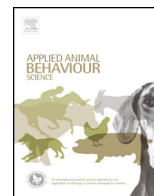




Contents lists available at ScienceDirect

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

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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.

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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

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.

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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 non-contingent 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, 2-phenylethanol (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 ~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™, 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 5 s 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 programmed 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

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