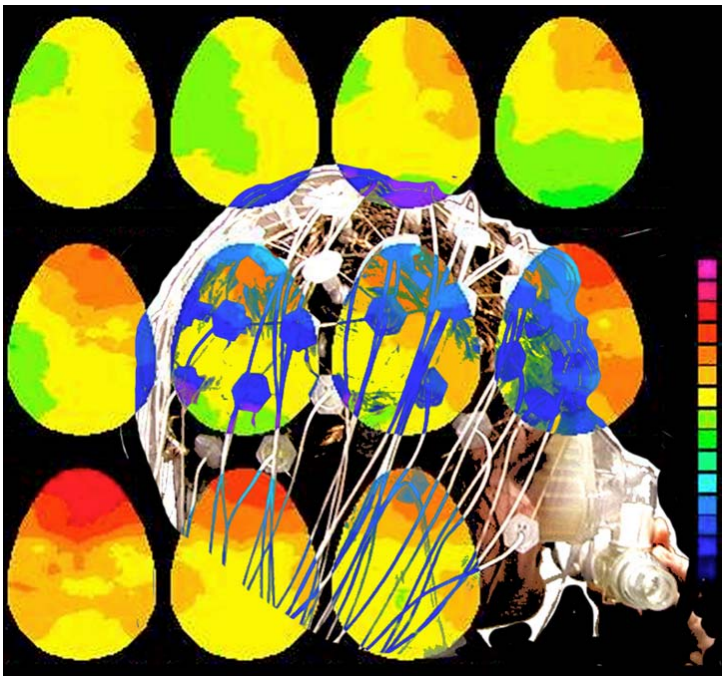
OBJECTIVE SENSORY EVALUATION USING ADVANCED NEUROPHYSIOLOGICAL TECHNIQUES FOR FLAVOUR AND TAINT EVALUATION IN THE WINE INDUSTRY



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: SUT 99/1

Principal Investigator: Dr Caroline Owen

Research Organisation:
SWINBURNE UNIVERSITY OF TECHNOLOGY

Date: 30 September 2002

OBJECTIVE SENSORY EVALUATION USING ADVANCED NEUROPHYSIOLOGICAL TECHNIQUES FOR FLAVOUR AND TAINT EVALUATION IN THE WINE INDUSTRY

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1. Abstract

Research linked traditional sensory techniques with brain activity recordings using panels of consumers, untrained in sensory analysis, to investigate differences in responses to β-damascenone, ethanol and 2,4,6-trichloroanisole (TCA).

Sensory ratings provided evidence of enhanced fruity/sweet qualities in response to TCA versus ethanol, and a low concentration of TCA was more liked than the higher concentration. Brain activity responses to all the odours revealed significant differences associated with liking, independent of conscious detection of the odour. This novel approach to sensory evaluation provides a new objective technique to measure of flavour quality and attributes in wine.

2. Executive Summary

Acceptance of wine is dependent on sensory appreciation, but this is strongly influenced by emotional and experiential aspects. Techniques are needed which can assist in determining differences between and within individuals to flavour responses at different concentrations (sub- and supra-threshold). Knowledge of individual differences in levels of discrimination and threshold detection for wine flavours will assist the wine industry in gaining an understanding of the factors, both chemical and sensory, affecting wine flavours and consumer perceptions.

At the most basic level, it is the sensory experience which determines the success of a product, and the on-going success of the wine industry. Techniques are required to improve the accuracy and ease with which the actual consumer's responses to underlying olfactory stimuli can be resolved. This study linked traditional sensory with electrophysiological techniques to facilitate a new understanding of consumer responses to flavours by studying changes in brain electrical activity associated with individual wine components. In an effort to determine what consumers like or dislike and the effect of variations in the range of influencing parameters, we investigated how people reacted to different odours by making comparisons of subjective (reported) and objective (brain activity) responses.

This project was an investigation into high-risk, innovative and strategic research to culminate in a new approach to determining the factors involved in the pleasure of wine. Large-scale sensory evaluations are extremely expensive and the applicability of the results is not guaranteed. All sectors of the wine industry would benefit from access to faster, more reliable, simpler and cheaper ways of determining consumer preference. A system which can determine preference independent of language or experience, obtain results from small subject pools, determine preferences of sub-groups in the community (while using very small quantities of product) will benefit the wine industry. Such a system could provide a new approach to optimising assessment of wine flavours and preference, and establish methods for assessing international preferences for wines while assisting in the rapid and cost-effective development of new wine flavours.

The **general aims** of the project were:

- To develop a new strategic approach to the complex problem of wine grading and development, with the view to enhancing the pleasurable experience of wines.
- To develop a validated, cost effective, objective and sensitive flavour measurement technique to quantify preference responses for the Australian Wine Industry.

Method and Results

Panels of consumers, untrained in sensory analysis, were screened using an olfactory performance screening test to determine their overall smell ability. Brain recording (EEG) sessions were conducted for 5-minute recording trials, with up to 6 trials conducted per session, and 3 or 4 trials per odour stimulus. Either a control sample or an equivalent volume of the target odour was delivered synchronous with breathing. Between trials, panellists completed rating scales and provided descriptions of the perceived odour. Comparisons were made between subjective ratings (liking and strength) and descriptor responses, and objective brain activity responses, with particular reference to reported liking for the test odour.

The project was divided into three studies, based on the test odour(s) used:

- Study 1: β-damascenone
- Study 2: Ethanol and 2,4,6-trichloroanisole (TCA)
- Study 3: Concentration effects of 2,4,6-trichloroanisole (TCA)

Study 1: β-damascenone

A group of 36 subjects participated in repeated trials of delivery of either air or β-*damascenone*. EEG signals were averaged for left and right frontal electrode sites. Relative % power was calculated for the frequency bins of interest (1–4 Hz, 5–8 Hz, 9–12 Hz). Comparisons were made between the left and right frontal regions relative to the liking response group (*Like*, *Neutral*, *Dislike*), and data were subjected to statistical analysis.

The major findings of this study were that significant power differences in frontal activity were associated with reported Liking for the odour, with reduced power in the alpha and theta ranges evident for the *Like* group in comparison to the *Dislike* group. Further analysis revealed this difference was significant in the left frontal region, with reduced alpha and theta for the *Like* group and increased alpha and theta for the *Dislike* group relative to the *Neutral* group. These results provided evidence of changes in brain activity associated with different hedonic responses to the same odour.

Study 2: Ethanol and 2,4,6-trichloroanisole (TCA)

The degree to which consumers vary in response to TCA has rarely been investigated using objective physiological techniques. This study was undertaken to provide preliminary information concerning the degree to which consumers perceive and respond to TCA, even when not consciously aware of its presence.

A group of 27 subjects participated in repeated trials of delivery of either air or odour (TCA in 100% ethanol or ethanol alone). The concentration of TCA delivered to the participants was calculated to be 10 – 20 ng/L. The concentration of the ethanol was calculated to be 31.6 mg/L, present in both the TCA and ethanol odour syringes.

The same procedure was used as in Study 1, with comparison of subjective ratings and descriptors provided during the EEG recording trials. The results were analysed for comparisons of TCA versus ethanol and for Liking group responses to each odour, resulting in Like and Neutral groups only. Subjective responses provided evidence of enhanced alcohol qualities associated with the TCA responses in comparison with ethanol.

Significant power differences in frontal activity were associated with TCA responses *cf* ethanol responses, with reduced power in the beta range. Beta wave bands are more prominent during intense mental activity (alert or anxious), and may be a reflection of a decrease in arousal or attention in response to the TCA stimuli, relative to the ethanol stimuli. Analysis of the Liking group responses to both TCA and ethanol supported the findings of the previous study, with a decrease in alpha activity associated with Liking responses interpreted as an increase in activation associated with a more positive (liked) odour response.

Study 3: Concentration effects of 2,4,6-trichloroanisole (TCA)

Variations in sensitivity to TCA will result in only portions of the population even being aware of its presence in a wine, and variations in concentrations of TCA will have different manifestations. This study was designed as a preliminary assessment of the concentration effects of TCA by investigating changes in brain activity and subjective responses to different concentrations of TCA.

A group of 13 subjects participated in repeated trials of delivery of either air, ethanol or two concentrations of TCA. The low concentration of TCA (hereafter referred to as TCA1) was calculated to be 10–20 ng/L. The stronger concentration of the TCA (hereafter referred to as TCA2) was four times the strength of TCA1, calculated to be 40-80 ng/L. The same procedure was used, with comparison of subjective ratings and descriptors provided during the EEG recording trials. The results were analysed for comparisons of TCA1 versus TCA2 and for Liking responses associated with the two odours: TCA1 (like) and TCA2 (neutral).

There was a change in perception of TCA2 characteristics associated with the increased use of musty/damp descriptors, and this change was complemented by the change in intensity ratings for TCA2 (with slightly stronger mean responses) and served as a further indication of the change in subjective liking responses to TCA2 *cf* TCA1.

The analysis of the EEG responses to the two concentrations of TCA (one near threshold, the other above threshold) revealed differences in responses associated with liking for the lower concentration (TCA1), providing further evidence of changes in brain activity associated with stronger like responses, comparable to the results of the damascenone study.

Project Implications and Conclusions

This study met the proposed performance targets and outputs in successfully correlating differences in subjective and objective measures of flavour responses with untrained consumers.

- The use of different wine flavour compounds and different concentrations attained the performance targets, with successful evaluation of the brain activity responses associated with concentration and preference (liking) responses.

The sensory and physiological techniques used in this project provided evidence of reliable differences in brain activity processing between like and dislike and between the presence of alcohol and an odour plus alcohol. Although this research still contains substantial unknowns in terms of the fine detail of the nature of the relationship between brain activity and preference, the potential for the application of objective techniques to contribute valuable information about flavour responses has been demonstrated.

Research and Communication Strategies

This study has taken important steps in demonstrating the variety of human preferences and experiences which contribute to the complexity of flavour responses. The reported results provide unassailable evidence of a link between EEG structure and preference for odours.

Further development of these objective measures of flavour responses (contributing to the wine industry's understanding of actual consumer responses) has the potential to improve the accuracy and ease with which the individual's responses to the underlying factors involved in the pleasure of wine can be determined.

Further research will assist in determining the consistency of these responses across a larger subject group, by correlating with measured sensitivity and subjective reports of responses to wine odours.

- This research could relate to: the interaction of taints in wine with different flavour components; focus on the interaction of wine flavours in different styles of wines; study the degree to which different concentrations of components affect flavour attributes.
- Variations in sensitivity to wine flavours and taints should be further investigated in the consumer population, to establish a greater understanding of the subjective assessment of such flavours associated with their presence at different concentrations in wines.
- This consumer-based work could be extended to investigate differences in age groups and/or different cultural groups (nationally and internationally) with practical implications for the development of new wines and markets and for the marketing of Australian wines both nationally and internationally.

The results of this project will be communicated to the scientific and wine industry communities through scientific publications, conference presentations and industry seminars.

3. Background

The wine industry is a major local and export industry with the potential to expand into novel local markets and to major international markets. The effects of odours (aromas, flavours and taints) on individuals, and the population, determine the satisfaction and pleasure of the product, and contribute to the success of the industry. At the most basic level, it is sensory experience which determines the success of a product, and the on-going success of the wine industry. In Australia, as is the case in many situations overseas, techniques for revealing the actual consumer responses are still in the development stage. Techniques are required which can improve the accuracy and ease with which the individual's responses to underlying olfactory stimuli can be resolved. Knowledge acquired using such techniques can be applied to all process optimisation and marketing areas concerned with improving the flavour experience. They have the potential to expand into novel local markets and to major international markets.

There is a growing appreciation that experience may strongly influence the ability to perceive and discriminate odours and thus play a significant role in preference responses (Ayabe-Kanamura *et al*., 1997). Differences in odour responses could reveal variations in odour and flavour preferences that may be related to experience. Developing from this is the question of what would be the effect of differences in experience on perceptual and preference responses. To realise its full potential, the Australian wine industry must focus on the development of both internal and external markets. The expansion of external markets may involve difficulties related to the potential language barriers. A research system which does not rely on verbal communication, and can provide objective, quantitative information about odour responses, can therefore assist in overcoming this problem.

The perceptual experience of a flavour is the combination of a cascade of physiological responses involving odour, taste, texture, temperature, with the odour component providing approximately 75% of the flavour experience. In attempting to gain a greater understanding of the factors contributing to the success or failure of a flavour, it is therefore of great relevance to flavour research to further investigate differences in responses to odours and flavours and their interactions with emotional or hedonic responses which may ultimately contribute to the enjoyment, and subsequent success, of a product.

Our sense of smell provides us with a great deal of information, most of which can be transparent to our consciousness. Personal experience shows us that we ignore smells after a short period of time unless we choose, or are obliged, to notice them. Changes in our electrical brainwaves relate to what our brain is doing. It is clear that some aspects of brain electrical activity changes must relate to processing of a flavour or odour. It has been established that electroencephalographic (EEG) recordings alter when an odour is present or when the concentration of an odour is altered (Owen, 1998; Patterson *et al*., 1998; Owen *et al*., 2002a), and even when the individual is not consciously aware of the presence of the odour (Owen, 1998). There is also evidence that the nature of some components of the EEG differ between those who like a flavour compared with those who dislike the flavour (Owen & Patterson, 2002). The diversity of human preferences and the wide variety of experiences within and between individuals combine so that any individual is almost unique in terms of their attitude to a flavour or odour.

The complexity of human preferences and variety of experiences add together to make studies of flavour responses complicated and their results need further verification. While there is unassailable evidence of a link between EEG structure and preference for odours, the precise nature of the link has not yet been revealed in these studies.

Project Aim and Output

The overall aim of the research conducted at the Sensory Neuroscience Laboratory (SNL) was to link traditional sensory with electrophysiological techniques to facilitate a new understanding of consumer responses to flavours. More specifically, research is conducted to investigate the ability of the sensory rating and brain recording techniques to reveal changes in responses associated with

differences in experience, preference, age, gender or any other factors which may influence the flavour response.

The **general aims** of the project were:

- To develop a new strategic approach to the complex problem of wine grading and development, with the view to enhancing the pleasurable experience of wines.
- To develop a validated, cost effective, objective and sensitive flavour measurement technique to quantify preference responses in the Australian Wine Industry.

The research aimed to assist wine producers into the next phase of industrial development by exploring the potential of a neurophysiological technique to deliver quantitative and qualitative information about the like or dislike of a flavour and the concentration of chemicals in a flavour or mixture. The range of concentrations to be tested were to cover sub- to above-threshold levels.

In a series of experiments conducted at the SNL, changes in brain electrical activity associated with individual wine components was investigated. In an effort to determine what consumers like or dislike and the effect of variations in the range of influencing parameters, the study investigated how people reacted to different odours by making comparisons of subjective and objective responses.

4. Project Aims and Performance Targets

The planned outputs and performance targets as they appeared in the original approved application are outlined below in Targets 1 – 4.

Outputs	*Performance Target*
1. To correlate differences in subjective and objective measures of flavour responses with groups of subjects with different experience of wines (i.e. naïve vs. experienced).	1. A group of at least 50 naïve consumers subjects and a minimum of 10 industry experts will be established for testing. All subjects will complete the same testing paradigm, including screening for overall olfactory ability.
2. To create a database of differences in brain electrical activity topography caused by different wine flavour compounds.	2. Responses of the 50 subjects (naïve & experts) to two odorants (2,4,6 trichloroanisole & beta-damascenone) will be used to establish the database.
3. To evaluate the differences in both the psychological and brain electrical activation responses to flavours at different concentrations.	3. Differences in brain electrical activity associated with different concentrations of the odorants at sub- to above-threshold levels will be evaluated in comparison to subjectively reported responses.
4. To evaluate differences in brain activity responses that can be correlated with preference responses obtained during both traditional sensory evaluations electrophysiological recordings.	4. The electrophysiological responses of the naïve and expert subjects will be correlated with the subjective preference responses and sensory evaluations to determine possible objective indicators of preference.

5. Basic Methodology

There was a basic methodology common to all parts of the study which is outlined in the following section. However, this project was conducted in three parts, which will be outlined in detail in Sections 6.1, 6.2, and 6.3. Each of these sections will provide further details of specific methodological factors and then report the results and discussions associated with each study.

Code of Conduct and Ethics Assurance

All research was conducted within the parameters and procedures established by the Swinburne University of Technology's policies on Code of Conduct, Human Experimental Ethics Clearances and Intellectual Property.

Olfactory Performance Testing

All participants are screened for general olfactory ability using the *Sniffin' Sticks* Test of Olfactory Performance (Burghart, Wedel, Germany) which tests threshold, identification and discrimination abilities. This provides a screening system as a measure of an individual's olfactory performance for comparison and consideration when interpreting sensory and EEG performance.

This is a portable, commercially available test used internationally to assess olfactory performance, particularly in clinical and research settings (Kobal *et al*., 2000). This test of olfactory performance consists of a basic screening test and an advanced test. The screening test includes a basic smell identification task. The advanced test is composed of three sub-tests: the threshold test for one odorant (the "just noticeable difference" detection threshold; a forced choice triangle staircase test), discrimination test which assesses the ability to discriminate between two odorants at one time (a forced choice triangle test), and the odour identification test which assesses identification ability using a choice of four descriptors for each odour stimulus (Hummel *et al.,* 1997). The scores from the Threshold, Discrimination and Identification tests were summed to form the TDI score, with a total maximum score out of 48.



Figure 1. Subject performing the *Sniffin' Sticks* threshold test.

All participants were also screened for handedness (which can be an important variable when considering laterality of brain electrical activity) and complete health and food/flavour questionnaires.

Sensory Panel Odour Tests

Sensory panel responses are used for comparison with the objective EEG responses, and therefore attempts are made to replicate industry procedures in simple sensory rating sessions. Sensory ratings are obtained during the EEG recording sessions, and in separate sensory sessions as required.

During the EEG recordings, each participant completed a psychometric rating of the odour (hedonic or emotional response and perceived strength) after each odour delivery trial. These ratings were based on simple 10 cm Likert scales, usually designated as: *Liking* (strong dislike = 0; strong like = 10) and *Strength* (very weak = 0; very strong = 10). Subjects also provided descriptions of the odour and any associated responses or reactions. Liking ratings are then grouped according to these rating levels: *Dislike* (<4), *Neutral* (4–6) and *Like* (>6).

Odour delivery system: Continuous Respiration Olfactometer (CRO)

A 2-channel odour delivery system with removable syringes used in the studies was developed at Swinburne University of Technology. The continuous respiration olfactometer (CRO) system was devised for use with brain electrical recording techniques to monitor changes in brain electrical activity associated with odour responses induced during natural respiration (Owen, 1998). The odour syringe is filled with 50 mL of the odorous gas sample, with a similar volume of air or a control odour in the control syringe. The CRO delivers odour during normal respiration by closely monitoring the subject's natural respiratory cycle using a pneumotachograph mounted on a facemask with a two-way non-rebreathing valve. The computer-controlled delivery syringes use a pseudo-random sequence (usually at a ratio of 3:1 control:odour) to deliver odour or air/control stimuli, timed to the subject's inspiration, taking approximately 500 ms to deliver 1 mL of air/control or odour through ports in the facemask. This synchronous method of controlling delivery of the odorant permits the isolation of the periods of electrical activity associated with the odour response for comparison with electrical activity associated with breathing specific odour-free air or a specific control odour. The apparatus is described in detail elsewhere (Owen *et al.,* 1997*,* 1999, 2002b; Patterson *et al.,* 1998).

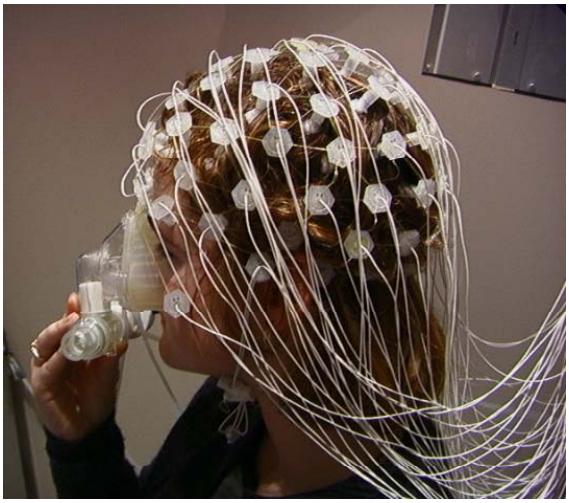


Figure 2. Subject wearing the 64-channel EEG net and holding in place the CRO odour delivery and respiratory monitoring mask.

Use of the CRO system, delivering known quantities of odour synchronous with the inspiratory phase of the natural breathing cycle, avoids the problems associated with sniff or blast techniques used elsewhere and provides the timing information required for analysis of the EEG response.

Electrophysiological Recordings

Brain electrical activity is recorded with a 64-channel ElectroGeodesic Inc (EGI) EEG sensor system (saline electrodes), referenced at Cz channel, with a sample rate of 500 samples/sec, and high and low-pass filter settings of 200 Hz and 0.1 Hz respectively. Stimulus presentation records are achieved by triggering the EGI through its interface port between the acquisition and task computers. The source of the trigger is a pair of specific outputs indicating syringe drive from the CRO. In this way the EGI-EEG record is marked when odour or air are delivered. A 2-button response box is used when subject responses during the EEG trial are required, marking the EEG file with event codes for odour detection.

Using the delivery technique with the above system, differences caused by the odour in comparison to air without odour are recorded and analysed. Analysis involves correlating the odour nature with the psychometric rating responses. Using this system, three-dimensional reconstructions of brain activity can be mapped onto digitised representations of an individual subject's head for more complete visualisation of the regional brain responses to stimuli. This provides a method of utilising different techniques to better quantify the electrophysiological effects of odours, reducing the problem of the subjectivity of responses found in much of the research into hedonic odour responses.

Experimental paradigm summary

- Brain recording sessions are conducted for 5-minute recording trials, with up to 6 trials conducted per session (typically 3 or 4 trials per target odour).
- Either a control sample or an equivalent volume of target odour is delivered synchronous with breathing.
- These control deliveries occur in a pseudo-random sequence so the subject is not able to predict the presence of the target.

- The number of control deliveries exceed the targets so that no target stimuli are presented consecutively (to avoid any potential adaptation effects). A control:target ratio of 3:1 is commonly used.
- EEG is recorded from 64 channels using a light electrode assembly (EGI Sensor Net: saline electrodes held in a geodesic frame of nylon lines).
- The EEG record is marked for stimulus delivery (target, control) and/or subject responses.

EEG analytical techniques

Initial analysis involves the extraction of the control EEG signals and the odour-related EEG signals from the continuous stream of 64 channels. After averaging the control and odour signals separately using the time of delivery event markers, these data can be processed by subtraction of the control from the odour signals leaving just the effects of the odour. As the individual is not aware of delivery sequence they treat every breath as novel. The perceptive subjects will realise that a long sequence of no smell must increase the likelihood of a smell being presented. To overcome this likely "expectancy" effect, we use low concentrations of odour which are around the threshold for the subject. In other words, the smells are rarely very obvious to the participant, and avoid potential adaptation effects.

Subsequent analysis involves traditional Fourier spectral analysis, examination of the averaged EEG time-series and frequency analyses, the individual electrodes, and the animated topographic maps. The subjective responses are correlated with the electrophysiological data.

EEG frequency responses

Normal EEG shows activity in the range of 1-30 Hz, with amplitudes in the range of 20-100 μV (Westbrook, 2000). Traditionally, EEG frequency has been separated into frequency bands: Delta from 0.1 to 4 Hz, Theta from 4 to 8 Hz, Alpha from 8 to 12 Hz and Beta from 13 Hz up. The EEG spectrum can be obtained when processed by Fourier analysis which separates the various rhythms and performs frequency estimations to quantify the amount of activity within a frequency band. Alpha and beta wave bands are commonly interpreted to be associated with changes in activation or arousal. Alpha and theta waves are typical of relaxed wakefulness and drowsiness - inversely related to cortical activity (Coan *et al*., 2001). Beta bands consist of fast waves of low amplitude and are more prominent during intense mental activity (alert or anxious). It is generally regarded as a normal rhythm, the dominant rhythm when eyes are open, and is the typical EEG of the awake adult

Suppression of alpha activity and an increase in beta has been equated to an increase in activation in response to stimulation (Westbrook, 2000). Decreases in alpha are common in EEG research, and are taken to be suggestive of cognitive activity, but are more commonly associated with cortical arousal due to sensory stimulation (Lorig *et al*., 1990). This has been demonstrated in response to visual and auditory stimulation (Brauchli *et al.*, 1995) and has more recently been reported by EEG studies in response to olfactory stimulation, particularly in frontal brain regions (Lorig *et al*., 1990, 1991; Klemm *et al*., 1992; Kobal *et al*., 1992; Schwartz *et al*., 1992; Van Toller *et al.*, 1993; Lorig, 1994; Brauchli *et al.*, 1995; Martin, 1998; Kline *et al*., 2000; Kobal & Kettenmann, 2000). Stimulation with an unpleasant odour may lead to stronger cortical deactivation than stimulation with a pleasant odour, as also suggested by previous research (Ehrlichman & Bastone, 1992; Miltner *et al.*, 1994). The trend for reduced left frontal activation in response to odours in the 4-8Hz or theta range has been reported, with findings of reduced theta activity in response to pleasant food odours such as chocolate (synthetic and real), spearmint (synthetic) and spiced apple (Lorig & Schwartz, 1988; Lorig *et al.,* 1990; Martin, 1998). It has also been proposed that the olfactory effects on theta may be specific to different odours possessing similar psychological properties (Martin, 1998). Recent studies have demonstrated changes in alpha and theta activity in the left frontal region associated with liking responses to odour (Owen & Patterson, 2002) which may reflect the shift in attention associated with liking an odour.

Chemical Analysis Techniques

Analysis of flavours is conducted using Solid Phase Microextraction (SPME) and GC-MS and GC-olfactometry (GC-O). These techniques are used to optimise aroma profiling, using chemical ionisation, and MS/MS to identify key flavour components.

SPME-GC-O analysis is conducted to identify the key compounds present in the flavours used for the EEG recordings. Once identified, the major impact odours can be used in EEG recordings and sensory ratings. The focus of the current project was to investigate responses to β-damascenone and 2-4-6-Trichloroanisol (TCA). However, in order to validate the results of analysis of these component, additional GC-MS analysis of wines was conducted to validate the chemical analysis protocol used in this laboratory against other industry findings.

Chemical Analysis Gas Chromatograph/Mass Spectrometer

Gas chromatography: Varian Chrompack 3800 with 1079 temperature programmable split/splitless injector, cryogenic oven cooling to -10˚C, Electronic Flow Control, flame ionisation detector (FID). 'Merlin-Microseal' septumless injector port.

Mass spectrometry: Varian Saturn ion trap mass spectrometer, electronic and methanol chemical ionisation and MS/MS analysis.

Olfactory Analysis

GC-FID-Olfactometry: 1:10 FID/ olfactory port split. GC-MS is conducted separately. Odour compounds are matched up with mass spectra using a retention mix of straight chain alkanes. Almost 100% of compounds pass through olfactory port, no loss in intensity.

GC-MS-Olfactometry with the ODO II (SGE Australia): 1:1 split MS/olfactory port. Both MS and olfactory analysis are conducted simultaneously, so odours can be identified in real time. Less sensitive than the above method. Currently improving the performance in collaboration with SGE.

Solid Phase Microextraction Analysis of Aroma

Solid Phase Microextraction (SPME) of aroma volatiles. Most olfactometry and general volatile analyses in the laboratory have been performed using SPME. In most cases the most suitable fibre is the 75 μm PDMS-Carboxen phase. Techniques for the olfactory profiling of wine, beer and cheese have been successfully developed. A polar WAX column is invariably used for chromatographic separation of odour volatile compounds.

Wine aroma analysis

SPME fibres have also been applied successfully for the analysis and characterisation of wine aroma. Amongst the chemical compounds found to contribute to the aroma of wine are:

acetic acid	benzyl alcohol	beta-decalactone	butan-1,3-dione
butanoic acid	n-butyl furan	carbon disulphide	β-damascenone
diethyl succinate	dimethyl sulphide	dimethyl trisulphide	2,2-dimethyl butanol
ethanol	ethyl acetate	ethyl benzoate	ethyl benzene acetate
ethyl butanoate	2-ethyl butanoate	ethyl cinnimate	ethyl isovalerate
eugenol	ethyl methoxy	ethyl hexanoate	ethyl-2-methyl benzoate
furaneol	isoterpenolene	3-hexen-1-ol (Z)	isoamyl acetate
linalool	methionol	methional	methoxy methyl phenol
octanoic acid	methoxy phenol	methyl salicylate	3-methy butyl octanoate
sotolon	octen-3-one	pyrazine	phenyl acetaldehyde
undecanol	phenyl ethyl alcohol	2-phenyl ethyl acetate	trichloroanisole (TCA)

6. Results and Discussion

This section will outline the three studies undertaken as part of this project. Each section will describe methods specific to the study, followed by the results and a discussion of these results. These sections will then be followed by a general discussion of the outcomes and conclusions in Section 7, bringing together the results of all three studies and providing a more general assessment of the implications of this research relative to the wine industry's interests.

Section 6.1 β-damascenone Study

Introduction

The bulk of the research investigating hedonic odour responses has focussed on comparisons of brain activity between pleasant and unpleasant odours (Kobal *et al.*, 1992; Brauchli *et al.*, 1995; Ehrlichman *et al.,* 1995; Roescher *et al.*, 1998; Kobal & Kettenmann, 2000), such as the comparison of responses to pleasant (vanilla), neutral (water) and unpleasant (valerian) odours undertaken by Kline *et al.* (2000). This research supported left-frontal involvement in positive/approach-related emotion, finding increased left frontal activation associated with the pleasant odour, but failing to demonstrate the expected right frontal effect associated with the unpleasant odour. Studies such as this comparison of frontal activation in response to odours of different valence have supported the hypothesis of left frontal activation in response to pleasant versus unpleasant stimuli, however little research has examined differences in frontal activation associated with different responses (pleasant versus unpleasant) to the same odour.

This study was designed to use electrophysiological techniques to further investigate differences in responses between those who "liked" a compound compared with those who "disliked" the compound. Subjective odour liking responses were correlated with the objective physiological (EEG) responses to investigate trends in brain activation associated with hedonic responses to a low concentration odour delivered during natural respiration.

Method

Participants

Thirty-six subjects (males = 18, females = 18) participated in the study, with a mean age of 24.03 (±6.59: range 15 - 43 yrs). All were right handed, as assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). All subjects were profiled using the *Sniffin' Sticks* Olfactory Performance Test (Burghart, Wedel, Germany) as described previously. Subjects were divided into Liking groups, based on rating responses obtained during the EEG recording. Liking group demographic details and *Sniffin' Sticks* Olfactory Performance TDI scores are presented in Table 1.

Table 1. Demographic details and *Sniffin' Sticks* TDI scores (±SD) for the Total Group (n = 36) and liking response sub-groups: *Like* (n = 15), *Neutral* (n = 13) and *Dislike* (n = 8).

	Group (n = 36)	Like (n = 15)	Neutral (n = 13)	Dislike (n = 8)
Mean Age	24.03 ±6.59	24.53 ±6.89	23.77 ±5.97	23.50 ±7.76
Age Range	15-43	15-43	18-39	18-36
Gender: Males	18	7	8	3
Females	18	8	5	5
TDI Score	34.34 ±3.72	35.10 ±3.29	31.90 ±3.35	36.56 ±3.22

Odour Stimulus Preparation

A gas form of the test stimulus β-damascenone ($C_{13}H_{18}O$; a sweet, fruity, rose, berry odour) was obtained from a pure liquid source (Product Makers; Melbourne, Aust.) by bubbling room air through 8 mL of liquid in a 20 mL glass vial sealed by a rubber stopper. The rubber stopper was pierced by two blunt 18 G hypodermic needles (Terumo; Melbourne, Aust.), sealed by three-way disposable

stopcocks (Discofix®; Braun; Melsungen,) fitted with Luer lock terminations. A two metre length of Tygon™ tubing was fitted to the taps by ways of plastic tubing adapters and then inserted into the pump head of a Cole-Palmer™ roller pump. Air was then pumped and recirculated through the liquid for 20 minutes. At the conclusion of the recirculation, the air was removed from the tubing into a teflon gas sample bag and the tubing flushed out with 350 mL of room air using plastic 50 mL Luer syringes. In this way odour-rich air was prepared for the damascenone stimulus. In GC/MS analysis, the concentration of the odorous gas was determined to be 1.99 ppm (reported threshold: 0.009 ppm; Ohloff, 1994).

Procedure

All subjects were screened using the *Sniffin' Sticks* Olfactory Performance Test prior to attending an EEG recording session. EEG during stimulus delivery (air or odour) was recorded for five-minute trials, and each subject participated in 4 repeated trials resulting in a total of 20 minutes of EEG recorded. Following each trial, participants completed a rating sheet, using the 10-point Likert scale to indicate their perceived liking and strength ratings, and describing the odour they detected during the recording. Using the CRO delivery technique with the EEG system, differences caused by the test odours in comparison to air without test odours were recorded and analysed, correlating the odour nature with the hedonic/emotive responses.

Data Processing and Statistical Analysis

The EEG data obtained in the 20 minutes of recording were epoched for 1024 msec periods around the stimulus point for air or odour delivery (100 msec pre-delivery and 924 msec post- delivery), and averaged for air or odour. During off-line analysis, the EEG signals were processed for artefact reduction and digitally filtered using a 45 Hz low-pass filter to reduce electrical noise contamination (50 Hz mains). In addition to studying the EEG signals at specific anterior electrode sites, the signals were averaged for left and right frontal electrode sites (grouped at sites equating to around F3 and F4 in the International 10/20 system), as shown in Figure 3.

The averaged signals for each subject were also subjected to FFT analysis for the 1024 ms epoch period (512 samples). Following the FFT analysis, each subject's air averages and odour averages were normalised such that the total power in each set totalled 100%. Relative % power was then obtained for the frequency bins of interest (1-4Hz, 5-8Hz, 9-12Hz) for each condition odour *vs* air). The use of relative % power in this study was justified due to the protocol adopted, which incorporated both air and odour stimuli randomised throughout the same time frames (each 5 minute recording period). It could therefore be assumed that within each time frame the total power for each epoch would remain relatively constant. In view of the use of a no-odour control (air stimulus only), the relative % power was also calculated for the odour-air difference, based on the assumption that such a calculation would account for any common brain responses and isolate the odour-related responses. From Excel, comparisons were made between the left and right frontal regions relative to the different liking response groups at different frequencies, and subjected to statistical analysis.

Statistical analysis was conducted using SPSS (version 10.0 for Macintosh; Release 10.0.7a; SPSS Inc., 2000). Analysis of variance (ANOVA) was used, with the Newman-Keuls post-hoc test and $p<0.05$ for identifying significant results.

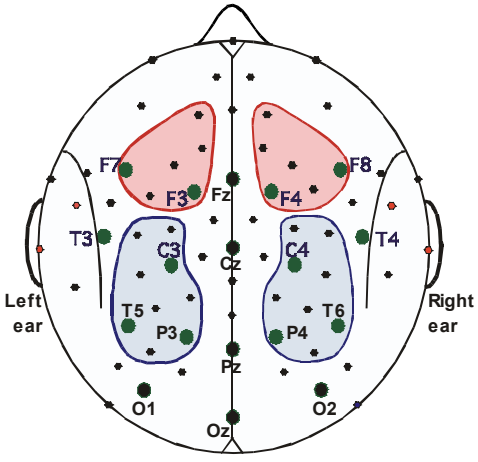


Figure 3. Illustration of electrode site locations, viewed from above (nose at top, right and left ears as indicated). The EGI electrode sites have been labelled for the sites equalling the International 10/20 system.

Results

Olfactory Performance

The mean TDI score of the *Sniffin' Sticks* Test of Olfactory Performance screening test (M=34.34 ±3.72; range 22.25 – 40.50) indicated that all subjects were within the range for normal olfactory function (Kobal *et al.,* 2000). Oneway ANOVA with Liking Group (Like, Neutral, Dislike) as the between-subjects factor was conducted for each olfactory performance task. Results revealed that the *Threshold* scores differed significantly across liking groups ($F(2,33) = 3.644$, $p<0.01$) and there was a significant difference in the *TDI* scores across liking sub-groups ($F(2,33) = 5.751$, $p<0.05$), but there was no significant difference in the mean scores of the liking sub-groups for the *Discrimination* and *Identification* tasks. Newman-Keuls post-hoc comparisons revealed that the mean *Threshold* and *TDI* scores for the *Neutral* response group were significantly lower than the scores for both the *Like* and *Dislike* response groups, as seen in Table 2.

Table 2. Mean *Sniffin' Sticks* sub-test and TDI scores (± SD) for the liking response sub-groups: *Like* (n = 15), *Neutral* (n = 13) and *Dislike* (n = 8).

	Like n = 15	Neutral n = 13	Dislike n = 8
Threshold	8.70 (±2.35)	6.98 (±2.34)	9.81 (±2.75)
Discrimination	12.80 (±1.47)	11.85 (±2.73)	13.13 (±1.73)
Identification	13.60 (±1.50)	13.08 (±1.19)	13.50 (±1.31)
TDI Score	35.10 (±3.30)	31.90 (±3.35)	36.56 (±3.22)

Odour Liking

The mean liking responses were calculated from the subjective odour ratings provided following each EEG recording trial, where the participants had to rate their liking for the odour on a scale of 0 (strong like) to 10 (strong dislike). Liking sub-groups were formed based on the mean liking score for all four trials, with mean liking ratings for each sub-groups summarised in Table 3.

Independent groups One-Way ANOVAs with Liking Group (Like, Neutral, Dislike) as the between-subjects factor were computed for the mean Liking and Strength responses, revealing significant differences in mean Liking scores between the designated Liking sub-groups ($F(2,33) = 75.778$, $p<0.001$, $\eta^2 = 0.82$) as shown in Table 3. This analysis supported the post-hoc formation of these Liking sub-groups. There was no significant difference in Strength scores between the three Liking sub-groups ($p>0.05$).

Table 3. Mean Liking and Strength Response Ratings ((± SD) for the liking response sub-groups: *Like* (n = 15), *Neutral* (n = 13) and *Dislike* (n = 8). (Like= <4, Neutral = 4-6, Dislike= >6)

	Like n = 15	Neutral n = 13	Dislike n = 8
Liking Mean	2.64 (±0. 91)	4.99 (±0.58)	7.05 (±1.06)
Liking Range	(0.6 – 3.7)	(4.1 – 5.8)	(6.0 – 9.2)
Strength Mean	4.15 (±1.99)	4.40 (±1.50)	5.54 (±1.65)
Strength Range	(0.7 – 7.38)	(0.8 – 6.35)	(3.28 – 8.70)

EEG Responses

Frequency and frontal EEG responses to the odour were examined for each liking response group with a 3 x 3 x 2 mixed design ANOVA conducted on the Odour relative % power responses, with Liking group (Like, Neutral, Dislike) as a between subjects factor, and frequency range (1-4 Hz, 5-8Hz, and 9-12 Hz) and frontal location (Left, Right) as within subject factors.

Table 4. Frontal (Left *vs* Right) mean relative % power odour responses (± SD), grouped in frequency bins, for the odour liking response sub-groups: *Like* (n = 15), *Neutral* (n = 13) and *Dislike* (n = 8).

Relative % power odour response	Like n = 15	Neutral n = 13	Dislike n = 8
1 – 4 Hz Left mean	67.20 (±5.84)	71.86 (±6.28)	62.38 (±8.00)
1 – 4 Hz Right mean	65.20 (±5.53)	70.20 (±5.94)	74.43 (±7.57)
5 – 8 Hz Left mean	9.69 (±2.41)	10.13 (±2.59)	14.99 (±3.30)
5 – 8 Hz Right mean	10.59 (±2.45)	12.47 (±2.64)	9.94 (±3.59)
9 – 12 Hz Left mean	2.83 (±1.22)	5.58 (±1.32)	8.95 (±1.68)
9 – 12 Hz Right mean	2.37 (±1.21)	5.13 (±1.30)	6.82 (±1.65)

There was no significant interaction between liking group, frequency and frontal location ($F(4, 66) = 2.433$, $p=0.098$, $\eta^2=0.13$). Figure 4 depicts the means for the relative % power odour responses for the three frequency bands for liking sub-groups and left versus right frontal responses. Although there were no significant differences associated with frontal region of activation in these frequency bands, a study of the means reveals a trend associated with liking group responses for left versus right frontal locations, as shown in Table 4.

In general, there was an increase in theta activity in the left frontal location and an increase in alpha activity in the both the left and right frontal locations associated with *Dislike* for the odour (*cf Like* or *Neutral* responses).

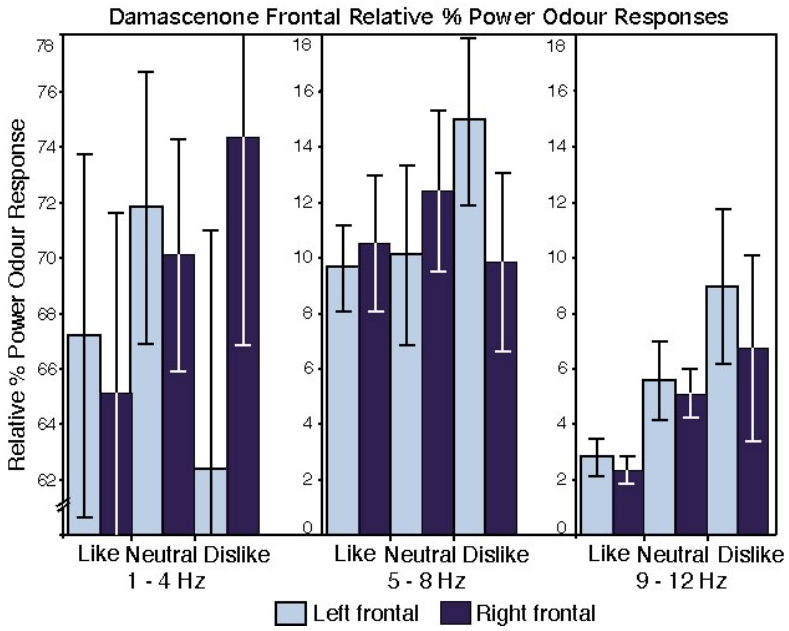
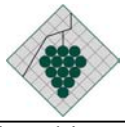


Figure 4. Relative percent power for the 1-4 Hz, 4 – 8 Hz, and 8 – 12 Hz power bands (with standard error bars) in response to the odour stimulus (damascenone) for liking response sub-groups: *Like* (n = 15), *Neutral* (n = 13) and *Dislike* (n = 8). The columns represent Left (light) and Right (dark) frontal activation. The Y-axis shows power normalised as a relative percentage. In the 5- 8 Hz and 9- 12 Hz bands there was a trend for reduced left frontal activation for the *Like* and *Neutral* groups and increased activation for the *Dislike* group relative to the right frontal activation. [Note the different Y-axis values for the 1- 4 Hz range in comparison to the 5- 8 Hz and 9- 12 Hz ranges.]

Relative % power was also calculated for the odour-air difference, to determine if the significant differences observed for the odour responses were consistent across the odour-air difference condition. A 3 x 3 x 2 mixed design ANOVA was conducted on the relative % power difference (Odour minus Air) responses, with Liking group (Like, Neutral, Dislike) as a between subjects factor, and with



frequency range (1- 4 Hz, 5- 8 Hz, and 9- 12 Hz) and frontal location (Left, Right) as within subject factors.

There were no significant within or between subject effects for frontal location or frequency range for the liking groups ($p>0.05$). However, the mean responses for the difference frequency ranges in the left versus right frontal locations suggested a trend in difference (odour-air) responses associated with liking group. The increase in theta activity in the left frontal location and an increase in alpha activity in the both the left and right frontal locations associated with *Dislike* for the odour (*cf Like* or *Neutral* responses), observed for the odour responses, was again evident in the difference responses.

Discussion

Although there were no significant interactions between liking responses, frontal location and relative % power evident in the current study, there was evidence of a trend for differences in left and right frontal power differences associated with *Like* or *Dislike* for the odour. In addition, there was evidence to suggest an increase in theta activity in the left frontal location and an increase in alpha activity in the both the left and right frontal locations associated with *Dislike* for the odour (*cf Like* or *Neutral* responses).

The hedonic effect of odours on brain electrical activity has been described in the literature as differences in anterior brain activity associated with pleasant and unpleasant odours (Kobal *et al.,* 1992; Brauchli *et al*., 1995; Ehrlichman *et al.,* 1995; Roescher *et al*., 1998). A particular association between left frontal activation and pleasant odours has been described (Kline *et al*., 2000), supporting previous studies of positive emotional responses activating the left frontal region (Davidson, 1992; Lane *et al*., 1997; Sutton & Davidson, 2000). This study approached this research question from a different direction by investigating different liking (pleasant, unpleasant) responses to one particular odour. While failing to find significant differences in the relative power of frontal responses associated with reported like or dislike for the odour, there was a trend for differences in theta and alpha responses for *Dislike* group *cf* the *Like* and *Neutral* groups, particularly in the left frontal location. The presentation of damascenone resulted in a lower relative power (non-significant) for those who reported liking the odour and higher relative power for those who disliked the odour when compared to those with a neutral response. This extends previous findings from electrophysiological and neuroimaging studies (Klemm *et al.*, 1992; Kobal *et al*., 1992; Kline *et al*., 2000; Kobal & Kettenmann, 2000) which reported differences in left frontal activation associated with stimulation by a pleasant odour in comparison with stimulation by an unpleasant odour.

The trend for reduced left frontal activation in the 5-8 Hz or *theta* range associated with a like response also support previous findings of reduced theta activity in response to pleasant food odours such as chocolate (synthetic and real), spearmint (synthetic) and spiced apple (Lorig & Schwartz, 1988; Lorig *et al*., 1990; Martin, 1998). However, it has been proposed that the effect of odours on theta activity may actually be a reflection of the odour's ability to attract or distract and therefore reflect shifts in attention associated with the emotional reaction to the odour (Martin, 1998). This has been supported previously by findings of an apparent association between changes in theta activity and self-reports of tension and anxiety responses (Lorig & Schwartz, 1988). The olfactory effects on theta may be specific to different odours possessing similar psychological properties (Martin, 1998). In the current study, the evidence (non-significant) of increased theta activity in the left frontal region evident in the *Dislike* responses in comparison with the *Like* and *Neutral* responses may reflect the shift in attention associated with a dislike response common to different odours and associated with the similar psychological (hedonic) response to the odours.

As cortical alpha power has reported to be inversely related to cortical activity (Coan *et al*., 2001), suppression of alpha activity has been equated to an increase in activation in response to stimulation. This has been demonstrated in response to visual and auditory stimulation (Brauchli *et al.*, 1995) and has more recently been reported by EEG studies in response to olfactory stimulation (Lorig *et al.*, 1990, 1991; Schwartz *et al*., 1992; Van Toller *et al.*, 1993; Lorig, 1994; Brauchli *et al.*, 1995). Due to the protocol used in the current study, it may have been expected that there would be an increase in alpha due to the test conditions (darkened room, calming breathing), similar to the increase in alpha associated with a 'restful' state (Klemm *et al.*, 1992). Such an effect would be common to both the air and odour conditions due to the experimental design. Although it was not significant, the consistent

trend in alpha band left frontal decreases associated with liking for an odour (*cf* dislike) was found in both the relative % power of the odour responses and the odour-air differences, supporting the assumption that these decreases are related to the odour stimulus.

The approach-withdrawal theory (Davidson, 1987, 1992) proposed that approach-related positive responses would be evident in changes in anterior EEG activation in the left frontal region, seen as decreased alpha activation (increased arousal or attention) in response to the positive stimuli and a corresponding increased alpha activation in response to negative stimuli. Changes in right anterior activation would be predicted with withdrawal-related negative responses, seen in decreased alpha activation right frontal response to negative stimuli, and a corresponding increased alpha activation right frontal response to positive arousal. Although there was evidence of a trend in alpha changes associated with like *vs* dislike, the current study did not find the significant differences in left or right frontal activation predicted by the approach-withdrawal theory. This may have been due in part to the range of hedonic responses evoked by the odour, and in particular to the degree of negativity involved in the hedonic response. The perceived *Dislike* response to the test odour was possibly a less potent negative response than that used in the emotional studies. This may therefore have reduced the effect of the negative response which was predicted by the approach-withdrawal theory (Davidson, 1987, 1992). A lack of significant right frontal differences was also reported by Kline *et al*. (2000), with a possible explanation offered associated with the period of the FFT was computed - over an entire minute - which may have caused the phasic right frontal activation during behavioural withdrawal to be missed. In the current study, the FFT was computed over a 900 ms post-stimulus period in an attempt to analyse the cortical arousal due to sensory stimulation, and again the right frontal activation did not reveal the negative withdrawal response. It would therefore appear that the duration of the FFT analysis, as proposed by Kline *et al*. (2000), may be a potential dominant contributor to this lack of right frontal negative effect.

While not fully supporting the predictions for hedonic-related EEG changes, this study has extended olfactory research by demonstrating the consistency of the left frontal response associated with liking an odour. In taking a different approach and using the same odour to evoke different hedonic responses, these results have provided further [non-significant] evidence of changes in left frontal activation associated with the hedonic response. Future studies with greater numbers of subjects and different odours (particularly using odours which may evoke stronger negative reactions) will provide evidence of the significance or robustness of these changes in frontal activation associated with the hedonic response. This application of objective physiological techniques to sensory studies has the potential to contribute to a general understanding of the sensory qualities associated with odour perception and liking.

Section 6.2 TCA versus Ethanol Study

Introduction

The occurrence of taints or off-flavours in wine which cause unpleasant flavours or odours are a significant economic problem to the wine industry (Taylor *et al*., 2000; Silva Periera *et al*., 2000). While there are different sources of taints in wines, the most commonly reported is the compound 2,4,6-trichloroanisole (TCA; Fuller, 1995; Taylor *et al*., 2000; Silva Periera *et a*l., 2000). The effect of TCA on consumer responses to wines is a serious problem exacerbated by the difficulty of its assessment. It is generally recognised that TCA can adversely affect the sensory properties of wine, although recent research has indicated that wines exhibiting cork taint may have low or chemically undetectable concentrations of TCA (Soleas *et al*., 2002). Individual responses to wines vary to such a degree that even experienced tasters may have conflicting opinions about the tainting of a wine (Simpson and Veitch, 1993; Butzke *et al*., 1999; Casey, 1999). Variations in sensitivity to TCA will result in only portions of the population even being aware of its presence in a wine, and variations in concentrations of TCA will have different manifestations. Even when not consciously detected, TCA is reported to suppress and alter the aroma and flavour of wine (Casey, 1999). It is also reported that we quickly become adapted to the smell of TCA, which in turn affects the subjective assessment of its presence or effect on a wine.

The degree to which consumers vary in responses to TCA has rarely been investigated using objective physiological techniques. This study was undertaken to provide preliminary information concerning the degree to which consumers perceive and respond to TCA, even when not consciously aware of its presence. Responses to TCA and ethanol were assessed using subjective and objective techniques to monitor perceived and physiological responses in an attempt to assess differences in perceptual responses to TCA in a population of wine consumers, untrained in taint (TCA) detection.

Method

Participants

A panel of 27 subjects participated in the study (12 males and 15 females, mean age 24.63 years ± 7.3; age range 18-43 years). All volunteer participants were screened for olfactory performance using the *Sniffin' Sticks* Olfactory Performance Test (Burghart, Wedel, Germany), with test results indicating all participants had total olfactory performance scores (TDI score: the sum of Threshold, Discrimination and Identification scores) within a normal range for this age group (Hummel *et al*., 1997; Kobal *et al*., 2000): TDI = 34.65 ±3.83.

Odour Stimuli and Gas Chromatography/Mass Spectroscopy analysis of odour samples

The TCA odour sample was prepared by dissolving the TCA in 100% ethanol. Equivalent volumes of TCA in ethanol and ethanol alone were placed in the 50 mL glass odour syringes incorporated into the syringe delivery system of the CRO.

SPME-GC analysis of the generated TCA and ethanol odour samples was conducted using a Chrompack 3800 gas chromatograph (Varian, Australia) fitted with a 1079 injector and flame ionisation detector. These odour samples were separated on an EC-WAX capillary column (Altech, Australia) (30 m, i.d. 0.32, film 0.25 µm), and concentrated using solid phase micro extraction (SPME) fibres (Supelco, Bellafonte, PA, USA). This analysis was conducted to determine concentration and consistency of sample odour preparation throughout the experimental period.

After taking the dilution factor of the facemask into account (variable with face size and shape), the concentration of TCA delivered to the participants was calculated to be 10-20 ng/L. The concentration of the ethanol was calculated to be 31.6 mg/L, present in both the TCA and ethanol odour syringes.

Procedure

All subjects were screened using the *Sniffin' Sticks* Olfactory Performance Test. Participants then attended an EEG recording session consisting of 6 x 5-minute trial recording periods (3 TCA trials and 3 ethanol trials) resulting in a total of 15 minutes of EEG recorded for each test odour. Order of presentation for the ethanol or TCA trial series was counterbalanced across the group. Following each trial, participants completed a rating sheet, using the 10-point Likert scale to indicate their perceived liking and strength ratings, and describing the odour they detected during the recording. Differences caused by the test odours in comparison to air without test odours were recorded and analysed.

Results

Subjective Liking and Strength Responses to TCA and Ethanol

Analysis was conducted to examine differences in Liking and Strength (Intensity) ratings for the two odours (TCA and ethanol). The ratings acquired following each EEG trial for each odour were averaged for each participant and are summarised in Table 5.

Table 5. Summary of subjective *Liking* (0 = strong dislike; 10 = strong like) and *Strength* (0 = very weak, 10 = very strong) rating responses (± SD) to TCA and ethanol (n= 27).

	TCA	Ethanol
Liking mean	6.53 ±1.60	6.22 ±1.67
Liking range	4.60 - 9.80	3.83 - 8.97
Strength mean	3.85 ±2.40	3.11 ±2.17
Strength range	0.50 - 7.70	0.23 – 6.75

Small differences in ratings were evident, and the range of Liking and Strength responses to both odours were comparable. Paired samples t-tests revealed no significant differences in mean Liking responses ($p>0.05$), but there was a significant difference between mean Strength responses for the odours (TCA: $\underline{M} = 6.53$, $\underline{SD} = 1.60$; ethanol: $\underline{M} = 6.22$, $\underline{SD} = 1.67$; $\underline{t}(27)= 2.64$, $\underline{p}<0.05$), with the mean Strength responses indicating that TCA was rated as significantly stronger than the ethanol. Correlation analysis revealed no significant correlations between preference and strength ratings of TCA or ethanol ($p>0.05$).

Descriptor Responses to TCA and Ethanol

Following each recording trial, participants provided as many descriptors as they deemed appropriate to describe the qualities of the odour they had perceived in the preceding trial. There was no restriction on the number of descriptors provided for each odour. For the 6 trials (3 trials of each odour), a total of 253 descriptors were provided to define the qualities of the TCA and ethanol stimuli. The frequency of all descriptor responses for trials of TCA and ethanol are summarised in Table 6.

Odour descriptors provided for TCA and ethanol included sweet, fruity, fermented, wine, alcohol, chemical, heavy. All participants reported perceiving an odour in both the TCA and ethanol trials. However, due to the low concentration of the TCA odour stimulus, participants were rarely able to identify or describe it in the terms commonly associated with TCA. Terms such as musty, mouldy or stale were provided by several participants in response to both TCA and ethanol; the use of damp and heavy descriptors was only evident with the TCA trials.

There was a trend for participants to report stronger wine, alcohol and chemical qualities in the TCA trials *cf* the ethanol trials, as evident in the increased use of wine/alcohol and chemical (chemical, solvent, methylated spirits) descriptors associated with TCA trials. Beer and brewing descriptors were more commonly applied to ethanol. Table 7 provides a summary of the main descriptors provided by each participant, and serves to summarise the perceived changes in descriptors associated with TCA versus ethanol for each participant.

Table 6. List of main descriptors and the number of times the descriptors were provided for TCA and ethanol. A total of 253 descriptors were provided by participants for 3 trials for each odour. Participants were instructed to provide as many descriptors they thought appropriate to describe the odour. There was no restriction on the number of descriptors provided for each odour.

Descriptor	TCA	Ethanol
Chemical, solvent, methylated spirits	29	23
Fermented	5	5
Wine, alcohol	15	9
Beer, brewery, malt	4	8
Sour, vinegar, sharp, acidic	7	7
Fruity, apple	18	16
Citrus	2	2
Sweet, vanilla	23	23
Mint	1	1
Floral	2	3
Woody	Nil	2
Damp, heavy, stale	4	1
Musty, mouldy	4	6

Table 7. List of descriptors provided by each participant for TCA and ethanol. Included in this table are the main descriptors which varied for each participant (n = 27). There was no restriction on the number of descriptors provided by participants for each odour. The descriptors which varied between odours are highlighted in *italics*.

	TCA Descriptors	Ethanol Descriptors
1	fermented fruit, wine	fermented fruit
2	chemical, thin, fruity	chemical, sweet, sharp
3	no description	no description
4	alcohol, citrus	alcohol, sweet
5	sweet, musty, chemical,	sweet, malt, beer
6	chemical, vinegar, acidic	chemical, sour, synthetic
7	heavy, sweet, solvent	musty, woody, weak
8	chemical (texta)	chemical (texta), citrus
9	chemical (texta), red wine	chemical (texta)
10	sweet, fruity	sweet, fruity
11	sweet, alcohol	sweet, sugary
12	floral, glue, chemical	floral, glue
13	sweet, mint	sweet, mint
14	apple, fermented, sweet	apple, fermented
15	fresh, fruity, sweet, green apple	fresh, fruity, sweet, clear
16	methylated spirits, sharp, chlorine	methylated spirits, sharp
17	alcohol, damp, musty	alcohol, fruity, sweet
18	sweet, fruity, alcohol, mouldy	sweet, fruity, alcohol, mouldy
19	chemical, ether, rose, floral	chemical
20	chemical (texta)	chemical (texta), methylated spirits
21	fruity, ketone, rotting/ripe fruit	fruity, ketone, alcohol
22	apple cider, old grapes	methylated spirits, soap, grain, wine
23	chemical (texta), fruit	chemical (texta), weak
24	acetone	acetone
25	fruity, sweet, orange	fruity, sweet, weak
26	alcohol, spirits, acetone	alcohol, spirits, sweet, vanilla
27	chemical (texta), sickly	sweet, pleasant, nice

Interactions of Descriptor and Liking/Strength Responses to TCA and Ethanol

Cross-tabulations were used to examine the relationship between descriptors and liking or strength ratings for TCA and ethanol. The descriptors provided by each participant were grouped into the following categories: fruity/sweet; alcohol; musty (damp); combination of previous categories; other descriptors. As shown in Table 8, while only 7.6% of the participants thought TCA smelled musty or damp (*cf* 1.3% in response to ethanol), 44.3% used fruity/sweet descriptors to describe TCA (*cf* 34.2% in response to ethanol). This has been interpreted to suggest that TCA enhances the fruity/sweet qualities of the alcohol.

An examination of the use of different categories associated with liking and strength responses revealed that participants who described the TCA odour as more fruity and sweet also tended to rate the odour as more pleasant (M = 6.81, SD = 1.85) than those participants who described the odour as alcoholic (M = 6.55, SD = 1.68) or musty/damp (M = 5.88, SD = 1.22). As shown in Table 9, a similar pattern was evident in the strength ratings: participants who rated TCA as fruity and sweet also rated it as weaker (M = 3.50, SD = 2.36) than those who rated it as alcohol (M = 4.37, SD = 2.26) or musty/damp (M = 5.08, SD = 2.03). Similar trends were evident in the ethanol responses, however any consideration of the musty/damp responses must take into account the small number of times these descriptors were applied to the odours (TCA: n= 6; ethanol: n= 1).

Table 8. Percentage of descriptors provided within the different descriptor categories used for TCA and ethanol subjective responses.

Descriptor category	TCA	Ethanol
Fruity/sweet	44.3 %	34.2 %
Alcohol	20.3 %	20.3 %
Musty/damp	7.6 %	1.3 %
Combination of above categories	5.1 %	6.6 %
Other	22.8 %	31.6 %

Table 9. Mean Liking and Strength ratings (±SD) and the number of times the descriptor was provided for different descriptor categories used for TCA and ethanol subjective responses.

Descriptor category	Liking		Strength	
	TCA	Ethanol	TCA	Ethanol
Fruity/sweet	6.81 (±1.85) (n=35)	6.76 (±1.92) (n=26)	3.50 (±2.36) (n=35)	3.19 (±2.22) (n=26)
Alcohol	6.55 (±1.68) (n=15)	5.42 (±1.12) (n=20)	4.37 (±2.26) (n=15)	3.41 (±2.14) (n=20)
Musty/damp	5.88 (±1.22) (n=6)	6.40 (n=1)	5.08 (±2.03) (n=6)	0.60 (n=1)
Combination of above categories	6.92 (±1.14) (n=4)	6.16 (±1.92) (n=5)	2.95 (±2.73) (n=4)	2.32 (±1.22) (n=5)
Other	5.97 (±1.53) (n=17)	6.15 (±1.54) (n=24)	4.45 (±2.19) (n=17)	2.88 (±2.01) (n=24)

Electrophysiological Power Analysis: Odour – Air difference comparisons

Relative % power odour -air difference responses, grouped in three frequency bins (5-8 Hz, 9-12 Hz and 13-16 Hz), were submitted to statistical analysis to determine the significance of differences in responses associated with electrode location and odour type. Anterior electrode locations were selected for this analysis to provide corresponding left and right hemisphere sites: Left and Right frontal groups, Left and Right temperoparietal groups, and individual electrode sites: F3, F4, F7, F8, C3, C4, T3 and T4 (see Figure 3).

Paired samples t-tests were conducted to evaluate the impact of odour type (TCA *vs* ethanol) on relative % power odour minus air difference responses at the different frontal electrode sites. There was a significant decrease in 13-16 Hz frequency responses at the left frontal electrode group associated with TCA (M= -0.62, SD = 1.82) *cf* ethanol (M = 0.28, SD = 1.29), $t(27)= 2.25$, $p<0.05$)

and in particular at the left frontal site F7 associated with TCA (M= -0.58, SD = 1.56) *cf* ethanol (M = 0.61, SD = 1.51), t(27)= 2.81, p<0.01).

These responses suggest a decreased left frontal beta response associated with TCA *cf* ethanol. This decrease was further demonstrated in the non-significant trend observed for the corresponding right frontal responses, with a decrease in TCA responses *cf* ethanol (see Figures 5 and 6). There was also a similar [non-significant] trend in the left frontal responses in the 5-8 Hz (theta) range, but no trends evident in the 9-12 Hz (alpha) range.

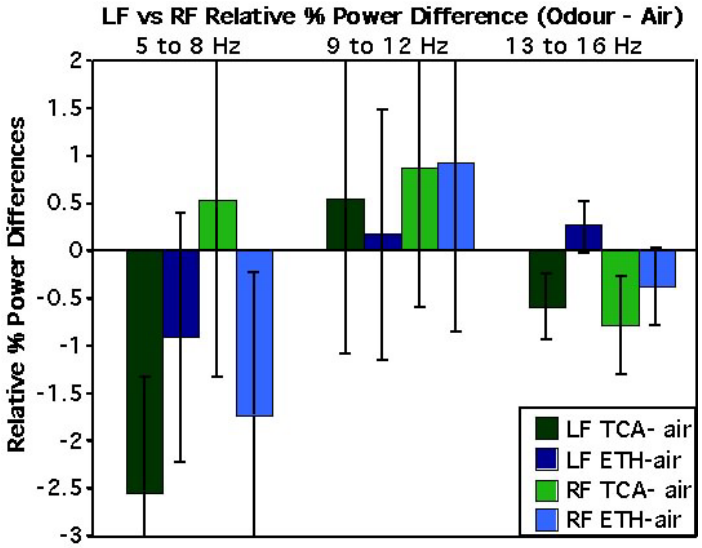


Figure 5. Frontal relative percent odour-air power difference (with standard error bars) for the 5-8 Hz, 9-12 Hz, and 13-16 Hz power bands in response to TCA and ethanol (n= 27). The columns represent activation at Left and Right frontal electrode groups (see Figure 3). The Y-axis shows power normalised as a relative percentage. There was a significant difference in odour responses in the 13-16 Hz (beta) band, with greater reduction in TCA responses *cf* ethanol.

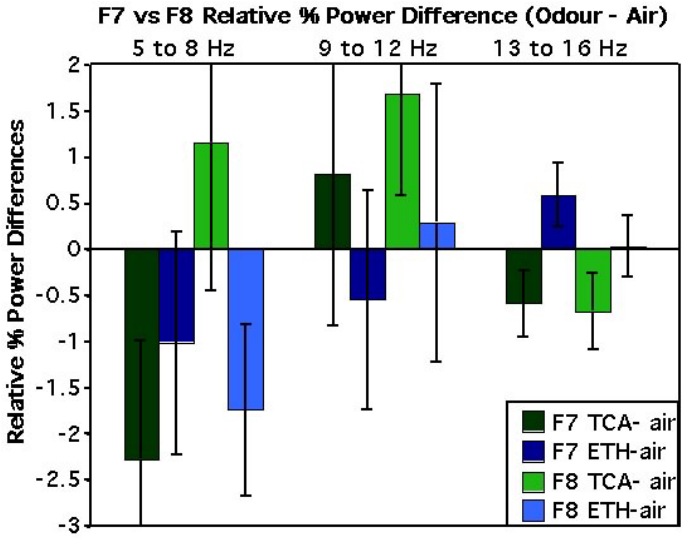


Figure 6. Frontal relative percent odour-air power difference (with standard error bars) for the 5-8 Hz, 9-12 Hz, and 13-16 Hz power bands in response to TCA and ethanol (n= 27). The columns represent activation at Left and Right frontal electrode sites equating to F7 and F8 in the 10/20 system (see Figure 3). These electrodes are within the frontal groupings illustrated in Figure 5. The Y-axis shows power normalised as a relative percentage. There was a significant difference in left (F7) odour responses in the 13-16 Hz (beta) band, with greater reduction in TCA responses *cf* ethanol. There was a similar trend in the right (F8) odour responses, but this was not significant.

Electrophysiological Power Analysis: Odour – Air difference comparisons for Liking groups

The same statistical procedures were adopted for the relative % power odour-air difference responses, grouped in three frequency bins (5-8 Hz, 9-12 Hz and 13-16 Hz). Independent samples t-tests were conducted to evaluate the impact of liking group on relative % power odour minus air difference responses for ethanol and TCA at the different frontal electrode sites. Note that analysis of the liking responses resulted in Like (n=13) and Neutral (n=14) groupings only, with no reported Dislike responses within this study's particpant group.

There was a significant difference in 8-12 Hz frequency responses at the left frontal electrode group associated with Liking group for ethanol, with a decrease in alpha responses for Like *cf* Neutral responses (*Like*: $\underline{M}$= -2.28, $\underline{SD}$ = 4.28; Neutral: $\underline{M}$ = 3.14, $\underline{SD}$ = 8.43; $\underline{t}(25)$= -2.08, $\underline{p}$<0.05). This difference was supported by a non-significant trend at the F7 (left frontal) electrode site ($\underline{p}$= 0.07). This difference was also observed in a non-significant trend for TCA responses ($\underline{p}$> 0.05). These responses suggest a decrease in left frontal alpha responses associated with odour liking responses for ethanol and TCA.

Discussion

This study explored the potential for the monitoring of physiological changes to provide an objective and quantitative method for determining differences in flavour responses between two odours, with the second odour (TCA) being presented in the first (ethanol) for comparison with responses to the ethanol alone. The results of this preliminary study revealed differences in both sensory and electrophysiological responses to TCA versus ethanol.

Although small differences in subjective Liking and Strength ratings were evident, statistical analysis revealed no significant differences in mean Liking responses. However, there were significant differences in mean Strength responses, with stronger TCA ratings *cf* ethanol. There was no significant correlation between Liking and Strength ratings for TCA or ethanol.

Evidence that the low concentration of TCA used in this study was close to threshold level for many of the participants was provided by the difficulty experienced by some participants in identifying or describing the odour, although all participants did report perceiving an odour in both the TCA and ethanol trials. There was a tendency for participants to report an enhanced alcohol quality (evident in the increased use of wine, alcohol and chemical qualities) in the TCA trials *cf* the ethanol trials. Analysis of the rating responses in comparison with descriptors also revealed a trend for stronger Like responses and a corresponding reduction in intensity rating responses associated with fruity/sweet ratings for TCA and ethanol.

Anecdotal evidence of apparent TCA thresholds in this study appear to be conflicting with the sensitivities reported in the wine industry literature. TCA recognition thresholds have been reported to be as low as 6 ppt (ETS Laboratories, Cork Quality Council, USA). While definitive experiments of the concentration delivered by our delivery system have yet to be conducted, the best estimate that can be made at this stage of TCA concentrations delivered in this study is in the range of 10-20 ppt, varying with respiratory cycles, at which levels the participants were able to detect an odour but were unable to recognise or label the odour.

The results of the power analysis of the EEG responses to TCA and ethanol revealed differences in responses to the low concentration flavour component odours delivered during natural respiration. In particular, there were significant differences in responses in the frontal locations, with a decrease in the 13-16 Hz band (beta) response associated with TCA *cf* ethanol, and a similar [non-significant] trend in the left frontal responses in the 5-8 Hz (theta) range, but no trends evident in the 9-12 Hz (alpha) range. This trend in the theta range has been previously reported in association with food odours, with reduced theta activity in response to pleasant food odours such as chocolate (synthetic and real), spearmint (synthetic) and spiced apple (Lorig & Schwartz, 1988; Lorig *et al*., 1990; Martin, 1998), as reported in Section 6.1. However, it has been proposed that the effect of odours on theta activity may actually be a reflection of the odour's ability to attract or distract and therefore reflect shifts in attention associated with the emotional reaction to the odour (Martin, 1998). This may also be an explanation for the changes in the beta responses, as beta wave bands are commonly interpreted to

be associated with changes in activation or arousal. Beta bands consist of fast waves of low amplitude and are more prominent during intense mental activity (alert or anxious). It is generally regarded as a normal rhythm, the dominant rhythm when eyes are open, and is the typical EEG of the awake adult. The observed changes in beta responses in response to TCA *cf* ethanol may be a reflection of a decrease in arousal or attention in response to the TCA stimuli, relative to the ethanol stimuli.

The results of the TCA and ethanol responses associated with liking group (*Like vs Neutral*) provide further evidence of the alpha changes associated with stronger like responses described in Section 6.1, with a decrease in alpha associated with Liking *cf* Neutral. As cortical alpha power has reported to be inversely related to cortical activity (Coan *et al*., 2001), suppression of alpha activity has been equated to an increase in activation in response to stimulation, suggesting an increase in response to Liking for the odour. The trend in alpha band left frontal decreases associated with liking for an odour was also found in the damascenone study, further supporting the assumption that these decreases are related to the Liking response for the odour stimulus.

This study was undertaken to provide preliminary information concerning the degree to which consumers perceive and respond to TCA, even when not consciously aware of its presence. These results provided preliminary evidence of changes in brain activity associated with stimulation by TCA (delivered in ethanol) versus stimulation by ethanol alone. Analysis of the subjective responses provided preliminary evidence of differences in perceptual responses to TCA versus ethanol in untrained consumers, with suggestions of enhanced fruity/sweet qualities and more positive Liking ratings associated with the presence of low concentrations of TCA in ethanol. The significant findings in these objective measures, independent of the relatively non-significant subjective data, provide evidence of the increased sensitivity of objective monitoring techniques to determine differences in flavour responses between different odours.

Section 6.3 TCA Concentration Study

Introduction

As described previously, variations in sensitivity to TCA will result in only portions of the population even being aware of its presence in a wine, and variations in concentrations of TCA will have different manifestations. This study was designed as a preliminary assessment of the concentration effects of TCA by investigating changes in electrophysiological and subjective responses to different concentrations of TCA.

Method

Participants

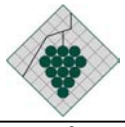
A panel of 13 subjects participated in the study (4 males and 9 females, mean age 21.31 years ± 4.37; age range 16-34 years), all of whom had previously participated in the initial TCA study, reported in Section 6.2. As with the previous study, all volunteer participants were screened for olfactory performance using the *Sniffin' Sticks* Olfactory Performance Test (Burghart, Wedel, Germany), with test results indicating all participants had total olfactory performance scores (TDI score: the sum of Threshold, Discrimination and Identification scores) within a normal range for this age group (Hummel *et al.,* 1997; Kobal *et al*., 2000): TDI = 34.40 ±3.73.

Odour Stimuli and Gas chromatography/mass spectroscopy analysis of odour samples

The TCA odour samples were prepared by dissolving the TCA in 100% ethanol. Equivalent volumes of TCA in ethanol and ethanol alone were placed in the 50 mL glass odour syringes incorporated into the syringe delivery system of the CRO.

SPME-GC analysis of the generated TCA and ethanol odour samples was conducted using the procedure described previously.

After taking the dilution factor of the facemask into account (variable with face size and shape), the low concentration of TCA delivered to the participants was calculated to be 10-20 ng/L, hereafter



referred to as TCA1. The stronger concentration of the TCA (hereafter referred to as TCA2) was four times the strength of TCA1. Taking into account the same dilution factors, TCA2 was therefore calculated to be 40-80 ng/L. The concentration of the ethanol was calculated to be 31.6 mg/L, present in both the TCA and ethanol odour syringes.

Procedure

All subjects were screened using the *Sniffin' Sticks* Olfactory Performance Test. All participants attended two EEG recording sessions, each consisting of 6 x 5-minute trial recording periods (3 TCA trials and 3 ethanol trials) resulting in a total of 15 minutes of EEG recorded for each test odour. All participants completed the TCA1 trials in the first session, and the TCA2 trials in the second session. Order of presentation for the ethanol or TCA trial series within each recording session was counterbalanced across the group. Following each trial, participants completed a rating sheet, using the 10-point Likert scale to indicate their perceived liking and strength ratings, and describing the odour they detected during the recording. Differences caused by the test odours in comparison to air without test odours were recorded and analysed.

Results

The same analysis procedures used in the first TCA study (Section 6.2) were employed in this study. As ethanol trials were conducted in both sessions, ethanol results were also labelled to indicate the appropriate session: ethanol_1 and ethanol_2.

Subjective Liking and Strength Responses to TCA and Ethanol

Analysis was conducted to examine differences in Liking and Strength (Intensity) ratings for the two sessions. The ratings acquired following each EEG trial for each of these odours were averaged for each participant and are summarised in Table 10.

Table 10. Summary of subjective *Liking* (0 = strong dislike; 10 = strong like) and *Strength* (0 = very weak, 10 = very strong) rating responses to TCA1 and TCA2 and the corresponding ethanol sessions: ethanol_1 and ethanol_2 (n= 13).

	TCA1	Ethanol_1	TCA2	Ethanol_2
Liking mean	6.33 ±1.56	6.45 ±1.65	5.53 ±1.66	5.41 ±1.59
Liking range	4.77 - 9.80	3.80 - 8.30	3.57 - 7.23	3.10 - 7.73
Strength mean	3.67 ±2.19	2.64 ±1.92	3.28 ±2.09	3.13 ±2.65
Strength range	0.23 - 6.80	1.70 - 6.20	1.07 - 7.70	0.20 - 6.83

Paired samples t-tests revealed a significant difference in mean Liking responses between TCA1 and TCA2 (TCA1: $\underline{M}$ = 6.33, $\underline{SD}$ = 1.56; TCA2: $\underline{M}$ = 5.53, $\underline{SD}$ = 1.66; $\underline{t}(34)$= -2.05, $\underline{p}<0.05$), and between ethanol_1 and ethanol_2 (ethanol_1: $\underline{M}$ = 6.45, $\underline{SD}$ = 1.65; ethanol_2: $\underline{M}$ = 5.41, $\underline{SD}$ = 1.59; $\underline{t}(29)$= -2.40, $\underline{p}<0.05$), but there was no significant difference between mean Strength responses for the same odour pairings ($\underline{p}>0.05$). These significant differences can be interpreted as a stronger like response to both the first session odours when compared to the second session corresponding odour. This would initially suggest that there were differences in responses associated with the different sessions (and different concentrations) but the lack of significant differences in the strength responses across the sessions supports that the session number was not the influencing factor, as only preference responses varied with the session days.

Descriptor Responses to TCA and Ethanol

As with the previous study, following each recording trial participants provided as many descriptors as they deemed appropriate to describe the qualities of the odour they had perceived in the preceding trial. There was no restriction on the number of descriptors provided for each odour. With the focus of this part of the study being the difference in perceptual responses to different concentrations of TCA, the descriptors provided for each odour stimulus were compared, with particular attention to the differences in responses to TCA1 versus TCA2. The frequency of all descriptor responses for trials of TCA and ethanol are summarised in Table 11.

In general, there was a reduction in the overall number of descriptors and in the number of fruity descriptors provided in the TCA2 and ethanol_2 trials. Terms such as musty, mouldy or stale were provided by several participants in response to both TCA and ethanol, and the use of stale, damp and heavy descriptors was ore prevalent with the TCA trials.

There was a trend for participants to report stronger wine, alcohol and chemical qualities in both TCA1 and TCA2 trials *cf* the ethanol (1 and 2) trials, as evident in the increased use of wine/alcohol and chemical (chemical, solvent, methylated spirits) descriptors associated with TCA trials.

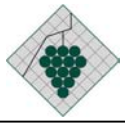
Table 11. List of main descriptors and the number of times the descriptors were provided for TCA1, TCA2, ethanol_1 and ethanol_2. Participants were instructed to provide as many descriptors they thought appropriate to describe the odour. There was no restriction on the number of descriptors provided for each odour.

Descriptor	TCA1	Ethanol_1	TCA2	Ethanol_2
Chemical, solvent, methylated spirits	29	23	10	11
Fermented	5	5	Nil	2
Wine, alcohol	15	9	11	4
Beer, brewery, malt	4	8	Nil	Nil
Sour, vinegar, sharp, acidic	7	7	Nil	3
Fruity, apple	18	16	7	3
Citrus	2	2	Nil	Nil
Sweet, vanilla	23	23	7	6
Mint	1	1	Nil	Nil
Floral	2	3	1	Nil
Woody	Nil	2	1	3
Damp, heavy, stale	4	1	8	4
Musty, mouldy	4	6	6	6

Table 12 provides a summary of the main descriptors provided by each participant, and serves to summarise the perceived changes in descriptors associated with TCA1 versus TCA2 for each participant.

Table 12. List of descriptors provided by each participant for TCA1 and TCA2. Included in this table are the main descriptors which varied for each participant (n = 13). There was no restriction on the number of descriptors provided by participants for each odour. The descriptors which varied between TCA concentrations are highlighted in *italics*.

	TCA1 Descriptors	TCA2 Descriptors
1	no description	damp rainforest, damp socks
2	texta, red wine	texta, alcohol
3	sweet, alcohol	sweet, alcohol, fruity, wine, honey
4	apple, fermented, sweet	complex
5	sweet, fruity, fresh, green apple	sweet, fruity, glue, pungent, light
6	alcohol, damp, musty	alcohol, damp, musty, fruity
7	mouldy, fruity, alcohol, sweet	mouldy, fruity, alcohol
8	chemical (white board markers)	chemical, alcohol
9	nail polish, acetone	methylated spirits
10	fruity, sweet, orange	fruity, sweet, stale
11	alcohol, nail polish remover, spirits	alcohol, nail polish remover
12	permanent marker, sickly	different
13	wine, yeasty	wine, fermented, sweet



Interactions of Descriptor and Liking/Strength Responses to TCA and Ethanol

Cross-tabulations were used to examine the relationship between descriptors and liking or strength ratings for TCA2 and ethanol_2, with the descriptors provided by each participant grouped into the same categories as in Section 6.2.

As shown in Table 13, 22.2% of the participants thought both TCA2 and ethanol_2 smelled musty or damp. Only 13.9 % of the participants provided fruity/sweet descriptors to describe TCA2 *cf* 29.6% in response to ethanol_2. The enhanced use of alcohol descriptors with TCA was again revealed, with 27.8% of the participants using alcohol descriptors for TCA2, *cf* 18.5% in response to ethanol_2.

Again, an examination of the use of different categories associated with liking and strength responses was conducted, revealing that participants who described the TCA2 odour as more alcoholic also tended to rate the odour as more pleasant (M = 5.20, SD = 1.49) than those participants who described the odour as fruity and sweet (M = 4.86, SD 0.97) or musty/damp (M = 5.35, SD = 2.32). However, it should be noted that all these ratings were within the *neutral* range on the rating scales.

Table 13. Percentage of descriptors provided within the designated categories in response to TCA2 and ethanol_2 (n = 13).

Descriptor category	TCA2	Ethanol_2
Fruity/sweet	13.9 %	29.6 %
Alcohol	27.8 %	18.5 %
Musty/damp	22.2 %	22.2 %
Combination of above categories	11.1 %	3.7 %
Other	25.0 %	25.9 %

As shown in Table 14, participants who rated TCA2 as fruity and sweet also rated it as weaker (M = 2.92, SD 1.56) than those who rated it as alcohol (M = 3.06, SD = 2.32) or musty/damp (M = 3.23, SD = 3.23). Similar trends were evident in the ethanol_2 responses. However, note that all these strength ratings were within the weak range on the rating scale.

A comparison of the descriptors associated with Liking and Strength ratings across the two TCA concentrations also reveals a decrease in liking associated with fruity/sweet and alcohol descriptors for TCA2 *cf* TCA1, and an increased number of musty/damp descriptors associated with neutral Liking responses. The strength ratings reveal a decrease in the perceived intensity of the TCA2 stimulus *cf* TCA1 for all descriptors except for the musty/damp descriptors, which were associated with slightly stronger mean responses, (although still within the weak range; see Table 14).

Table 14. Mean Liking and Strength ratings (±SD) for different descriptor categories used for TCA1, TCA2, ethanol_1 and ethanol_2 subjective responses (n= 13).

Descriptor category	Liking				Strength			
	TCA1	Eth_1	TCA2	Eth_2	TCA1	Eth_1	TCA2	Eth_2
Fruity/sweet	7.21 (± 1.57) (n=15)	7.39 (± 1.61) (n=14)	4.86 (± 0.97) (n=5)	5.09 (±1.62) (n=8)	4.17 (± 2.26) (n=15)	3.29 (±2.40) (n=14)	2.92 (±1.56) (n=8)	1.86 (±2.25) (n=5)
Alcohol	7.17 (±1.56) (n=7)	5.41 (±1.17) (n=12)	5.20 (±1.49) (n=10)	5.88 (±1.29) (n=5)	4.86 (±2.52) (n=7)	3.41 (±2.38) (n=12)	3.06 (±2.32) (n=10)	3.60 (±2.31) (n=5)
Musty/damp	4.10 (n=1)	nil	5.35 (±2.32) (n=8)	4.55 (±1.78) (n=6)	2.30 (n=1)	nil	3.23 (±2.59) (n=8)	4.20 (±2.86) (n=6)
Combination of above categories	7.60 (n=1)	6.02 (±2.14) (n=3)	6.10 (±1.03) (n=4)	8.30 (n=1)	6.60 (n=1)	2.73 (±0.61) (n=3)	3.10 (±2.70) (n=4)	8.20 (n=1)
Other	5.81 (±1.74) (n=8)	6.02 (±1.58) (n=15)	5.99 (±1.63) (n=7)	5.83 (±1.61) (n=7)	5.21 (±2.17) (n=8)	3.43 (±2.03) (n=15)	3.43 (±1.97) (n=7)	2.93 (±1.86) (n=7)

Electrophysiological Power Analysis: Odour - Air difference comparisons

The same procedure was applied to analyse the relative % power odour minus air difference responses, grouped in three frequency bins (5-8 Hz, 9-12 Hz and 13-16 Hz), which were submitted to statistical analysis to determine the significance of differences in responses associated with electrode location and odour type.

Paired samples t-tests were conducted to evaluate the impact of odour type (TCA1 *vs* TCA2) on relative % power odour minus air difference responses at the different frontal electrode sites. There were no significant differences in relative % power odour-air frequency responses between TCA1 and TCA2 ($p>0.05$).

Due to the small subject group participating in this study, the participants were not divided into subjective liking groups. However, in view of the differences in reported liking for TCA1 *vs* TCA2 (TCA1: $M = 6.33$, $SD = 1.56$; TCA2: $M = 6.33$, $SD = 1.56$), the results were studied to determine if there were any trends in responses associated with liking responses comparable with the results of studies one and two (Sections 6.1 and 6.2).

In keeping with the findings of the damascenone study (Section 6.1) of a Left frontal decrease in theta and alpha response associated with reported liking for the odour, there was a [non-significant] trend for a decrease in 5-8 Hz (theta) activation associated with TCA1 (*like* response) *vs* TCA2 (*neutral* response) in the left frontal region, also evident at individual left frontal electrode sites (F3 and F7).

Discussion

This study explored the use of objective physiological monitoring techniques to measure changes in brain activity associated with a concentration difference in the odour stimulus. The lower concentration was around threshold level for the participants, although all reported the ability to detect some odour during the trials, but were not always able to recognise it, suggesting it was above their detection threshold level but below their recognition threshold level. The higher concentration was above recognition threshold level for all participants, as evident in their ability to suggest a description of what they were able to smell as part of the subjective ratings.

There was a significant difference in mean Liking responses for TCA1 *vs* TCA2, but no significant difference in strength ratings. There was a more positive Liking response to TCA1 (*liked*) than for TCA2 (*neutral*). The reduced liking response to TCA2 was further reflected in the increased use of musty/damp/stale descriptors for TCA2, and the lessening of the alcohol, fruity/sweet descriptors for TCA2. The change in perception of TCA2 characteristics associated with the increased use of musty/damp descriptors was complemented by the change in intensity ratings for TCA2 (with slightly stronger mean responses) and served as a further indication of the change in subjective liking responses to TCA2 *cf* TCA1.

The results of the power analysis of the EEG responses to the two concentrations of TCA revealed differences in responses associated with the stronger liking for the lower concentration (TCA1). The significant difference in liking responses, as determined using the subjective rating scales, was investigated to determine if the brain activity would also reveal differences, particularly in view of the changes observed in the damascenone and TCA versus ethanol studies (Sections 6.1 and 6.2).

Again, there was a [non-significant] trend in the left frontal responses in the 4-8 Hz (theta) range associated with increased liking for the odour, which has previously been associated with a like response to pleasant food odours such as chocolate (synthetic and real), spearmint (synthetic) and spiced apple (Lorig & Schwartz, 1988; Lorig *et al*., 1990; Martin, 1998). The results of the TCA1 (*liked*) *vs* TCA2 (*neutral*) and the damascenone responses associated with liking group for each odour described in Sections 6.1 and 6.2 provide further evidence of brain activity changes associated with stronger like responses, with a decrease in theta associated with Liking *cf* Neutral, and again provide further support for the assumption that these decreases are related to the Liking response for the odour stimulus.

7. Outcomes/Conclusion

This project was an investigation into high-risk, innovative and strategic research to culminate in a new approach to determining the factors involved in the pleasure of wine.

Outputs and Performance Targets

- This study succeeded in meeting the proposed performance targets and outputs in successfully correlating differences in subjective and objective measures of flavour responses with untrained consumers.
    - The use of different wine flavour compounds and different concentrations attained the performance targets, with successful evaluation of the brain activity responses associated with concentration and preference (liking) responses.
- The only aspect of the current project which did not succeed was the aim to incorporate a small panel of industry experts - ie panellists trained in sensory/wine analysis - to provide a contrast in experience of wine flavours and therefore the opportunity to investigate differences in sensory and brain activity responses associated with expertise. Due to difficulties in accessing a group of industry experts (associated with the wine experts' demanding schedules), this aspect of the study was not completed.
    - However, a separately funded study has been completed using brewing flavours (reported at the *Institute & Guild of Brewing 2002 Conference*: Owen *et al*., 2002c) which demonstrated significant differences between expert and naïve participants for both sensory and brain activity responses in response to beer odours.
    - This independent study supported the expectation that differences in flavour analysis responses would be evident associated with expertise and flavour experience.
    - Future studies will provide the opportunity to investigate this in wine flavour responses.

Project Implications and Conclusions

- The sensory and physiological techniques used in this project provided evidence of reliable differences in brain activity processing between like and dislike and between the presence of alcohol and an odour plus alcohol.
- Although this research still contains substantial unknowns in terms of the fine detail of the nature of the relationship between brain activity and preference, the potential for the application of objective techniques to contribute valuable information about flavour responses has been demonstrated.
- Further development of these objective techniques will provide the Australian Grape and Wine Industry with a system with the potential to determine preference independent of language or experience, obtain results from small subject pools, and determine preferences of sub-groups in the community while using very small quantities of product will be the successful outcome of the current project.

Research and Communication Strategies

The application of objective measures of flavour responses which contribute to the wine industry's understanding of actual consumer responses has the potential to improve the accuracy and ease with which the individual's responses to underlying the factors involved in the pleasure of wine can be identified, thereby providing all sectors of the wine industry with access to faster, more reliable, simpler and cheaper ways of determining consumer preference.

Further research will assist in determining the consistency of these responses across a larger subject group, while correlating with measured sensitivity and subjective reports of responses to wine odours.

This research could:

- Be related to he interaction of taints in wine with different flavour components
- Focus on the interaction of wine flavours in different styles of wines

- Focus on the degree to which different concentrations of components affect flavour attributes.
- Variations in sensitivity to TCA and other wine flavours and taints should be further investigated in the consumer population, to establish a greater understanding of the subjective assessment of such flavours and in particular associated with their presence at different concentrations in wines.

The results of this project will be communicated to the scientific and wine industry communities through scientific publications, conference presentations and industry seminars. There is also the potential to produce an industry information CD for circulation to the wider industry community, as outlined in Appendix 5.

Practical Implications for the Australian Grape and Wine Industry

This study has taken important steps in demonstrating the complexity of human preferences and variety of experiences which contribute to the complexity of flavour responses. The results reported here provide unassailable evidence of a link between EEG structure and preference for odours.

The combined objective and sensory techniques used in this research provide access to subjective reports from untrained consumer groups which can be correlated with the objective and language independent odour-induced brain responses, independent of the participant's conscious awareness of the odour.

This consumer-based work can be extended to investigate differences in age groups, different cultural groups (nationally and internationally) with practical implications for the development of new wines and markets.

Greater understanding of consumer flavour responses will assist the industry in developing new wine markets, with the potential to tailor different wines to different markets, both nationally and internationally. This then has further implications and potential benefits for the marketing of Australian wines both nationally and internationally.

Project Benefits

This project used subjective and objective techniques to monitor perceived and physiological responses to wine-related odours in a preliminary investigation of the variations in human sensitivities and the perceived effects of concentration of TCA on consumer responses to wine odours.

While advances have been made in chemical analysis, the extent and variety of responses to wine flavours is still largely reliant on subjective reports and awareness of the odour. Individuals vary greatly in sensitivity to odours, both positive (enhancers) or negative (taints). This is in part due to physiological differences and to differences in experiences. In addition, subjective responses to odours and taints are strongly influenced by the degree and quality of experience with the odour, and its associated emotional and memory responses. Electrophysiological (EEG) responses have been found to change in association with reported preference, providing objective measure of responses, independent of language and experience.

This study has demonstrated that, together with traditional sensory techniques, the use of objective EEG measures has provided evidence of differences in responses to wine flavours independent of conscious awareness or recognition of the flavour and therefore has potential for application in further research to provide the Australian wine industry with a greater understanding of variability in sensitivity and perceptual responses to wine flavours.

8. Recommendations

This project was designed to use sensory, psychophysiological and EEG recordings to establish if detailed EEG signal changes correlate with subliminal or conscious detection of wine odours (enhancers and taints) together with either, or both, hedonic response to, or preference for, an odour. As indicated, the sensory and physiological techniques used in this project provided evidence of reliable differences in brain activity processing between like and dislike and between the presence of alcohol and an odour plus alcohol.

Future Research Directions

Although our research still contains substantial unknowns in terms of the fine detail of the nature of the relationship between brain activity and preference, we are in a position to do routine research with valid and useful conclusions to be drawn or benefit to the wine industry about objective assessment of threshold.

- The physiological evidence of changes induced by the odours (β-damascenone, ethanol and TCA) independent of subjective awareness supports the need for further research to determine the consistency of these responses across a larger subject group, and correlation with measured sensitivity and subjective reports of responses to the odour.
  - The alcohol and flavour interaction with TCA and the variability of responses in consumers is of great interest for future research.
- Additional research is also needed to investigate the interaction of different flavour components, and the degree to which masking of different flavour components influences consumer responses to wine flavours. This research could investigate:
  - The interactions of taints in wine with different flavour components
  - The interaction of wine flavours in different styles of wines
  - The degree to which different concentrations of components affect flavour attributes.

Broader Industry Practices

This study has taken important steps in demonstrating the variety of human preferences and experiences which contribute to the complexity of flavour responses. The reported results provide unassailable evidence of a link between EEG structure and preference for odours.

This research has important implications for broader industry practices associated with the assessment of quality and flavour management. As stated in the *GWRDC Five Year Research & Development Plan (2002-2007)*, the industry needs quality and flavour measurement technologies which enable greater control of quality and more potential for differentiating wine to meet market preference. Further development of this research techniques reported here will provide access to information which will contribute to a greater understanding of flavours and qualities associated with different market preferences.

Priorities for Future R&D

Future research is needed:

- To investigate variations in sensitivity to TCA and wine flavours in the consumer population
- To establish a greater understanding of the subjective assessment of wine flavours and the interaction of different flavours and concentrations of flavours in wines.

9. Appendix 1: Communication

Conference Presentations and Industry Seminars

Preliminary results have been reported in platform and poster presentations at the following scientific meetings:

- *ASP 2000*: Patterson J. & Owen C.M. (2000) A comparison of electrophysiological activity induced by two preferred odours. *Proceedings: Australasian Society for Psychophysiology*, Adelaide.
- *ISOT 2000*: Owen. C.M. & Patterson, J. (2000) Comparison of preference-related brain electrical activity in response to odour. *Proceedings: International Symposium of Olfaction and Taste*, Brighton, UK.
- *AIFST 2001*: Owen. C.M. & Patterson, J. (2001) EEG and its application in sensory analysis. *Victorian Sensory Group Meeting,* Melbourne, VIC.

A poster presentation of the TCA concentration study results will be made at the following scientific meeting:

- *AACSS 2002*: Owen C.M., Patterson J., Frank D. & Smith R. Mouldy, musty, or marvellous? Differences in consumer perceptions of 2,4,6-trichloroanisole (TCA). *Australasian Association for Chemosensory Science*, Heron Island, December 2002.

Publications

- Preliminary results of the damascenone study have been published as follows:
  Owen, C.M. & Patterson, J. (2002) Odour liking physiological indices: a correlation of sensory and electrophysiological responses to odour. *Food Quality and Preference*. 13, 307-316.
- A paper discussing preliminary findings associated with TCA *vs* ethanol responses is currently under preparation, to be submitted to the *Australian and New Zealand Wine Industry Journal.*
  Patterson, J., Owen, C.M., Smith, R. & Frank, D. Consumer sensory and electrophysiological (EEG) responses to 2,4,6-trichloroanisole (TCA).

Further Communication Activities

Industry seminars and presentations will be undertaken by the researchers to provide industry with access to the developments of this project and the potential to become involved in further investigations of consumer flavour and preference responses.

Such interactions will also provide the researchers with valuable information about the future and developing research interests of the industry.

An industry reference group consisting of wine makers, judges and industry consultants has been established to advise the researchers for future projects.

The researchers also propose the development of an industry information CD to provide access to these results and to provide background information aimed at increasing the wine community's understanding of the physiology and techniques which are the basis of this research. A demonstration of this CD has been included and explained in further detail in Appendix 5.

Further development and mass production of this Information CD will require some additional funds to ensure the CD is professionally presented and accessible to the wider community in the Australian Wine Industry.

10. Appendix 2: Intellectual Property

This research has extended olfactory and flavour research in general by investigating the interaction of sensory and electrophysiological responses to wine odours and further demonstrating the consistency of the left frontal response associated with liking an odour, together with changes in concentration responses associated with a liking response.

Previous studies have demonstrated the effect of odours on responses (and subsequently behaviour) even when the individual is not aware of the odour. This has implications in wine quality assessment, with the complexity of the wine flavours and quality incorporating the subtle and subconscious interaction of the complex flavours, associated with the psychophysiological aspects of the flavour responses (variations in thresholds, adaptation and masking effects).

These objective measurement techniques have not previously been explored in the Australian Wine Industry, and now provide the Industry with an indication of the valuable information which is potentially accessible in consumer responses.

- The valuable information arising from this research is based on the measurement of flavour responses using techniques which are language and experience independent.
- The variations in responses to different wine odours and the great sensitivity of brain recording techniques to provide additional evidence of flavour responses at the basic sensory level (prior to the formation of conscious responses) provide significant information to the industry associated with the emotional and physiological responses involved in the complexity of the flavour response.

11. Appendix 3: References

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12. Appendix 4: Staff

Dr John Patterson BSc, MSc, PhD.

Director, Sensory Neuroscience Laboratory, Swinburne University of Technology.

Dr Patterson pioneered the development of the novel delivery system technology, allowing subjects to breathe normally and be exposed to very small quantities of odour with over 200 successful recordings. With this, innovative work investigating EEG changes to normally inspired odours is being undertaken. This methodology offers a significant opportunity to advance knowledge in this field to Australia's benefit. Dr Patterson is currently Coordinating Supervisor for 3 PhD and 2 Masters (by research) students.

Dr Caroline Owen. BA (Psychology & Psychophysiology), PhD.

Research Fellow, Sensory Neuroscience Laboratory, Swinburne University of Technology.

Dr. Owen is an Early Career Researcher, having completed her PhD in 1998.

As project leader in the olfactory research conducted at the laboratory, Dr Owen is involved in coordinating and directing the psychophysiological and electrophysiological recordings, initiating new research and industry contacts, preparation of publication and presentations.

Dr Damian Frank. BSc (Hons), PhD.

Research Fellow, Sensory Neuroscience Laboratory, Swinburne University of Technology.

Dr. Frank is an Early Career Researcher, having completed his PhD in 2000.

He has been involved in the analysis of the wine flavour components, and preparation of the odour stimuli for the EEG recording and sensory rating sessions.

Research Assistants:

Involved in electrophysiological and psychophysiological recording sessions and data analysis:

- Simon Danckert. BAppSc (Psychology & Psychophysiology)
- Jo Fitzsimons. BAppSc (Psychology & Psychophysiology), BAppSc (Hons)
- Rebecca Smith. BA (Psychology & Psychophysiology), BSc (Hons)
- Andrew Tucker. BAppSc (Psychology & Psychophysiology), BAppSc (Hons).

13. Appendix 5: Preliminary Preparation of an Industry Information CD (Demonstration Copy)

The researchers propose the development of an industry information CD to provide access to the results of this project and to provide background information which will increase the wine community's understanding of the physiology and techniques which are the basis of this research.

Such an information CD would need to be produced in large numbers for circulation to the greater Australian Wine Industry community .

This Appendix provides the background to the demonstration CD which has been incorporated into this report.

The Proposed CD would include the following:

- Final Report for this project:
  Objective sensory and objective evaluation using advanced neurophsyiological techniques for flavour and taint.
  - An Adobe Acrobat format (pdf) version of this report.
- Animations of brain activity responses monitored during this research.
  - Presentations for each study: damascenone, TCA *vs* ethanol, TCA1 *vs* TCA2.
- Tutorial: *Physiology of the Flavour Response*
  - An Adobe Acrobat format (pdf) Powerpoint presentation describing the physiology of the flavour response, particularly associated with the interaction of the smell, taste and trigeminal systems.
  - To provide industry people with a greater understanding of the interactions of the physiology, psychology and psychophysiology of flavour responses.
- *Introduction to GC-O analysis of wine flavours*: providing a visual explanation of the differences between odour responses and GC-MS analysis of wine odours. This file has been prepared as a demonstration of the potential in GC-O GC-MS analysis of wines, and is viewed using an Internet browser.

14. Appendix 6: Budget Reconciliation

GRAPE & WINE RESEARCH & DEVELOPMENT CORPORATION
Statement of Receipts and Expenditure - **FORM B**
For the year ending 30 June 2002
Final Project Budget Reconciliation

Trust Fund :	RESEARCH TRUST FUND	FUNDING	(excludes GST)
Project No :	SUT99/1	Salaries	5, 000
Grantee :	Swinburne University of Technology	Travel	1,500
Title of Project :	Objective sensory evaluation using	Operating	6,840
	advanced neurophysiological techniques for	Capital	3,000
	flavour and taint evaluation in the wine industry	Total Funding	$16,340

EXPENDITURE										
	Salaries		Travel		Operating		Capital		Total	
	$	¢	$	¢	$	¢	$	¢	$	¢
A Uncommitted (c/f 1 July)	NIL		NIL		NIL		NIL		NIL	
B Outstanding Commitments (c/f 1 July)	NIL		1,500		4,180		NIL		5,680	
C Refunds of funding	NIL		NIL		NIL		NIL		NIL	
D Cash Received From Trust Fund	5,000		1,500		6,840		3,000		16,340	
E Approved transfers (from Form C)	NIL		NIL		NIL		NIL		NIL	
F Cash available (A+B-C+D±E)	5,000		3,000		11,020		3,000		22,020	
G Expenditure	5,000		3,000		11,020		3,000		22,020	
H Outstanding Commitments (30 June)	NIL		NIL		NIL		NIL		NIL	
I Total funds Committed (G-H)	5,000		3,000		11,020		3,000		22,020 (excluding GST)	
J Uncommitted (30 June) (F-I)	NIL		NIL		NIL		NIL		NIL	
K Other income (Paid to Trust Funds)	N/A		N/A		N/A		N/A		N/A	

Note : *Row B should be the same as Row H from the previous year and Row A the same as Row J from the previous year.*

I hereby certify that this statement of expenditure is correct.

Signature	Caroline Owen Printed Name	30th September 2002 Date