Visual Event Related Potentials Modulated by Contextually Relevant and Irrelevant Olfactory Primes

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Abstract

Visual evoked potentials were recorded from 16 scalp locations on 10 young subjects during presentation of a series of high-quality photographs on a computer screen. The photographs consisted of equal numbers of pictures of fruit (citrus and non-citrus fruits), flowers (roses and other flowers) and objects (e.g. buildings, vehicles, animals etc.). Every picture was different in order to avoid repetition effects. The pictures were presented under four odour conditions: no odour, rose odour, jasmine odour and citrus odour. In order to keep the subjects alert they were asked to make categorizing decisions for the visual stimuli (e.g. flower or fruit). No decision was required concerning the relationship between the visual stimulus and the odour. As expected, the N400 peak was more negative when the picture stimulus did not match the odour. It is hypothesized that the N400 peak can be used as a measure of relatedness of a sensory stimulus to a previous or on-going prime, irrespective of the mode of the stimuli.

Introduction

It is possible to record brain electrical activity associated with olfactory stimulation. The principal methods include either recording spontaneous EEG activity or recording event-related potentials (ERP) following presentation of an odour. Since Moncrieff (1962) first reported EEG effects due to odour stimulation, considerable advances have been made in both investigative techniques and theoretical exposition. Several researchers have reported direct effects of odours on EEG and have attempted to understand the underlying emotional and cognitive events of fragrance perception (e.g. Klemm et al., 1992; Van Toller et al., 1993). Lorig and Schwartz (1988a, 1989) found that a particular odour (spiced apple) increased the number of waves in the theta (4–7 Hz) band when compared with a resting baseline or with other odours (lavender and eucalyptus). They also found that a number of other odorants, most of which were food-related, reduced higher frequency EEG activity. The same group (e.g. Lorig and Schwartz, 1989) also indicated, however, that imagery of food affected the EEG in a similar way to the food-related stimulus. These findings were interpreted as suggesting that the mechanism by which some odours decrease EEG arousal is cognitively mediated rather than direct. Klemm et al. (1992) measured EEG response to a selection of pleasant and unpleasant odours, and found consistent responses to odours, across subjects, in the theta waveband. These were not clearly associated with perceived strength, degree of arousal or pleasantness. Van Toller et al.

(1993) investigated a diverse range of odours and found an increasing alpha amplitude of cortical activity related to psychometric response, irrespective of the nature of that response (i.e. intensity, pleasantness or familiarity). A more recent study by Brauchli et al. (1995) reported increases in alpha2 power after stimulation with an unpleasant odour, while no effect was found with a pleasant odour. These studies highlight the complexity of brain response to olfactory stimulation and the scope for interaction with other modalities.

Other measures of brain activity used in investigations of olfactory response include chemosensory (olfactory) event-related potentials (CSERPs) (e.g. Kobal and Hummel, 1988; 1992) or contingent negative variation (CNV), which is DC, as opposed to the AC current of the EEG (e.g. Torii et al., 1988). Both of these have shown olfactory responses modified with changing hedonics and mood. Kobal and Hummel (1988, 1992) found differences in olfactory and chemosensory evoked potentials (OEPs and CEPs) to olfactory stimuli. They demonstrated that the latency and magnitude of the response peak was dependent on whether or not the odour presented was considered pleasant or unpleasant and whether it was presented to the left or to the right nostril. Ehrlichman (1986) had previously also reported hemispheric differences in processing pleasant and unpleasant odours. The experimental techniques employed nasal cannulae and a sophisticated olfactometer

which warmed and humidified the 5.6 l/min airstream passing over the olfactory mucosa. In our experience, many subjects find such odour administration techniques uncomfortable.

The interpretation of observed effects of odour on measures of CNV have been debated by Lorig and Roberts (1990). They suggested that cognitive effects (such as associations and memories) can be involved in CNV changes. Torii *et al.* (1988) studied the effect of odour stimulation on CNV. They interpreted their results as indicating that an increase in CNV signalled subject stimulation and a decrease signalled relaxation. However, although Lorig and Roberts (1990) found changes in the CNV similar to those reported by Torii, they also found that comparable changes could be induced by expectations of odour qualities.

Another avenue of research has utilized the effect of odour on traditional visual and auditory ERPs. For example, Lorig (1991) noted changes in P300 amplitude, i.e. a positive peak detected ~300 ms after presentation of the stimulus. He used a classic 'oddball' paradigm, in three different experiments, in which the subject was presented with a series of stimuli (e.g. different tones were presented in an auditory experiment or different words were presented together with 'non-words' in a visual experiment). In their work 80% of the stimuli perhaps were common and 20% rare, and the subjects counted the rare occurrences. A difference in amplitude of the P300 was detected between the two conditions (ie. common or rare). In the third experiment subjects were presented with three pairs of odours and labels; ERPs were recorded to the visual stimulus. In this latter case subjects' odour thresholds were found to be significantly correlated with the amplitude of the P300 peak but the data were not interrogated further.

Lorig *et al.* (1993), in their consideration of the use of psychophysiological measures for investigations of olfaction, expounded the use of traditional ERP paradigms evoked with a visual or auditory stimulus, modulated by odours. Again they used a classical 'oddball' paradigm wherein an olfactory stimulus was administered and followed by a visual label. In the most frequently occurring trials ($P = 0.75$), the label correctly identified the odour stimulus. In the rarer trials, an incorrect label was presented ($P = 0.25$). ERPs were recorded to these congruent and incongruent stimuli. A large positive component occurred ~300 ms following the stimulus. The amplitude of this P300 wave has been found to be affected by the subjective certainty that the rare and frequent stimuli differ. This is consistent with other observations in association with novel stimuli or stimuli that contradict one's expectations and enhance P300 amplitudes (Pritchard 1981; Coles *et al.*, 1990).

The concept of a priming effect has been investigated extensively, at both the behavioural and physiological levels, using linguistic (words) and non-linguistic (pictures and diagrams) stimuli. Studies have tested the hypothesis that a target stimulus is 'primed' and the subsequent recognition of the target is facilitated when it is preceded by a prime with which it has one or more features in common. This effect may be manifested, for example, by a faster reaction time to the target. It has also been found that an ERP component, N400, is sensitive to the congruence between the target stimulus and a previously established context (Pratarelli, 1994). For example, changes to the N400 have been reported by Kutas and Hillyard (1980) using sentences to establish context.

Subsequent investigations have focused on expanding the types of contextual information that can elicit priming effects. Of particular interest are experiments where cross-modal paradigms have been used. For instance, Barrett and Rugg (1989) followed previous semantic experiments with experiments of their own using semantic stimuli and then further experiments using pictures. In the second of their reported experiments using pairs of pictures of faces subjects were required to press a button to indicate whether the faces were congruent or incongruent. Their results showed that the time taken to press the button when faces were incongruent was slower and the ERPs more negative than their related counterparts. This negativity peaked at ~450 ms following presentation of the target. Barrett and Rugg concluded that their N450 component was similar to the N4 (or N400) evoked by linguistic stimuli and that N4 effects could be obtained with a variety of meaningful stimuli so long as an associative relationship between primes and targets could be established.

Barrett *et al.* (1988) have offered a general explanation which suggests that the class of N4 (or N400) ERPs reflect the processing of some aspect of a representational system that includes (but is not limited to) semantic information. It is possible that the N4 (or N400) responses are the result of a common conceptual processor that is indifferent to the mode of input or the representational system accessed.

The development of an argument for a common conceptual memory system valid across modes of stimuli was continued by Pratarelli (1994), whose work paired colour photographs with spoken words. Each of the 26 words and pictures appeared in both the matched and non-matched conditions. Therefore there was the potential for repetition priming in which subjects might be able to recall seeing a subset of the pictures or hearing a subset of the words. To compensate, the pseudorandom order of trials was carefully checked to ensure that any one item was placed as far as possible from its second occurrence. The ERPs elicited to unrelated picture targets and unrelated word targets were more negative than their related counterparts and fit the characteristics of the N400 in all but the initial phase of differential processing. Pratarelli concluded that the N400 is a member of a class of negativities that are evoked in response to stimuli that violate a previously established context.

Nigam *et al.* (1992) also compared the processing of line

drawings to the processing of words. They found larger negativities (N400) to anomalous words and anomalous pictures than to semantically congruous words and pictures. The authors reported no differences in amplitude, latency or scalp distribution between the negativities elicited by words and those elicited by pictures. They also concluded that these N400 effects reflect activity in an amodal, abstract semantic system.

One characteristic of many 'oddball' experiments and also of relatedness judgement tasks (cf. Barrett and Rugg, 1990) is that subjects are required to make an overt semantic decision. Holcomb and McPherson (1994) tested whether the N400-like effects were present in a picture-pair ERP experiment in which the task did not overtly require the subject to semantically compare the prime and the target. On each trial subjects were presented with two line drawings (prime and target). A total of 150 pairs were presented with one-third of the target pictures being related to the prior prime picture, one-third being unrelated to the prime picture and the remaining one-third being non-objects that were unrelated to the prime picture. The recorded ERPs varied with the type of target picture, showing a substantially larger negative peak, between 325 and 550 ms for unrelated than related target pictures. The non-object pictures, which were designed to be the picture equivalent of pseudo-words, also produced a large negative response that was significantly larger than the negativity elicited by unrelated pictures. Holcomb and McPherson (1994) concluded that their results, and those of Barrett and Rugg (1990), supported the position proposed by Nigam *et al.* (1992) that the N400 reflects activity in an amodal, abstract semantic system.

A previous study investigating cross-modal effects with olfactory stimuli was reported by Grigor (1995). She used a series of common food odours as odour primes for a series of photographs of foods. Subjects were presented with an olfactory prime followed by a visual target and were then required to decide whether the target was congruent or incongruent with the previously presented odour. The findings supported the prediction that the N400 component is more negative in the non-primed condition than in the primed condition.

The main objective of the present study was to extend the findings of Grigor (1995) using odour stimuli as primes and measuring N400 amplitude in a visual evoked potential and, using a modified experimental procedure, to address procedural issues discussed earlier. Specifically, the issue raised by Holcomb and McPherson (1994) concerning the effect of conscious decisions about the relatedness of the targets and primes was addressed. In order to eliminate this type of judgement task and to minimize contamination of the priming effect with a conscious memory task subjects were not asked to make any decision concerning the relatedness of the target visual stimulus to the odour prime. However, in order to keep the subjects alert each subject was asked simply to press one of two buttons on a hand-held box to indicate whether or not the picture belonged to a designated category (e.g. flowers or fruits). The current study also avoids short duration odour stimuli by using an odour that is continuously present throughout a series of visual stimuli. In addition, the current design also eliminates the potential for repetition priming as discussed by Pratarelli (1994) by using each visual stimulus only once for each subject. It was predicted that if the N400 effect is an amodal effect generated through non-conscious priming, and not dependent on conscious matching decisions, then the unrelated target pictures would elicit more negative N400s than related target pictures.

Materials and methods

The objective of this work was to record visual ERPs during olfactory stimulation. The experiment was designed to manipulate the level of relevance of the visual stimuli to odour primes by using a series of pictures of objects with varying degrees of relevance to the odour primes.

Visual ERPs were measured in four separate odour conditions: no odour; rose odour; jasmine odour; and citrus odour. Within each odour situation there were four different types of visual stimuli: the visual stimuli were congruent with the odour prime (e.g. rose odour with rose pictures) or semi-congruent (e.g. rose odour with flower pictures); or they were incongruent to the odour prime. There were two levels of incongruent pictures: one class in which the picture could be related to an odour but not the same odour as the odour stimulus (e.g. rose odour with pictures of fruit); and a second class with no such odour connotation (e.g. rose odour with pictures of a car or a building). The method employed for collection of the ERPs, where relevant, followed the principals of Evans *et al.* (1993).

Subjects

Participants were all volunteers recruited via advertisements on hospital and university notice boards. Two females and eight males aged between 21 and 23 took part in the experiment and were offered a small remuneration for their time. All were right-handed non-smokers and were screened for coryza and nasal allergy; all had bilateral nasal patency. None of the participants were taking part in any concurrent experimental trial. The recording procedure was explained verbally to the subjects who provided written consent prior to participation. Prior ethical approval had been obtained from the hospital ethical committee which operates in accordance with the Helsinki Declaration.

Apparatus

A simple olfactometer was employed to prepare and deliver the odour stimuli. Clean bottled air was further purified using charcoal filters and then switched by computer
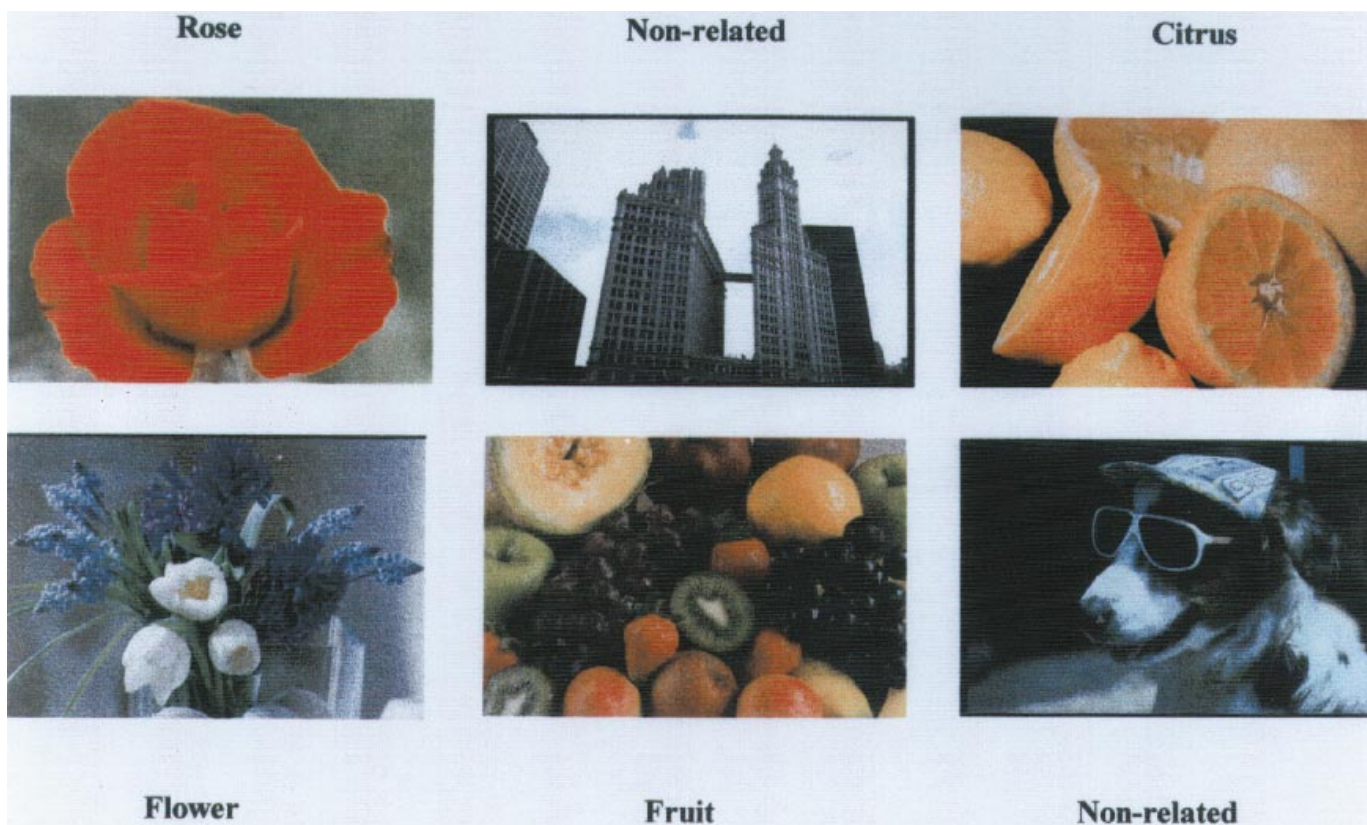
Figure 1 Examples of the three types of visual stimuli: flowers, fruit and non-related objects.

controlled solenoid valves between the different odour generators of the olfactometer as appropriate. Each odour generator consisted of a glass boat containing one of the odour stimuli. The air was passed over the odorant at a rate of 1 l/min and the resulting odour stream was presented directly to the subject at a sniffing port. Provision was also made to deliver clean air directly to the subjects at the appropriate times. The air was delivered to the subject via Teflon tubing to a facial mask.

During each phase of the experiment the appropriate odour-laden air was presented to the subject for 1 min and thereafter clean air, without odour, was delivered for 1 min. Computer control of the solenoid valves enabled precise timing of the odour changes. The computer also synchronized the timing of the presentation of the pictures, picture type (i.e. fruit or flower etc.) and marking of the EEG traces (to show the onset of each visual stimulus). Only data recorded during the perfume presentations were analysed. Previous experience had demonstrated that providing successive periods of odour and clean air reduced the effects of odour habituation in subjects. Debriefing of the subjects at the end of the recording period verified that the subjects were aware of the presence of odours intermittently throughout the trial period, and could still perceive the sample odorants with ease.

Olfactory stimuli

The olfactory stimuli were three pleasant synthetic odorants prepared by professional and experienced perfumers at Quest International and were considered to be accurate representations of the true smell of roses, jasmin or citrus fruit. A simple olfactometer (described above) was designed to deliver odours significantly above sensory threshold during the period of recording visual evoked potentials. The odours were judged to be iso-intense by a panel of experienced assessors, following the methods of Van Toller *et al.* (1993). The oils were placed in the glass olfactometer boats, as described previously, over which the air was passed during the 'odour on' periods.

Visual stimuli

The visual stimuli were high quality (super VGA 256 colours) photographs from a sequence of 1170 projected onto a computer screen. The pictures were of three types: flowers, fruits or other unrelated objects such as buildings or animals. The flowers and fruits also had subcategories of roses and citrus fruits.

Each picture was unique; none of the subjects were presented with the same picture a second time. Examples of the pictures are shown in Figure 1.
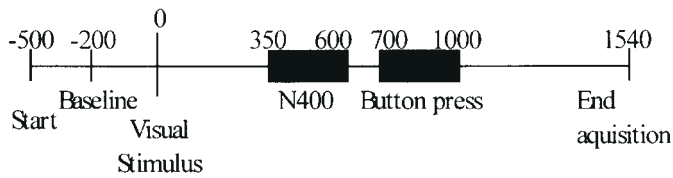
Figure 2 The timing of events during presentation of each visual stimulus. N400 occurs during the 350–600 ms window after the visual stimulus presentation and the subjects pressed the button on the hand-held box between 700 and 1000 ms. A new picture was presented every 4 s and signal averaging was terminated at 1540 ms. A blank screen replaced the picture with a fixation cross after 2000 ms.

Procedure

Subjects sat in a reclining chair in a small room (2 × 1.5 × 3.5 m), and were fitted with an electrode cap for recording EEG responses from the scalp and a facial mask through which the odours were delivered. The room was ventilated to avoid odour accumulation. The solenoid valves and other operating systems were located in the main laboratory outside the low-odour room and subjects were unable to hear the various operating sounds.

The subjects were asked to observe a series of pictures presented on a visual display unit. A picture was presented every 4 s and subjects were asked to classify them as fruit, flower or neither fruit nor flower. The subjects were instructed simply to observe the pictures on the screen and press the left hand button on a hand-held box when the picture was a fruit or a flower and the right hand button for any other object. They were not required to match the object to the odour nor to distinguish a stimulus class, e.g. citrus fruit. They were informed that odours would be presented from time to time but they were not required to identify the odour, and were asked to breathe normally and regularly throughout the testing period.

Pictures were presented in random order and individual pictures were not repeated. They were displayed for 2 s. The sequence of events during the EEG recording period is represented in Figure 2.

The experimental design splits the duration of measurements on a single subject into four distinct periods. The odour primes for each period are detailed in column 3 of Table 1. To avoid fatigue and adaptation to the odour prime the prime was alternated with filtered air every 1 min. Within each odour period an equal number of pictures from the five categories, namely rose, other flower, citrus, other fruit and neither fruit nor flower, were presented to each subject (see Table 1 for details). A new picture was presented every 4 s and displayed for 2 s. Pictures were presented during both the presence and absence of an odour prime, but only those responses in the presence of an odour were analysed.

After the last experimental session, the electrodes were removed and the subjects participated in a short debriefing. Subjects reported no difficulty with the categorization task

Table 1 Experimental order of procedure

Trial	Duration (min)	Odour	Pictures
1	10	none	roses, other flowers, fruit, unrelated objects
2	20	rose	roses, other flowers, fruit, unrelated objects
Rest	15		
3	20	citrus	citrus fruit, other fruits, flowers, unrelated objects
Rest	15		
4	28	jasmine	roses, other flowers, citrus fruits, other fruits, unrelated objects

using the hand-held box and none was evident. The computer recorded their reaction times and response. These responses served as a check on attention and possible fatigue; in fact there were only 1% incorrect or absent responses.

The class (flower etc.) of each stimulus was also recorded by the computer with each picture so that EEG responses for each class could be averaged separately.

ERP recordings

Recordings were obtained using a fabric cap fitted with 16 electrodes in locations defined by the 10–20 system, four central (Fz, Cz, Pz, Oz) and 12 lateral (F3, F4, C3, C4, P3, P4, T3, T4, T5, T6, O1, O2). Linked mastoid electrodes served as the reference. The electro-oculogram was recorded from electrodes situated above the right eyebrow and on the outer canthus of the left eye. Electrode impedances were <5 kΩ, and signals were amplified using Nihon–Kohden EEG amplifiers with a bandwidth of 0.03–30 Hz. Signal acquisition was achieved using NeuroScan analog-to-digital converters and software. Each trace was stored separately and checked off-line for correct subject response and other extraneous artefacts, e.g. eye blinks and noise level.

Figure 2 illustrates the sequence of events during the recording periods. Recordings of the visual evoked potentials were obtained during the 10–20 min epochs in which the subject was exposed to no odour, or to rose, jasmin or citrus odours. Each EEG recording began 500 ms (–500) before the presentation of the visual stimulus (time 0). The N400 wave of interest occurred between 350 and 600 ms. Subjects generally recorded their categorizations on the hand-held box between 700 and 1000 ms (i.e. post-N400). Data acquisition ceased at 1540 ms, when the stimulus was replaced by a fixation cross on the screen for 2 s. During this period 512 data points per channel were collected at 4 ms intervals per point, thus allowing alias-free recording of signal frequencies up to 40 Hz.

Signal analysis

The EEG recording for each picture consisted of 16 scalp potentials, an eye movement monitor and the class of stimulus (flower, fruit or incongruent). After recording, each EEG trace was visually inspected by an experienced neurophysiologist for artefacts such as excessive noise, eye blinks or other eye movement. EEGs were rejected on these criteria. They were also rejected where responses made by the subjects concerning the picture class were either absent or incorrect.

The combinations of pictures and odours were classified as congruent, semi-congruent or incongruent as follows. For the rose odour, the rose pictures were considered congruent; other flowers were classed as semi-congruent and fruit as incongruent. For the jasmine perfume all flowers, except roses, were classed as congruent, roses as semi-congruent and fruit as incongruent. For the citrus odour, citrus pictures, other fruits and flowers were congruent, semi-congruent and incongruent respectively. Accepted signals were averaged according to the congruence of the picture with the prevailing odour. EEG traces were obtained for 40 pictures of each congruence category and averaged.

The N400 potential amplitude was defined as the negative peak occurring between 350 and 600 ms after the visual stimulus. The amplitude was taken as the mean of the peak amplitude with five recorded points on either side, ie. the mean of 11×4 ms epochs or the mean amplitude over a 44 ms period.

Statistical analysis

The data were analysed by ANOVA using the Greenhouse Geiser factor ε for those terms which included the electrode locations. The error structure was nested: subjects were split by odour period, which in turn was split by the presentation of the pictures. The responses were analysed as repeated measures on each subject (thus splitting the picture periods into smaller subplots). The treatment structure for the odours and pictures was crossed.

Results

Subjects were judged to have remained alert throughout the EEG sessions. Only 1% of the subject responses to the pictures (recorded by a button press) were incorrect or missing, and reaction times also remained consistently within a range of 720–770 ms.

Each subject was debriefed after recording was completed. They were asked to identify those odours that had been used during the recording session from a group of five stimuli. All of the subjects recognized the rose odour, 9/10 subjects recognized the jasmine odour and 7/10 subjects recognized the citrus odour with absolute certainty. In all other cases the odour was recognized as familiar but could not be identified as the same odour with certainty. Subjects were also asked to match the odours with a subset of the pictures. All quickly matched the rose odour with a rose picture, citrus with a picture of citrus fruit and jasmine odour with a picture of flowers, though not necessarily with pictures of jasmine. This confirmed that the experimenter's classification of congruent and incongruent stimuli matched that of the subjects.

The omnibus ANOVA treating all 16 electrodes as repeated measures and classifying pictures as congruent, semi-congruent and incongruent produced the following. Odour main effects (i.e. no interactions) were significant ($P = 0.038$) but the odour by electrode site interaction was insignificant. Both the congruence main effect and the congruence by electrode site interaction were significant ($P = 0.042$ and 0.014 respectively). On inspection (using principal component analysis; 75.04% of variance accounted for in the first two principal components) it was found that the central and frontal electrode groups and the occipital and parietal electrode groups formed two distinct clusters. As the central and frontal electrodes had the greatest signal-to-noise ratio a second analysis was performed on the six electrodes C3, C4, Cz, F3, F4 and Fz. Congruent pictures were significantly different from incongruent pictures for these electrodes, with a P-value of 0.040. It is notable that these electrodes are topographically adjacent to one another. All further analyses were conducted on an odour by odour basis since the main effects for odour and order of presentation could not be independently resolved.

Analysis of the N400 potential amplitude showed a significant picture by site interaction effect when citrus odour was present and an analogous result for the rose odour ($P = 0.017$ and 0.075 respectively; Table 2). As semi-congruent pictures are an intermediate step between congruent and incongruent pictures, the test statistics for

Table 2 ANOVA of N400 amplitude including three picture categories (congruent, semi-congruent and incongruent)

Term	df numerator	df denominator	F-statistic	P
No odour				
Picture	1.00	9.00	0.00	0.949
Site	2.54	22.83	13.07	<0.001
Picture by site	2.21	19.89	3.31	0.530
Rose				
Picture	2.00	18.00	2.30	0.129
Site	1.59	14.35	6.01	0.017
Picture by site	2.36	21.26	2.81	0.075
Jasmine				
Picture	2.00	18.00	2.41	0.119
Site	1.41	12.70	2.40	0.140
Picture by site	2.64	23.80	1.07	0.373
Citrus				
Picture	2.00	18.00	0.86	0.442
Site	1.59	14.35	3.32	0.074
Picture by site	4.61	41.61	3.25	0.017

Table 3 ANOVA of N400 amplitude including two picture categories (congruent and incongruent)

Term	df numerator	df denominator	F-statistic	P
Rose				
Picture	1.00	9.00	3.27	0.104
Site	2.00	17.99	4.93	0.020
Picture by site	2.78	25.01	4.31	0.016
Jasmine				
Picture	1.00	9.00	17.29	0.002
Site	1.45	13.02	2.42	0.137
Picture by site	1.65	14.82	0.96	0.390
Citrus				
Picture	1.00	9.00	0.75	0.408
Site	1.63	14.66	3.36	0.071
Picture by site	3.15	28.38	4.60	0.009

Table 4 Average N400 amplitude with no odour prime

Picture	F3	F4	Fz	C3	C4	Cz
Flower	−4.53	−3.28	−5.98	−2.31	−0.54	−0.68
Fruit	−5.98	−4.05	−5.98	−1.92	0.59	−0.98

SED = 0.640; df = 19.89; LSD = 1.34.

Table 5 Average N400 amplitude with rose odour prime

Picture	F3	F4	Fz	C3	C4	Cz
Rose	−1.88	−0.91	−3.26	−1.19	0.85	−1.15
Flower	−4.44	−3.31	−4.38	−2.64	0.51	−1.12
Fruit	−5.12	−3.82	−5.68	−2.20	−0.35	−2.39

SED = 0.644; df = 21.26; LSD = 1.34.

picture type include comparisons of like with like diluting the effect of picture type. For this reason we have focused on the contrast between congruent and in congruent pictures (Table 3). In Table 3 the effect of picture is significant for jasmine odour ($P = 0.002$) and the P-values for the picture by site interactions for both citrus and rose odours have decreased ($P = 0.009$ and 0.016 respectively).

Tables 4–7 give the amplitudes for the N400 peak for each odour, picture and electrode averaged across all the subjects. Where there was no odour present and subjects viewed a series of randomly presented pictures there was no significant difference in the mean amplitudes calculated for the different picture types (Table 4).

When the subjects were primed with a rose odour and shown rose, flower and fruit pictures a significantly more negative amplitude was recorded for N400 evoked by the fruit pictures than by the rose pictures on all of the frontal electrodes ($P < 0.001$). The flower pictures evoked an N400

Table 6 Average N400 amplitude with jasmine odour prime

Picture	F3	F4	Fz	C3	C4	Cz
Rose	−0.02	−0.38	−1.69	−0.47	2.29	0.32
Flower	0.38	1.11	0.24	1.39	3.54	1.87
Citrus	−2.23	−1.86	−2.08	−0.62	1.50	0.35
Fruit	−2.79	−2.45	−3.07	−1.23	1.38	−0.28

SED = 0.673; df = 23.80; LSD = 1.39.

Table 7 Average N400 amplitude with citrus odour prime

Picture	F3	F4	Fz	C3	C4	Cz
Flower	−1.23	−1.04	−1.62	0.33	0.50	−0.32
Citrus	−2.48	−0.18	−0.72	0.94	4.16	2.05
Fruit	0.13	−0.22	−0.71	1.07	3.05	1.45

SED = 0.684; df = 41.52; LSD = 1.38.

peak with a less negative amplitude than that evoked by the fruit pictures but more negative than that evoked by rose pictures. This difference, for the rose and flower pictures, was significant on the two lateral electrodes (F3, $P < 0.001$; F4, $P < 0.01$). No significant differences were recorded from the central electrodes for different pictures in the presence of rose odour except C3 (rose versus flower pictures, $P < 0.05$; Table 5). Figure 3 provides a representation of the resulting EEG trace for the Fz electrode.

When the priming odour was jasmine there was a significant difference between the N400 amplitudes evoked by the flower and fruit pictures ($P < 0.01$), and also a difference between the potentials evoked by the flower and citrus pictures ($P < 0.05$), across all six of the electrodes analysed. In every case the potential evoked by the congruent visual stimulus (i.e. flowers) was the least negative. Differences in the ERP response between the flower and rose pictures were also evoked from the F4, Fz, C3 and Cz electrodes (Table 6 and, for Fz, Figure 4).

When a citrus odour was presented to the subjects a similar pattern of differences for congruent and incongruent pictures was recorded for the F4 and Fz electrodes, but these differences were not statistically significant. Significant differences ($P < 0.01$) were detected from the C4 and Cz electrodes, reflecting a more positive peak with the congruent picture than was also observed with the central electrodes and the jasmine odour. This may reflect a later overlapping component not investigated in our current analysis and will be the subject of further study (Table 6 and, for Fz, Figure 5).

Discussion

The results show a negative deflection in ERPs recorded between 350 and 600 ms after stimulation with a visual
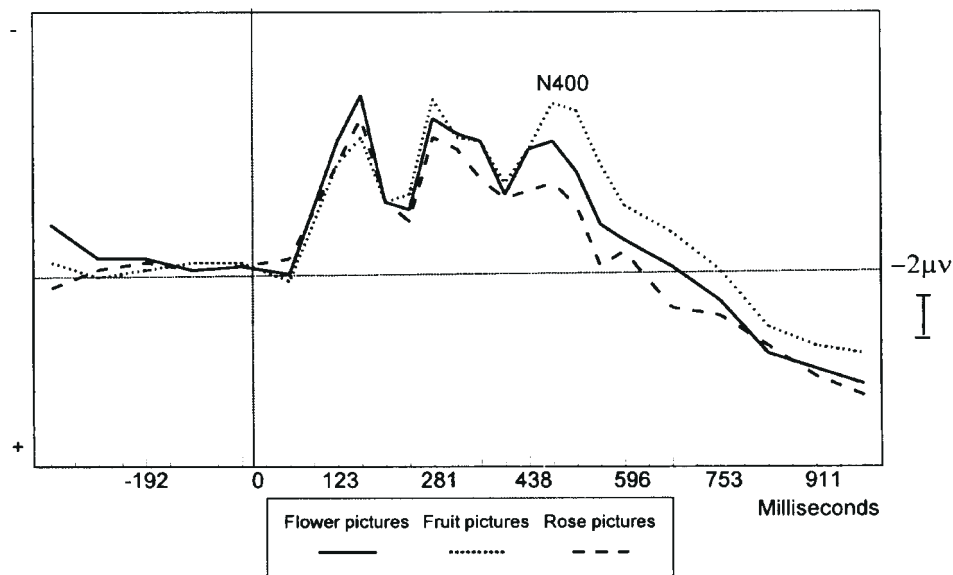
Figure 3 A graphical representation of the average of event related potentials (*n* = 10) recorded from Fz are shown with negativity denoted by an upward deflection in µV. The baseline was taken as the mean amplitude of the 200 ms preceding the visual stimulus, which was presented at time 0 ms. This figure shows the evoked potential from rose, fruit and flower pictures with rose odour present.
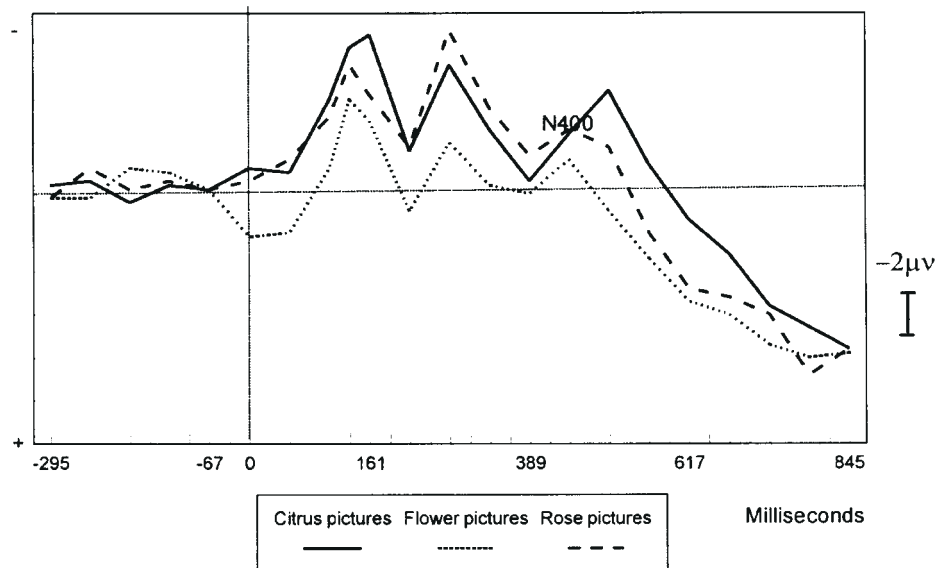

Figure 4 A graphical representation of the average of event related potentials (*n* = 10) recorded from Fz are shown with negativity denoted by an upward deflection in µV. The baseline was taken as the mean amplitude of the 200 ms preceding the visual stimulus, which was presented at time 0 ms. This figure shows the evoked potential from rose, citrus and flower pictures with jasmine odour present.

image. The amplitude of this negative peak was not affected by different types of pictures when no odour was present. However, when an odour was introduced, the amplitude of the negative peak was found to be related to the degree of congruity of the picture to the odour in nearly all cases.

The ERPs recorded in this study show a similar time latency to those identified as N400 in priming lexical decision tasks. Previous studies had investigated changes in the amplitude of this peak related to deviations in semantic expectancies (Kutas and Hillyard, 1980), contradictions in other linguistic and non-linguistic expectancies (e.g. Barrett and Rugg, 1990; Nigam *et al.*, 1992; Holcomb and McPherson, 1994), and across visual and auditory modalities (Holcomb and Neville, 1990). The present study, along with that of Grigor (1995), now extends this proposition to include olfactory and visual modalities; furthermore, it provides evidence that this effect is not dependent upon matching decisions.
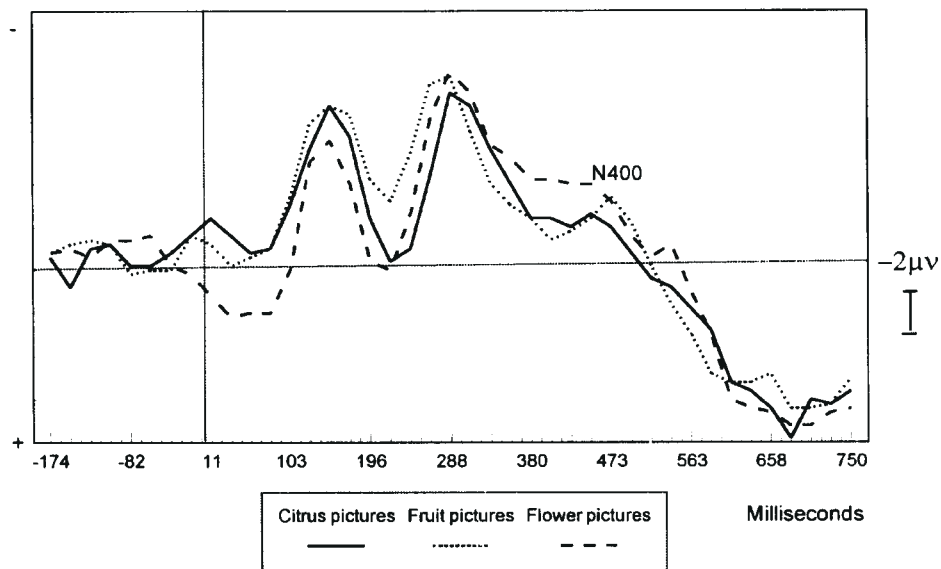
Figure 5 A graphical representation of the average of event related potentials (*n* = 10) recorded from Fz are shown with negativity denoted by an upward deflection in μV. The baseline was taken as the mean amplitude of the 200 ms preceding the visual stimulus, which was presented at time 0 ms. This figure shows the evoked potential from citrus, fruit and flower pictures with citrus odour present.

There are important differences between these experiments and those of Lorig *et al.* (1993) and Grigor (1995), who showed ERPs modified by olfactory stimuli. Both Lorig and Grigor used 'oddball' experimental designs, required semantic decisions to be made concerning the relatedness of the stimuli and used discrete odour stimuli presented for short intervals. Lorig *et al.* analysed the P300 potential and in addition described a long duration frontal negativity in association with odour primed stimuli. Similar frontal negativity was not detected in this study, so it could be speculated that the observed negativity was related to semantic processing.

The topographical distribution of the N400 observed in the current study showed a greater negative amplitude in the anterior electrodes, with greater negativity on the left than on the right hand side. This distribution is similar to that observed by Holcomb and McPherson (1994) but was not reported in the Barrett and Rugg studies (1990). These differences may result from differences in the task that the subjects were required to carry out. In our study, as in that of Holcomb and McPherson (1994), subjects were given a task which did not involve a memory decision as required in lexical decision tasks or in the visual/olfactory 'oddball' designs of Lorig and Grigor described above. Any uncertainties arising from odour memory effects were also removed in the current study by providing a constant odour stimulus throughout each recording period. It is possible that some aspects of the processing associated with the decision task are more strongly reflected by a different configuration of neural generators.

The object decision task engages a different set of preparatory and/or short-term memory processes than those employed in the matching task used by Barrett and Rugg (1990), and also by Lorig *et al.* (1993) and Grigor (1995). For example, the maintenance of information concerning the prime through the prime/target interval would not seem as crucial in the object decision task where the appropriate response can be made without even dealing with the prime. Not withstanding these differences in topography, and possible differences in mental processing involved in these two different tasks, the N400 still demonstrates sensitivity to the congruity of paired sensory stimuli which appears to be valid across different modes of sensory stimuli, whether or not semantic processing is involved in the task.

These results support the hypothesis that visual–olfactory interaction may occur in the frontal lobes but this is by no means supported unequivocally. Zatorre and Jones-Gotman (1991) demonstrated reduction in human olfactory discrimination (but not odour detection) in patients with temporal and frontal lobe lesions and identified the right orbito-frontal cortex as particularly important. This was elegantly confirmed using positron emission tomography. One might speculate that primary visual processes occur in the posterior cortex, but subsequent processing involves progressively more frontal regions and visual attention is dependent on the frontal lobe acting as an integrated unit. Precise localization of the source of late ERPs is probably an unrealistic aim with this protocol as the waveforms almost certainly represent activity in a distributed population of neurons. However, further examination should indicate any lateral asymmetry.

To show the N400 phenomenon with olfactory priming stimuli for visual ERPs confirms N400 as a common event in higher sensory processing. Because the olfactory system

is organized quite differently from the visual, auditory and somatosensory systems, whose input has to ascend the neuraxis from lower levels, intuitively one might not have expected the results that have been obtained in this and other studies. However, finding direct evidence that brain activity to a visual stimulus is altered in the presence of an odour, as in this current study, does indeed support the notion that N400 reflects neuronal activity during an amodal cognitive process.

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