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Discrimination of ''Odorless'' Mineral Oils Alone and as Diluents by Behaviorally Trained Mice

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Abstract

Odorant diluents are generally chosen because of their odorless qualities, allowing them to dilute a target odorant without otherwise altering its perception. Unpublished observations from our laboratory, however, suggest that mineral oil (MO), a common diluent for oil-based odorants, may possess a distinct odor when used in the behavioral testing of mice. To test this, mice were trained to discriminate between 4 brands of MO, using a commercial, liquid-dilution olfactometer and a 2-odorant discrimination task. The results demonstrate that mice were able to detect MOs and to discriminate between MO pairs obtained from different sources. Additionally, we sought to determine if mice could discriminate different MOs when used as a diluent for suprathreshold levels of cineole. Mice were required to discriminate between bottles containing identical concentrations of cineole diluted in different brands of MO. The results showed that the mice readily discriminated each cineole/MO pairing. These data demonstrate that mice are able to detect and discriminate MOs obtained from different sources, both when presented alone and in mixtures. The results also indicate that MO is not an odorless diluent and should be used with caution in olfactory experiments, as the perception of odors being diluted may be unintentionally altered.

Key words: odor diluent, odorant, odorant discrimination, odorant mixtures, olfaction, olfactometer

Introduction

Control over experimental stimuli is an essential aspect of psychophysical research, and adequate control can only be realized with a thorough understanding of all dimensions of the stimuli being applied. In olfactory research, it is necessary to rigorously control the target odorants being tested, but it is equally important to consider the nature of the substances in which the test odorants are diluted.

Most odorants are soluble in water, alcohol, or oil. Because an odorant is mixed with and is therefore perceived simultaneously with a diluent, properties and choice are critical. On a practical basis, diluents are typically assumed to be inherently odorless, serving the purpose of diluting an odorant, or mixture of odorants, without otherwise adding to or altering the original perceptual characteristics of the target odorant itself. If a diluent, such as mineral oil (MO), has an odor, mixture interactions can occur and the manner in which the target odorant, or odorants, is perceived may be altered in an unintended and complex manner (Laing et al. 1989; Smith 1998; Rospars et al. 2008). For example, an odorous MO would cause the percept of the target stimulus to be inadvertently altered, as the perception of an odorant in a mixture is different from the percept produced by that odorant presented in isolation.

MO, a common diluent for oil-based odorants, is generally described as an odorless substance and has been used in many olfaction experiments (Bodyak and Slotnick 1999; Wiltrout et al. 2003; Abraham et al. 2004; Kay et al. 2005; Mandairon, Stack, Kiselycznyk, and Linster 2006; Mandairon, Stack, and Linster 2006; McNamara et al. 2007), though most studies fail to report what vendor was used (Bodyak and Slotnick 1999; Wiltrout et al. 2003; Kay et al. 2005; Li et al. 2006; Mandairon, Stack, Kiselycznyk, and Linster 2006; Mandairon, Stack, and Linster 2006; McNamara et al. 2007; Yoshida and Mori 2007). Recent, unpublished observations from our laboratories suggest, however, that different MOs possess distinct odors when used in behavioral studies with mice. In those unrelated studies, we conducted habituation tests to measure the amount of time mice spent investigating various odorants. In general, habituation studies show that repeated exposure to a given odorant results in a progressive reduction in exploratory sniffing (Linster et al. 2001; Yadon and Wilson 2005; McNamara et al. 2007; Wesson et al. 2008). In our studies, using

a habituation paradigm described by Linster et al. (2001), we found that mice took longer to habituate to MO than they did to other target odorants (i.e., corn oil, tangerine oil, and an artificial ''apple blossom'' odorant), suggesting that the MO possessed a distinct odor (Gamble KR, Smith DW, unpublished data).

The possibility that MO may have an odor raises the concern that, when used as a diluent, it might influence or alter the perception of a target odorant by creating an odorant mixture or producing complex stimulus interactions, either of which might confound interpretations of the data. In this study, we sought to determine whether MOs from different vendors (Sigma-Aldrich, CVS, Fisher Scientific and Wal-Mart) could be discriminated alone in pairwise comparisons and when used as a diluent for a suprathreshold level target odorant (cineole). Our results demonstrate that mice are able to detect and discriminate different MOs not only when presented alone but also in mixtures with a suprathreshold odorant.

Materials and methods

Animals

Five C57Bl/6J mice were used in this study. Mice were obtained from Jackson Laboratories and from an in-house breeding colony maintained at the McKnight Brain Institute, University of Florida. The animals were male, 15–21 months old, and had been previously used in behavioral studies. Mice had ad libitum access to dry LabDiet mouse chow (Purina Mills, LLC/PMI Nutrition International) and were maintained on a 23-h water restriction schedule. The animals were weighed daily and were maintained at 85–90% of their free-body weight (26.5–31.5 g). During testing, mice received no more than 3 mL of a liquid nutrient supplement (Ensure, Abbott Laboratories) as a reward. At the end of each testing session, they also received supplementary water, up to a combined total of 3 mL of water/Ensure per day (National Research Council 1996). Animals were tested once daily, 7 days a week. All animals were individually housed in order to regulate the consumption of fluids. All procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

Odorants

MO used in these studies was obtained from 4 different distributors: CVS (CVS Pharmacy, Inc.), Wal-Mart (Cumberland Swan), Sigma-Aldrich (light), and Fisher Scientific (light). Cineole was purchased from Sigma-Aldrich and was of 99% purity.

Apparatus

Testing of behaviorally trained mice was accomplished with a commercial liquid-dilution rodent olfactometer (Knosys Olfactometers). The use of the Knosys olfactometer has been described in detail elsewhere (Bodyak and Slotnick 1999; Laska et al. 2007; Smith et al. 2007) and will only be described briefly here. Olfactometers had a 15-cm deep, 20-cm wide, and 13-cm tall ventilated Plexiglas operant chamber. The chamber was fitted with a conductive metal floor and a glass sampling port containing a metal licking tube. Ventilation within the chamber provided a steady stream of fresh room air and maintained positive pressure so that odorants remained within the sniffing port air stream and did not enter the testing box.

A photo beam was broken when the animal inserted its head into the sampling port, initiating a trial sequence. Mice were required to keep their noses within the port and sample the stimulus air stream for a minimum of 200 ms, at which time a stimulus, either the S+ or S–, target, or control stimulus (as defined below), was introduced through the bottom of the sampling port. The air stream and odorant were drawn through the sampling port in which the mouse positioned its nose and were then exhausted out of the top by an in-line exhaust fan and fed into a central room-evacuation system.

In this study, animals were trained to discriminate between a target odorant (S+) and a control stimulus, which they were trained to ignore (S–). Reinforcement was contingent upon the animal reporting detection of the S+ odorant by licking on the metal lick tube (correct detection), which completed an electrical circuit with the metal floor and registered the response with the computer-based olfactometer control program. A correct detection was rewarded by the presentation of 5 μL of Ensure through the lick tube. Failure to report the presence of the S+ (a miss) and licking the response tube during presentation of an S– stimulus (false alarm) were recorded as incorrect responses and put the animal in a 5-s time-out during which it was not able to initiate a new trial.

Trials were presented in blocks of 20, consisting of 10 S+ and 10 S– trials presented in a quasi-random order. The percent correct was calculated for each block, with 85% accuracy required to pass a block. Animals were tested in 2 olfactometers simultaneously, being randomly switched between olfactometers each day.

Procedure

MO discrimination

Because the mice were not naive at the beginning of these experiments and had been previously used in similar behavioral paradigms, training time was minimal. The animals were required to sample the odor stream within the sampling port for incrementally longer intervals before responding, progressing from 0.2- to 1.2-s requirements over 140 trials. It took the mice approximately 1 week to become fully trained on this program, reaching 85% or higher accuracy in responding to the target odorant (S+) and not responding to the S–. Mice were initially trained to detect CVS MO as the S+, and for the training phase of this experiment, a clean,

empty bottle containing filtered air served as the S–. Each of the other 3 MOs (Wal-Mart, Sigma-Aldrich, and Fisher Scientific) was then also used in the training paradigm to ensure that each could be discriminated from clean air and thus had an odor. Once the animals successfully completed training, they were run on a 2-odorant discrimination program, where CVS MO (S+) was sequentially tested against 3 different brands of MO (S–). The mice were required to pass 3 consecutive blocks of 20 trials of different MO brand discriminations with 85% or higher accuracy in order to pass a discrimination pair. Similarly, the animals were tested on pairwise comparisons of all 3 brands of MO, one MO being the S+ and another being the S–, in order to determine if each MO was discriminable from the others.

Cineole in MO discrimination

Although discrimination between different types of MOs would be a compelling evidence for different MOs having distinct olfactory notes in testing with mice, findings would hold the most practical significance if MOs were shown to affect the perception of odors for which it is used as a diluent. To address this issue, we tested mice on a discrimination task pairing identical suprathreshold levels of cineole diluted in 2 different brands of MO. Levels of cineole used in these trials were 10^{-5}% and 10^{-4}% v/v, 3–5 log concentrations above previously determined threshold levels (10^{-8}% and 10^{-9}%, Gamble KR, Smith DW, unpublished data; Kelliher et al. 2003). In this experiment, the only difference between the S+ and the S– stimuli was the source of the MO diluent, either CVS brand or Sigma-Aldrich MO. As with the previous discrimination task, mice had to reach 85% or higher accuracy on 3 blocks of 20 trials in order to pass the discrimination pair.

Results

MO discrimination

Animals were readily trained to discriminate the headspace of a bottle containing MO from a bottle of clean, filtered air. Seven different bottles from 4 different vendors were used, and all animals were able to discriminate each MO bottle from a bottle containing clean air, indicating that all the MOs possessed a detectable odor. Each animal required only one training session in 1 day for each MO to obtain these acquisitions.

Subsequent pairwise comparisons of different MOs showed that all the MO pairs were discriminated from one another by at least 2 of 5 mice with 85% or higher accuracy in 3 consecutive blocks (Figure 1). CVS MO was discriminated from Sigma-Aldrich and Wal-Mart MOs by 4 of the 5 mice and from Fisher Scientific MO by 2 of 5 mice. Discrimination for each MO pairing was acquired in 5 or fewer days with an average of 13 blocks (standard deviation [SD] = 8.09). Animals that failed to discriminate by remaining at chance discrimination levels, 45–55% accuracy, were given an equal number of days to attain discrimination but were stopped being run when they showed no improvement in their discrimination accuracy.

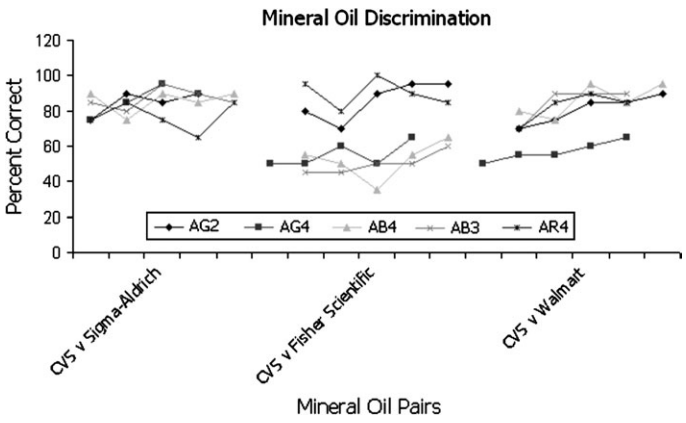

Figure 1 Acquisition of discrimination for 3 pairs of 4 brands of MO. Percent correct for each block of 20 trials is plotted as a function of successive blocks for 3 different MO comparisons.

Following training with and testing of the CVS MO against the other 3 MOs, pairwise comparisons of all MOs were conducted employing the Knosys training program; all 5 mice discriminated each MO from a second MO, regardless of source, in only one training session (not shown).

Discrimination of different MOs as cineole diluents

When CVS and Sigma-Aldrich MOs were used as diluents for cineole to concentrations of 10^{-5}% v/v, all 5 mice were able to discriminate CVS MO from Sigma-Aldrich MO with 85% or higher accuracy in 3 consecutive blocks, taking an average of 9 blocks (SD = 8.66) to reach acquisition. When used as diluents for a higher cineole concentration of 10^{-4}%, 4 of 5 mice were able to discriminate the CVS and Sigma-Aldrich MO diluents with an accuracy of 85% or higher in 3 consecutive blocks over an average of 9 blocks (SD = 4.29), as well (Figure 2). Discrimination of acquisition took an average of 2 days for mice to reach criterion; 1 mouse that was unable to pass the discrimination within 2 days was tested for an additional 2 days, though showed no improvement in discrimination accuracy.

Discussion

MO is a common diluent for oil-based odorants. Most studies using MO as a diluent and/or control stimulus in discrimination studies do not mention the supply vendor or types of MO used, but merely refer to it as ''odorless.'' The results presented here, however, demonstrate that MOs obtained from 4 different sources were easily discriminated from one another by behaviorally trained mice, suggesting that, in terms of olfactory-guided behavior, not all MOs are the

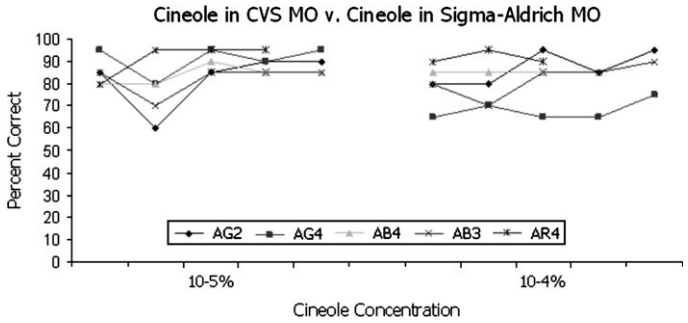

Figure 2 Acquisition of discrimination for 2 MOs (CVS or Sigma-Aldrich) used as diluents for suprathreshold levels of cineole. Percent correct discrimination for each block of 20 trials is plotted for successive blocks for 2 different cineole concentrations with each diluent.

same. The present data demonstrate that mice are able to detect and discriminate different MOs not only when presented alone but also in mixtures with suprathreshold odorants. The data also suggest that MO is not an odorless diluent and should, therefore, be used with caution in olfactory experiments, as the perception of odors being diluted may be altered in unknown ways.

Animals were originally trained to respond to presentation of 4 brands of MO and to ignore presentation of filtered air (through an identical saturation bottle) as the control stimulus. Animals were able to discriminate between purified air and MO, suggesting that the MOs had detectable odors (i.e., that they were perceived differently from the filtered carrier air stream alone). Once trained, mice were also able to discriminate each MO from the 3 other brands of MO in pairwise comparisons, suggesting that not only does MO have an odor but also MOs from different vendors each possess distinct notes that allow them to be discriminated from one another.

Although this finding is important, the main concern with a diluent being odorous is how it may affect the perception of a target odorant, or odorants. In our second experiment, cineole, an intense and trigeminal odorant (Laska et al. 1997), was diluted to identical concentrations of 10^{-5}% and 10^{-4}% v/v by use of 2 different types of MOs, such that the source of MO was the only difference between the 2 stimuli. Even in the presence of an intense odorant like cineole, mice were still able to discriminate between the 2 stimuli by using the distinct note of the MO diluent—or by use of an unintended alteration in the perception of cineole produced by the different MOs. These findings suggest that different MOs have distinct, discriminable notes.

The present data also suggest that an odorant, simple or complex, when diluted with MO becomes a more complex mixture of 2 or more odorous substances whose perception may be inadvertently influenced by odorant interactions (Laing et al. 1989; Livermore and Laing 1998; Smith 1998; Kay et al. 2005; Rospars et al. 2008). As a consequence, MO, when used as a diluent in olfactory studies, should be used with an awareness of possible unintended odorant interactions or odorant cues. The present findings indicate that consistent use of a specific MO within an experiment is essential and that changing brands or sources of MO during an experiment may introduce confounding variables to interpretation of data.

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