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The Influence of Training on Chemosensory Event-related Potentials and Interactions between the Olfactory and Trigeminal Systems

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Abstract

It is not possible to accurately predict the perceptual response to odorants and odorant mixtures without understanding patterns of suppression and facilitation that result from interactions between the olfactory and trigeminal systems. The current study extends previous findings by exploring the effect of intensive training on the interaction between these systems and also by using a different mixed chemosensory stimulus to examine whether the principles established in earlier studies generalize to different odorants. Stimuli were chosen so as to selectively activate the olfactory (H_2S) and trigeminal (CO_2) nerves. In addition, linalool was included as a stimulus that activated both systems. Thirty-five participants (19 men, 16 women) rated the intensity of each stimulus when presented both alone and in binary mixtures (linalool + H_2S, and linalool + CO_2). Chemosensory event-related potentials were obtained from three recording positions. Analysis of intensity ratings showed that linalool was significantly less intense than the other stimuli when presented alone. In binary mixtures, H_2S was strongly suppressed by linalool. One week of intensive odor training produced significant and specific reductions in the intensity of linalool and H_2S, both alone and in their mixture. Training with a different odor (champignol) had no effect. Chemosensory event-related potential data confirmed previous findings showing changes in topographical distribution that reflected the degree of trigeminal activity. Binary mixtures generally produced larger amplitudes than single stimuli. Latencies clearly differentiated between the three single stimuli and the binary mixtures. Changes were observed in event-related potentials that reflected those obtained for intensity ratings in that they were observed for linalool and H_2S in the linalool trained group only. The amplitude of the late 'endogenous' component (P3) was significantly decreased for these odors at frontal recording sites. In summary, strong and specific training effects were observed in intensity ratings for participants trained with the test odor (linalool), but not for those trained with a different odor. This was supported by a significant decrease of amplitudes of the event-related potentials at frontal recording sites following training with the test odor only

Key words: chemosensory event-related potential, mixtures, olfaction, olfactory event-related potential, olfactory nerve, training, trigeminal nerve

Introduction

The contribution of the olfactory and trigeminal sensory systems to the sense of smell has been of research interest for a long time (e.g. Fröhlich, 1851; Cain, 1976; Cain and Murphy, 1980). Suppressive interactions between the two systems have been found, for example, by Kobal and Hummel (1988), in that CO_2 suppressed the intensity of olfactory sensations produced by vanillin. Cain and Murphy (1980) reported that suppression between the two systems was mutual. Specifically, the odor of amyl butyrate was suppressed by CO_2, and CO_2-induced irritation was suppressed by some concentrations of amyl butyrate. These suppressive effects were found to occur regardless of whether the mixture was presented simultaneously to both nostrils or if a different odorant was presented to each nostril, suggesting that the suppression was centrally mediated. Thus, the interaction between the olfactory and trigeminal systems is not straightforward, and may be difficult to predict, but has a powerful influence on odor perception both at different concentrations of a single stimulus and between different chemosensory stimuli. The

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pattern of interaction depends on stimulus quality (Livermore *et al.*, 1992), stimulus concentration and the relative intensity of olfactory and trigeminal components (Hummel *et al.*, 1992).

Prolonged exposure or training with sensory stimuli has been shown to result in either an increase, or a decrease in responsiveness to that stimulus. Intense stimuli, or stimuli that are reinforced or associated with meaningful objects/events, become more clearly discriminated and result in larger responses (Schwartz and Robbins, 1995). In contrast, less intense stimuli, or those that are not meaningful or reinforced, tend to result in habituation, i.e. a gradual decrease in responsiveness with repeated exposure. For the olfactory modality, several studies have found that training and corrective feedback to subjects with accurate (veridical) labels enhances the recognition and identification of the intensity and quality of odors (Engen and Pfaffmann, 1959; Desor and Beauchamp, 1974; Engen, 1977; Cain, 1979; Schemper *et al.*, 1981; Rabin and Cain, 1984). Rabin and Cain (1984, 1986) have shown that familiarity with an odor can be more important than intensity in determining how well one component will be discriminated from another in a mixture. Low intensity minor components in binary mixtures could be detected if they were of high but not of low familiarity.

While this increase in discrimination and sensitivity may be a result of associative or perceptual learning, there is evidence that it may also result from an enhanced representation for the odor. Grajski and Freeman (1989) found that stable patterns of neural activity are not observed in the olfactory bulb for unfamiliar or novel stimuli. However, continued reinforced presentation results in stable odor specific burst amplitudes over the surface of the bulb, which reorganize with training and are correlated with behavioral changes (Coopersmith and Leon, 1984; Roman *et al.*, 1987; Kay and Laurent, 1999). It has been demonstrated that sensitivity can also be increased at the level of the olfactory epithelium as a result of odor exposure (Wang *et al.*, 1993; Nevitt *et al.*, 1994; Youngentob and Kent, 1995; Hudson and Distel, 1998).

The primary aims of the present study were twofold. Firstly, it was hypothesized (hypothesis 1) that previous results would be replicated and that the mixed olfactory/trigeminal stimulant, linalool, would suppress both pure olfactory (H_2S) and trigeminal (CO_2) activity. Secondly, it was hypothesized (hypothesis 2) that training with the mixed stimulant used for testing, linalool, would result in both enhancement of cortical and perceptual responses to the stimulus and its suppression of the other stimuli. This enhancement of responses should be specific to the stimulus used for testing and should not generalize from a different odor (champignol) to any other stimulus.

In addition, due to the recent finding of sex differences in sensitivity in response to repeated odor exposure (Dalton *et al.*, 2002), it was considered important to include 'sex' as a factor in the analyses.

Materials and methods

Participants

The participants were 35 healthy volunteers (19 men, 16 women; age range 18–44 years, mean age 23.7 years) who provided written informed consent. All participants reported normal smell and taste sensitivity and no history of sinu-nasal disease or extensive exposure to chemicals with potential olfactory or trigeminal toxicity. Normal olfactory function was verified by applying validated olfactory tests ('Sniffin' Sticks'; Kobal *et al.*, 2000). The participants were instructed to avoid alcohol, coffee, and tobacco smoking 1 h prior to testing. The study was conducted in accordance with the Declaration of Helsinki on biomedical research in human subjects; the protocol was reviewed by the ethics committee of the University of Dresden Medical School (approval number EK136092000).

Procedure

Following the order of enclosure into the study subjects received either linalool (P4347; Sigma, Taufkirchen, Germany) or champignol (1-octen-3-ol; O-528-4; Aldrich, Taufkirchen, Germany) as the odor to be trained with. The two different odorants are of similar intensity, familiarity, distinctiveness, and trigeminal activation (as assessed through a lateralization paradigm in highly trained observers; Berg *et al.*, 1998).

Eighteen subjects (nine female, nine male) received champignol, five of them smoked. Seventeen subjects (seven female, 10 male) received linalool; 4 of these subjects were smokers. These odorants were given out in brown glass bottles (250 ml). They contained 10 ml of either linalool or champignol. To prevent spillage of the odorant the bottles also contained three cotton pads (size 10×10 cm) of gauze (Gazin®, Lohmann+Rauscher, Rengsdorf, Germany). Subjects were instructed to store the bottles in an upright position. They were asked to sniff the odor three times a day (in the morning, at noon, and in the evening); when sniffing the odor subjects should try to think of an event or a place that would be associated with this odor. Further, they were asked to keep a 'smell diary' where they should note down any special observations associated with the training procedure. In addition, the diary contained numerous visual analogue scales which subjects used to rate odor intensity every time they sniffed the odor (10 cm length; left hand end: no odor perceived; right hand end: maximum odor intensity). This 'smell diary' was also used to as a means of monitoring the subjects' compliance with instructions. Specifically, all participants commented on their olfactory experience at different days throughout the training period. Subjects trained for an average of 7.6 days (minimum: 5.5 days; maximum: 11 days). They visited the lab before

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and after the training period where olfactory sensitivity was measured on a psychophysical (odor intensity ratings) and an electrophysiological level (chemosensory event-related potentials, ERP).

Stimuli

Stimuli were chosen to selectively activate the olfactory (H_2S; 4.0 p.p.m.) and trigeminal (CO_2; 40% vol/vol) nerves. Linalool (20% vol/vol; L260-2; Sigma, Taufkirchen, Germany), diluted in propylene glycol (P4347; Sigma) was included due to its activation of both neural systems as a mixed olfactory/trigeminal stimulant. Linalool has a clear and distinctive citrus-like odor which previous work (Doty *et al.*, 1978) has shown to be discriminated by anosmic subjects on the basis of its trigeminal activity. Further, it has been demonstrated that at concentrations used in the present study linalool produces activity related to activation of nociceptors in the respiratory epithelium (Frasnelli and Hummel, 2003).

All stimuli had a duration of 200 ms and were generated by a dynamic olfactometer based on air-dilution olfactometry that does not alter the mechanical or thermal conditions at the mucosal level (Kobal, 1981). In essence, two gaseous streams, odorless and odorized air, are being switched by application of vacuum so that during stimulation odorized air reaches the olfactory region, and during interstimulus intervals only odorless air is applied. Stimuli were administered non-synchronously to breathing; the technique of velopharyngeal closure was used to restrict breathing to the mouth (Kobal and Hummel, 1989). The stimuli were presented to the right nostril in a constantly flowing air stream of 7.8 l/min with controlled temperature and humidity (36.5°C, 80% RH). In each session, five different stimuli [H_2S, CO_2, linalool, binary mixture of linalool and H_2S (LH), binary mixture of linalool and CO_2 (LC)] were presented 16 times in a randomized order, with an average interstimulus interval of 30 s (range 24–34 s).

ERP recording and analysis

Electroencephalographical records of 2048 ms including a 512 ms prestimulus period were obtained from 3 midline positions [Fz (frontal), Cz (central) and Pz (parietal)] of the international 10–20 system, referenced to linked earlobes (A1+A2). Eye-blinks were monitored from Fp2 (frontopolar, right)/A1+A2, and single recordings with artifacts >40 μV were discarded. Records were amplified and filtered (Schabert Instrumente, Röttenbach, Germany; band pass 0.02–30 Hz), digitized (sampling rate 250 Hz), and averaged off-line separately for the 5 stimuli and for each recording position. Averaging of the single responses obtained within a session yielded late near-field ERP (Hummel and Kobal, 2001). Base-to-peak amplitudes and peak latencies of N_{411} and P_{617} were evaluated. To render the current presentation of results more compatible with previous publications where components with similar latencies have been investigated, throughout the rest of the manuscript these peaks will not be termed N_{411} and P_{617} (according to peak polarity and latency at position Cz—in this example results for responses to CO_2 are used) but N_1 and P_3, respectively.

All participants received standardized instructions to estimate overall stimulant intensity evoked by each chemosensory stimulus. The ratings were performed by means of a continuous visual analogue scale displayed on a computer monitor. Its left hand end was defined as 'no stimulant perceived' (0 estimation units), its right hand end as 'maximum strength stimulant' (100 estimation units); the two ends were identified on the screen with '0' and '+++', respectively. The intensity of each individual stimulus was indicated by means of a marker which was adjusted by means of a joystick. Participants were seated comfortably in an air-conditioned room; movements were monitored through a video camera. White noise was used to mask acoustical stimulation from switching valves. To avoid a low state of vigilance during ERP recordings, participants were instructed to perform a tracking task on a video screen (Kobal and Hummel, 1989): a smaller square, controlled by a joystick, was to be kept inside a larger square which moved unpredictably.

Statistical analyses

Analyses of variance (ANOVA) were used to examine differences in diary data, psychophysical measurements, and ERP. 'Group' (training with linalool or champignol) was a between-subjects factor in all analyses. 'Repeat' (before versus after training) was a within-subjects factor for each analysis. For the smell diary data, the three intensity ratings made for the first complete day of home training were averaged separately for each participant and compared with the average of the ratings for the last complete training day. For the psychophysiological measurements, the within-subjects factor was 'stimulant' (linalool, CO_2, H_2S and the two binary mixtures LC and LH). Separate analyses were conducted to investigate linalool, H_2S and their binary mixture LH, and linalool, CO_2 and their binary mixture LC. Each ERP parameter (amplitude and latency of peaks N1 and P3) was analyzed via two ANOVAs. Separate ANOVAs were conducted for each mixture and its components, i.e. one ANOVA was conducted for linalool, H_2S and LH (linalool analysis), and the second for linalool, CO_2 and LC (CO_2 analysis). Within-subjects factors were 'recording position' (Cz, Pz, Fz) and 'stimulant' (linalool, CO_2 and LC, or linalool, H_2S and LH, respectively).

Separate ANOVAs were run to examine effects of the between-subjects factor 'sex' in the behavioral data. Where no differences were found analyses were run on the combined data for males and females.

A Greenhouse–Geisser Epsilon adjustment was used in univariate repeated measures when the sphericity assumption was violated. Significant main effects and interactions are indicated, mean differences are interpreted in light of

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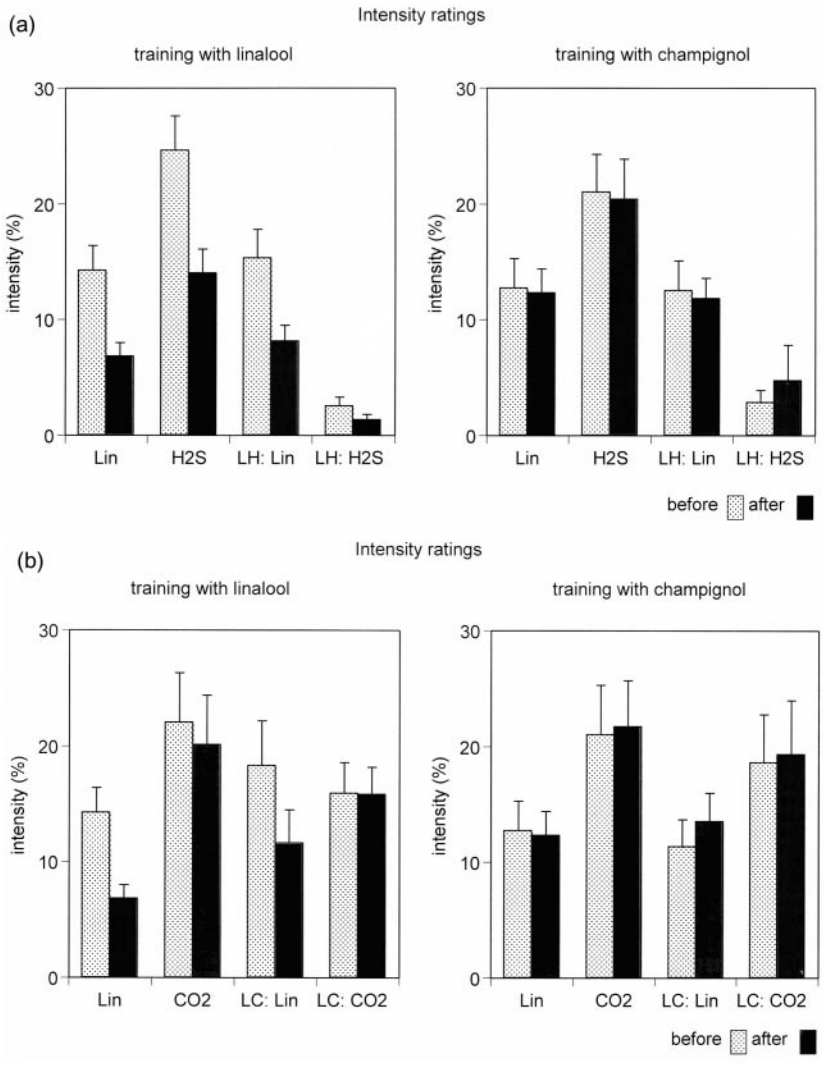

Figure 1 Intensity ratings (mean, standard errors of means) for **(a)** linalool and H_2S, when presented as a single stimuli (linalool or H_2S) or as linalool component (LH: linalool) or H_2S component (LH: H_2S) in a binary stimulus LH; **(b)** linalool and CO_2, when presented as single stimuli (linalool or CO_2) or as a linalool component (LH: Lin) or CO_2 component (LH: CO_2) in a binary stimulus LH. Ratings were obtained before and after training with either linalool (left) or champignol (right).

one-tailed *t*-tests with Bonferroni correction. Planned comparisons were used to compare means for within-subjects factors. Contrasts for psychophysical measures and ERP components compared each unmixed stimulant with the other stimulant and with its rating in the binary mixture. Contrasts among ERP components obtained at the different recording sites were performed comparing Cz versus Fz and Cz versus Pz. The software package SPSS version 10 (SPSS Inc., Chicago, IL) was used to analyze the data.

Results

Smell diary data

All subjects completed their home diaries on every day between the first and last testing sessions. No significant differences were found between intensity ratings for the main effects of 'sex', 'group' or 'repeat', nor were there significant interactions.

Psychophysical measurements

No significant main effect or interactions involving the factor 'sex' was found. Figure 1a shows intensity ratings for linalool, H_2S and LH, Figure 1b shows ratings for linalool, CO_2 and LC. Despite attempts to balance intensities prior to commencement, there were significant differences between the unmixed stimulants [H_2S: $F(3,31) = 43.6$, $P < 0.001$, $ETA^2 = 0.66$; CO_2: $F(3,31) = 8.34$, $P = 0.001$, $ETA^2 = 0.35$]. Linalool was perceived as being the least intense unmixed stimulus with both H_2S ($F = 48.9$, $P < 0.001$) and CO_2 ($F = 17.8$, $P < 0.001$) being perceived as significantly stronger before training. While linalool intensity was unchanged when mixed with H_2S or CO_2, both other stimuli were

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significantly suppressed in the binary mixtures (H_2S: F = 58.1, $P < 0.001$, CO_2: $F = 5.8$, $P = 0.022$).

In addition, there was a significant main effect of 'Repeat' for the H_2S analysis [$F(1,33) = 12.2$, $P = 0.001$, $ETA^2 = 0.27$] and a significant interaction between 'group' and 'repeat' for both H_2S and CO_2 analyses [H_2S: $F(1,33) = 12.7$, $P = 0.001$, $ETA^2 = 0.28$; CO_2: $F(1,33) = 5.49$, $P = 0.025$, $ETA^2 = 0.14$]. Training with linalool, but not champignol, resulted in a significant overall reduction in intensity. For the H_2S analysis there was also a significant interaction between 'stimulant' and 'repeat' indicating that the influence of training varied between stimulants [$F(3,31) = 5.84$, $P = 0.021$, $ETA^2 = 0.16$]. This effect appears to have resulted from a 'floor effect' due to very low ratings for H_2S in the LH mixture.

In order to compare differences between the effect of training on LH and LC mixtures, Bonferroni adjusted within-subjects t-tests were conducted contrasting session 1 and session 2 ratings for each stimulant (linalool, H_2S, CO_2 and the intensity of each stimulant in its corresponding mixture). Each group was treated as a separate family of tests giving seven comparisons for each group and leading to an adjusted alpha level of 0.007 for each comparison. No differences were significant for the champignol-trained group. In contrast there were significant differences between sessions for both linalool [$t(16) = 3.8$, $P = 0.002$] and H_2S [$t(16) = 3.7$, $P = 0.002$] for the linalool-trained group, while the difference approached significance for linalool intensity in the LH mixture [$t(16) = 2.8$, $P = 0.012$].

Chemosensory event-related potentials (ERP)

Descriptive statistics of ERP amplitudes and latencies are presented in Tables 1–4.

Table 1 ERP parameters [means and SEM of amplitudes N1 and amplitudes P3 (in μV)] at recording positions Cz, Fz and Pz before and after training with linalool, separately for the five stimulus qualities linalool, H_2S, CO_2, the binary mixture of linalool and H_2S (LH), and the binary mixture of linalool and CO_2 (LC)

			Position Cz		Position Fz		Position Pz	
			Mean	SEM	Mean	SEM	Mean	SEM
Amplitude N1	before training	linalool	–3.18	0.55	–3.59	0.60	–2.48	0.58
		H_2S	–2.80	0.72	–2.61	0.84	–3.68	0.88
		CO_2	–3.96	1.15	–3.07	1.04	–2.43	1.04
		LH	–2.39	1.07	–1.96	1.08	–2.22	1.00
		LC	–4.14	1.29	–3.09	0.94	–2.77	1.04
	after training	linalool	–3.11	0.65	–3.57	0.69	–2.34	0.79
		H_2S	–3.03	0.68	–3.45	0.96	–2.73	0.63
		CO_2	–3.16	0.93	–1.76	1.08	–2.00	0.93
		LH	–3.89	0.78	–4.02	0.88	–3.25	0.74
		LC	–4.10	1.47	–3.13	1.11	–3.16	0.96
Amplitude P3	before training	linalool	8.49	0.88	7.68	1.26	9.20	0.94
		H_2S	10.25	1.45	9.42	1.07	10.80	1.34
		CO_2	13.35	1.88	11.33	2.11	15.23	2.48
		LH	9.56	0.94	8.99	1.04	9.96	0.95
		LC	13.74	2.02	13.16	2.43	14.39	1.79
	after training	linalool	7.16	0.67	5.68	0.72	8.46	0.69
		H_2S	11.81	0.71	9.66	0.89	12.51	0.91
		CO_2	13.68	1.42	10.68	1.13	14.43	1.42
		LH	8.41	0.42	6.43	0.93	9.99	0.52
		LC	11.48	1.36	9.41	1.41	12.58	1.46

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Table 2 ERP parameters [means and SEM of amplitudes N1 and amplitudes P3 (in μV)] at recording positions Cz, Fz and Pz before and after training with champignol, separately for the five stimulus qualities linalool, H_2S, CO_2, the binary mixture of linalool and H_2S (LH), and the binary mixture of linalool and CO_2 (LC)

			Position Cz		Position Fz		Position Pz	
			Mean	SEM	Mean	SEM	Mean	SEM
Amplitude N1	before training	linalool	–2.22	0.63	–2.32	0.67	–2.49	0.54
		H_2S	–1.96	0.66	–2.81	0.78	–1.93	0.74
		CO_2	–4.06	0.73	–2.96	0.67	–2.88	0.38
		LH	–3.07	0.86	–3.46	0.98	–2.50	0.63
		LC	–4.36	0.61	–2.80	0.67	–3.30	0.59
	after training	linalool	–3.47	0.66	–3.82	0.94	–3.00	0.49
		H_2S	–2.56	0.49	–2.20	0.59	–2.71	0.51
		CO_2	–4.93	1.03	–3.13	0.80	–4.43	1.15
		LH	–4.15	0.72	–4.90	0.72	–3.68	0.76
		LC	–5.52	0.99	–3.35	1.06	–3.85	0.86
Amplitude P3	before training	linalool	6.87	0.82	5.28	0.71	8.25	0.64
		H_2S	10.07	1.09	8.39	0.81	11.44	1.30
		CO_2	11.39	1.41	10.37	1.44	12.50	1.74
		LH	7.05	0.85	4.57	1.10	8.51	1.18
		LC	11.89	1.52	8.93	1.23	12.22	1.49
	after training	linalool	6.89	0.92	5.55	0.74	7.44	0.88
		H_2S	10.39	1.01	9.35	0.87	10.75	0.90
		CO_2	13.73	2.30	12.51	1.80	14.93	2.07
		LH	6.63	0.74	6.18	0.68	7.59	0.71
		LC	11.69	1.72	10.23	1.74	12.19	1.64

Amplitude N1

Statistical analysis for linalool, H_2S and their mixture LH indicated differences between recording positions [$F(2,23) = 4.67$, $P = 0.014$, $ETA^2 = 0.16$] in addition to a 'Position × Stimulant × Group' interaction [$F(4,21) = 2.54$, $P = 0.045$, $ETA^2 = 0.10$]. However, *a priori* comparisons confirmed that these differences were due to significant differences for the contrast between Fz and Pz for the linalool versus LH comparison ($F = 4.88$, $P = 0.037$).

Statistical analysis for linalool, CO_2 and LC revealed a significant difference between recording positions [$F(1,25) = 5.91$, $P = 0.005$, $ETA^2 = 0.29$], Cz amplitudes being significantly greater than Pz ($F = 9.17$, $P = 0.006$) and Fz ($F = 6.97$, $P = 0.014$) and a significant 'Position × Stimulant' interaction [$F(4,22) = 3.77$, $P = 0.015$, $ETA^2 = 0.39$]. Contrasts revealed that the effect of stimulant was significantly different at Fz compared with Cz. Linalool produced significantly smaller Cz amplitudes than both CO_2 and LC (linalool versus CO_2: $F = 14.6$, $P = 0.001$; linalool versus LC: $F = 10.0$, $P = 0.004$).

Latency N1

Mean latencies for N1 appeared to differentiate between the 3 single stimuli and the binary mixtures. Generally, single stimulants produced longer latencies than their mixtures. This effect was especially exaggerated for the LC mixture whose latency was much shorter than either linalool or CO_2. This stimulant effect was significant for CO_2 and LC [$F(2,24) = 11.1$, $P < 0.001$, $ETA^2 = 0.47$] with responses to LC having significantly shorter latencies than either linalool ($F = 20.0$, $P = 0.001$) or CO_2 ($F = 10.5$, $P = 0.003$).

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Table 3 ERP parameters [means and SEM of latencies N1 and latencies P3 (in ms)] at recording positions Cz, Fz and Pz before and after training with linalool, separately for the five stimulus qualities linalool, H_2S, CO_2, the binary mixture of linalool and H_2S (LH), and the binary mixture of linalool and CO_2 (LC)

			Position Cz		Position Fz		Position Pz	
			Mean	SEM	Mean	SEM	Mean	SEM
Latency N1	before training	linalool	445	32	438	33	440	33
		H_2S	424	20	431	22	419	21
		CO_2	411	23	417	24	410	26
		LH	412	29	410	29	408	27
		LC	369	17	379	20	367	17
	after training	linalool	451	22	450	22	450	22
		H_2S	438	11	441	11	443	11
		CO_2	425	17	436	17	422	19
		LH	413	17	418	17	413	17
		LC	366	22	376	24	367	24
Latency P3	before training	linalool	673	34	668	33	665	33
		H_2S	662	30	663	27	657	25
		CO_2	617	34	607	34	614	30
		LH	637	31	641	32	639	30
		LC	569	30	564	28	563	27
	after training	linalool	701	34	697	34	700	30
		H_2S	643	15	644	13	634	15
		CO_2	625	29	626	30	622	28
		LH	657	21	658	22	657	24
		LC	594	27	596	31	603	27

Amplitude P3

Largest amplitudes for all stimuli were found at Pz [H_2S analysis: $F(2,23) = 34.7$, $P < 0.001$, $ETA^2 = 0.63$; CO_2 analysis: $F(2,24) = 23.9$, $P < 0.001$, $ETA^2 = 0.56$]. Both analyses suggest that the stimulus differences mirror those found for ratings [H_2S analysis: $F(2,23) = 13.7$, $P < 0.001$, $ETA^2 = 0.42$; CO_2 analysis: $F(2,24) = 16.6$, $P < 0.001$, $ETA^2 = 0.49$]. For the H_2S analysis, largest amplitudes were found for H_2S with no significant difference between linalool and LH (LH versus H_2S: $F = 14.0$, $P = 0.001$), reflecting the perceived suppression of H_2S by linalool. For LC smallest amplitudes were found for linalool with no significant difference between CO_2 and the mixture (LC versus linalool: $F = 20.5$, $P < 0.001$).

For linalool, H_2S and LH, training resulted in changes across recording sites for the two training groups ['Repeat × Position × Group' interaction; $F(2,23) = 8.63$, $P = 0.001$, $ETA^2 = 0.33$]. For the linalool group, activity in frontal sites decreased significantly relative to that at central ($F = 11.6$, $P = 0.002$) and parietal sites ($F = 5.57$, $P = 0.027$).

Latency P3

Corresponding to amplitude changes there was decrease in H_2S latency and an increase in linalool and LH latency in both groups. These observations were supported by a significant 'Repeat × Stimulant' [$F(2,23) = 4.38$, $P = 0.01$, $ETA^2 = 0.34$] interaction for linalool, H_2S and their mixture. Contrasts revealed that this was due to a significant decrease in H_2S and increase in response latency to LH following training ($F = 5.16$, $P = 0.032$).

There was a significant effect of stimulant for the CO_2 analysis [$F(2,24) = 17.9$, $P < 0.001$, $ETA^2 = 0.57$]. Latencies were significantly shorter for the LC mixture than for either linalool or CO_2 ($F = 31.3$, $P < 0.001$).

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Table 4 ERP parameters [means and SEM of latencies N1 and latencies P3 (in ms)] at recording positions Cz, Fz and Pz before and after training with champignol, separately for the five stimulus qualities linalool, H_2S, CO_2, the binary mixture of linalool and H_2S (LH), and the binary mixture of linalool and CO_2 (LC)

			Position Cz		Position Fz		Position Pz	
			Mean	SEM	Mean	SEM	Mean	SEM
Latency N1	before training	linalool	434	27	433	27	429	25
		H_2S	422	27	414	29	421	26
		CO_2	402	28	401	28	396	27
		LH	412	23	422	24	415	23
		LC	363	20	353	20	366	19
	after training	linalool	435	33	446	37	422	30
		H_2S	432	21	437	21	433	21
		CO_2	416	20	413	20	409	18
		LH	414	24	411	26	407	23
		LC	391	14	396	13	390	13
Latency P3	before training	linalool	659	31	658	24	657	29
		H_2S	735	27	710	33	724	26
		CO_2	672	29	679	33	662	29
		LH	646	22	654	21	652	22
		LC	588	24	596	23	583	21
	after training	linalool	680	30	692	29	676	27
		H_2S	665	29	656	27	664	29
		CO_2	626	24	627	23	628	23
		LH	676	31	670	28	677	31
		LC	617	27	620	26	613	28

Discussion

In the present experiment two hypotheses were tested. Hypothesis 1 was that previous results would be replicated and that the mixed olfactory/trigeminal stimulant, linalool, would suppress both pure olfactory (H_2S) and trigeminal (CO_2) activity. Hypothesis 2 was that training with the olfactory/trigeminal stimulant would result in both enhancement of responses to this stimulus and its suppression of the other stimuli. This enhancement of responses should be specific to the stimulus used for testing and should not generalize from a different odor to any other stimulus.

With regard to hypothesis 1, psychophysical ratings revealed significant interactions between linalool, H_2S and CO_2. Linalool was perceived as being less intense than the other stimuli. However, it was found to strongly suppress the intensity of H_2S in the binary mixture while being itself unchanged. Similarly, linalool suppressed the intensity of CO_2.

The results also provided support for previous findings with regard to the interaction between CO_2 and mixed olfactory/trigeminal stimuli. Livermore *et al.* (1992) found that the intensity of H_2S was powerfully suppressed in mixtures with both the trigeminal stimulant CO_2 and more strongly by the mixed olfactory/trigeminal stimulant carvone. In contrast, when CO_2 was mixed with carvone the intensity of CO_2 was suppressed, while that of carvone was enhanced slightly in the same mixture. In the current study, there was a decrease in CO_2 intensity when it was mixed with linalool, but there was no consistent increase across groups and conditions for linalool when it was mixed with CO_2. However, like the earlier experiment, the current study did provide evidence for a 'dominance' of mixed olfactory/trigeminal stimulation over either system alone. This may

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reflect a stronger memory trace for the stimulus which is encoded in both systems, hence providing for less interference and more pathways for retrieval, or redundancy in encoding (Paivio, 1971; Lyman and McDaniel, 1990). The relatively weak suppression of CO_2 may have resulted from the unmixed intensity of linalool being lower than that of CO_2. It is possible that a much stronger suppression of CO_2 would have been observed had the intensities been more evenly matched.

ERP recordings supported those of previous studies (Hummel and Kobal, 1992; Hummel *et al.*, 1992) indicating that the degree of olfactory versus trigeminal sensation produced by a stimulus can be predicted on the basis of the topographical distribution of ERP. Trigeminal stimuli (single stimuli or mixtures containing linalool or CO_2) produced larger N1 amplitudes at Cz than they did at Pz. In contrast, the olfactory stimulant (H_2S) produced either higher amplitudes at Pz or no clear difference between amplitudes at Cz and Pz. Stimulation with binary mixtures generally produced larger ERP amplitudes than single stimuli; as expected, they were much smaller than the sum of the responses to single stimuli (Livermore *et al.*, 1992). Mean latencies for N1 differentiated between the three single stimulants and the binary mixtures (compare Livermore *et al.*, 1992).

Interestingly, the results extend previous findings indicating that amplitude N1 does not simply reflect stimulus intensity (Kobal and Hummel, 1988). While there was a substantial decrease in the intensity of linalool, both alone and in its mixtures, in the linalool trained group, there was no decrease in N1 amplitudes. In fact, there was an increase in the amplitude of the LH mixture. This supports previous work (Livermore *et al.*, 1992) in suggesting that N1 reflects more than stimulus intensity alone (see also Krauel *et al.*, 1998).

Hypothesis 2, that training with linalool would result in both the enhancement of cortical and perceptual responses to linalool and of the other stimuli, was only partially supported. There was a strong and specific training effect. However, rather than an increase, training with linalool resulted in a substantial decrease in intensity of linalool and H_2S when they were subsequently presented both alone and in mixtures. While training lead to the intensity of linalool being reduced in its mixture with CO_2, the latter was unaffected either in the mixture or when presented alone. Interestingly, following training the pattern of suppression was virtually identical, i.e. linalool intensity was unchanged by mixing while H_2S was strongly suppressed. Further, CO_2 was not released from suppression when training reduced linalool intensity. In contrast, training with the odorant not used for testing, champignol, produced no change in intensity of either linalool or H_2S or to the interaction between them. This indicates that the changes in the linalool-trained group were due to a stimulus specific learning effect that produced a decrease in intensity of linalool and H_2S. Further, while the decrease in intensity transferred from the mixed olfactory/trigeminal stimulus to the pure olfactory stimulant, they did not transfer to CO_2. The question then arises as to why there was a decrease in H_2S, but not CO_2 intensity following training.

Training effects were found for the later, more cognitive, ERP component P3. This effect was significant with the analysis of linalool, H_2S and LH and approached significance with the CO_2 analysis. While there was a small change merely as a result of training or retesting, there was a significant change in ERP scalp distribution specifically as a result of training with linalool. Increased P3 amplitudes and decreased latencies for H_2S following linalool training were combined with decreased amplitudes and increased latencies for linalool and the binary mixture LH. There was also a decrease in amplitudes at the frontal recording site relative to the parietal site as a result of linalool training. CO_2 was the only stimulus in which Fz amplitude did not decrease after linalool training, reflecting the observation that only CO_2 ratings were unaffected by linalool training. Thus, the results indicate that recordings at Fz are a good indicator of perceptual changes following training as it reflects the perceptual changes observed. This may relate to previous findings indicating that learned changes to sensory stimuli occur primarily in frontal regions, particularly the orbitofrontal cortex (Royet *et al.*, 1999). Future research using source localization techniques (e.g. Kettenmann *et al.*, 1997) is needed to further investigate this phenomenon.

An associative explanation may be proposed to account for the generalization of learning from linalool to H_2S after training with linalool. Many associative theories (e.g. Rescorla, 1972; Kehoe and Graham, 1988) predict that memories for events are laid down in an associative network. Stimuli are present in this network as representations that are linked or associated as a result of experience. With experience and repeated presentations, increasingly stronger links are formed among the features of a single stimulus or between different stimuli making up a unitary event in a process known as unitization. As a result of repeated pairing of H_2S in session 1, associative links may have been formed between linalool and H_2S. Decreased responsiveness to linalool after training may then have generalized to H_2S through associative processes formed in session 1. This may explain why there was generalization from linalool to H_2S but no generalization from champignol to linalool, i.e. no associative links had been formed with champignol as it was not present in session 1. However, this associative explanation does have problems. While paired presentation of stimuli may lead to the formation of associative links between them, presentation of the stimuli in isolation, or in combination with other stimuli, as occurred in this study, should lead to the formation of inhibitory links. In turn, this should result in a weaker association between stimuli and hence greater discrimination (Livermore *et al.*, 1997).

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This generalization appeared to be specific to the olfactory system and did not extend to the trigeminal sensory system. While this appears to be contradictory, there is ample evidence for preferential learning in which some stimuli are more easily associated than others (Garcia and Koelling, 1966). The prototypical example of this is taste aversion in which a taste may be effectively associated with illness on a single trial. Thus, it may be proposed that odor-odor, or within-system associations, such as that found between linalool and H_2S, are formed more readily than between-system (odor-trigeminal) associations. Alternatively, the neural pathways involved in trigeminal stimulation may not be susceptible to short term desensitization in the same way as the olfactory pathways, or the amount or type of trigeminal stimulation provided by linalool may have been insufficient to produce sensitization.

The presently observed decrease in intensity seemed to be stimulus specific. Buonviso and Chaput (2000) proposed that generalized learning effects may be a result of overlapping patterns of neural activation in the olfactory bulb. Hence it is possible that generalization occurred from overlapping representations for H_2S and linalool. It would also be necessary to propose the absence of overlap for champignol with linalool and H_2S. This could also explain why CO_2 was not affected, i.e. being a purely trigeminal stimulant, when presented alone, it might produce little activation of the olfactory bulb and hence could not be influenced by an overlapping activation pattern with linalool.

Surprisingly, while we found a specific learning effect, as hypothesized, it was not in the direction predicted. As discussed above, training that produces an enhancement of the emotional or motivational salience of an odor (or any stimulus) should result in an enhancement of its perceptual representation. Despite using a training strategy designed to enhance stimulus salience, we observed a decrease in odor intensity. The most probable reason for this is that, rather than enhancing stimulus salience, the presently used training technique may have resulted in either habituation as a result of changes in memory or in sensory adaptation. It is possible that participants chose to ignore instructions given for training or, more likely, that they may have come to do their encoding 'chores' automatically rather than processing the stimulus to a sufficiently deep level.

Decreased intensity could also reflect a purely associative process as a result of the interaction of the representations of the stimuli in memory (Schwartz and Robbins, 1995). The short duration of training does not preclude a low level sensory change. Buonviso and Chaput (2000) have shown neural changes in the bulb to occur in the time frame of this study. These hypothetical, bulbar changes are likely to be functional and may reflect modulatory feedback from higher processing centers, or could represent short term inhibitory processes within the olfactory bulb (Kay and Laurent, 1999). Associative and neural explanations are not necessarily mutually exclusive as bulbar processes or modulatory feedback may reflect associative learning at cortical levels, and could, in the long term, produce mitral desensitization or enhancement.

In summary, strong and specific training effects were observed with the odorant used for testing, but not with a different training odor. These effects were reflected in both ratings and in ERP. Future research may include investigations in the area of associative learning paradigms and enhanced stimulus training techniques to see if they produce an enhancement rather than a degeneration of perceptual representations.

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