a no/go trial (no odorant). In addition, at the end of all testing, dogs were given a session in which all jars were filled with mineral oil only, to test whether they could respond appropriately in the absence of an explicit target odorant.

### 2.8. Statistical analyses

Statistical analyses were conducted using the lme4, lmerTest, and multcomp packages in R (R Core Team, 2013). To assess change from baseline to post-test we computed a change score by subtracting the lowest dilution reached during post-test from the lowest dilution reached in baseline for each dog and odorant tested. To test whether there was an effect of the exposure type (Pavlovian, noncontingent exposure, or no exposure control) we fit a linear mixed effect model with the change score as the dependent variable and the subject ID as a random effect term (intercepts only). For fixed effects, we included the exposure type, age, sex (intact male, fixed male, intact female, spayed female), prior training history, and odor identity (isoamyl acetate or phenylethanol). This model was then subjected to backward elimination to remove non-significant variables using the step function in the ImerTest package leaving a more parsimonious model. This function eliminates non-significant variables, one at a time, based on an F test with p-values calculated using a Sattethwaite's correction for degrees of freedom. The effect of the exposure conditions was compared using Tukey corrected post-hoc comparisons implemented in the *multcomp* package.

### 3. Results

Fig. 3 shows the performance for each dog on each odorant (control or exposed/Pavlovian conditioned) across the series of half dilutions. The left column shows dogs in the non-contingent exposure group whereas the right column shows dogs in the Pavlovian conditioning group. First, looking at the control odorant, dogs showed similar performances and reached similar dilution steps for the pre and post-test. The Pearson correlation between pre- and post-test thresholds for the control odorant was 0.94 (p < 0.001), indicating good repeatability of the sensitivity measure across the two testing time points. For the exposure only group, there was little change in the sensitivity curve from baseline to post-test for the control odorant, except for Bessa and Rex who showed slight improvement in post-test. For the exposed odor, however, only Sonya showed a minor improvement from baseline. Overall, the change from baseline to post test looked similar between the non-contingently exposed odorant and the control odorant. In contrast, for the Pavlovian conditioned odorant, all dogs except Atlas showed an increase in sensitivity following Pavlovian conditioning, but showed little change in sensitivity for the control odorant.

Fig. 4 shows the mean change in the lowest concentration reached for the control, non-contingent exposure, and Pavlovian conditioned odorants with 95% Confidence Intervals, which indicates that only Pavlovian conditioning lead to a significant change from baseline. The full linear mixed effect model described in Section 2.8 was subjected to backwards elimination. Sex ( $F_{2,12} = 0.13$ , p = 0.88), odor type (isoamyl acetate vs. 2-phenylethanol;  $F_{1,14} = 0.26$ , p = 0.63), prior training ( $F_{1,15} = 0.75$ , p = 0.40), and age ( $F_{1,16} = 1.52$ , p = 0.24) had no effect on the change in odor dilution reached and were removed from the model in the order listed above. There was, however, a significant effect of the exposure type ( $F_{2,17} = 7.16$ , p = 0.01) and was therefore retained as the only significant variable in the model. Post-hoc comparisons indicated that Pavlovian conditioning lead to a greater change in

#### N.J. Hall et al. / Applied Animal Behaviour Science xxx (2016) xxx-xxx

### 6 Table 2

Experimental design. Table shows the exposure for each phase of the experiment for both experimental groups. For both groups, the pre-test consisted for assessing sensitivity to odors A and B. Each group then received their respective exposure (contingent or non-contingent) to odor A. For both groups, the post-test consisted of the same sensitivity assessment conducted in pre-test.

	Pre-test	Exposure	Post-test
Exposure	Sensitivity assessment to odors A & B	Non-contingent exposure to odor A	Sensitivity assessment to odors A & B
Pavlovian	Sensitivity assessment to odors A & B	Pavlovian conditioning to odor A	Sensitivity assessment to odors A & B



Fig. 3. Proportion correct responses at each dilution step for each dog. Figure shows the proportion correct for each dog on each dilution for both the control and exposed/Pavlovian conditioned odorants.

#### N.J. Hall et al. / Applied Animal Behaviour Science xxx (2016) xxx-xxx



**Fig. 4.** Mean change in dilution reached. Shows the mean change from pre-test to post-test in the dilution step reached. Positive values indicate increased sensitivity from baseline (detecting a lower concentration). Error bars show the 95% Confidence Interval.

the dilution step reached compared to the exposure only (z=2.99, p<0.01) and control (z=3.62, p<0.01). The change in dilution reached was not different between the exposure only and control conditions (z=-1.19, p=0.45).

On control trials dogs made a go response on only 8% of the trials throughout testing (solenoid activated to produce sound only). In addition, during the final control session in which all saturation jars were filled only with mineral oil, mean percent correct was 48%. Most dogs did not pass the first 'pseudo-dilution' step, with no dogs succeeding past dilution step 2.

### 4. Discussion

The results suggest that Pavlovian conditioning led to dogs being able to reach a lower binary dilution step compared to baseline, and this change was significantly greater than any change observed for a non-exposed odorant, or an odorant exposed without explicitly associated consequences. This result follows from our prior research that found that Pavlovian conditioning facilitated acquisition of an odor discrimination, but exposure alone did not (Hall et al., 2014). Our results also follow studies that found that repeated exposure to an odorant can have no effect (Cunzeman and Slotnick, 1984; Laing and Panhuber, 1980), or that suggests exposure may lead to adaptation and higher detection thresholds (e.g. Dalton and Wysocki, 1996; Wysocki et al., 1997). Our findings, however, do not directly support research that has found that exposure alone can enhance threshold detection (Wang et al., 1993; Yee and Wysocki, 2001). Unfortunately, several variables differ across the various studies including, for example, species, age of exposure, and types of long-term exposures, making it difficult to isolate the variables that may be related to whether enhancement, no effect or adaptation is likely to be observed following repeated exposure to an odorant.

We did, however, see a clear effect of Pavlovian conditioning. One parameter we did not manipulate, but may have a significant effect, is the concentration of the Pavlovian conditioned stimulus (CS+). Perhaps using a lower concentration CS+ may lead to even lower detection thresholds, as it would be conditioning dogs to lower concentrations. In the present study, however, the Pavlovian conditioning was conducted in an open environment (the dog's crate), making the exact concentration that the dog was being exposed to during the Pavlovian and non-contingent exposure phases uncertain. Future research could conduct the Pavlovian conditioning in a closed environment that would allow for manipulation of the concentration of the CS+.

There are several important considerations regarding the absolute threshold values reported in the present study. The first is that we utilized a liquid-dilution olfactometer instead of an air-dilution olfactometer. Thus it is important here to consider the relative changes from baseline, rather than the absolute stimulus levels (Slotnick and Restrepo, 2001). In addition, because we were interested in the effects of experiences on sensitivity, we only assessed sensitivity with a descending series of dilutions instead of assessing the full psychometric function which would have required giving dogs substantially more exposure to lower concentrations of the odorant. In addition, it should be noted that the present withinsubject design, in which each subject was tested with both the experimental and control odorant, there may have been effects of manipulating one odor on the detectability of the other odorant. Importantly, however, with the present procedures we did observe excellent test-retest correlations for the control odorant (r=0.94) indicating that the sensitivity assessment was relatively consistent.

The present research may have potential applied applications for detection dog training. We have previously shown that Pavlovian conditioning may facilitate acquisition of later odor detection training (Hall et al., 2014), and enhance resistance to disruption of an olfactory discrimination (Hall et al., 2015). Together, these results suggest that Pavlovian conditioning may be a simple way to facilitate detection dog training. It has the benefits that it does not require expert skills to implement and the procedure can be readily automated, as was done in the present experiment. In addition, it can be readily utilized in situations where the dog may otherwise simply be in a crate. Further research will be needed to see how and whether Pavlovian conditioning facilitates detection dog training in an applied setting using more task-relevant odorants.

### 5. Conclusion

In sum, the present study indicates that Pavlovian conditioning can enhance detection sensitivity to that odorant. In contrast, we saw no effect of repeated exposure without associated consequences compared to a no-exposure control. These results indicate that pairing an odorant with food can lead to significant changes in sensitivity for that odorant. Future research will be needed to further evaluate the future applications of the present finding.

### Acknowledgments

We thank the owners and research assistants who made this research possible. This research was supported in part by a College of Liberal Arts grant from the University of Florida to NJH.

### References

- Bower, G., Grusec, T., 1964. Effect of prior Pavlovian discrimination training upon learning an operant discriminaiton. J. Exp. Anal. Behav. 7, 401–404.
- Cablk, M.E., Heaton, J.S., 2006. Accuracy and reliability of dogs in surveying for desert tortoise (*Gopherus agassizii*). Ecol. Appl. 16, 1926–1935.
- Cunzeman, P.J., Slotnick, B.M., 1984. Prolonged exposure to odors in the rat: effects on odor detection and on mitral cells. Chem. Senses 9, 229–239, http://dx.doi. org/10.1093/chemse/9.3.229.
- Dalton, P., 2000. Psychophysical and behavioral characteristics of olfactory adaptation. Chem. Senses 25, 487–492, http://dx.doi.org/10.1093/chemse/25.4. 487.
- Dalton, P., Doolittle, N., Breslin, P.A.S., 2002. Gender-specific induction of enhanced sensitivity to odors. Nat. Neurosci. 5, 199–200, http://dx.doi.org/10.1038/ nn803.
- Dalton, P., Wysocki, C.J., 1996. The nature and duration of adaptation following long-term odor exposure. Percept. Psychophys. 58, 781–792.
- Dean, E.E., 1972. Training Dogs for Narcotic Detection.
- Furton, K.G., Myers, L.J., 2001. The scientific foundation and efficacy of the use of canines as chemical detectors for explosives. Talanta 54, 487–500, http://dx. doi.org/10.1016/S0039-9140(00)00546-4.

8

## **ARTICLE IN PRESS**

N.J. Hall et al. / Applied Animal Behaviour Science xxx (2016) xxx-xxx

- Goldblatt, A., Gazit, I., Terkel, J., 2009. Olfaction and explosives detector dogs. In: Helton, W. (Ed.), Canine Ergonomics: The Science of Working Dogs. CRC Press, Boca Raton, FL, pp. 135–175.
- Hall, N.J., Smith, D.W., Wynne, C.D.L., 2015. Pavlovian conditioning enhances resistance to disruption of dogs performing an odor discrimination. J. Exp. Anal. Behav., http://dx.doi.org/10.1002/jeab.151.
- Hall, N.J., Smith, D.W., Wynne, C.D.L., 2014. Effect of odor preexposure on acquisition of an odor discrimination in dogs. Learn. Behav., 1–9, http://dx.doi. org/10.3758/s13420-013-0133-7.
- Laing, D.G., Panhuber, H., 1980. Olfactory sensitivity of rats reared in an odorous or deodorized environment. Physiol. Behav. 25, 555–558.
- Moser, E., McCulloch, M., 2010. Canine scent detection of human cancers: a review of methods and accuracy. J. Vet. Behav. Clin. Appl. Res. 5, 145–152, http://dx. doi.org/10.1016/j.jveb.2010.01.002.
- R Core Team, 2013. R: A language and environment for statistical computing.
- Slotnick, B., Restrepo, D., 2001. Olfactometry with mice. In: Current Protocols in Neuroscience. John Wiley & Sons Inc.

- Wang, H.W., Wysocki, C.J., Gold, G.H., 1993. Induction of olfactory receptor sensitivity in mice. Science 260, 998–1000, http://dx.doi.org/10.1126/science. 8493539.
- Wysocki, C.J., Dalton, P., Brody, M.J., Lawley, H.J., 1997. Acetone odor and irritation thresholds obtained from acetone-exposed factory workers and from control (occupationally unexposed) subjects. Am. Ind. Hyg. Assoc. J. 58, 704–712, http://dx.doi.org/10.1080/15428119791012342.
- Wysocki, C.J., Dorries, K.M., Beauchamp, G.K., 1989. Ability to perceive androstenone can be acquired by ostensibly anosmic people. Proc. Natl. Acad. Sci. U. S. A. 86, 7976–7978.
- Yee, K.K., Wysocki, C.J., 2001. Odorant exposure increases olfactory sensitivity: olfactory epithelium is implicated. Physiol. Behav. 72, 705–711.