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The effect of odour priming on cortical EEG and visual ERP responses

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Abstract

Two studies are reported that were designed to examine the way in which subjects, when primed with an olfactory stimulus, make perceptual judgements regarding subsequent congruent/incongruent information. The paradigm used was based on a modified version of a standard ERP semantic priming procedure. Subjects watched a computer screen and made a decision as to whether an image was congruent or incongruent with a priming olfactory stimulus. Results of the two studies showed significant effects in the N400 component of the visual ERP between congruent and incongruent stimuli are reported when a complex malodour accord was used as a prime but not when a complex pleasant odour accord was used as a prime. © 2000 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

In 1993 Lorig and his colleagues reported a visual event-related potential study in which subjects were asked to take note of an initial, visual or olfactory stimulus, followed 3 s later by a visual label, presented on a video screen (Lorig et al., 1993). The authors used a standard oddball

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paradigm (75:25 presentation ratio) in order to investigate the P300 component. However, traditionally, event-related potentials, elicited in response to semantic incongruity have primarily nested in the domain of psycholinguistics (Kutas and Hillyard, 1980; Bentin et al., 1985; Fischler et al., 1985; Rugg and Barrett, 1987; Osterhout and Holcomb, 1995). For example, the N400 component is a negative deflection occurring anywhere between 300 and 600 ms and reflects the degree of mismatch between a presented stimulus and an

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expected stimulus. The 'prime' is followed by either: a related target; an unrelated target; or a non-target. Meyer and Schvanaveldt (1971), demonstrated the phenomenon using pairs of letter strings.

Although the majority of research has been carried out in the visual modality (Kutas and Van Petten, 1988) comparisons have been made of N400 between the visual and auditory modalities in lexical decision tasks using a semantic priming paradigm (Holcomb and Neville, 1990). In light of these psycholinguistic studies, Grigor (1995) used olfactory primes to investigate the effects of food odours on subsequent visual images. Further studies have examined these effects in relation to non-food odours (Castle and Van Toller, 1997; Grigor and Van Toller, 1997; Grigor et al., 1999; Castle, 1999) Using a different paradigm, Sarfarazi et al. (1999) confirmed these findings.

The two studies reported in this paper extend Grigor's exploration of the N400 component, by manipulating the nature of the olfactory and visual stimuli presented. Whereas the odours used by Grigor could be considered as primary notes to perfumers, we chose to investigate odour accords that were complex mixtures producing early and late perceptual components and are more typical of fragrances used to scent commercial products. Experiment 1 investigated the effect of complex olfactory primes, readily identifiable with 'daily life' (e.g. laundry powder fragrance). Experiment 2 investigated the effect of hedonically negative olfactory primes, in order to discover how, or indeed whether, the N400 component would be affected.

2. Method

2.1. Subjects

Forty male and female subjects were drawn from staff and students at the University. Ages ranged from 18 to 40 years. Subjects were screened prior to testing and non-smokers were selected for participation in the experiment. They completed a variation of the Edinburgh Handed-

ness Inventory (Oldfield, 1971) in order to confirm hand preference. All were dextral and had normal or corrected-to-normal vision. On the day of testing, they were free of respiratory complaints.

2.2. Task

Subjects were required to view a series of images presented on a computer screen and make a decision regarding whether each image was congruent or incongruent with a priming odour.

2.3. Experimental setting and sensory stimuli

The experiments were carried out in a specially constructed, sound attenuated, low odour room (LOR) having a high air throughput. The ERP recording equipment and the odour delivery system were located in an adjacent ante-chamber. The ambient temperature in the LOR remained constant at 19°C and humidity levels ranged between 57 and 62%. Lighting was subdued to a level of 8 lux units during testing.

2.4. Odours

Four synthetic, commercially prepared washing powder derivatives were selected from various international market fragrances (Quest International, UK). During a pilot study, the odours were selected for similarity. Prior to testing, the odorants were diluted to supra-threshold levels, i.e. those which would be experienced in a normal home environment. The odours were also matched to isointensive levels. As in previous research (Grigor et al., 1999), delivery of odorants was via an olfactometer at a flow rate of 1 1/min (White, 1997). In order to standardise tactile stimulation when odours were not being presented, a stream of humidified air was delivered via the odorant nozzle. The multi-delivery nozzle was positioned below the subject's nares. Odours were delivered for 4 s. A small extraction unit, placed 4 cm away from the subject's face, helped to remove residual environmental odours. Each odour was presented eight times, constituting 32 trials overall.

Visual stimuli were presented using a Power-Mac 7500/100 computer (Milligan, 1996). Images were presented for a duration of 750 ms in order to allow subjects sufficient time to process the information (Grigor et al., 1999). The images were preceded by a single flash from a low-wattage orange warning light. This was incorporated into the experiment in order to warn subjects to ready themselves for the presentation of the image, providing a cue for the subject to avoid eye-blinking or gross muscle movement. Whenever an image was absent, subjects viewed a fixation dot, located in the centre of the screen. All images were adjusted to be comparable in terms of viewing size and overall luminance (images @ 10 lux units; fixation dot @ 7 lux units; and ambient lighting level @ 8 lux units). The screen was at a distance of 0.58 m from the subject. A configuration setting of 256 colours allowed for the image to be presented on screen within an epoch of 33 ms. The overall size of each image was 180×125 mm, presented on a pale blue background.

Each odour was paired with a subsequent congruent or incongruent image. A pilot study highlighted that the odours used were readily identifiable as laundry fragrances. On six occasions, the odour and the image sequence was congruent and, on two occasions, the odour and image sequence was incongruent. For example, a congruent trial would consist of a washing powder fragrance paired with an image of clean washing. An incongruent trial would consist of the same fragrance paired with an image of a book case.

2.5. ERP measures

Visual ERPs, time-locked to image onset, were recorded using a NeuroScience Series III Brain Imager. Recordings were made using a bandpass of 1.05–80 Hz and were sampled at 256 Hz for 100 ms prior to presentation and continued for 1400 ms after image onset. The Imager required waveforms to be averaged off-line. A subset of electrodes was selected for data analysis. FZ, CZ and PZ were selected due to their qualities in producing maximally recorded ERPs in many studies (Osterhout and Holcomb, 1995). In addi-

tion, electrode sites F3, F4, C3, C4, P3, P4, TCP1 and TCP2 were selected in order to provide information concerning hemispheric localisation. Johnson (1986) suggests that activation in these regions might be indicative of specialisation for the perception and processing of olfactory/visual information. Trials containing ocular or myogenic artefact were removed during an initial visual inspection of the records. The criterion for rejection was an amplitude in excess of 75 μ V (Byrne et al., 1994; Connolly et al., 1995).

2.6. Procedure

Before the experimental session, subjects were asked to avoid: ingesting caffeine products for at least 3 h prior to testing; avoid eating spicy food in the 24 h prior to testing; refrain from wearing any fragranced products; and avoid using hair products.

At the start of the session, subjects were given a forced-choice odour discrimination test in order to determine their discriminative ability. The discrimination test required subjects to select a 'blank' from four possible choices (three contained floral odours). After the screening tasks had been completed, subjects were told that they were to participate in an experiment investigating visual perception and brain activity. A 28-loci, International 10/20 electrode cap was fitted. Electrodes were injected with Electrogel[©], providing high conductivity with the scalp. Impedance levels were less than 10 k Ω . The electrodes were referenced to linked ears and a ground. An elastic chest strap was used to hold the cap in place for the duration of the experiment. Following headcapping, subjects were made comfortable in the LOR before practice trials were started.

The need to place their chin on the adjustable chin-rest and sit as still as possible during each trial was explained to subjects. It was emphasised that muscle movement would produce artefacts invalidating the trial. Subjects were asked not to blink following the second warning light, which signalled image onset. The odour nozzle was adjusted for each subject. Subjects were instructed in the required method of breathing in through

the nose and out through the mouth. It was explained that retronasal olfactory stimulation would introduce an additional unwanted variable. It was stressed that the breathing technique should be carried out in a natural and relaxed manner.

The instructions were given to subjects whilst the electrode cap was being fitted and the electrodes prepared. They were reiterated orally when subjects were in the LOR. Three practice trials were then presented to the subject. After acknowledging that they were familiar with the procedure the 32 trials commenced. A small, orange warning light was illuminated for a duration of 1 s, just prior to delivery of an odour. The light served as a cue for subjects to start the required breathing pattern. Odours were delivered for a period of 4 s. The orange light was then briefly illuminated for a second time, signalling the impending presentation of a visual image. Visual images were presented for 750 ms. In the absence of an image a visual fixation dot was present in the centre of the screen.

During presentation of an image, subjects were required to subvertly note whether the odour and the subsequent image were congruent or incongruent. Following removal of the image, subjects were provided with a two-button response option on a semi-covered keyboard. They were instructed to indicate either congruence or incongruence. If subjects were unsure, they were instructed to make an informed 'best effort'. An inter-trial interval (ITI) of 25 s served to reduce subject fatigue, eye strain and to avoid olfactory habituation.

2.7. Data quantification and analysis

The peak identification method of waveform assessment was adopted, for both amplitude and latency measurements (Connolly et al., 1995). The N400 component was designated as the most negative peak occurring between 300 and 600 ms. We selected this timing window because of uncertainty in the literature. Byrne et al. (1994) used 350–600 ms and Connolly et al. (1995) 300–500 ms. Amplitude was designated as being the great-

est difference in voltage between the mean activity for the 100-ms baseline period and the chosen timing range. Latency was measured from stimulus onset to the point showing the greatest negativity within the timing range.

A repeated analysis of variance ANOVA (priming × electrode) was carried out on the data set. The Greenhouse–Geisser conservative degrees of freedom procedure (Greenhouse and Geisser, 1959) was used in the adjustment of significance levels for the analyses reported here.

3. Results

Analysis of variance of the overall data set showed a significant main effect of electrode for both amplitude ($F_{10,280} = 5.302$, P < 0.004) and latency data $(F_{10,280} = 1.959, P < 0.038)$. As there was no significant main effect of odour priming no further analysis was carried out on the overall dataset. However, further analysis was carried out on a subset of data to investigate cortical lateralisaton. Three regions, corresponding to left/ right/midline and front/central/rear electrodes were used in a three-way analysis of variance (priming \times laterality \times anterior/posterior). The analysis revealed a significant main effect of anterior/posterior for amplitude ($F_{2.56} = 15.936$, P <0.0004) but not for latency. There were no significant main effects of priming or laterality, nor were any of the interactions significant. This appears to reflect the common tendency for ERP signals to be stronger at some electrode sites.

4. Experiment 2

Although Brauchli et al. (1995) reported alterations in EEG using unpleasant odours all the previous studies reported in the literature relating to ERPs have examined hedonically positive odours. It was, therefore, decided to examine the effects of malodours using the same paradigm and procedure. The chosen malodours had been judged to be hedonically negative in a pilot study. The odour accords, which were similar in com-

plexity to those used in experiment 1 were: 'fishy', 'bathroom', 'mildew', and 'kitchen' smells. The vERP images were contextually similar to those used in the first study. For example, a congruent trial consisted of a trimethylamine odour ('fishy') paired with an image of a fish tray in a fish market. An incongruent trial would consist of the same odour paired with an image of a fireplace. Except for the odours and visual stimuli, methods and subject characteristics were identical to experiment 1.

5. Results

5.1. Latency

An analysis of variance (priming \times electrode) was carried out on the overall dataset. There was a trend towards a significant main effect of electrode ($F_{10,310}=1.813,\,P=0.058$) but, as explained above in experiment 1, no further analysis was carried out.

5.2. Amplitude

An analysis of variance (priming × electrode) was carried out on the overall dataset. There were significant main effects of electrode ($F_{10,310} = 4.115$, P < 0.005) and priming ($F_{1,31} = 13.929$, P < 0.005) and a significant interaction between priming and electrode ($F_{10,310} = 4.097$, P < 0.005) indicated that there was a difference between the two odour priming conditions across electrode sites. To explore the simple main effect of priming for each electrode site, a post hoc examination of the interaction between priming and electrode was carried out using t-tests. With 11 pairwise comparisons, it was necessary to apply Bonferroni adjustment to the alpha level, i.e. $\mu' = 0.05/11$.

Table 1 shows mean amplitudes to primed and non-primed stimuli across subjects, for each electrode.

Fig. 1 shows mean amplitude differences (μV) between primed and non-primed conditions for each electrode site. Amplitude differences increase progressively towards posterior regions.

Table 1 Showing mean amplitudes, Bonferoni *t* values and significance levels at individual electrode loci^a

Electrode	Congruent (μV)	Incongruent (µV)	t (d.f. = 31)	Р
FZ	0.725	1.431	3.141	0.001*
CZ	0.196	1.102	4.030	0.0004*
PZ	-0.400	1.358	7.821	0.0004^{*}
F3	0.605	1.352	3.323	0.0004^{*}
F4	0.546	0.960	1.842	0.033
C3	0.228	1.025	3.546	0.0004^{*}
C4	0.099	1.027	4.128	0.0004*
P3	-0.385	1.260	7.318	0.0004^{*}
P4	-0.594	0.825	6.313	0.0004*
TCP1	-0.185	1.528	7.621	0.0004^{*}
TCP2	-0.214	1.056	5.650	0.0004^{*}

^aEach mean has been collapsed across groups, in order to further examine the simple main effects of priming for each electrode, i.e. the electrode × priming interaction.

Although no priming effect was observed in experiment 1, the data suggested marginally greater negative deflections to incongruent stimuli. This is illustrated in the example at electrode CZ (Fig. 1).

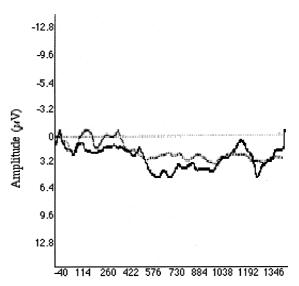


Fig. 1. Graph showing an example of primed and non-primed waveforms in response to olfactory/visual stimuli, averaged across trials for one subject at electrode site CZ. Latency (ms) dotted line = congruent condition; solid line = incongruent condition.

^{*} Significant at Bonferroni adjusted $\mu' = \frac{0.05}{11} = 0.004$.

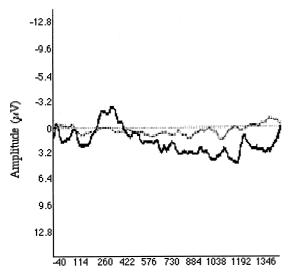


Fig. 2. Graph showing an example of primed and non-primed waveforms in response to olfactory/visual stimuli, averaged across trials for one subject at electrode site CZ. Latency (ms) dotted line = congruent condition; solid line = incongruent condition.

As reported in the first experiment, two electrodes were removed from the dataset [TCP1, TCP2], in order that laterality and the anterior/posterior dimensions could be investigated. A three-way analysis of variance (priming × laterality × anterior/posterior) was carried out on the latency data. There were no significant main effects of odour priming, laterality, or anterior/posterior; nor were there any significant interactions. A three-way analysis of variance (priming × laterality × anterior / posterior) was carried out on the amplitude data. There was a significant main effect of odour priming ($F_{1,31} = 10.964$, P <0.05); laterality ($F_{2,62} = 3.320$, P < 0.05); and, anterior/posterior ($F_{2,62} = 9.551$, P < 0.0004). The mean amplitudes for the main effect of priming are shown in Fig. 2.

Unlike the findings with pleasant odours, the malodours provided evidence of a significant effect for priming. However, like the findings with pleasant odours, no latency effect was found. As the contextual nature of odours and images was reasonably similar across the two experiments, this suggests that hedonic qualities of priming appears to be an important variable (Fig. 3).

6. General discussion

The experiments reported here extend earlier investigations (Grigor, 1995; Grigor et al., 1999; Sarfarazi et al., 1999) by highlighting that nonfood-related odours can evoke differences in congruent/incongruent N400 vERP amplitudes. The finding that malodour priming to incongruent pictures served to attenuate N400 amplitudes in the second experiment suggests the possible importance of hedonically negative odours. Brauchli et al. (1995) reported increases in alpha power bands after stimulation with a malodour but no effect with a pleasant odour. In the studies reported here changing the hedonic properties of odours also elicited differences. On the basis that the visual stimuli used in experiments 1 and 2 (Tables 2 and 3) were of a comparable nature, it is possible to infer that failure to elicit significant N400 components in the first experiment may have been a consequence of using complex odour accords rather than single odour notes. This finding might suggest the possibility of a primary and secondary odour hierarchy. The odours used in Grigor's studies were basic odour notes. In contrast, the odours used in these experiments were complex odour accords. Such an odour hierarchy suggests further avenues for investigation. It might be possible to examine congruency effects of the N400 component in relation to various generic odour notes (floral, spicy, green, etc.). If the congruent/incongruent N400 effect were to be shown

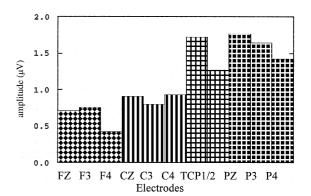


Fig. 3. Graph showing mean amplitude differences for each electrode site (variation in shading indicates frontal, central, temporal and parietal regions).

Table 2 Experiment 1. Showing presentation order, odour type and status for a typical subject a

Image	Odour	Status
Washing line 1	Washing powder 1	Congruent
Washing machine 1	Washing powder 2	Congruent
Mandolin 1	Washing powder 3	Incongruent
Shampoo 1	Washing powder 4	Congruent
Shampoo 2	Washing powder 1	Congruent
Washing line 2	Washing powder 2	Congruent
Deodorant 1	Washing powder 3	Congruent
Fireplace 1	Washing powder 4	Incongruent
Soap 1	Washing powder 1	Congruent
Bookcase 1	Washing powder 2	Incongruent
Clean clothing 1	Washing powder 3	Congruent
Washing machine 2	Washing powder 4	Congruent
Soap 2	Washing powder 1	Congruent
Telephone 1	Washing powder 2	Incongruent
Shampoo 3	Washing powder 3	Congruent
Deodorant 2	Washing powder 4	Congruent
Washing line 3	Washing powder 1	Congruent
Clean towels 1	Washing powder 2	Congruent
Telephone 2	Washing powder 3	Incongruent
Soap 3	Washing powder 4	Congruent
Clean towels 2	Washing powder 1	Congruent
Mandolin 2	Washing powder 2	Incongruent
Clean clothing 2	Washing powder 3	Congruent
Washing line 4	Washing powder 4	Congruent
Bookcase 2	Washing powder 1	Incongruent
Shampoo 4	Washing powder 2	Congruent
Clean towels 3	Washing powder 3	Congruent
Deodorant 3	Washing powder 4	Congruent
Fireplace 2	Washing powder 1	Incongruent
Clean clothing 3	Washing powder 2	Congruent
Washing machine 3	Washing powder 3	Congruent
Soap 4	Washing powder 4	Congruent
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^aImage/odour counterbalancing was taken into consideration across subjects. Odour codes available upon written request.

to be confined to specific odours then this might provide insight about a subject's odour lexicon (Fig. 4).

The 'basic vs. complex' odour proposition might also be explained along the lines of Cabanac's allocentric and autocentric modes of processing. The odours used in Grigor's studies might be perceived as allocentric, or object-centred. In contrast, the complex odour accords used in these experiments may be processed in an autocentric, or subject-centred manner. The underlying reason for considering this mode of processing would

be a conceptual change to, 'how does this odour affect me?' rather than 'what is this odour?' (Cabanac, 1971; Engen, 1982). A change of emphasis to feeling states rather than semantic naming might more accurately describe how odours are processed by the brain.

Although the results obtained in the first experiment failed to provide significant support for Grigor's studies, it is emphasised that less complex visual images were used in her investigations. Grigor used photographs in which the object was very clear and obvious, i.e. roses or grass. Para-

Table 3 Experiment 2. Showing presentation order, odour type and status for a typical subject a

Image	Odour	Status
Fish market 1	Trimethylamine	Congruent
Toilet 1	'Bathroom' malodour	Congruent
Mandolin 1	'mildew/mould' malodour	Incongruent
Dirty Kitchen 1	'Kitchen' malodour	Congruent
Fish market 2	Trimethylamine	Congruent
Dirty bathtowels 1	'mildew/mould' malodour	Congruent
Dirty Kitchen 2	'Kitchen' malodour	Congruent
Fireplace 1	'Bathroom' malodour	Incongruent
Dirty Kitchen 3	'Kitchen' malodour	Congruent
Bookcase 1	Trimethylamine	Incongruent
Dirty bathtowels 2	'mildew/mould' malodour	Congruent
Toilet 2	'Bathroom' malodour	Congruent
Dirty bathtowels 3	'Mildew/mould' malodour	Congruent
Telephone 1	'Kitchen' malodour	Incongruent
Fish market 3	Trimethylamine	Congruent
Dirty Kitchen 4	'Kitchen' malodour	Congruent
Dirty bathtowels 4	'mildew/mould' malodour	Congruent
Fish market 4	Trimethylamine	Congruent
Telephone 2	'mildew/mould' malodour	Incongruent
Toilet 3	'Bathroom' malodour	Congruent
Dirty bathtowels 5	'Mildew/mould' malodour	Congruent
Mandolin 2	'Kitchen' malodour	Incongruent
Fish market 5	Trimethylamine	Congruent
Toilet 4	'Bathroom' malodour	Congruent
Bookcase 2	'Bathroom' malodour	Incongruent
Dirty bathtowels 6	'Mildew/mould' malodour	Congruent
Toilet 5	'Bathroom' malodour	Congruent
Toilet 6	'Bathroom' malodour	Congruent
Fireplace 2	Trimethylamine	Incongruent
Dirty Kitchen 5	'Kitchen' malodour	Congruent
Dirty Kitchen 6	'Kitchen' malodour	Congruent
Fish market 6	Trimethylamine	Congruent

^aImage/odour counterbalancing was taken into consideration across subjects. Odour codes available upon written request.

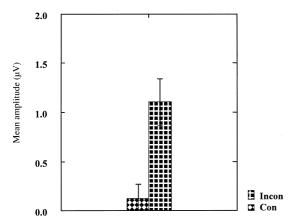


Fig. 4. Showing the main effect of priming for congruent vs. incongruent stimuli. Incon = incongruent; Con = congruent.

doxically the results from experiment 2 using malodours do offer support. Sarfarazi et al. (1999), using a continuous stream of odour and measuring vERPS in subjects viewing photographs, also reported increased negativity in the N400 waveform.

The investigations support the acknowledged position, that N400 is not language-specific. It has been shown that the olfactory sense is equally effective in eliciting a cross-modal N400 component. However, it is not known if the exact nature of the olfactory stimulus is a determining factor in terms of whether or not an N400 component will be elicited.

Although a significant increased negative deflection was not found in experiment 1, it was in experiment 2. This suggests that the methodology was effective. Within the latency range used one may be observing a negative N400 deflection in some but not all instances. Therefore, it would be prudent to talk in terms of an N400-like component, until further exploratory studies provide supportive evidence.

Using picture priming followed by a word recognition task, Vanderwart (1984) concluded that distinctive, form-specific semantic systems did not exist. He argued for a single system of semantic representations, accessed by pictures and words. The findings reported here, using olfactory priming and picture recognition, supports this view. We suggest that Vanderwart's proposition

should be revised to include a specific reference to the olfactory sense within this 'single system of semantic representations'.

An event-related potential is a harmonic of complex brain electrical activity and it is not possible to state its precise occurrence. It might fall outside of our chosen latency window and this possibility requires further investigation. The essential problem in this area of research is the lack of sufficient studies. This neglect is slowly being overcome but we are still some way from reaching a critical mass where progress can be made. The investigations reported here have confirmed that olfactory stimuli may successfully be used in investigations of cross-modal priming on vERPs and experiments 1 and 2 have highlighted what appears to be an N400-like component.

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