Pemenone and Androstenone do not Cross-adapt Reciprocally

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Abstract

Explorations of the qualitative and quantitative differences between the odors of pemenone (PEM), androstenone (AND) and isovaleric acid (IVA) show that they share a number of common perceptual characteristics. Among these are similarities in their odor quality and relative intensity ratings. PEM is also an efficient cross-adaptor and modulator of a subject's AND sensitivity. Here we evaluate the reciprocal efficacy of AND adaptation to alter the perceived intensity and quality of PEM, IVA and AND. Twenty-three people, including both those osmic and allosmic (n = 11) for the putrid odor quality of PEM, were tested. Following training in odor quality and intensity rating techniques, subjects sampled a selected substance for 2 min to obtain adaptation and then reported quality and intensity ratings for the three test stimuli. There was significant self-adaptation by PEM and IVA in all subjects, but self-adaptation by AND was only observed in the PEM-osmic subjects. AND did not cross-adapt PEM or IVA to any significant extent. Collectively, these results contrast with our earlier study in which PEM was an efficient cross-adaptor of AND. Here, AND was no more efficient than the control as an adapting substance for PEM, despite significant self-adaptation of PEM by itself. This lack of reciprocity in the effectiveness of PEM and AND as cross-adapters is not related to differences in odor intensity, as the PEM and AND concentrations were adjusted for each subject to elicit comparable intensity reports. These results support the notion that PEM, AND and IVA share certain perceptual characteristics, but interact differentially with three or more sets of perceptual channels that are now thought to result in a putrid odor quality. **Chem. Senses 21: 711–717, 1996.**

Introduction

We continue to explore the perceptual relations among the odors of the diastereoisomeric ketone, *cis*-4-(4′-t-butylcyclohexyl)4-methyl-2-pentanone (pemenone, PEM), 5α-androst-16-en-3-one (androstenone, AND) and isovaleric acid (IVA), all of which share, in individuals osmic for pemenone, a pronounced urine–sweaty type odor (Ohloff *et al.*, 1983a, b; Wysocki and Beauchamp, 1984; O'Connell, 1991). We previously determined intensity ratings and quality reports for suprathreshold (O'Connell *et al.*, 1989) and threshold concentrations (Stevens and O'Connell, 1991) of these compounds and several other materials described as urinous or which were said to exhibit specific anosmias or allosmias (Ohloff *et al.*, 1983b; O'Connell *et al.*, 1994). Principal-component analyses of these data revealed significant relationships between near-threshold and suprathreshold intensity scores for PEM, AND, IVA and several other odorants. An analysis of the verbal odor descriptors used to characterize these materials by subjects judged to be osmic or allosmic for the urinous note showed a corresponding clustering. Similar clusters were also generated with a semantic-free scaling technique (Stevens and O'Connell, 1996). We also demonstrated that PEM is an efficient cross-adaptor of a subject's AND sensitivity (O'Connell *et al.*, 1994) and that regular PEM exposure increases the AND sensitivity of some subjects (Stevens and O'Connell, 1995).

These observations reveal that single compounds, like PEM, AND or IVA, may share a general odor quality label but may still elicit widely different intensity and specific quality reports among individual observers. These differences may arise because several different interactions are possible between a stimulus molecule and the process responsible for its detection and coding (Beets, 1970, 1974; Griffiths and Patterson, 1970; Polak, 1973; O'Connell, 1991). Thus, the number of secondary odor qualities reported by osmics and allosmics exposed to a particular compound could be an indirect measure of the total number of different molecular interactions that the odorant may normally elicit across the whole population of olfactory afferents (Guillot, 1948). One could then argue on the basis of these psychophysical observations that PEM normally interacts with multiple perceptual channels which collectively give rise to odor quality descriptions labeled urinous, floral or herbal. Thus allosmic individuals who, for example, perceive a floral quality for PEM or AND may do so because they lack one or more of the perceptual channels which normally contribute to the perception of putrid odors or because they have a proportionately greater number of channels sensitive to floral odors than are typically found in osmic individuals who normally characterize these materials as urinous (Amoore, 1966).

Adaptation techniques, which involve the presentation of strong stimuli for prolonged periods of time, have long been employed to characterize sensory systems, especially those in which multiple afferent pathways are responsible for the perception of particular stimuli (Titchener, 1910; Moncrieff, 1956; Cain and Engen, 1969; Cain and Polak, 1992). These techniques are most often evaluated in the intensive domain (Berglund *et al.*, 1978), where the adapting stimulus subsequently reduces the behavioral effectiveness or perceived intensity of the same (self-adaptation) or another (cross-adaptation) test stimulus (Boring, 1942; Amoore, 1966; Cain, 1970; Pierce *et al.*, 1993). Sensory adaptation is typically interpreted as evidence that the adapting and adapted stimuli are processed by the same perceptual channels (Titchener, 1910; Engen, 1982; Ohloff *et al.*, 1983b; Todrank *et al.*, 1991; Cain and Polak, 1992). Both reciprocal and non-reciprocal cross-adaptation have been found (Moncrieff, 1956; Cain, 1970). We interpret reciprocal cross-adaptation between two odors as evidence that both stimuli are encoded by the same set of perceptual channels. In a like fashion, non-reciprocal cross-adaptation is taken as evidence that both stimuli are encoded by overlapping but non-identical sets of perceptual channels. Differences in the magnitude and relative effectiveness of one odor as an adaptor of another provides information about the extent and degree of overlap in the perceptual channels involved with their processing.

The present study continues our intensive and qualitative investigations of the adaptive effects of PEM and AND, both on themselves and on IVA. Given the differences already noted among these urinous odorants, including an indication that AND elicits fewer odor descriptors than PEM and thus can be considered a less complex odor, we reasoned that cross-adaptation between PEM and AND might not be reciprocal. We hypothesized that a compound (like AND) that elicits a smaller number of odor descriptors across a population of subjects should be less effective in cross-adapting an odorant (like PEM) that shares these and additional odor descriptors because the simpler odorant appears to interact with fewer communication channels. We had already demonstrated a lack of adaptation of IVA by PEM, suggesting that these two urinous compounds did not share common communication channels (O'Connell *et al.*, 1994). Here we investigated whether AND would be as efficient a cross-adaptor of PEM as PEM was of AND, and moreover, if it would cross-adapt IVA. A finding of reciprocal cross-adaptation between AND and PEM or between AND and IVA would require a revision in our multi-channel model of the processing of urinous odor qualities.

Materials and methods

Subjects

Volunteers were first screened by asking them to sniff a swab containing 150 μl of 390 μM PEM and to report the quality and intensity of its odor. Suggested quality labels were provided with an open-ended list of 51 odor quality descriptors (Stevens and O'Connell, 1991) with a space to write in additional descriptors. Intensity judgements were indicated on a nine-point line scale with the labels 'no odor, faint, moderate, strong and very strong' marked at points 1, 3, 5, 7 and 9 respectively. The subjects sniffed the PEM swab, circled (or wrote in) the selected descriptor of the odorant's quality and then circled a point on the line scale representing its intensity. As a result of this screening, 12 subjects were found who reported a putrid odor for PEM (e.g. rancid, sweaty, urine) and were classed as osmics. Eleven additional subjects reported a non-putrid odor quality (usually floral, fruity or green-vegetable-like) and were classed as allosmic (O'Connell *et al.*, 1994). All of the subjects were students at Clark University. They ranged in age from 19 to 45 years and included 19 females.

Stimuli

All of the compounds used as stimuli were presented to subjects as liquid dilutions on polyester swabs, each holding ~150 µl of odorant, sealed in individual test tubes (Falcon no. 2078). A set of 14 odor quality training odorants were diluted in water or mineral oil to produce stimuli of moderate and approximately equal odor intensity (Table 1). Each of the three test compounds were prepared as binary dilutions in mineral oil. The concentrations available for testing were: PEM (100 mM), AND (0.34, 1.35, 2.70 and 5.40 mM) and IVA (0.24 mM). The stimuli were stored at 4°C and allowed to reach room temperature (22°C) before each testing session.

Table 1 Odorants used in odor identification training

Training set	Verification set	Quality category
1. Butyric acid	8. Caproic acid	Putrid
2. Orange	9. Lemon	Fruity
3. Melon	10. Strawberry	Fruity
4. Wintergreen	11. Camphor	Minty
5. Galbanum	12. Pinene	Woody
6. Lavender	13. Muguet	Floral
7. Green Bean	14. Celery	Vegetable

Testing procedures

Each subject was evaluated in three stages.

Stage 1 provided practice in odor quality identification for the 14 odorants to insure that all of the subjects were familiar with and capable of providing appropriate odor quality and intensity reports (O'Connell *et al.*, 1994). Subjects rated the intensity of each odorant on a nine-point scale which ranged from 'no odor' to 'very strong' and gave a quality label from a list of odor names (Table 2) which included both specific (e.g. rose) and general terms (e.g. floral). Either type of descriptor was allowed. During all stages of testing a minimum 30 s pause was enforced between sampling individual swabs to avoid adaptation effects. The 14 odorants were presented in two sets of seven odorants. The first set was used as a training tool by providing selected feedback to the subject concerning an appropriate label for each test compound. This insured that the subject used internally consistent labels for their individual odor perceptions. One or two passes through the training set were generally required before all of the subjects were providing modal quality descriptors. The second set of odorants was then presented, without feedback, to verify that modal descriptors continued to be employed.

Stage 2 of testing was designed to confirm the subject's osmicity for PEM and to determine the concentration of AND which most closely matched the intensity of the odor report for the PEM test sample evaluated. After providing a quality descriptor and an intensity rating for the 100 mM

Table 2 Quality categories and odor descriptors available during training and testing sessions

Putrid	Vegetable	Floral	Woody	Minty	Fruity
Sweaty	Green pepper	Rose	Pine	Wintergreen	Lemon
Urine	Cucumber	Lavender	Cedar	Peppermint	Orange
Rancid	Celery	Violet	Sandalwood	Camphor	Strawberry
Sour	Green bean	Lilac	Hickory	Eucalyptus	Cherry
Fecal	Cabbage	Muguet	Balsam	Menthol	Melon
Other	Other	Other	Other	Other	Other

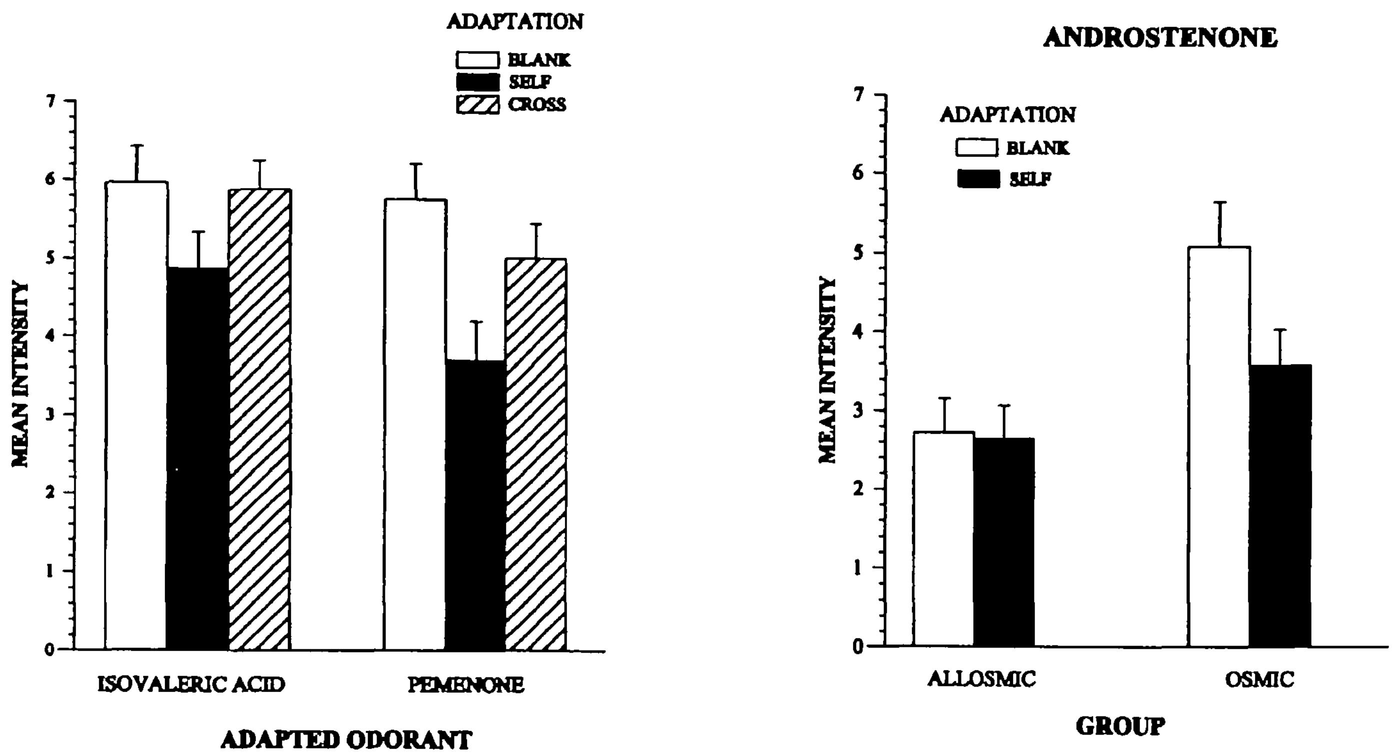

Figure 1 **(A)** Mean odor intensity ratings for IVA and PEM following the three adaptation treatments. Blank, following 2 min exposure to the diluent (mineral oil); Self, following exposure to the same compound (self-adaptation); Cross, following exposure to AND (cross-adaptation). Error bars are SEMs. **(B)** Mean odor intensity ratings for AND in PEM allosmic and osmic subjects following 2 min exposure to the Blank and following exposure to AND (Self). Error bars are SEMs.

PEM sample, the AND concentration series was evaluated in ascending order. The concentration of AND which best matched the intensity rating of the PEM sample was then used as the adapting stimulus in the cross-adaptation trials for that subject. Again, there was a minimum 30 s wait between stimuli.

Stage 3 consisted of three blank (mineral oil) adaptation trials, three self-adaptation trials and two AND cross-adaptation trials. Each type of adaptation was presented as a block of trials presented to each subject in random order. The first swab of a block (blank, test odor or AND) was given to the subject, who was instructed to sniff it quickly two or three times, first rating the odor intensity of the swab on the nine-point scale and then recording the odor quality using the list of 51 odor descriptors (Stevens and O'Connell, 1991). The subject continued to sniff this adapting swab for 2 min, after which the intensity and quality of the last few sniffs were noted. The subject returned the adapting swab to its tube and immediately sniffed a test swab that contained one of the three odorants, again rating the intensity and recording the perceived odor quality. Following these judgements the test swab was resealed and a 1 min interblock intermission began. The subjects were reminded periodically about their task as testing proceeded. The three stages of testing were normally accomplished in a single 40 min test session.

Results and discussion

The mean intensity scores for each of the test odorants in the different adapting conditions are shown in Figure 1. Two-way ANOVAs, done separately for the three test odorants (IVA, PEM and AND), with the adaptation condition and PEM osmicity classification as the main sources of variance, are summarized in Table 3. While there was self-adaptation of PEM, there was no significant cross-adaptation of it by AND. The same results were found for IVA—significant self-adaptation of IVA but no cross-adaptation with AND (Figure 1A). PEM-osmic subjects gave significantly higher intensity reports for PEM than did the allosmic subjects, but this variable did not interact with

Table 3 Summaries of analyses of variance

Source	Isovaleric acid			Pemenone			Androstenone		
	df	MS	*F*	df	MS	*F*	df	MS	*F*
Classification	1	0.41	0.04	1	32.85	5.16*	1	31.31	8.06**
Ss w/class.	21	10.76	21	6.37	21	3.89			
Adaptation	2	8.43	5.76**	2	23.91	9.41***	1	7.26	5.66*
Class. adapt.	2	0.23	0.16	2	4.00	1.57	1	5.70	4.44*
Class. adapt. w/Ss	42	1.46	42	2.54	21	1.28			

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

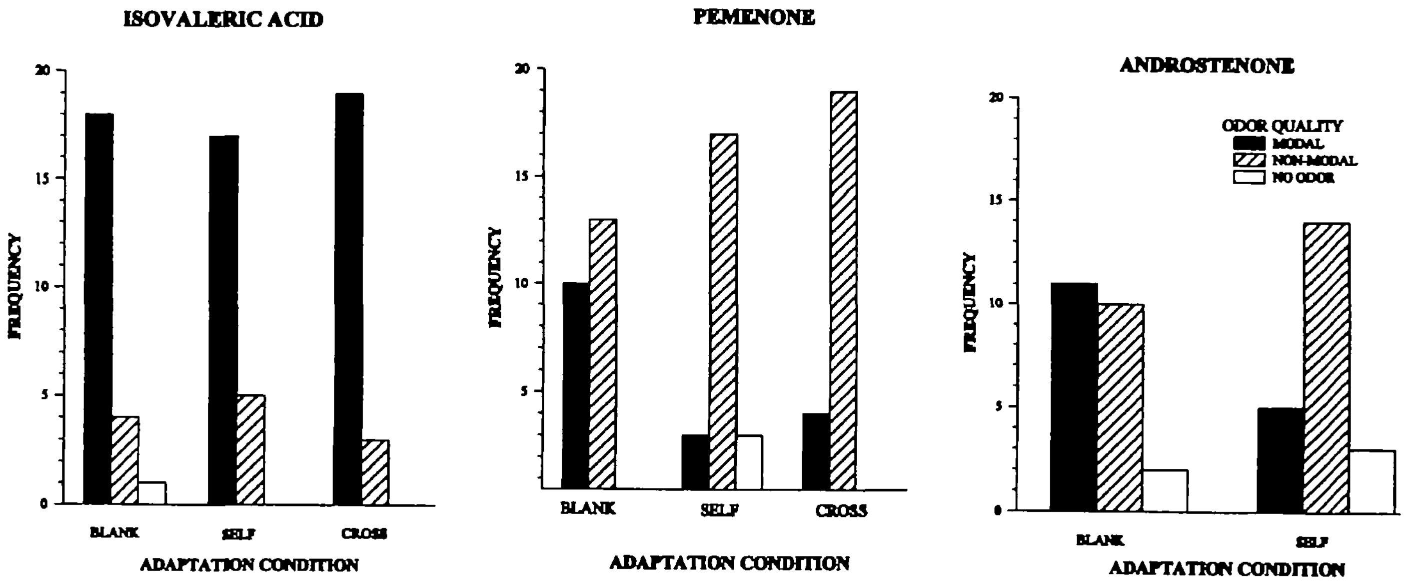

Figure 2 Frequency histograms of the odor quality reports for IVA **(A)**, PEM **(B)** and AND **(C)** following the various adaptation treatments. Modal descriptors included those subsumed under the putrid category in Table 2, Non-Modal descriptors include all of the remaining descriptors listed in Table 2. No Odor is the descriptor used for odor qualities too faint to classify.

the adaptation condition. For IVA, the intensity reports of PEM-osmic and allosmic individuals were comparable. PEM-osmic subjects gave significantly higher intensity reports for AND than did the allosmic subjects. This variable had a significant interaction with the adapting condition as self-adaptation of AND was only observed in subjects initially classified as PEM-osmic (Figure 1B).

Collectively these results reveal that AND failed to elicit significant amounts of cross-adaptation in the intensity ratings for either PEM or IVA. All of these compounds share the putrid odor quality label in osmic individuals. This observation is in contrast with certain aspects of our earlier findings where a significant amount of cross-adaptation was observed in the intensity rating of AND following PEM exposure. Here, exposure to AND was found to affect the intensity ratings of PEM and IVA to a far smaller extent, if at all, than exposure to PEM affects the intensity ratings of AND.

These adaptation techniques thus provided a window into the mechanisms underlying odor perception and the results collectively suggest that the three modally putrid smelling compounds evaluated here must interact with at least three different sets of perceptual channels. One set is involved with the perception of the urinous notes apparently shared by PEM and AND. In the case of PEM, this shared set accounts for only a small proportion of the total urinous intensity. Another set of channels represents those urinous notes apparently unique to PEM and are revealed by the lack of a symmetrical relationship in the cross-adaptation potency of these two compounds. A third set of channels must also exist to account for the urinous qualities of IVA, nearly all of which appear to be independent of those associated with PEM and AND.

Adaptation treatments generally lead to reductions in odor intensity reports (Berglund *et al.*, 1978; Todrank *et al.*, 1991; Cain and Polak, 1992). The odor descriptors used by subjects do not usually shift appreciably after adaptation, except for shifts to the 'no odor' category. This initially suggests that adaptation operates largely in the intensity domain and that the majority of the shifts seen in the selection of an odor quality descriptor after adaptation are a concomitant feature of the reduction observed in perceived intensity (Pierce *et al.*, 1993). However, in cases where odorants interact with multiple perceptual pathways, adaptation in one set might be expected to alter both the perceptual quantity and quality of another.

Figure 2 shows the frequency of modal, non-modal and 'no odor' odor quality reports across subjects for the three test odorants in each of the adapting conditions. Neither self- nor cross-adaptation affected the odor quality of IVA, as the relative proportion of modal (putrid) and non-modal responses was unchanged by either adaptation condition (Figure 2A). In contrast, of the 20 subjects that reported an odor quality for PEM after self-adaptation and the 23 that did so after cross-adaptation (Figure 2B), six subjects in each set reported a different quality after those treatments than after blank-adaptation. In each case, all six osmics gave a modal (putrid) descriptor after blank- and a non-modal descriptor after self- and cross-adaptation. McNemar's Test of Change indicated that this uniform shift in descriptor was statistically significant: Yates' $\chi^2 = 4.17$, $P < 0.05$ in both cases (Siegel, 1956). Of the 17 subjects that gave quality reports for the odor of AND in both the blank- and self-adaptation conditions (Figure 2C), seven gave different quality descriptors. However, the direction of these shifts (to a modal or non-modal descriptor) was not sufficiently uniform to yield a significant result.

These data are all consistent with the notion that AND is a 'simpler' urinous smelling compound than PEM, at least to the extent that there appears to be less individual variation in the quality reports it elicits among subjects. In contrast, the odor of PEM seems more 'complex' than AND, again to the extent that there is more individual variation in odor quality reports among subjects. When coupled with the observation of asymmetrical cross-adaptation between these compounds it seems clear that there must be at least three different sets of perceptual channels involved with their processing. Some of these appear to be shared to varying degrees and together with those identified as independent channels collectively account for the total urinous character of the three test substances. Explorations of additional compounds with these odor characteristics will make it possible to outline the maximum size of the domain for this odor class. Further, compounds for which asymmetrical cross-adaptation can be demonstrated may well reveal odor quality classes which differ in complexity.

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