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The Role of Perceptual and Structural Similarity in Cross-adaptation

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Abstract

Cross-adaptation, the decrease in sensitivity to one odorant following exposure to a different odorant, is affected by odorant similarity, both perceptual and structural, but the precise relationship is obscure. The present series of studies was designed to explore various aspects of perceptual and structural similarity as they relate to cross-adaptation. In Experiment 1, cross-adaptation was assessed between androstenone and five odorants that share a common urinous note with androstenone, but retain unique perceptual characteristics; only the compound judged most perceptually similar to androstenone cross-adapted it. In Experiment 2, odorants both perceptually and structurally similar (androstenone and androstanone) displayed significant, mutual cross-adaptation. Furthermore, magnitude estimates for androstanone were significantly reduced following exposure to 3-methylidene-5α-androstane (3M5A), a structurally similar, perceptually odorless compound. This finding appears to be the first demonstration that an odorless compound can affect, via cross-adaptation, the perception of an odorous compound. Finally, in Experiment 3, significant, asymmetric cross-adaptation was observed between compounds that are perceptually and structurally dissimilar (4-cyclohexylcyclohexanone [4-CHCH] and androstenone). These findings indicate that the role of similarity in cross-adaptation is difficult to quantify and emphasize the numerous odorant characteristics that can affect cross-adaptation. **Chem. Senses 21: 223–237, 1996.**

Introduction

Most current models of olfactory transduction postulate a lock-and-key mechanism by which different odorants stimulate particular receptors; however, the precise manner by which odorant structure determines receptor activity is unknown. One approach to addressing structure-activity relationships is via studies of cross-adaptation using psychophysical methods. Cross-adaptation, the decrease in sensitivity to one odorant following exposure to a different odorant, may represent the degree to which odors share common sensory receptors or mechanisms (Moncrieff, 1956; Todrank *et al.*, 1991; Pierce *et al.*, 1993) and is affected by similarity in olfactory perception or chemical structure. The present studies were designed to explore various aspects of similarity as they relate to cross-adaptation.

An important distinction can be made between perceptual and structural similarity (Pierce *et al.*, 1993). Perceptual analogs have similar odors, but may have dissimilar chemical structures; structural analogs are compounds with similar chemical structures, but may have different odors. In general, perceptual analogs cross-adapt (Moncrieff, 1956; Engen, 1982; Todrank *et al.*, 1991; Cain and Polak, 1992; Pierce *et al.*, 1993), but there is no clear relationship between

perceptual similarity and cross-adaptation. Compounds so similar in perception as to be indistinguishable in triangle tests of discrimination can cross-adapt, yet odorants need not be perceptually indistinguishable for cross-adaptation to occur. Thus, strong, mutual cross-adaptation has been observed between the perceptually indistinguishable compounds 5α-androst-16-en-3-one (androstenone), a volatile steroid found in human sweat (Brooksbank *et al.*, 1974; Claus and Asling, 1976) and saliva (Bird and Gower, 1983), and a non-steroid perceptual analog, DMCMC (Pierce and Wysocki, 1992; Pierce *et al.* 1993). By contrast, two synthetic musks, Galaxolide® and Thibetolide®, are perceptually distinguishable, yet show mutual, albeit asymmetric, cross-adaptation (Todrank *et al.*, 1991; Pierce and Wysocki, 1992), as do other discriminable odorants (Cheesman and Townsend, 1956; Moncrieff, 1956; Engen, 1982).

A similar uncertainty surrounds the role of structural similarity in cross-adaptation. In one study (Pierce *et al.*, 1995), a 10:1 mixture of (E)- and (Z)-3-methyl-2-hexenoic acid (3M2H), a principal component of the characteristic odor of human sweat (Zeng *et al.*, 1991, 1992), was cross-adapted by its ethyl esters (EE3M2H), which possess a fruity odor. Cross-adaptation was asymmetric (adaptation to 3M2H did not significantly affect the perceived intensity of EE3M2H) and specific; no cross-adaptation was noted when subjects were adapted to the ethyl esters of 3-methyl-2-octenoic acid (EE3M2O) and 3-methyl-2-pentenoic acid (EE3M2P). Cain (Cain and Engen, 1969; Cain, 1970) has likewise shown cross-adaptation between the structurally similar odorants, *n*-propanol and *n*-pentanol. Engen and Lindstrom (1963) reported cross-adaptation among a homologous series of aliphatic alcohols, but the degree of cross-adaptation did not covary with the degree of chemical similarity. Thus, as with perceptual similarity, the relationship between structural similarity and the extent of cross-adaptation remains unclear.

In the present series of studies, the role of perceptual and structural similarity in producing cross-adaptation was examined from several different perspectives. First, structurally distinct compounds that share a perceptual note but differ in overall perceptual quality were assessed for cross-adaptation (Experiment 1). Secondly, compounds that are both perceptually and structurally similar were assessed for cross-adaptation (Experiment 2). Experiment 2 also tested whether a compound must be odorous to cross-adapt an odorant. Finally, cross-adaptation by structurally similar compounds having different perceptual qualities was examined in Experiment 3.

Experiment 1

The extent of mutual cross-adaptation noted between androstenone and DMCMC (Pierce and Wysocki, 1991; Pierce *et al.*, 1993) is not surprising, because these compounds are perceptually very similar. In Experiment 1, we assessed specificity of cross-adaptation by testing five odorants that share a urinous note with androstenone, but differ in their overall perceptual quality.

Method

Subjects

Subjects were recruited from the Monell Chemical Senses Center, and the surrounding University of Pennsylvania and Drexel University communities. All were screened for sensitivity to the odorants used in the experiment by an olfactory threshold procedure. Only those subjects able to detect androstenone at a concentration level equivalent to step 4 or less (2.4 p.p.m. in oil) were used. Subjects were paid to participate.

Twelve subjects (six males and six females; mean age of 27.6 years) participated in the cross-adaptation procedure. A total of 25 subjects (11 males and 14 females; mean age of 26.9 years) participated in the single-session similarity ratings procedure.

Stimuli

Odorants were diluted in odorless, light, white, mineral oil and presented in 270-ml, polypropylene squeeze-bottles with plastic, flip-top caps. Each bottle contained 10 ml of the prepared solution.

A 12-step binary dilution series was prepared for each of the six odorants tested for cross-adaptation. Concentrations were selected to represent a range from weak to strong. The dilution scheme for androstenone (MW = 272.4; odorant no. 6 in Figure 1) ranged from 3.67 mM (step 12: 1.0×10^{-1}% w/v; 1 g/l) to 1.79 μM (step 1: 4.88×10^{-5}% w/v; 0.488 mg/l). The other odorants, selected because they share a urinous note with androstenone in odor profiles supplied by International Flavors and Fragrances, included aldron [Dragoco; MW = 248.4; (1)], bacdanol [IFF: MW = 222.4; (2)], cassis ether [IFF; MW = 134.2; (3)], sandiff [an isomer mix; IFF; MW = 236.4; (4)] and timberone [Hercules; MW = 220.4; (5)]. The dilution scheme for these odorants represented a range of concentrations similar in intensity to the androstenone series. Initial concentrations (step 12) were as follows: aldron (4.0×10^{-1}% v/v; 14.5 mM),

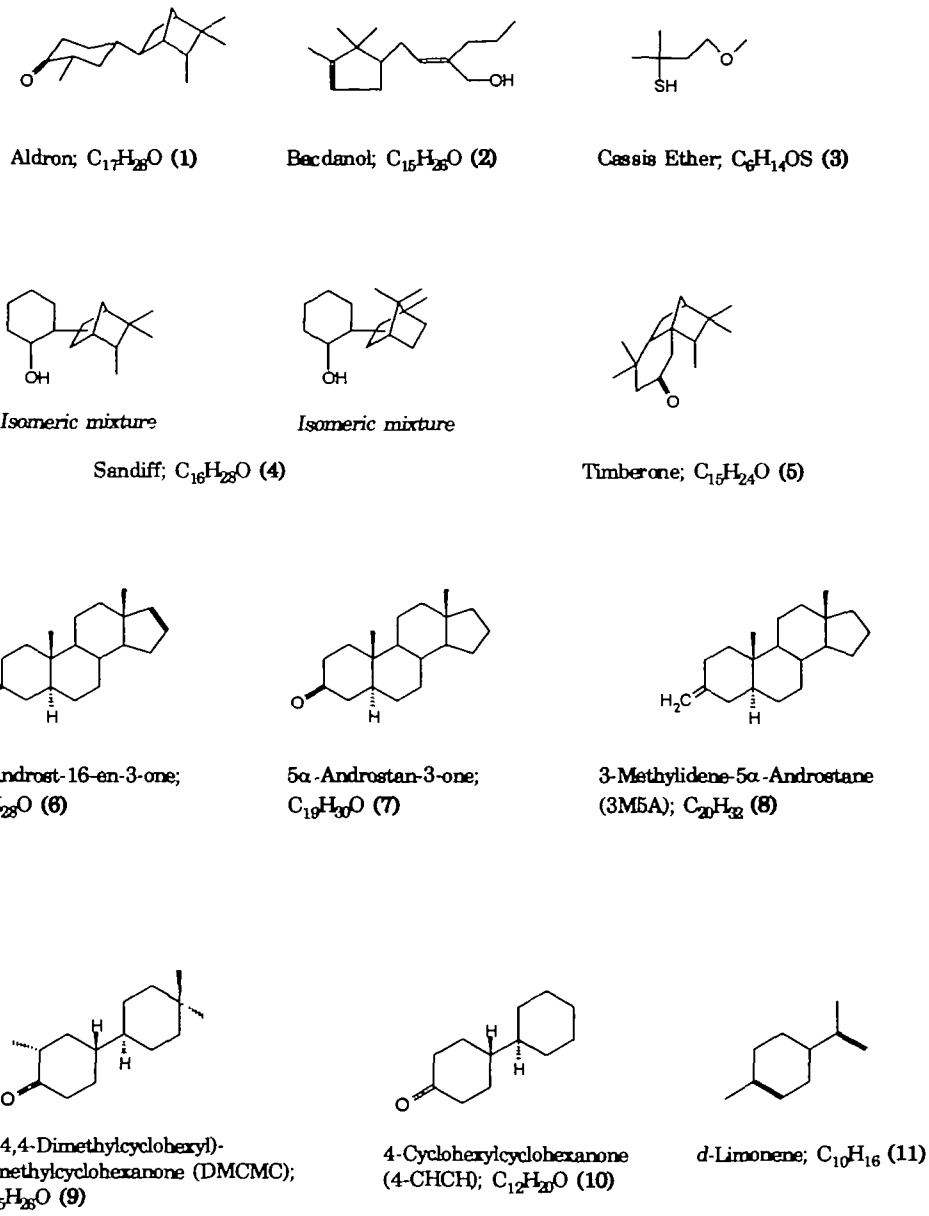

Figure 1 Chemical stimuli used in the present studies.

bacdanol (40% v/v; 1.62 M), cassis ether (1% v/v; 67.1 mM), sandiff (100% v/v; 3.81 M), and timberone (20% v/v; 0.817 M).

For the similarity ratings, the step 12 concentration of each of the six tested odorants was used, along with a 6.73 mM (1.0×10^{-1}% v/v) concentration of amyl acetate (Fisher).

Procedure

Cross-adaptation procedure

Subjects were tested in ten 30-min sessions spaced at least 24 h apart. Each session used androstenone and one of the other five compounds as the test odorants. Androstenone served as the adapting odorant in five sessions; the other compounds served in one session each as the adapting odorant. Choice of the adapting odorant was counter-balanced across sessions.

For each subject, at the beginning of each session, a forced choice staircase procedure was used to equate stimulus intensities. Each trial consisted of a step 10 concentration of androstenone and an alternating concentration of one of the perceptually related compounds (starting at step 8). Subjects were instructed to identify the perceptually stronger of the two bottles. Each pair of stimuli was presented twice, with trials separated by 1 min. If the subject selected

androstenone on each of the two trials, the subsequent trial used the next stronger concentration of the perceptually related compound. Similarly, if the perceptually related compound was selected twice, the next weaker concentration of it was used. The concentration at which subjects failed to identify the same stimulus as stronger on two consecutive trials was selected as the perceptually equivalent intensity to the step 10 concentration of androstenone. The next higher concentration of the perceptually related compound and step 11 of androstenone were used as the adapting stimuli.

A 2-min rest was imposed following intensity matching. Subjects then rated, using magnitude estimation, the intensities of androstenone step 10 and the intensity-matched stimulus of the perceptually related compound. If magnitude estimates were dissimilar (greater than 20% discrepancy), the matching procedure was repeated. This procedure ensured that the two stimuli were of approximately equivalent intensity for each subject during each test session.

After making the initial magnitude estimates, subjects began to sniff repeatedly the adapting stimulus (either androstenone or the perceptually related compound). Subjects were instructed to breathe at a normal rate, taking a sniff of the adapting stimulus with each incoming breath. Every 15 s during this adaptation period, subjects sniffed and rated a test stimulus between sniffs of the adapting stimulus. The test stimulus, either androstenone or the perceptually related compound, alternated on sequential trials so that subjects made a total of 20 ratings (10 for each test compound) during the 5-min adaptation period. The adapting stimulus was then removed and subjects continued to rate test stimuli every 15 s for the next 5 min to chart any recovery of olfactory function. Subjects thus made a total of 20 ratings during this recovery period.

Similarity ratings procedure

In a single 30 min session, subjects provided similarity ratings comparing androstenone to each of the other tested compounds. Subjects were presented with a horizontal scale 19.0 cm (575 pixels) in length on a monochrome monitor connected to an IBM-compatible computer. Endpoints on the scale were labeled 'No Similarity' and 'Identical'; there were no other markings on the scale. Subjects moved a mouse-driven cursor along the length of the scale to indicate the degree of similarity.

Subjects were instructed to concentrate on individual perceptual notes in common as well as the overall perceptual quality. On a given trial, subjects were presented with two bottles from which they were allowed to sniff *ad libitum*. Subjects then moved the cursor to indicate the extent of similarity and pressed a button on the mouse to enter the response and terminate the trial. The next pair of bottles was presented following a 30 s inter-trial interval.

Subjects rated the degree of similarity for each comparison between androstenone and the other compounds. Androstenone was compared to itself and each of the other compounds twice each, for a total of 14 comparisons. The order of presentation was randomized, with the stipulation that subjects rated each possible comparison once before any repetition of a trial.

Results

Tests of cross-adaptation

Figure 2 indicates for each odorant the concentration step judged perceptually most similar to androstenone step 10 by each subject; there was substantial inter-subject variability. The perceptual matching of sandiff to androstenone illustrates this variability. Overall, the mean sandiff concentration judged perceptually equivalent was step 8; however, the range extended from step 2 to step 12, a difference exceeding three orders of magnitude. Similarly, perceptual matching for the other odorants was highly variable across subjects, with the matching for aldron being most consistent.

Magnitude estimates, plotted as a percentage of the initial magnitude estimates, are presented in Figures 3–5. Self-

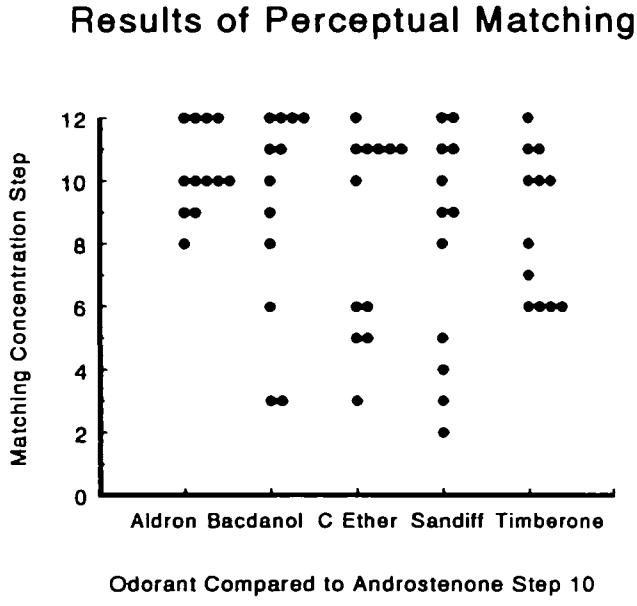

Figure 2 Inter-subject variability in the concentration step of the five urinous odorants in Experiment 1 judged perceptually most similar to androstenone step 10.

adaptation was noted in each session; estimates during adaptation significantly differed from pre-adaptation estimates for each adapting odorant. Androstenone displayed the strongest self-adaptation (Table 1); estimates were reduced to a mean of 33.7% of original estimates in all sessions (range = 27.6–48.1%).

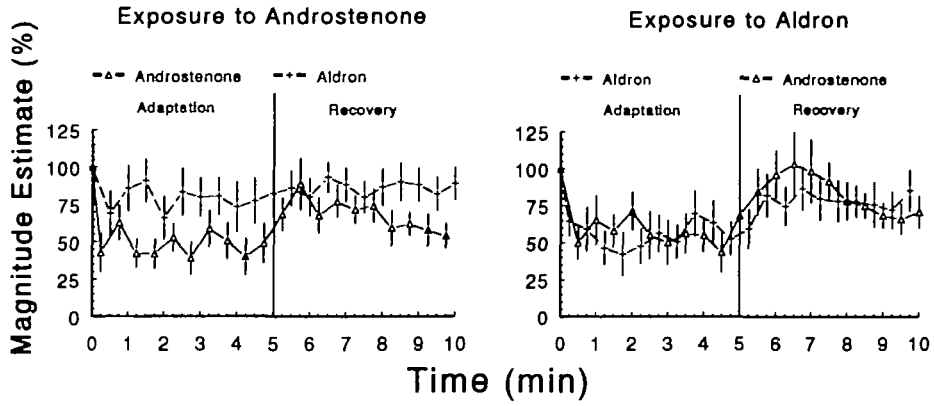

Figure 3 Mean magnitude estimates (with standard errors) as a percentage of the initial estimates for aldron and androstenone following self- and cross-adaptation

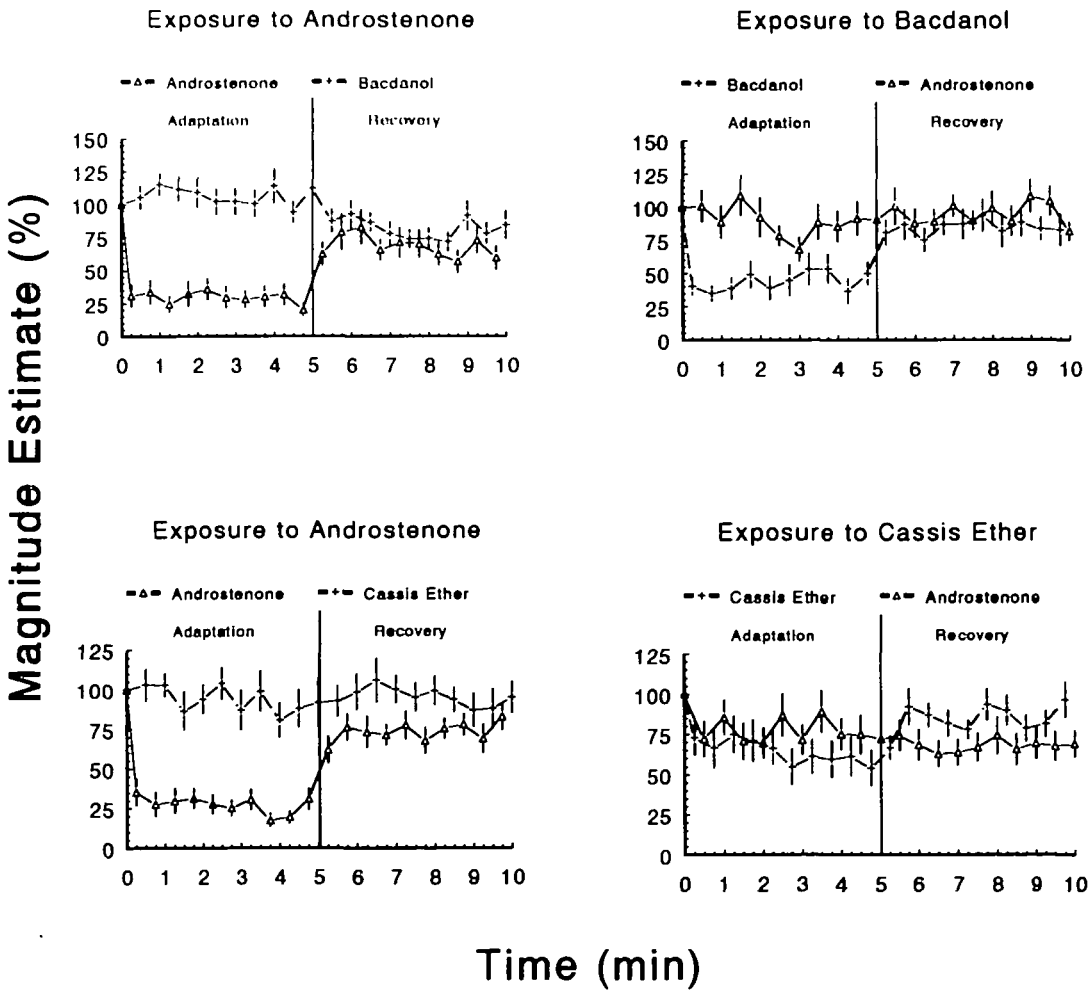

Figure 4 Mean magnitude estimates (with standard errors) as a percentage of the initial estimates for bacdanol, cassis ether, and androstenone following self- and cross-adaptation.

Despite the shared urinous note, significant cross-adaptation was observed only between androstenone and aldron. Androstenone magnitude estimates were significantly reduced following adaptation to aldron (Figure 3; Table 1), but were unaffected by adaptation to the other compounds (Figures 4 and 5; Table 1). Similarly, adaptation to androstenone did not significantly influence magnitude estimates for the other compounds (Figures 3–5; Table 1). Timberone estimates following adaptation to androstenone showed a modest increase, but this cross-facilitation was not statistically significant (Figure 5).

Each compound, with the exception of androstenone, generally showed quick recovery following self-adaptation (Figures 3–5). Androstenone estimates following self-adaptation remained significantly depressed in all sessions, except for the session involving timberone (Figure 5; Table 1). For odorants tested for cross-adaptation, estimates during recovery were not significantly changed from pre-adaptation levels, except for androstenone following adaptation to cassis ether (Figure 4; Table 1) and for sandiff following exposure to androstenone (Figure 5; Table 1).

Similarity ratings

For statistical analysis, all similarity ratings were expressed as a percentage of the scale length. Subjects who failed to rate the androstenone-androstenone comparisons as at least 50% similar were dropped from the analysis. Of 28 individuals tested, 25 subjects met the criterion for inclusion.

Mean similarity ratings, presented in Figure 6, indicate that aldron was rated perceptually most similar to androstenone (74.5%). The other urine-note compounds did not differ in their perceived similarity to androstenone; each was rated only moderately similar (range = 34.4–42.0%). The androstenone-androstenone comparison was rated strongly similar (87.0%), whereas there was little perceived similarity between androstenone and amyl acetate (8.1%; Figure 6).

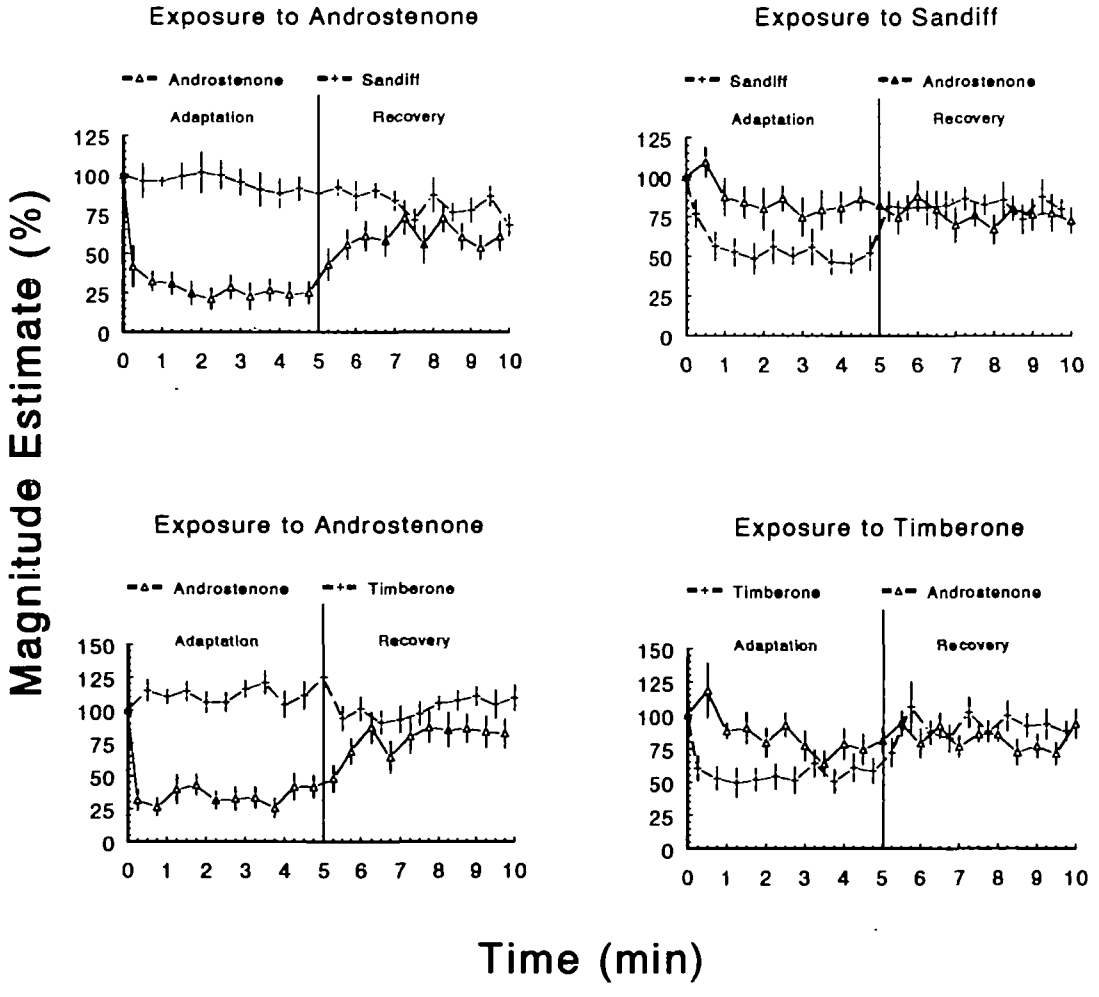

Figure 5 Mean magnitude estimates (with standard errors) as a percentage of the initial estimates for sandiff, timberone, and androstenone following self- and cross-adaptation.

Discussion

Despite sharing a urinous note, four of the five compounds tested in Experiment 1 did not cross-adapt with androstenone in any session; the exception was aldron which cross-adapted androstenone. This finding suggests that simply sharing a common note is insufficient to predict cross-adaptation, provided the compounds retain unique perceptual character-

Table 1 Mean magnitude estimates as a percentage of initial estimates and associated *F*-values for each test odorant during and following adaptation to each of the adapting odorants in Experiment 1

	Adaptation		Recovery	
Condition	*X* (SE)	*F*	*X* (SE)	*F*
Androstenone-aldron				
Androstenone				
Self-adaptation	48.1% (9.17)	32.05***	68.1% (7.58)	17.70***
Cross-adapted	57.4% (12.11)	12.38**	83.3% (12.32)	1.83
Aldron				
Self-adaptation	55.4% (10.18)	19.18***	77.3% (11.88)	3.66
Cross-adapted	79.0% (13.33)	2.49	86.4% (9.86)	1.91
Androstenone-bacdanol				
Androstenone				
Self-adaptation	29.8% (5.99)	137.38***	68.5% (8.37)	14.20**
Cross-adapted	89.4% (8.97)	1.41	95.2% (6.58)	0.54
Bacdanol				
Self-Adaptation	44.2% (7.02)	63.36***	84 9% (8.79)	2.97
Cross-Adapted	106.8% (7.03)	0.93	82.0% (6.70)	7.19
Androstenone-cassis ether				
Androstenone				
Self-Adaptation	27.6% (4.79)	228.31***	73.4% (6.36)	17.45**
Cross-Adapted	77.0% (8 93)	6.66	68.4% (7.04)	20.12**
Cassis ether				
Self-adaptation	64.4% (9.18)	15.00**	84.8% (6 16)	6.13
Cross-adapted	93.7% (9.53)	0.44	95.6% (8.07)	0.30
Androstenone-sandiff				
Androstenone				
Self-adaptation	28.0% (6.64)	117.73***	59.5% (7.05)	33.04***
Cross-adapted	85.1% (7.52)	3.95	76.1% (7.82)	9.31
Sandiff				
Self-adaptation	54.2% (7.56)	36.76***	82.0% (6.84)	6.90
Cross-adapted	94.7% (6.51)	0.65	81.9% (3.61)	25.11***
Androstenone-timberone				
Androstenone				
Self-adaptation	34.9% (7.13)	83.40***	77.1% (7.57)	9.15
Cross-adapted	84.6% (7.55)	4.14	83.0% (6.99)	5.95
Timberone				
Self-adaptation	55.4% (7.79)	32.79***	91.4% (8.17)	1.10
Cross-adapted	113.0% (6.69)	3.78	101.2% (5.51)	0.05

**$P < 0.005$; ***$P < 0.001$.

Note. Table values represent the mean magnitude estimate for an odorant expressed as a percentage of the initial baseline estimates. Each *F*-test compares this mean magnitude estimate with the initial magnitude estimates for that odorant. Degrees of freedom for all *F*-tests = (1,11). The significance level was set at $P < 0.01$ because multiple *F*-tests were performed

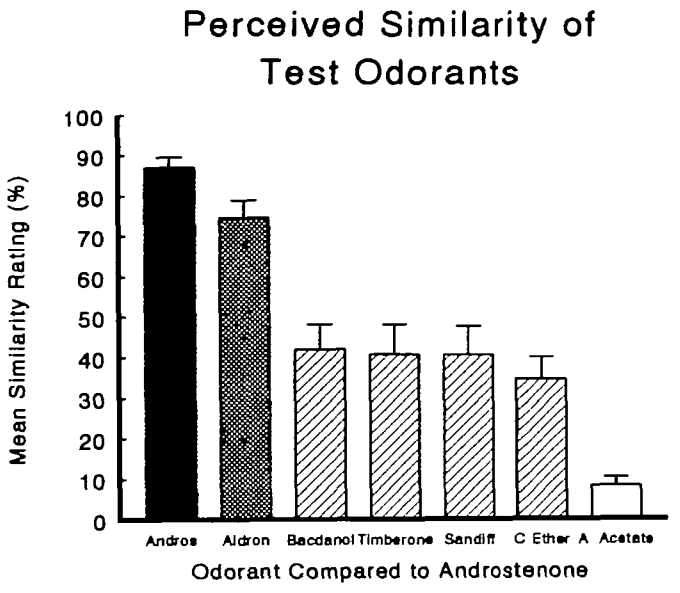

Figure 6 Mean similarity rating (with standard errors) for each comparison between androstenone and the test odorants.

istics. A similar conclusion was reported by Todrank *et al.* (1991) who failed to observe cross-adaptation between Galaxolide and androstenone, although both share a musky note for many people. Rather, cross-adaptation between structurally dissimilar compounds appears to be specific to compounds that share all or many of their perceptual characteristics rather than a single trait.

Aldron, which cross-adapts androstenone, conforms to the structure-activity model of androstenone proposed by Beets and Theimer (e.g. Beets, 1982), suggesting that key structural characteristics may have influenced cross-adaptation. However, sandiff and timberone also show reasonable alignment with this model, yet failed to cross-adapt androstenone. Importantly, of the five odorants sharing characteristics with androstenone, aldron was rated most perceptually similar to androstenone in direct similarity ratings. Whereas cross-adaptation among odorous compounds probably represents both peripheral interaction and central inhibition (see Discus-

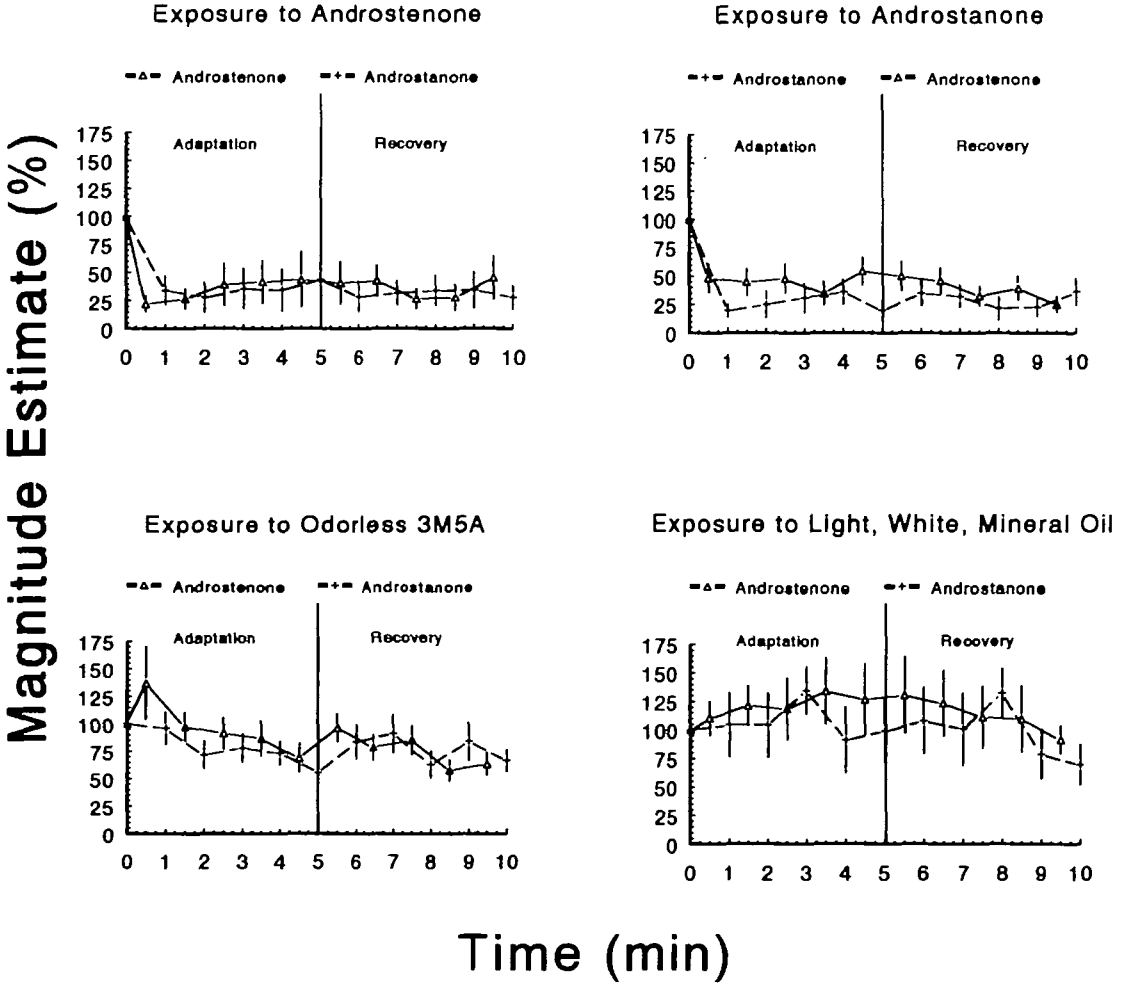

Figure 7 Mean magnitude estimates (with standard errors) as a percentage of the initial estimates for each test odorant following adaptation to each compound.

sion for Experiment 2), it is this strong perceptual similarity that likely underlies the cross-adaptation noted between aldron and androstenone.

Experiment 2

In Experiment 1, compounds differing in their overall perceptual qualities did not cross-adapt. In Experiment 2, we examined the role of structural similarity in producing cross-adaptation by comparing androstenone with 5α-androstan-3-one (androstanone). We also assessed whether a compound must be odorous to cross-adapt an odorous compound, by examining the effects of exposure to 3-methylidene-5α-androstane (3M5A), an odorless structural analog (Zinkevich and Aronov, 1993), on the perception of urinous-smelling compounds.

Methods

Subjects

For tests of cross-adaptation, 12 subjects (six males and six females; mean age of 27.3 years) were recruited, screened, and selected as described above. Six of these subjects had previously served in Experiment 1. To confirm that 3M5A is essentially odorless, 16 subjects (eight males and eight females; mean age of 31.1 years) participated in triangle discrimination tests.

Stimuli

Odorants were synthesized in our laboratory, diluted in mineral oil and presented in polypropylene squeeze-bottles. Each bottle contained 10 ml of the odorant/mineral oil solution.

A 12-step binary dilution series starting at 0.1% w/v was prepared for each odorant: androstenone (step 12: 3.67 mM MW = 272.4; odorant no. 6 in Figure 1), 3M5A (step 12: 3.67 mM; MW = 272.5; no. 8) and androstanone (step 12: 3.64 mM MW = 274.5; no. 7). A 1% solution of androstanone (10 g/l) was prepared for use as an adapting stimulus.

The stimuli for the discrimination tests were 10 ml of a 0.1% solution of 3M5A diluted in mineral oil, and 10 ml of mineral oil.

Triangle discrimination tests

Subjects were tested in a single 15-min session for their ability to discriminate the odorless 3M5A from mineral oil. On a given trial, subjects were presented with three stimuli and instructed to identify which stimulus was different from the other two. Each subject received two blocks of four trials each. For one block of trials, two of the stimuli were 3M5A and the third stimulus was the mineral oil. For the other block, two stimuli were the mineral oil blanks and the third stimulus was 3M5A. Each trial was separated by a 30-s interval to minimize adaptation. Block presentation was counter-balanced across subjects.

Cross-adaptation procedure

Subjects were tested in four 30-min sessions spaced at least 24 h apart. Each session used androstenone (step 10) and androstanone (step 12) as the test odorants. A different adapting stimulus (either step 12 of androstenone, the 1% solution of androstanone, step 12 of 3M5A, or 10 ml of mineral oil) was used for each session. Choice of the adapting odorant was counter-balanced across sessions.

The testing protocol was similar to that of Experiment 1. Subjects rated, using magnitude estimation, the intensities of step 10 androstenone and step 12 androstanone prior to, during, and following exposure to an adapting stimulus. A 30-s interval between ratings, rather than the 15-s interval in Experiment 1, was used to minimize adaptation effects caused by sniffing the test stimulus.

Results

Triangle discrimination tests

To determine whether subjects could discriminate between 3M5A and a blank, a criterion of four correct discriminations in one block of four trials or seven of eight correct across both blocks of trials ($P < 0.05$) was used. By this criterion, 14 of 16 subjects failed to discriminate between the odorless 3M5A and mineral oil. The mean number correct was 3.6 of 8 trials. Tests when the 3M5A was the signal yielded slightly better performance (mean of 2.1 correct per four trials) than did tests when the blank was the odd stimulus (mean of 1.6 correct per four trials). Two subjects were correct on all eight trials; no other subject got more than five of eight correct.

Tests of cross-adaptation

Each magnitude estimate was converted to a percentage of the initial magnitude estimate for that odorant; the results are presented in Figure 7. Each odorous compound showed significant self-adaptation (Table 2) which occurred rapidly, continued through the adaptation period and persisted following the removal of the adapting stimulus.

Significant, mutual cross-adaptation was observed

between the structurally and perceptually similar androstenone and androstanone. Following exposure to androstenone, the perceived intensity of androstanone (Table 2) was quickly reduced (estimates were 34.4% of initial estimates within 1 min of exposure) and remained depressed for the duration of the adaptation period. Further, perceived intensity remained depressed following removal of the adapting stimulus. Estimates during the recovery phase did not differ significantly from estimates made during adaptation, and differed significantly from initial estimates.

Androstenone displayed a similar pattern of cross-adaptation following exposure to androstanone (Table 2). A significant reduction in magnitude estimates was noted within 1 min of exposure (48.3% of initial estimates), continued for the duration of the adaptation period and persisted following removal of the adapting stimulus.

Exposure to the odorless 3M5A resulted in significantly reduced estimates for androstanone over the course of the adaptation period (Table 2). In contrast to the cross-adaptation induced by the odorous compounds, the cross-adaptation induced by 3M5A was more gradual and less extensive. Thus, androstanone estimates displayed a mean reduction in intensity of 4% following 60 s of exposure to 3M5A and an overall reduction of 25% during the adaptation period. The effect of 3M5A was apparent only on androstanone; androstenone estimates following 3M5A exposure did not differ from initial estimates. During the recovery period, estimates for both odorants did not differ significantly from initial estimates (Table 2).

There was no effect of exposure to the odorless mineral oil on the perception of the intensity of either androstenone or androstanone during either the adaptation period or recovery (Figure 7; Table 2).

Discussion

Androstenone and androstanone displayed strong, symmetrical cross-adaptation. This finding was expected, given the extent of perceptual and structural similarity. Of particular interest, however, was the significant reduction in the perception of androstanone following exposure to the odorless analog 3M5A. This observation, that an odorless compound can affect via cross-adaptation the perceived intensity of an odorous compound, appears to be the first demonstration of a true olfactory antagonist.

Odorless compounds have been shown to affect the perception of odorous compounds. Hill (1977) noted that perfumers commonly add odorless components to change the fragrance of a perfume mixture, as 'fragrance is affected by molecules which in and of themselves have little or no aroma' (p. 3). Alterations in EEG activity have been noted following nasal inhalation of room air (Werntz *et al.*, 1983;

Table 2 Mean magnitude estimates as a percentage of initial estimates and associated *F*-values for each test odorant during and following adaptation to each of the adapting odorants in Experiment 2

	Adaptation		Recovery	
Odorant	*X* (SE)	*F*	*X* (SE)	*F*
Exposure to androstenone				
Androstenone	34.9% (14.47)	20.22***	36.9% (13.36)	22.33***
Androstanone	35.3% (15.58)	17.25**	31.5% (11.38)	36.28***
Exposure to androstanone				
Androstenone	46.2% (9.20)	34 22***	38.8% (8.81)	48.25***
Androstanone	26.4% (8.50)	74.99***	29.7% (8.72)	65.06***
Exposure to the odorless 3M5A				
Androstenone	96.3% (13.50)	0.07	76.2% (9.03)	6.93
Androstanone	75.0% (8.55)	8.57*	77.7% (9.86)	5.12
Exposure to light, white, mineral oil				
Androstenone	122.4% (22.18)	1.02	113.6% (24.98)	0.30
Androstanone	116.3% (40.90)	0.16	98.3% (30.64)	0.00

*$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

Note. Table values represent the mean magnitude estimate for an odorant expressed as a percentage of the initial baseline estimates. Each *F*-test compares this mean magnitude estimate with the initial magnitude estimates for that odorant. Degrees of freedom for all *F*-tests = (1,11) The significance level was set at $P < 0.01$ because multiple *F*-tests were performed.

Lorig and Schwartz, 1988), sub-threshold concentrations of odorants (Lorig *et al.*, 1990; Schwartz *et al.*, 1992b, 1993, 1994b), and perhaps of androstenone in androstenone-anosmic subjects (Schwartz *et al.*, 1992a, 1994a), suggesting that odors that are undetected can affect CNS activity.

The magnitude and time course of cross-adaptation observed between 3M5A and androstanone appears to be qualitatively different from that observed between two odorants; cross-adaptation was more gradual and less extensive following exposure to the odorless compound. Cross-adaptation produced by structural versus perceptual analogs may represent a peripheral versus central distinction. Cross-adaptation among odorous compounds entails a central component; unilateral adaptation leads to a reduction in perception in the contralateral naris (Koster, 1971). Perhaps the effect of the odorless 3M5A, as a structural analog, is solely peripheral (see General Discussion), whereas cross-adaptation among odorous compounds represents both peripheral interaction and central inhibition.

The finding that structural similarity, even in the absence of a perceptible odor, can result in cross-adaptation is consistent with the work of Pierce *et al.* (1995) which demonstrated that the perception of the sweaty-smelling 3M2H is reduced by exposure to the fruity-smelling ethyl esters of 3M2H. In Experiment 3, we further studied the role of structural similarity by testing structurally similar odorous compounds with different perceptual qualities.

Experiment 3

Aronov and Zinkevich (1993) performed a structure-activity analysis of molecular topology and geometry of androstenone and androstenone-like compounds. The results of this analysis led to a predicted model identifying several key molecular requirements for an androstenone-like odor, with one key requirement being the presence of an extra methyl group in an equatorial α-position of cyclic ketones.

The proposed model was confirmed by the synthesis of DMCMC, a racemic mixture of the isomers 4(R)-(4′,4′-dimethylcyclohexyl)-2(R)-methylcyclohexanone and 4(S)-(4′,4′-dimethylcyclohexyl)-2(S)-methylcyclohexanone. This compound, although structurally different from androstenone, possesses the extra methyl group and, thus, satisfies the key requirements of the model. Furthermore, DMCMC mutually cross-adapts and is perceptually indistinguishable from androstenone (Aronov and Zinkevich, 1993; Pierce *et al.*, 1993).

In further tests of their model, Aronov and Zinkevich (1993) synthesized a compound which lacks the extra methyl group but is otherwise identical in structure to DMCMC. This compound, 4-cyclohexylcyclohexanone (4-CHCH; odorant no. 10 in Figure 1), is perceptually distinct from DMCMC; subjects report a citrus-like odor. Since this methyl group is a key determinant of the resulting perceptual quality (Aronov and Zinkevich, 1993), it may also affect cross-adaptation between DMCMC and 4-CHCH. In Experiment 3, these odorants were tested for cross-adaptation.

Method

Subjects

Twelve subjects (five males and seven females; mean age of 25.7 years) were recruited, screened, and selected as described above. Four of these subjects previously had served in either Experiment 1 or Experiment 2.

Stimuli

Odorants were diluted in mineral oil and presented in polypropylene squeeze-bottles. Each bottle contained 10 ml of the odorant/mineral oil solution.

Androstenone, DMCMC, and 4-CHCH were synthesized in our laboratory and diluted in 12-step binary series with an initial concentration of 0.1% w/v (step 12: 1 g/l; 3.67 mM for androstenone [odorant no. 6 in Figure 1], 4.5 mM for DMCMC [9], and 5.14 mM for 4-CHCH [10]). Dilutions of *d*-limonene (IFF), started at 2.1% v/v (step 12: 0.21 mg/l; 139mM [11]).

Procedure

Subjects were tested in four, 30-min sessions spaced at least 24 h apart using the cross-adaptation procedure described in Experiment 1. The structurally similar compounds, DMCMC and 4-CHCH, were used as test odorants in two sessions with each of these compounds serving as an adapting odorant in one session. Another session used 4-CHCH and *d*-limonene as test odorants and androstenone as the adapting odorant. A fourth session used *d*-limonene and androstenone as test odorants and 4-CHCH as the adapting odorant. Choice of the adapting odorant was counter-balanced across sessions.

Results

Each magnitude estimate was converted to a percentage of the initial magnitude estimate for that odorant; the resulting percentages are presented in Figure 8 and Table 3. Strong self-adaptation occurred rapidly and continued for the duration of the adaptation period for both DMCMC and 4-CHCH.

There was significant recovery following self-adaptation for 4-CHCH, but not for DMCMC (Table 3).

There was no significant cross-adaptation observed between DMCMC and 4-CHCH (Table 3). Likewise, there was no significant cross-adaptation observed on *d*-limonene or 4-CHCH when androstenone was used as the adapting stimulus. Following adaptation to 4-CHCH, there was no effect on the perception of *d*-limonene, but, surprisingly, the perception of androstenone was significantly reduced (Table 3).

General discussion

A central tenet guiding much of the cross-adaptation research over the past century has been the underlying assumption that cross-adaptation reflects the degree to which odors share common sensory receptors or mechanisms (Moncrieff, 1956; Engen, 1982). As such, there should be a strong relationship between cross-adaptation and similarity, both perceptual and structural. Yet, whereas previous research has generated data consistent with this proposed relationship, numerous exceptions exist.

In part, the difficulty has been how best to define and quantify similarity. Perceptual similarity, for example, can entail more than a simple, unitary continuum based on overall perceptual quality. In many instances, an odorant contains different perceptual notes and thus elicits the perception of different odor qualities. In such cases, an odorant may share a common perceptual note with other odorants, yet still be perceptually distinct. A pair sharing a single note, but having other distinct perceptual characteristics, may be placed on the low end of a unidimensional scale, obscuring the strong similarity on one characteristic of the odor perception. Conversely, compounds may share

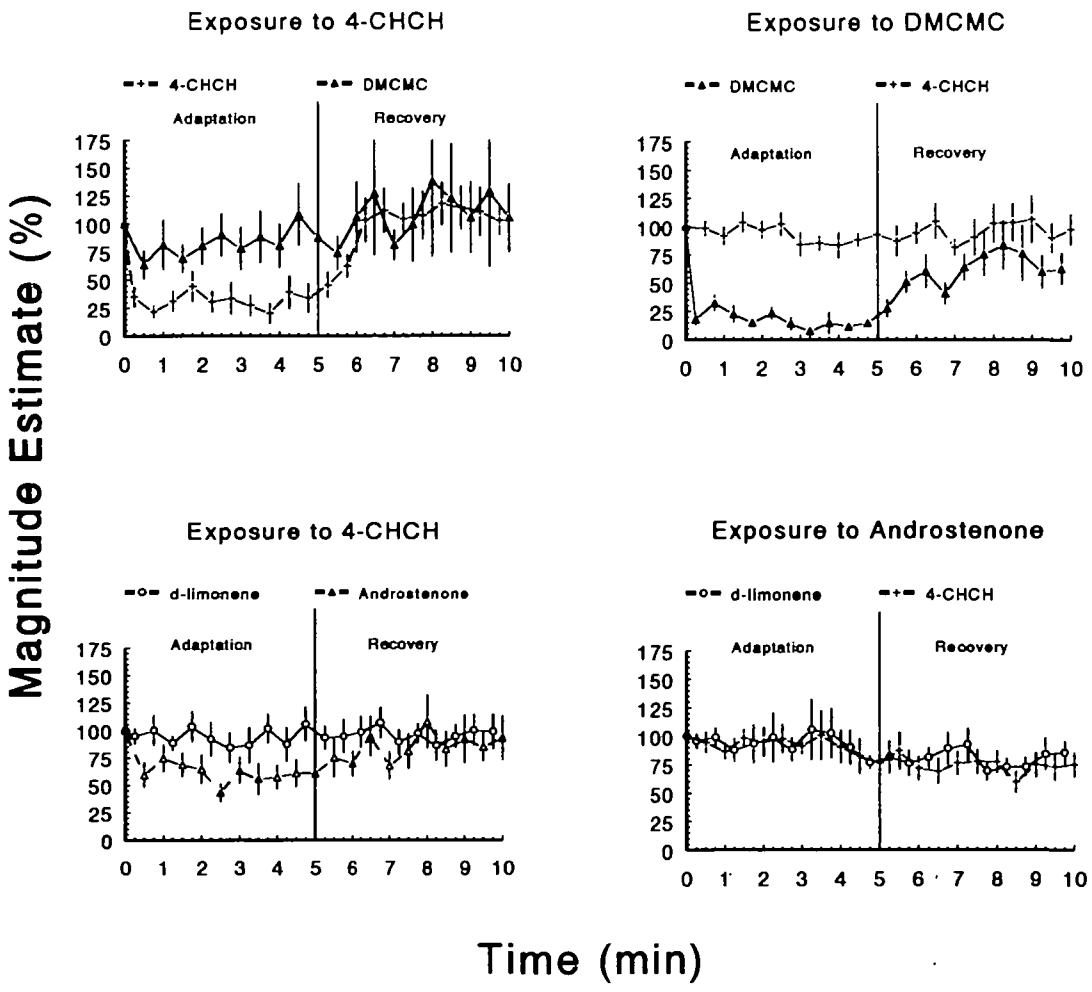

Figure 8 Mean magnitude estimates (with standard errors) as a percentage of the initial estimates for each test odorant following adaptation to each compound.

a dominant perceptual note but have important perceptual distinctions that may be overshadowed on a unidimensional scale. Structural similarity, likewise, encompasses numerous structural relationships among odorants (e.g. the size and shape of parts of the molecule, lipophilosity, electron charge distributions, etc.), and compounds may be similar on some characteristics while differing on others. The use of a unitary concept such as *similarity* to describe the complex relationship between odorants, then, belies the myriad facets of odor quality and odorant structure.

The present results can be viewed in this context. The results of Experiment 1 show that compounds that share perceptual qualities must do so to a significant degree in order to cross-adapt. Thus, the perception of the tested compounds (aldron, bacdanol, cassis ether, sandiff and timberone) was not affected by exposure to androstenone, in spite of their sharing a urinous note. The other dominant qualities of these compounds were retained following exposure to androstenone. Similarly, the primary urinous quality of androstenone was not affected by exposure to the tested compounds, except for aldron which, of the odors tested, is perceptually most similar to androstenone. The present results do not preclude the possibility that the tested compounds share some sensory receptors or mechanisms with androstenone; in fact, the urinous note is probably conveyed through similar pathways. However, continued responding via pathways that mediate the dominant odor qualities would be sufficient to maintain overall intensity, even given a reduction in responding through the shared receptors or mechanisms.

In a similar manner, the results of Experiment 3 suggest that differences in one key structural characteristic are sufficient to prevent cross-adaptation among otherwise structurally similar compounds. The structurally similar odorants DMCMC and 4-CHCH failed to cross-adapt, perhaps due to the attached methyl group present in DMCMC. This feature has previously been shown to be a critical molecular requirement for an androstenone-like odor (Aronov and Zinkevich, 1993) and may cause DMCMC and 4-CHCH to stimulate different receptor populations. Thus, as is the case for perceptual qualities, odorants must share substantial and key structural similarities in order to cross-adapt. Identifying the key molecular requirements necessary for cross-adaptation among structural analogs will have broad implications for structure-activity relationships in olfaction.

We further demonstrated that perceptual similarity is not necessary for cross-adaptation. Indeed, the results of Experiment 2 suggest that a cross-adapting compound need not even be perceptible to affect the perception of odorous compounds, as the structurally similar, odorless 3M5A cross-adapted the odorous androstanone. This cross-adaptation was more gradual and less extensive than that typically seen

Table 3 Mean magnitude estimates as a percentage of initial estimates and associated *F*-values for each test odorant during and following adaptation to each of the adapting odorants in Experiment 3

	Adaptation		Recovery	
Odorant	*X* (SE)	*F*	*X* (SE)	*F*
Exposure to 4-CHCH				
4-CHCH	31.8% (8.33)	67.04***	98.2% (14.72)	0.02
DMCMC	82.9% (15.66)	1.20	108.7% (38.51)	0.05
Exposure to DMCMC				
DMCMC	17 6% (3.71)	493.94***	60.3% (12.80)	9.61*
4-CHCH	92.9% (6.26)	1.30	95.9% (12.09)	0.11
Exposure to Androstenone				
4-CHCH	91.6% (7.71)	1.19	74.5% (9.64)	7.01
d-limonene	94.0% (11.64)	0.27	80.9% (10.15)	3.56
Exposure to 4-CHCH				
d-limonene	94.4% (10.70)	0.28	95.9% (11.02)	0.14
Androstenone	60.9% (7.78)	25.26***	84.8% (14.51)	1.09

*$P < 0.01$; **$P < 0.005$; ***$P < 0.001$

Note. Table values represent the mean magnitude estimate for an odorant expressed as a percentage of the initial baseline estimates. Each *F*-test compares this mean magnitude estimate with the initial magnitude estimates for that odorant. Degrees of freedom for all *F*-tests = (1,11). The significance level was set at $P < 0.01$ because multiple *F*-tests were performed.

for perceptual analogs and may represent a peripheral process; 3M5A may act as an antagonist, binding to receptors to make them non-functional in a manner similar to that noted in pharmacological investigations (e.g. Snyder, 1978). Alternatively, the odorless compound may modify receptor activity through potentiation effects. Similar effects may explain other cases of cross-adaptation between structurally similar compounds (Pierce *et al.*, 1995).

Finally, the results of Experiment 3 provide a sobering caveat; the relationship between similarity and cross-adaptation is neither straightforward nor simple. The absence of cross-adaptation between 4-CHCH and either *d*-limonene or DMCMC was somewhat surprising, given that these compounds shared either perceptual or structural similarities with 4-CHCH. More troubling was cross-adaptation between 4-CHCH and androstenone in the absence of any apparent structural or perceptual similarity. That this cross-adaptation was not due to any generalized fatigue resulting from exposure to 4-CHCH is evident from the fact that 4-CHCH failed to cross-adapt either *d*-limonene (in the same session) or DMCMC (in a different session). Similarly, the cross-adaptation is not attributable to a generalized tendency for androstenone to adapt quickly in response to any odorant stimulation; androstenone does not cross-adapt following exposure to either Galaxolide (Todrank *et al.*, 1991), amyl acetate (Pierce and Wysocki, 1991), or four of the five odorants with a urinous note tested in Experiment 1. Rather, the cross-adapting relationship between 4-CHCH and androstenone is a specific one, and difficult to explain by a similarity hypothesis.

The fact that cross-adaptation between 4-CHCH and androstenone was asymmetrical is a further difficulty for a similarity hypothesis (Cain and Polak, 1992; Pierce *et al.*, 1995). Asymmetries in cross-adaptation are relatively common (Cain and Engen, 1969; Cain, 1970; Koster, 1971; Todrank *et al.*, 1991; Pierce *et al.*, 1995) and may represent a situation of partial overlap of sensory pathways (Pierce *et al.*, 1995; Todrank *et al.*, 1991). Input via independent sensory pathways apparently was sufficient to maintain perceived intensity for 4-CHCH following exposure to androstenone, but insufficient for the perception of androstenone following exposure to 4-CHCH.

Cain and Polak (1992) have described a similarity hypothesis of cross-adaptation as one that 'neither will nor should die easily' (p. 488). Its persistence after decades of decidedly mixed support testifies to its elegance as an explanatory concept for the common perceptual experience of cross-adaptation. What continues to lie ahead is the daunting task of identifying the complexities involved in the diverse attributes of similarity.

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