Response Accuracy and Odor Sampling Time in Mice Trained to Discriminate between Enantiomers of Carvone and Those of Terpinen-4-ol

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Abstract
Response accuracy and odor-sampling times were used to compare the ability of mice to detect (+)-carvone and (+)-terpinen-4-ol and to discriminate between enantiomers of carvone and of terpinen-4-ol. Except for increased odor sampling when mice were first exposed to the (+)-carvone odor, there was no difference in odor-sampling time or response accuracy in tests of odor detection or in discriminating between enantiomers of these odorants. These results fail to support the suggestion that odorants that produce different patterns of olfactory bulb activation should be easier to discriminate than those that produce much more similar patterns of bulb activation.

Key words: enantiomers, odor detection, odor discrimination, odor-sampling time, olfactometer

Introduction
There is now considerable anatomical and physiological evidence that exposure to an odorant produces a spatially distributed pattern of activation in olfactory bulb glomeruli. The pattern produced is characteristic of the stimulus and varies in predictable ways as a function of several identified physical parameters of odorous molecules (Uchida et al. 2000; Leon and Johnson 2003). These patterns constitute an “odotopic signature” for the odorant, and it is generally assumed that this odotopy provides an important mechanism by which odor quality is coded (Xu et al. 2000). However, attempts to confirm this assumption at the level of behavior have not been particularly successful. Disruption of such patterns by surgical lesions of the olfactory bulbs or olfactory epithelial toxicants does not produce a specific anosmia or hyposmia, significantly disrupt the ability to discriminate the targeted odor from similar or different odors or, in tests of odor quality perception, to alter trained responses to such odors (e.g., Slotnick and Bodyak 2002). There are, of course, significant constraints on the use of such invasive methods for assessing the role of odotopy in odor coding. These include the possibility that trained animals may use odor cues that do not contribute to the maps produced by imaging studies and the difficulty in removing all parts of an identified odotopic pattern.

In what is an arguably more satisfactory test for the significance of odotopy for behavior, Linster et al. (2001, 2002), in combined anatomical and behavioral studies, examined the [14C]2-deoxyglucose (2-DG) patterns of glomerular activation produced by exposure to the enantiomers of limonene, carvone, and terpinen-4-ol and assessed the ability of rats to discriminate between the enantiomers of these odorants. Because enantiomers differ physically only in the chiral properties of the molecule, one may assume that this difference must be responsible both for any difference in bulbar activation patterns and the ability to discriminate between the enantiomers. They found that bulbar activation patterns produced by enantiomers of limonene were quite similar as were the enantiomers of terpinen-4-ol. In contrast, the patterns produced by the enantiomers of carvone were significantly different from each other and Linster et al. predicted that rats would more easily discriminate between the enantiomers of carvone than between those of limonene or terpinen-4-ol. This prediction was supported by a study of habituation (Linster et al. 2001) and a follow up study in which rats were trained to discriminate between enantiomers of each odorant (Linster et al. 2002). However, McBride and Slotnick (2006) failed to confirm these behavioral outcomes: in a psychophysical study, normal rats performed equally well in discriminating between the enantiomers of terpinen-4-ol and those of carvone. Further, olfactory bulb lesions that disrupted the patterns of activation for both odorants had little or no effect on ability to detect or discriminate between their enantiomers.

The present report provides another behavioral test for the hypothesis that the enantiomers of carvone are more easily discriminated than those of terpinen-4-ol. To assess the
generality of the results of McBride and Slotnick (2006), mice were used and, to provide an additional and, as reported (Slotnick 2007b), a more sensitive measure of discrimination, odor-sampling time was measured.

Materials and methods

Subjects

Ten 4- to 5-month-old male CF-1 strain mice were housed in groups of 3–4 in plastic cages in a temperature- and humidity-controlled vivarium. A water restriction schedule of 1–2 ml/day was in effect and served to maintain weights at 85–88% of prerestriction baseline weights. These mice were a subset of those used in the Slotnick (2007b) study, and the tests described in this report were conducted several days after the termination of tests described in that report. All experimental procedures were approved by the University of South Florida Institutional Animal Care and Use Committee.

Apparatus, odorants, and procedures

Three identical Knosys LD8-1 mouse olfactometers were used. The units, details of odor generation, and training procedures were identical to those described in detail by Slotnick (2007b).

The odorants used and their rated purities were ethyl acetate (99.5%), (+)-carvone (98%), (−)-carvone (99%), (+)-terpinen-4-ol (97%), and (−)-terpinen-4-ol (97%). Ethyl acetate was purchased from Sigma (St Louis, MO), and the carvone and terpinen-4-ol enantiomers were purchased from Fisher Scientific/Acros (Pittsburgh, PA). The purity levels were the highest available from these suppliers. All odorants were diluted v/v with odorless mineral oil to the desired concentration, and 10 ml of solution was used as the odorant source in the odor saturation tubes. Odorant concentrations are given as the liquid dilution of the odorant in the saturator tubes, and positive and negative stimuli used in training are named with regard to the odorant and its liquid dilution. As described previously (Slotnick 2007b), the 50-cc/min odorant vapor from the saturator tube was manifolded with 1950 cc/min of clean air before being introduced to the rat sampling port. Thus, the odor concentration delivered to the animal sampling port was approximately 2.5% of the concentration of the headspace above the liquid odorant. The odorant concentration of the headspace above the liquid solution is not known, but gas chromatographic analyses indicate that headspace concentrations of a wide variety of hydrocarbons from mineral oil dilutions are proportional to their liquid dilution (Cometto-Muniz et al. 2003).

The mice were arbitrarily divided into 2 equal sized groups (Groups CT and TC) and trained in 100- (for ethyl acetate detection tasks described below) or 200-trial daily sessions (all other tasks). Odorant concentrations were 0.5% for ethyl acetate and 1% for each of the enantiomers. In the first session, ethyl acetate served as the positive (S+) stimulus and the water solvent as the negative (S−) stimulus for all mice. Next, Group CT was given a 200-trial session in which (+)-carvone served as the S+ stimulus and the mineral oil solvent served as the S− stimulus. Training was continued in a 200-trial session in which (+)-terpinen-4-ol served as the positive stimulus and the mineral oil served as the S− stimulus. Discrimination training was initiated in the next session in which (+)-carvone served as the S+ stimulus and (−)-carvone served as the S− stimulus and a second session in which (+)-terpinen-4-ol served as S+ and (−)-terpinen-4-ol served as S−. These discrimination tests were repeated in the next 2 sessions.

Group TC mice were trained similarly except that the sequence of carvone and terpinen-4-ol tests was reversed: these mice were first trained on the terpinen-4-ol detection task, then on the carvone detection, terpinen-4-ol discrimination, and the carvone discrimination task. For all tests with carvone and terpinen-4-ol, 2 sessions were run each day, one in the morning and the second, 2–4 h later, in the afternoon.

Within- and between-group Student t-tests were used to evaluate all outcomes. Because multiple within and between t-test were performed, the adopted alpha level of 0.05 was adjusted to <0.01 using the Bonferroni correction. However, all P values that were equal to or less than 0.05 are reported.

Results

Performance accuracy

Mice performed at near maximal accuracy on the well-practiced 0.5% ethyl acetate (EA) detection task. The shift from the EA detection to the carvone detection task (Group CT) or terpinen-4-ol detection task (Group TC) resulted in an initial sharp drop in performance accuracy followed by a rapid acquisition of the respective detection tasks for each group (Figure 1). The decrease in accuracy from the last block of trials on the EA detection task to the first block of trials on new detection task was substantial for both groups (P < 0.02, within-group t-tests). However, accuracy in acquisition of the carvone detection task (Group CT) did not differ from acquisition of the terpinen-4-ol task (Group TC): between-group t-tests for accuracy on the first block of trials, mean of first 3 blocks of trials, or mean for all trials were not significant (P > 0.1, each case).

On the second enantiomer detection task, the performances of Group CT (now tested on detection of terpinen-4-ol) and Group TC (now tested on detection of carvone) were essentially identical, and accuracy on the first block of trials, mean of the first 3 blocks of trial, or mean for all trials did not differ from the prior detection task for either group.

In the first discrimination task, Group CT initially made somewhat fewer errors in discriminating between
enantiomers of carvone than did Group TC in discriminating between the enantiomers of terpinen-4-ol. However, differences in performance accuracy between groups were not significant for the first block of trials, mean of the first 3 blocks of trials, and mean performance on all blocks ($P < 0.1$, each test). Performance accuracy of both group improved in subsequent discrimination tests (Figure 1) and differences between groups on these tasks were smaller than on the first discrimination task.

**Odor-sampling time**

Odor-sampling time on the EA detection task was essentially identical for the 2 groups: mean sampling time on the first block of trials and for all trials on the EA task was 420 and 400 ms for Group CT and 390 and 380 ms for Group TC. The switch from the EA detection to the carvone detection task for Group CT and to the terpinen-4-ol detection task for Group TC resulted in an abrupt increase in odor-sampling time (Figure 1). Mean odor-sampling time for Group CT on the carvone detection task for both the first block of 20 trials (530 ms) or all trials (570 ms) was greater than that for corresponding scores on the prior EA task ($P < 0.04$ each case). A similar but somewhat less pronounced increase in sampling time also occurred for Group TC: mean odor-sampling time for Group CT on the carvone detection task for both groups were longer than on the terpinen-4-ol tasks ($P < 0.03$, each comparison). Sampling time on the subsequent enantiomer discriminations were consistently higher than on the EA detection task, but there were no differences in either the first block of trials or mean odor-sampling times between the carvone and the terpinen-4-ol discrimination problems for between- or within-group comparisons.

Because the sequence of tests for Groups CT and TC had no significant effect, the scores on the carvone detection and discrimination tasks and terpinen-4-ol detection and discrimination tasks were replotted after collapsing scores on similar tasks across groups. As shown in Figure 2, it is evident that, except for higher odor-sampling times on the first exposure to carvone, both performance accuracy and odor-sampling time were essentially identical on the carvone and the terpinen-4-ol detection tasks and discrimination tasks.

**Discussion**

The results of this study indicate that mice were at least as accurate in detecting the presence of (+)-terpinen-4-ol and in discriminating between the enantiomers of terpinen-4-ol as they were in their corresponding carvone detection and discrimination tasks. These outcomes are in agreement with those of a prior psychophysical study that found no differences in the ability of rats to discriminate between the enantiomers of carvone and between those of terpinen-4-ol (McBride and Slotnick 2006). In addition, the present results also demonstrate that odor-sampling time was essentially identical in discriminating between carvone enantiomers and between terpinen-4-ol enantiomers. Because odor-sampling...
time can provide a more sensitive measure of discrimination difficulty than does accuracy (Slotnick 2007b), that outcome further strengthens the conclusion that, in olfactometric tests, the enantiomers of terpinen-4-ol are no more difficult to discriminate than are those of carvone. These results fail to support the contention of Linster et al. (2001, 2002) that because the enantiomers of carvone produce overlapping but distinctly different sites of activation across the olfactory bulb, they should be more easily discriminated than those of terpinen-4-ol, odorants that produce almost identical sites of bulbar activation. The more general question addressed by these studies is whether maps of olfactory bulb sites that are activated by monomolecular odorants predict odor quality and the extent to which or the difficulty with which 2 odors can be discriminated. Because studies in the awake-behaving animal are essential for evaluating such issues, it is useful to reexamine the behavioral evidence of Linster et al. in support of a combinatorial view of odor coding in which perception of odors is mediated largely by a pattern of bulbar activation.

Linster et al. provided 2 lines of behavioral evidence in support of this view. In a habituation study, rats readily recognized (+)-carvone as being different from (−)-carvone to which they had been repeatedly exposed. However, those tested in a similar manner with terpinen-4-ol enantiomers did not show a differential response to the novel test odor (Linster et al. 2001). In a second study, rats trained using positive reinforcement in a digging test made fewer errors in discriminating between carvone enantiomers than did those trained to discriminate between enantiomers of terpinen-4-ol (Linster et al. 2002).

There are several reasons why our olfactometric tests failed to support the conclusion of Linster et al. regarding the discriminability of carvone and terpinen-4-ol enantiomers. For one, the failure to show dishabituation in a habituation test does not provide prima facie evidence that the test odors cannot be discriminated or are difficult to discriminate. The evidence for this is clear both from our own studies and that of Linster et al. (2002) who reported that rats learned to discriminate between the enantiomers of terpinen-4-ol and that their terminal performance was equivalent to those trained to discriminate between carvone enantiomers. The Linster et al. habituation test probably reflects that the odor of one enantiomer of carvone is more novel relative to another than are enantiomers of terpinen-4-ol. Indeed, this “novelty effect” may account for the increased sampling time on the carvone detection task in the present study (Figure 2), but as shown in this report, the McBride and Slotnick (2006) study and even in the discrimination study of Linster et al. (2002), there may be considerable independence between measures of odor novelty and odor discrimination.

The Linster et al. (2002) conclusion that rats learned to discriminate between the enantiomers of terpinen-4-ol more slowly than those of carvone was based on the finding that the terpinen-4-ol group made significantly fewer correct responses on trials 6–10 of a 20-trial test than did those trained to discriminate between carvone odors. The results of the present study provide some support for this finding because, in the initial trials on their first session in discriminating enantiomers of carvone, both Groups CT and TC made somewhat but not significantly fewer errors than in discriminating between the enantiomers of terpinen-4-ol. However, in 2 independent studies with intact rats, McBride and Slotnick (2006) found no difference in error scores on carvone versus terpinen-4-ol enantiomer discrimination tasks. Thus, if carvone confers any advantage in odor discrimination learning, it is, at best, subtle and, apparently, not reliable.

In conclusion, the present results confirm and extend the findings of McBride and Slotnick that the enantiomers of terpinen-4-ol are no more difficult to discriminate than are those of carvone. These outcomes are in agreement with those from a variety of studies in which selective lesions of the olfactory system have failed to confirm predictions based on patterns of bulbar activation as revealed by intrinsic imaging or 2-DG methods (e.g., Hudson and Distel 1987; Lu and Slotnick1988; Lu and Slotnick 1994; Slotnick et al. 1997; Youngentob et al. 1997; Slotnick and Bodyak 2002; Bisulco and Slotnick 2003; Slotnick and Bisulco 2003; Slotnick 2000a). The failure of these behavioral studies to support such predictions may be because these imaging methods do not have sufficient resolution to reveal the entire pattern of bulbar activation, because these patterns mediate functions not assessed by simple 2-odor discrimination tasks, or because odor identification and discrimination are mediated by very early events (i.e., within the first few hundred milliseconds) in odor sampling (Uchida and Mainen 2003; Abraham et al. 2004; Rinberg et al. 2006; Spors et al. 2006; Slotnick 2007b) that are not revealed by averaging activity over much longer intervals.

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References


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