

# The Effect of Odour Priming on Long Latency Visual Evoked Potentials of Matching and Mismatching Objects

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## Abstract

This study reports a cross-modal, olfactory/visual event related potential (ERP) using odours as olfactory primes. The results show a difference in the ERP waveform for the N400 waveform when a visual image does not match the priming odour. An N400 peak was produced for both the matched and mismatched conditions but the peaks were significantly more negative for the mismatched condition. By the use of non-food odours this study extends an earlier finding by Grigor, who, using the same ERP paradigm, obtained similar results for food odours and photographs of food.

## Introduction

It is difficult to establish the precise moment of receptor contact of an odorous stimulus and to control precise duration of stimulation. Whilst direct effects of odours on EEG have been reported (Klemm, *et al.*, 1992; Van Toller *et al.*, 1993), it is difficult to interpret EEG signals in the noisy electrical environment of the cerebral cortex (Van Toller *et al.*, 1992). Such difficulties have led to the development of highly technical equipment in a small number of specialist laboratories in order to study directly olfactory/trigeminal generated chemosensory evoked potential (CEP) (Kobal and Hummel, 1992). The necessary conditions and controls for such studies have been presented by Evans *et al.* (1993) and Van Toller (1994). The standard evoked potential (EP) paradigm requires averaging numerous presentations before the signal becomes discernible against the noisy cortical electrical background. Technical difficulties relating to olfactory research have constrained the application of such studies to detailed systematic parametric studies using a limited number of substances: namely, vanilla, hydrogen sulphide and carbon dioxide. In order to extend olfactory investigations into more psychological studies it is necessary to use alternative methods. This experiment concerns the use of one such technique called event related potential (ERP). The ERP is a well established technique in auditory and visual experiments where these two sensory systems have an advantage in that they allow very precise control of stimulus onset and duration.

The so-called ERP 'oddball' study has the advantage of allowing the investigation of odours using olfactory primes. The task normally involves a subject counting the number

of odd stimuli occurring during a series of standard presentations. Odour priming has also been used by Torii *et al.* (1988) using an EEG technique called contingent negative variation (CNV) which measures direct current baseline changes rather than the oscillating alternating current displayed in normal EEG waveforms. Torii's subjects were primed with an odour and were then presented with an initial stimulus (S1) which served as a signal for an impending second signal (S2) which required the subject to make a response. Torii reported a post-S1 increase in the CNV baseline between the period 500–1000 ms when jasmine was used as the priming odour and a decrease in the CNV baseline when lavender was used as the priming odour. In a carefully controlled CNV study using odour priming Lorig and Roberts (1990) reported that the positive and negative CNV effects could be shown to match subject expectations. This suggested that cognitive factors influenced the results. It is interesting to note that four of the seven subjects used in the Torii study were said to be perfumers, which might suggest an influence of cognitive factors. These studies suggest that it would be of value to attempt evaluations of cognitive factors when a subject is presented with odours.

Earlier studies have reported semantic priming, which refers to the facilitation of a stimulus by a related preceding semantic stimulus (Myer and Schvaneveldt, 1971). Semantic priming is an ERP paradigm which has been investigated since Kutas and Hillyard (1980, 1984) first reported it. In the Kutas and Hillyard experiment subjects were primed with a sentence like, 'The pizza was too hot to \_ \_ \_'. The missing word at the end of the sentence was either 'eat' (congruous)

or 'cry' (incongruous). The authors found that the N400 component was characterized as a negative deflection occurring ~400 ms after presentation of a word with a larger deflection following an incongruous word. These authors were also able to show that the N400 was sensitive to the degree with which the target stimulus was in agreement with the prior semantic context. The greater the degree of incongruity the larger the N400 peak.

Subsequently the N400 component has been identified in a variety of visual priming tasks using verbal stimuli (Polich, 1985; Bentin, 1987), non-verbal stimuli using faces (Barrett and Rugg, 1989, 1990a) and pictures (Barrett and Rugg, 1990b). The non-verbal findings suggest that the N400 peak may not be as specifically related to linguistic stimuli as was initially suggested. Additionally an N400 peak has also been identified for the auditory modality but it appears to have different amplitude and latency characteristics (Holcomb and Neville, 1990). This latter finding suggests that the priming processes may be different across modalities. Evidence of cross-modal priming has been reported in two recent studies. Pratorelli (1994) found evidence of cross-modal semantic priming on the N400 peak using pictures and spoken words. Domalski *et al.* (1991) found evidence of cross-modal transfer for semantic repetition priming between auditory-verbal and visual-verbal stimuli.

A recent investigation using the cross-modal paradigm (Lorig *et al.*, 1995) examined the effects of odour on visual ERP responses generated by an 'oddball' paradigm. Three odour names were repeatedly presented and were preceded on 25% of the trials by a non-matching odour. Lorig and his colleagues found that the P300 waveform elicited was not differentially related to subjects' olfactory sensitivity. However, an increase in negativity of frontal electrodes was found when the subjects were required to match olfactory stimuli to labels. The authors suggested that the significant differences found for N100, P200 and P300 indicated that the neural processes used to evaluate odour labels differed from those used to evaluate labels from visual stimuli. Lorig and his colleagues did not report on the N400 waveform.

An examination of the relevant literature shows that few investigations to date have systematically examined the effects of olfactory stimuli on human brain activity using a cross-modal paradigm. The study reported in this paper is the first to examine the indirect effects of odour on visual ERPs using an odour priming paradigm. The N400 waveform component was chosen because it has been reported to be reliably elicited using a variety of stimuli across different sensory modalities. As indicated above (Domalski *et al.*, 1991; Pratorelli, 1994), it has also been found in cross-modal investigations.

In an earlier study Grigor (1995) used common food odours as primes. These were argued to be more readily identifiable and therefore likely to generate neural semantic networks. N400 peaks are thereby elicited when paired with

an incongruous (non-primed) target. The food smells preceded targets, which were photographs of foods. In some cases (the primed condition) the odour and the photograph consisted of the same food item. In others the odour and food photograph were different. The study was designed as a cross-modal design using the visual and olfactory senses. The main finding supported the prediction that the N400 component was greater for the incongruous (non-primed) than for the congruous (primed) condition.

The present study was designed to extend the earlier experiment by investigating the effects on long latency EPs (N400) using non-food odours and photographs of objects which were matched or mismatched in terms of the priming odour.

## Materials and methods

### Subjects

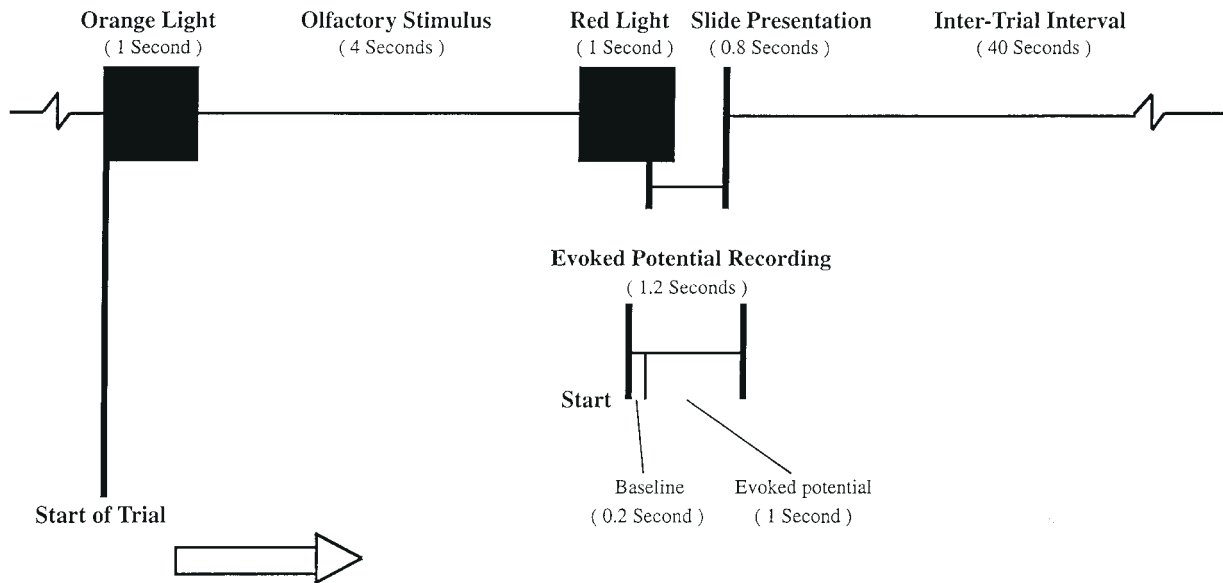
All participants were volunteers recruited via poster, computer mail-shots and adverts from within the university and external colleges (a few external volunteers came forward following an interview given on the local BBC radio station). A total of 21 volunteers took part in the study of which 11 were males and 10 were females. The mean age of the subjects was 25 years, with an age range of 20–35 years. All were right handed as assessed by the shortened version of the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects gave written consent for the study which had received approval by the Ethical Committee of the Psychology Department. Each subject was paid (£4.00) for participating in the study. They were screened for suitability using an in-house questionnaire relating to sleep habits, beverage consumption and medication. Subjects were also asked to confirm that they were not suffering from colds, sinus problems, asthma or hay fever on the day of testing.

### Apparatus

The study used an olfactometer with a multi-input nozzle (White, 1991, 1997). The nozzle involves an arrangement whereby separate odour lines are brought together in a cone which has a central opening that is placed below the nares of subjects. A NeuroScan Inc 32 Syn-amps EEG Amplifier (model number 50835/N) was used to record the electrical activity from the cortex. Three Kodak slide projector carousels back-projected images onto a translucent screen. A white noise generator provided masking sounds via headphones worn by the subjects. Using an in-house program, an Apple Macintosh 11SI computer synchronized the equipment and the presentations used during the experiment. A schematic flow diagram showing presentation sequences of the stimuli are shown in Figure 1.

### Olfactory stimuli

The olfactory stimuli were four suprathreshold synthetic odorants—rose, lemon, grass and leather—provided by



**Figure 1** Schematic diagram showing the experimental paradigm. An orange light signalled presentation of the priming odour for 4 s. A red light signalled the slide presentation. During the slide presentation, and for 20 ms before and after the trial, the amplifier recorded the EP. The intertrial interval lasted for 40 s.

Quest International. The odours were diluted and judged to be iso-intense in a pilot study using 10 subjects. The rose, lemon and grass solutions were each diluted using equal volumes of dipropylene glycol. The leather odour was diluted 1/10. A total of 75 ml of each odour was placed in the olfactometer bottles. The odours were screened in a pilot study by subjects who rated them for familiarity, iso-intensity and match to the visual stimuli. The olfactometer presented each suprathreshold odorant in a stream of charcoal-filtered air at room temperature using a flow rate of 1 l/min. Presentation of the odours was for a period of 4 s. The control air and odour streams were delivered just below the subject's nose using a multi-input nozzle. Each odour was delivered via separate lines to the conical output nozzle which was placed below the subject's nares. During periods when an odour were not being presented a stream of humidified air at room temperature was delivered to the subjects via the nozzle. As mentioned earlier, odour deliveries were controlled by a Macintosh computer. Switching time for the odour delivery was fast relative to the total odour delivery time.

### Visual stimuli

Each odour was represented by a series of photographs. These consisted of 35 professionally taken high-quality coloured slides presented via three Kodak carousel projectors. The slide presentations were controlled by the computer and were carefully synchronized with the priming odours. Each of the four priming odours had a corresponding set of eight slides. For six of the slides the odour matched the image and for two of the slides the odour did not match the image. For example, an odour-matched image

**Table 1** Mismatched visual stimuli presented concurrently with odours

Odour	Slide 1	Slide 2
Grass	road surface	beach
Leather	stool	plant
Rose	baseball bat	cricket bat
Lemon	rugby ball	electrical plug

for the smell of grass was a slide showing a picture of grass. An odour-mismatched image was a slide showing an image of a road surface. The mismatched images were selected to be visually similar in terms of either shape or size. The mismatched visual stimuli are presented in Table 1.

In an attempt to reduce boredom and novelty effects, and to ensure that subjects looked at the projected pictures, each view was taken from a different angle. All the presented slides were slightly different.

The slides were back projected onto a 160 × 109 cm translucent screen. Each slide was 80 × 50 cm when projected onto the screen. Subjects were seated 1.5 m in front of the screen with their chins placed on a chin rest and the odour nozzle just below their noses. To help standardize overall illuminance the slides were projected through a neutral density filter. To ensure that each subject was comfortable the chin rest was adjusted each time. As shown in Figure 1, the slides were presented for a duration of 800 ms. A ratio of 75/25 matched/mismatched was used.

### ERP recordings

The ERPs were recorded using a commercially produced

electrode cap (Electro-cap, Eaton, OH) and a NeuroScan Inc, 32 channel Syn-amps machine, model no 50835/N (Neuroscience, Warwick, UK). The electrodes were referenced to linked mastoids using a forehead electrode as a ground. All impedances when recording were below 15 k $\Omega$  and typically below 7 k $\Omega$ . The total recording epoch was 1200 ms, with a 200 ms pre-stimulus and a post-stimulus period serving as pre-stimulus and post-stimulus baselines. The A/D rate was 1000 Hz, leading to a sampling rate of 1200. The bandpass was 0–20 Hz. ERP trials were recorded individually tagged in order to allow for later off-line averaging into matched and mismatched trials.

### Procedure

Following completion of various questionnaires, the electrode cap was fitted to the subject with Omni-prep used to prepare the scalp sites and a commercial (SLE) electrode gel inserted into the individual electrode cups. The multi-input nozzle from the olfactometer was then adjusted to a position just below and in front of the nares of the subject. Headphones were carefully placed over the subject's ears, to ensure no alterations in electrode positions or impedance levels. The white noise generator was switched on and adjusted to a level comfortable for the subject but above a minimal level masking apparatus sounds. The olfactometer was located in a nearby closed fume cupboard. The experimenter was able to communicate with the subject via the headphones. They were then asked to read the following experimental instructions and encouraged to ask any questions.

#### *Instructions to subjects*

This experiment relies upon your breathing technique. You are required to breathe in through your nose and out through your mouth. The brass nozzle in front of your nose may or may not deliver an odour. Please breathe through your nose evenly, there is no need to sniff. Try to remain as relaxed as possible throughout the experiment. An orange light will appear in order to alert you to the presentation of an odour. At this time place your chin in the chin rest and begin breathing in through your nose and out through your mouth and try not to move your head from side to side. Following the odour a second red light will appear to alert you to the presentation of a slide on the screen in front of you, showing a colour slide which will either be related to the odour you have just received or bear no relation to the odour. You are not required to make an oral verbal judgement during the experiment but simply make conscious decisions as to the relationship between the two stimuli. It does not matter if you recognize the odour but are unable to name it, simply concentrate on deciding if the two stimuli are related. Please try not to blink, swallow or make any motor movement following the second light until about 1 second after the slide has been switched off. Please ignore the

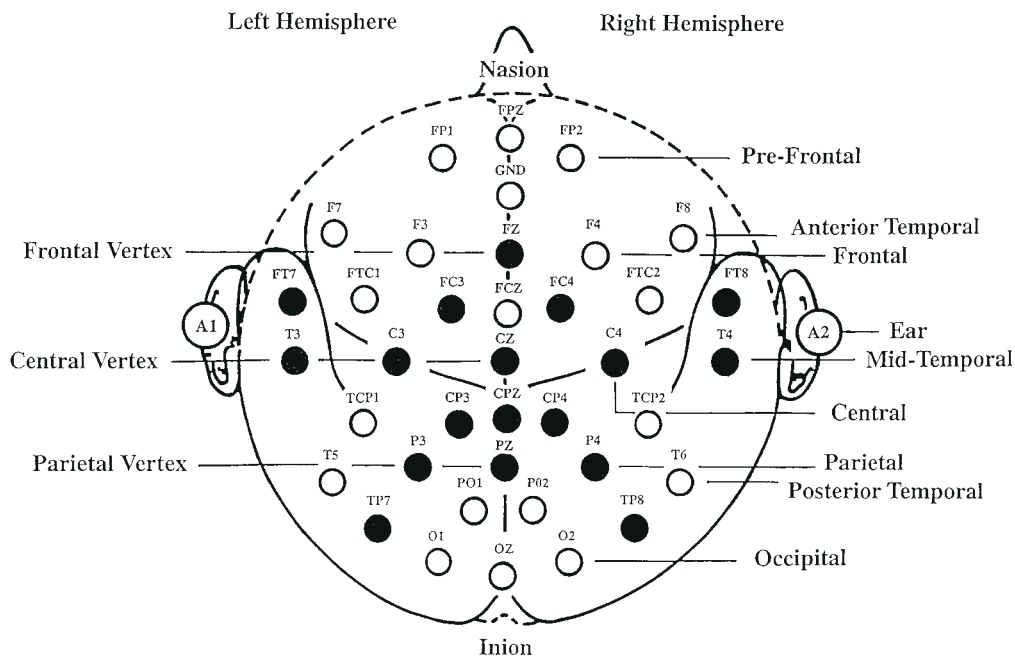
video camera which is recording your eye movements and try to keep your eyes focused on the centre of the screen. The headphones you are wearing will produce white noise in order to reduce the sounds from the various pieces of equipment used to run the experiment. Three practice trials will be given, followed by a short break, please ask any questions or make any comments you feel are relevant. There will be 32 experimental trials. Do you have any questions or require further information before you begin? Thank you for participating.

Subjects were instructed in the breathing technique and given the three warm-up practice trials. The breathing method was to prevent retronasal stimulation of the olfactory receptors. Once the initial phase had been satisfactorily completed, illumination level in the room was lowered and the computer programme and ERP recordings started. During the 32 experimental trials an orange warning light was illuminated for 1 s in order to alert subjects to the impending odour and the need for the required breathing pattern. An odour was presented for 4 s. A red light was then illuminated to warn the subject about an impending slide presentation. The warning lights helped the subjects reduce muscular movements during presentation of the stimuli. Subjects were asked to look at a fixation point on the screen. In order to do this a dot was present, other than during slide presentations, at the centre of the screen. Following a slide presentation, subjects were required to make a subvocal decision about the relationship, if any, between the odour and the slide. However, no oral or motor response was required during the experimental trials.

The EEG recordings were started 200 ms before the projection of the slide and lasted until 200 ms post-picture offset (see Figure 1 for details). Slides were projected for a total period of 800 ms. An intertrial interval of 40 s was used between each of the 32 trials in order to reduce response habituation. After the experimental trials had been completed subjects were presented with the same 32 stimuli in an identical order and asked say if the odour matched the slide. This procedure was carried out in order to check that the subjects had performed the task correctly.

### Treatment of results

Off-line editing of post-recording data included digital filtering at 0.03 and 30 Hz (24 dB/octave). Trials containing in excess of 75  $\mu$ V were rejected. Trials containing eye movements were corrected using the algorithm of Semlisch *et al.* (1986). Analysis was initially performed on a subject by subject basis on the NeuroScan Syn-amps by selecting the matched and mismatched ERP trials for each subject at each electrode site. These data were printed as two grand average waveforms (match and mismatch for each electrode for each subject; see Figure 2). The original NeuroScan ASCII data was then imported into SPSS and the statistical analyses



**Figure 2** The location of the 18 selected electrodes which were used in the SPSS statistical analysis. The four midline electrodes were Fz, FCz, Cz, CPz and Pz. The seven left and seven right hemisphere electrodes are to be found either side of the midline electrodes.

were performed on an Apple Macintosh computer using the SPSS statistical package version 6.1.

A latency window was selected based on the hypothesis that the N400 component would be differentially affected by the matched and mismatched conditions. The window selected for the ERP component analysis was between 300 and 600 ms post-stimulus onset, or 500–800 ms from the start of the recording (see Figure 1). The peak negativity values were selected from the 300 data points within this latency window.

## Results

The converted results were subjected to an SPSS repeated measures ANOVA with the factors being sex  $\times$  electrode  $\times$  condition. All significant results quoted are significant to at least  $P < 0.05$ . All reported ANOVA results are quoted using Greenhouse–Geisser tests for significance.

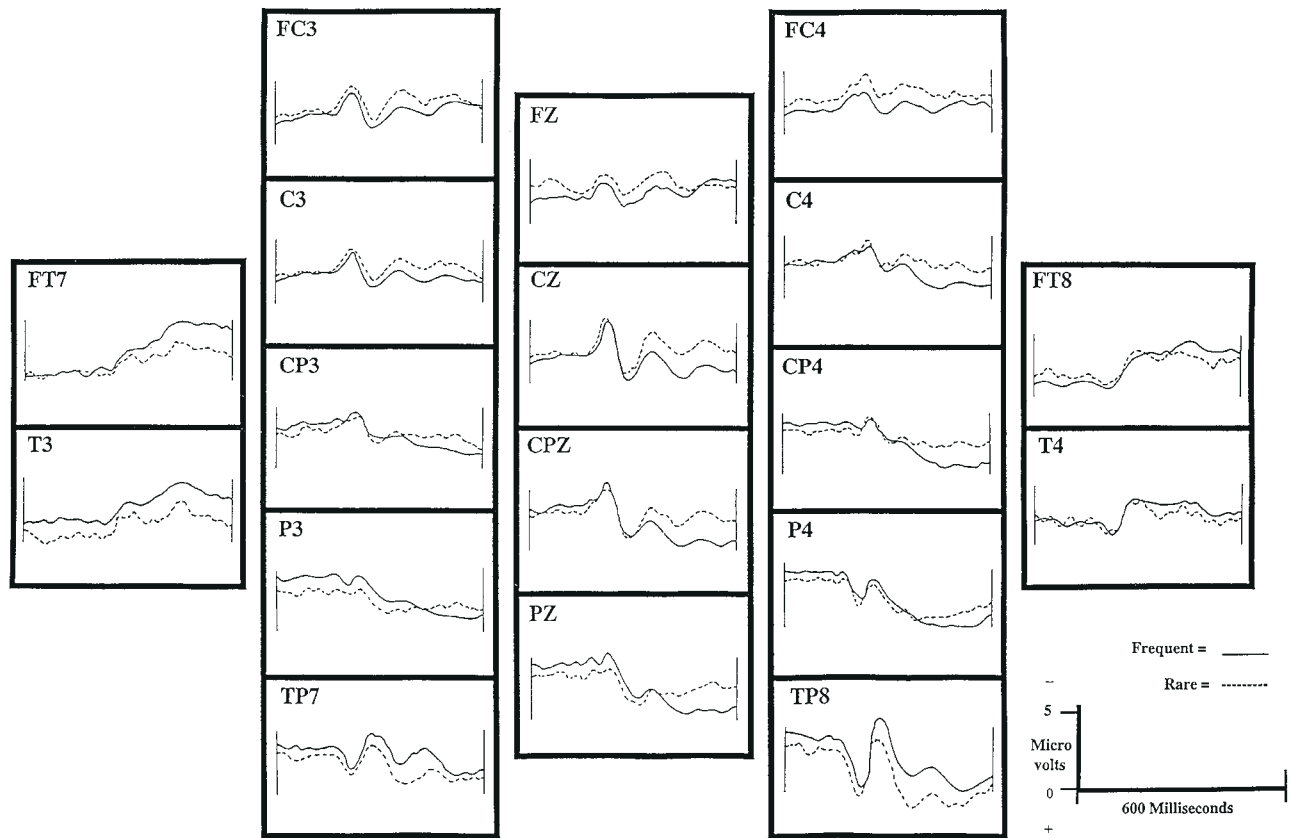
The waveforms were visually inspected and, in order to reduce the data to manageable proportions, 18 electrode sites were selected and used for the quantitative analysis. The selected electrodes were Fz, FT7, FT8, FC3, FC4, Cz, C3, C4, CP3, CP4, CPz, T3, T4, TP7, TP8, Pz, P3 and P4 (see Figure 2). The qualitative frequent and rare waveforms for each of the electrode are shown in Figure 3. Electrode grand means and standard deviations for the frequent and rare conditions are shown in Figure 4.

Overall there was a significant condition effect ( $F = 15.63$ ;  $df\ 20,1$ ;  $P = 0.001$ ) for the N400 waveform, showing a significant difference for the 21 subjects used in this study between the frequent (matched) and the rare (mismatched)

conditions. Electrodes reached significant levels ( $F = 2.44$ ;  $df\ 17,30$ ;  $P = 0.048$ ). The condition  $\times$  electrodes interaction showed a trend without quite reaching significance. Similarly, there was a near significant midline electrodes effect ( $F = 2.64$ ;  $df\ 1,20$ ;  $P = 0.081$ ) and a near significant interaction of condition by electrodes at the mid line sites ( $F = 2.91$ ;  $df\ 1,20$ ;  $P = 0.06$ ). Statistical testing of differences between the hemispheres showed a significant condition effect ( $F = 30.544$ ;  $df\ 1,20$ ;  $P = 0.002$ ). There was a significant electrode effect ( $F = 3.58$ ;  $df\ 1,20$ ;  $P = 0.02$ ), but no significant effect of hemisphere. An interaction between hemispheres and electrodes just reached significance ( $F = 2.65$ ;  $df\ 1,20$ ;  $P = 0.06$ ). Overall, female subjects showed a significant condition effect ( $F = 8.45$ ;  $df\ 1,10$ ;  $P = 0.016$ ). This was also the case for male subjects but it was more marginal ( $F = 5.59$ ;  $df\ 9,1$ ;  $P = 0.042$ ). The males and female subjects showed no significant hemispheric or electrode effects and no significant interactions.

## Discussion and conclusions

The results showed that there was a difference in the ERP waveform for the N400 waveform when a visual image did not match the odour used as a prime. A negative peak with a latency of  $\sim 400$  ms post-stimulus onset was produced for both matched and mismatched stimuli, with the latter condition being statistically significant. This study extends an earlier finding (Grigor, 1995) using food odours. This study, in common with earlier visual and auditory ERP experiments, found a significant cross-modal olfactory/visual effect using an olfactory prime. In contrast to the



**Figure 3** The grand mean waveforms for each of the selected electrodes. Electrodes are grouped into midline, left and right hemispheres. Up is negative.

Electrode	Condition	Average (Av.)	Standard Deviation (Sd.)
FC3	Freq.	0.20	6.55
	Rare	-5.12	6.22
C3	Freq.	-0.52	5.45
	Rare	-4.69	6.68
CP3	Freq.	-0.17	6.74
	Rare	-4.57	6.85
P3	Freq.	0.89	8.15
	Rare	-3.06	8.13
TP7	Freq.	-0.30	8.30
	Rare	-3.58	7.83
FT7	Freq.	-4.25	7.04
	Rare	-6.03	5.55
T3	Freq.	-1.49	9.09
	Rare	-3.66	10.03
FZ	Freq.	0.32	6.93
	Rare	-3.78	4.66
CZ	Freq.	-0.17	8.02
	Rare	-6.49	7.19
CPZ	Freq.	0.51	7.44
	Rare	-4.78	7.75
PZ	Freq.	1.27	8.22
	Rare	-3.47	8.21
FC4	Freq.	-0.20	6.86
	Rare	-4.91	5.62
C4	Freq.	0.63	6.63
	Rare	-4.76	6.24
CP4	Freq.	1.76	7.73
	Rare	-3.71	7.44
P4	Freq.	2.65	8.29
	Rare	-1.59	7.41
TP8	Freq.	2.69	10.48
	Rare	-2.35	6.53
FT8	Freq.	-4.27	9.08
	Rare	-6.47	6.08
T4	Freq.	-0.77	5.93
	Rare	-5.66	6.98

**Figure 4** Grand mean and standard deviation values for the frequent and rare trials for each of the 18 selected electrodes. At all sites the rare value is more negative than the frequent value.

previous reported work, no overall hemispheric effects were obtained and amplitude differences were greatest over the midline electrodes FZ, CZ, CPZ and PZ.

Both Lorig *et al.* (1993) and Grigor (1995) reported increased negativity over frontal electrodes. Lorig and his collaborators found increased negativity over the left frontal electrodes, whereas Grigor reported increased negativity over the right frontal electrodes. Kutas and Hillyard (1980) reported in their seminal study that N400 was maximal in sites over the right hemisphere. However, the present experiment failed to find such an effect. In the study conducted by Lorig and his colleagues (1993) the odour was provided with a verbal label. It could be argued that this would require left hemispheric processing, which may account for his findings. The earlier study by Grigor (1995) provided pictures of matching or mis-matching stimuli in response to the odours and is therefore more likely to require right hemispheric processing. An alternative explanation may be that both studies used food or food related odours. Lorig *et al.* (1993) noted that an incorrect label to an odour stimuli should result in a large P300 component because it is dependent upon the subjective likelihood of an event and therefore should produce an oddball effect. Whilst we would agree with this proposal, it is also true that both Lorig's study and the present one require semantic decisions regarding the degree of congruence between the odour and its subsequent label. This interpretation is supported by Lorig's finding of increased negativity when subjects were required to match olfactory stimuli to visual labels. Lorig *et al.* (1995) reported a slow wave elicited by an odour. In this study we did not measure the baseline changes and it is possible that slow wave changes account for the negativity reported here.

As has been noted earlier, the N400 is produced whenever a semantic decision is required but it is at its greatest amplitude when semantic incongruency is present. Decon *et al.* (1991) have suggested that what is critical for the N400 is not the relationship between the stimuli but whether or not the stimuli are processed semantically. An odour presented without cues is a stimulus requiring a high level of semantic processing. Subjects when tested after the experiment found no difficulty in identifying the odours and relating them to the photographs. Unlike Lorig, we did not attempt to assess the overall competence of our subjects but the odours were set at suprathreshold levels and presumably readily able to produce a contextual set.

The reported procedure shows a valid method of evaluating the linguistic and associative semantic networks and their relationships to odours. Although it was not attempted in this study, in any follow-up study it would be of interest to assess the odour lexicons of the subjects (van Toller *et al.*, 1992). It would also be interesting to extend the study by examining effects of emotional judgements on the N400 waveforms of an ERP waveform.

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Accepted November 6, 1998