Introduction

For many years two sensory modalities, sight and smell, when stimulated appropriately, have been shown to have a positive impact on mood and we hypothesised that a combination of light and smell stimuli might have a synergistic effect.

Seasonal Affective Disorder (SAD) is a sub-category of depression or mood disorders, in which people who have normal mental health throughout most of the year experience depressive symptoms at a certain time of the year, most commonly in the late autumn and winter. Bright Light Therapy (BLT) is an established treatment for SAD and mood disorders [1,2], having been successfully used for over 20 years. It has also been shown to be effective in other kinds of non-seasonal depression [3,4] and, in Major Depressive Disorder (MDD) a randomised, placebo controlled trial demonstrated that BLT was comparable to antidepressant medication in effectiveness [5]. In a systematic review and meta-analysis [6] BLT was associated with a small-to-moderate effect in reducing depressive symptoms as compared with placebo and control treatment. BLT is the recommended first-line treatment of the majority of cases of SAD, with improvements in symptoms observed with as little as 20 min of light exposure and a systematic review found BLT to be an excellent candidate for inclusion in the therapeutic inventory available for the treatment of non-seasonal depression [7].

Smell has been shown to have effects on mood, stress, anxiety and depression [8-12]. Certain odours can ameliorate depression and anxiety; for example, work on animals has shown that citrus fragrance can restore stress-induced immunosuppression [13,14] and lemon odour was found to be antidepressant in rats [15]. In normal human subjects, lemon odour reliably enhanced positive mood [16] and negative emotions became less intense during exposure to citrus odour [17]. Citrus fragrance was given to depressive human subjects and the results indicated that the doses of antidepressants necessary for the treatment
of depression could be markedly reduced. The treatment with citrus fragrance normalised neuroendocrine hormone levels and immune function and was rather more effective than antidepressants [15].

In order to test the effect of combining these two sensory stimuli, light and smell, on human psychophysiology, a physiological measure, EEG frontal alpha asymmetry (FA), and a psychological measure, emotional tone, were selected. Alphawaves have been shown to be inversely correlated with brain activity [18] and a reduction in left hemisphere activity is associated with anxiety and depression. The physiological measure, FA, was chosen because it has been used as an objective measure of depression and anxiety; depressed and anxious individuals tend to exhibit greater relative right anterior EEG or negative asymmetry [19-23], FA can predict future development of anxiety and depression [24], and this asymmetry has been shown to be a moderately stable individual difference in adults, irrespective of sex and history of depression [25,26]. A psychological measure, the Profile of Mood States (POMS) self-assessment questionnaire, was chosen because it is a widely used means of determining mood state [27-29]. The factor structure of the POMS, representing six dimensions of mood; anger-hostility (A/H), confusion-bewilderment (C/B), depression-dejection (D/D), fatigue-inertia (F/I), tension-anxiety (T/A) and the associated tables of normative values were derived from psychiatric outpatients and normal college students [30].

The study was designed to test new ways of delivering light and smell stimulation in an integrated manner. In particular, we wanted to develop a method that avoided the adaptation and habituation that occurs with longer term sensory stimulation. With this in mind we used physiological and psychological test parameters to evaluate the effectiveness of our method. Such an integrated sensory stimulation protocol might have beneficial applications in treating mood disturbances.

2 Methods

2.1 Subjects

Participants were students aged 18-28 years old from Cardiff University who had volunteered for the experiment; 24 subjects were used for stage 1 and 64 for stage 2. Each subject completed a consent form and medical questionnaire. Personal data were anonymised after the study. Exclusion criteria included olfactory dysfunction, allergies, epilepsy, respiratory disease, pregnant women, nursing mothers, and those taking prescription medications or exposed to chemicals on a regular basis in the workplace.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Local Research Ethics Committee (School of Biosciences, Cardiff University, UK).

2.2 EEG

EEG electrodes were positioned on the left and right lateral frontal areas - F7 and F8 respectively (International 10-20 System of Electrode Placement) – areas which have been shown to have large left-right alpha differences in relation to depression [31]. These were referenced to linked mastoids (A1 and A2). Another electrode placed in the centre of the forehead, at Fpz, was grounded. The electrodes were connected to a laboratory interface (CED1401, CED, Cambridge) via a preamplifier (CED1902, CED) and the data were stored on a computer for subsequent power spectrum analysis with Spike 2 (CED, Cambridge). In Stage 1, an EEG recording was made for the 90s of the administration of the stimulus and analysed per 10s époque to obtain the alphawave power in μV² per 10s époque. For Stage 2, recordings were made for 2 min prior to the start of the protocol (baseline), for the 15 min of the stimuli administration and for a further 2 minutes following a 5 min break. These recordings were also analysed per 10s époque and the average per 10s époque calculated. Subjects had their eyes open throughout. Ocular and other artefacts were removed by setting filters to exclude large voltage transients and, for the duration of the transient artefact, analysis was stopped.

Frontal alpha asymmetry (FA) was computed, following the methods of Henriques and Davidson [19], by measuring the power in the alpha waveband (8-12Hz), determined by power spectrum analysis (Spike 2; CED, Cambridge, UK) averaged over 10s époques and subtracting the value at the two frontal electrodes (F8-F7). FA can be positive or negative.

2.3 Psychometric testing

The Profile of Mood States (POMS-2) self-assessment questionnaire was administered before and after exposure to the stimuli. The effect of exposure of 64 subjects to
15 min stimulation with light and pleasant odour (lemon) was examined on the 6 psychological mood dimensions: anger-hostility (A/H), confusion-bewilderment (C/B), depression-dejection (D/D), fatigue-inertia (F/I), tension-anxiety (T/A) and vigour-activity (V/A).

2.4 Stimulus protocols

2.4.1 Protocol 1

For Stage 1 of this study the stimulus protocol consisted of short (90s) exposures to light, odour and light plus odour.

2.4.1.1 Light stimulus

The light stimulus was supplied by Seqinetic™ reverse sunglasses (http://www.seqinetic.com/). These supply UV-free light of 2,500 lux from a reflector 5.0 cm from the eye. The odour stimulus (see below) was presented by an odour diffusion helmet which fitted over the head. Odour was delivered to the odour helmet by an airflow generated by an in-line fan (15L/min) passing over an odour reservoir holding saturated vapour. The light and smell stimuli were delivered independently and then in combination, in random order to prevent order effects. The stimuli were delivered at a constant level for 90s in Stage 1 (c.f. Protocol 2 in which the stimuli were varied in a triangular wave). The EEG was recorded (see above) during each of these stimulus periods and for 90s control periods when no stimuli were delivered.

2.4.1.2 Odour stimulus

The odours used in Stage 1 were vanillin (99%, CAS 121-33-5, Sigma-Aldrich) dissolved in dipropylene glycol (5% w/v) and phenylethyl alcohol (CAS 60-12-8, Sigma-Aldrich) diluted in dipropylene glycol (10% v/v).

2.4.2 Protocol 2

For Stage 2 of the study, light and odour stimuli were delivered simultaneously as a triangular, non-sinusoidal wave with a 60s period (Fig. 1), using a specially designed set of goggles as described in Dong and Jacob [32] and the EEG recorded before, during and after the stimulation.

2.4.2.1 Light stimulus

The light source was an equivalent UV-free light stimulus emitting up to 2500 lux when in close proximity (2-4 cm) to the eyes. This light was delivered by white, 5700k LEDs (Radiospares, Corby, Northants, UK), 24 in total, 12 per eye fitted into specially constructed goggles. Total LED power $24 \times 3.2V \times 10mA = 0.77W$. A triangular wave light stimulus was applied with a 60s cycle time. The light was ramped up to a maximum of 2500 lux over 30s and then down to a minimum over 30s (Fig. 1). 2,500 lux from an area of 4.0 cm x 3.0 cm, 5.0 cm from the eyes is equivalent of sitting 80 cm from a rectangular light source of 10,000 lux with the dimensions 40 cm x 60 cm, the suggested conditions for a standard SAD light box.

During the up-ramp of the light, the odour (lemon essential oil) was delivered at two flow rates (see below). The odour stimulus was switched off at the peak of the light stimulus delivery (30s) and no odour was delivered during the down-ramp of the light. Fifteen such cycles were delivered in the course of an experiment.

2.4.2.2 Odour stimulus

Lemon oil (citrus limon (L.) burm. f.), obtained by expression (ISO 855:2003) was used as the odour stimulus in Stage 2. The odour-containing vapour was delivered to the nostrils at two flow rates (0.17 and 0.33 l/s) to coincide with the up ramp of the light stimulus. Fifteen cycles were used in each experiment (15 min total stimulation) in Stage 2.

**Figure 1.** The stimulus protocol. Diffuse full-spectrum white light (maximum 2500 lux) was presented (see 2.4.1.1 Light stimulus) as a triangular wave with a 60s cycle. An airstream containing odourised vapour was delivered to the nostrils at two flow rates (0.17 and 0.33 l/s) to coincide with the up ramp of the light stimulus. Fifteen cycles were used in each experiment (15 min total stimulation) in Stage 2.
2.4.2.3 Visual stimulus control

A control experiment was conducted in which the subjects were given a visual task while wearing ear protectors - to remove auditory stimuli - and sitting comfortably in the same chair as that used for the light-smell stimulus experiments. The visual task was to observe 60 neutral images presented on a computer screen for 15s and answer a few simple questions at the end of the 15 min session. A Profile of Mood States self-questionnaire was administered before and after the task.

2.5 Data analysis and statistics

A repeated measures ANOVA test was used to analyse changes in EEG frontal asymmetry (FA) in response to different stimuli (Stage 1) or before-during-after stimulation (Stage 2) with sign of FA as a between subjects factor. A paired t-test was used to compare scores of the POMS test before and after light-smell stimulation.

All statistical analyses were performed using SPSS20 (IBM SPSS Inc.) with α (significance level) set at $p < 0.05$. Where sphericity assumptions were violated, Greenhouse-Geisser corrections were applied.

3 Results

3.1 Stage 1

During the first part of this study the 4 treatments, light, smell, combined light+smell and no stimulus (control), were presented in random order. The stimuli were delivered at a constant level for 90s according to stimulus protocol 1 in Methods. EEG was recorded at left (F7) and right (F8) frontal positions and the frontal asymmetry (FA) was determined by subtracting the alphawave power at F7 from that at F8. Since we are not concerned with the differences between vanillin and PEA in this study, the data were combined to represent the response to “pleasant” odour and to increase the power of the analysis.

The sign of the FA was a statistically significant factor that varied between subjects, F(1,22) = 25.075, $p<0.0001$, $\eta^2_p = 0.533$ (power=0.998). Following the method of Davidson [34,21] and Sutton and Davidson [35] the asymmetry data were analysed by group, negative or positive, for the rest of the study. There were 9 subjects in the positive FA group and 15 subjects in the negative FA group. Thus, although these were normal, healthy subjects, they divided into two groups; positive and negative FA. Those exhibiting negative FA have a trait marker for depression/anxiety.

The subjects were exposed to four conditions delivered for 90s in random order; control, light, smell and light+smell (Fig. 2). There was a significant interaction between stimulus and frontal asymmetry (FA); F(3,66)=2.672, $p=0.05$, $\eta^2_p = 0.108$ (power=0.626) and both smell and light+smell caused a significant reduction in negative asymmetry ($p=0.027$ and 0.021 respectively in pairwise comparisons; see Fig. 2). In 9 out of 15 cases, light alone reduced the negative asymmetry from $-1.15\pm0.24 \mu V^2$ to $-0.88\pm0.31 \mu V^2$. However, the result did not achieve statistical significance.

3.2 Stage 2

In Stage 2 of this study, a new cohort of 64 subjects was given a combined (light+smell), cyclical stimulus protocol (see Fig.1) for 15min. This protocol reduces adaptation/habituation and is referred to as the nonadaptive stimulus. The EEG recordings were made from F7 and F8 and the alphawave power computed as described in Methods. The frontal asymmetry (FA) was determined (F8-F7) and the subjects were divided into two groups based on the sign of their asymmetry as described previously.

When subjects were divided into groups with positive and negative FA, the effect of the light-smell treatment was more pronounced on the negative FA group (blue bars, Fig.3). In other words, those with a greater negative affect (trait marker for depression/anxiety) exhibited greater changes in FA, in the direction of positive affect.

There was a significant effect of the light-smell treatment for the negative FA group (blue bars, Fig.3); within subjects repeated measures ANOVA indicated a significant effect of time F(1.53, 47 .4)=4.67 , $p=0.022$; $\eta^2_p=0.13$ (power=0.68) on FA and paired comparisons (adjusted using the Bonferroni correction for multiple comparisons) demonstrated a significant difference between before-after ($p<0.05$). There were no significant differences for the effect of light+smell on subjects with a positive FA (red bars, Fig.3). A between subjects test with FA sign as the between subjects factor demonstrated that there was a significant difference between the negative and positive FA groups; F(1,62)=30.45, $p<0.0001$; $\eta^2_p = 0.33$ (power=1.0) and there was an interaction between time (before-during-after) and sign; F(2,124)=5.61, $p=0.009$; $\eta^2_p = 0.083$ (power=0.78).

A negative asymmetry, the difference between alphawave activity in the right and left frontal hemispheres, is generally an indication of negative affect and has been suggested to be a trait marker for depression [36].

The power in frontal alphawave activity is shown for recordings from F7 and F8 separately in figure 4 for...
Figure 2. EEG alpha wave frontal asymmetry (FA). The effect of exposure to light, pleasant smell and light+smell (L + S) on frontal alpha wave asymmetry (FA) compared to control. The subjects were divided into positive (n=15) and negative (n=9) FA on the basis of their alpha wave asymmetry (F8-F7). Frontal alpha wave asymmetry ($\mu$V$^2$) is expressed as the average ± standard error (bars) per 10s époque for the 90s stimulation. *p<0.05 relative to control.

Figure 3. EEG alpha wave frontal asymmetry (FA). The effect of 15 min exposure to light and lemon odour on alpha wave asymmetry. The subjects were divided into positive (n=32) and negative FA (n=32) on the basis of their alpha wave asymmetry (F8-F7). Alpha wave power is expressed as the average ± standard error (bars) per 10s époque for 2 min before, during and after stimulation. *p<0.05.
3.3 Psychometric testing

The POMS test was given to the participants (n=64) before and after sensory stimulation. It is a 65 item test and takes 3-7 min to complete. The test asks “how do you feel right now” and can therefore be used as an indication of present state. The answers are compiled and contribute to six mood dimensions. The 15 min nonadaptive light-smell treatment induced a significant decrease in the scores for anger-hostility (A/H), confusion-bewilderment (C/B), depression-dejection (D/D), and vigour-activity (V/A) (Fig.5). When analysed by FA group, either negative or positive, both groups showed a reduction in most mood dimensions, but it was the negative FA group that demonstrated the most significant effects (Fig.6 and Table 2).

3.3.1 Control

A control experiment was conducted in which subjects were given a visual task while wearing ear protectors - to remove auditory stimuli - and sitting comfortably in the same chair as that used for the light-smell stimulus experiments. POMS questionnaire was completed before and after the task. There was no before-after difference in any of the POMS factors; Total Mood Disturbance difference = 0.79 ± 0.82 (mean ± SE; n=14, p=0.355).

![Figure 4. EEG alpha power in response to light+smell stimulus (15 min) in the right (red line; F8) and left (blue line; F7) frontal hemispheres for; (a) positive FA group and, (b) negative FA group. EEG alphawave power expressed in μV² per 10s época.](image)

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Two way 2(hemisphere) x 3(time), repeated measures ANOVA of data given in figure 4.
Combined Light and Smell Stimulation

Figure 5. Effect of light-smell stimuli on POMS scores. The exposure of subjects (n=64) to 15 min stimulation with light and pleasant smell (lemon) had a significant effect on, anger-hostility (A/H), confusion-bewilderment (C/B), depression-dejection (D/D) and vigour-activity (V/A). *p<0.05; **p<0.01. There was no effect on fatigue-inertia (F/I) or tension-anxiety (T/A).

4 Discussion

Light and smell stimuli have both been used independently in human studies to achieve positive psychophysiological benefit. For example, light and smell have been demonstrated to affect mood and alleviate depression (for reviews see [9,37]) and in this study we set out to develop an integrated stimulation protocol that combined both light and smell in order to try to obtain increased benefit from any synergistic effects that might occur.

Bright light therapy uses a constant light intensity presenting an unchanging stimulus to the retina and visual cortex. When a constant odour stimulus is presented to the olfactory system both physiological and psychological adaptation/habituation occur. Any prolonged, constant sensory stimulus is, after a certain period of time, ignored and therefore no longer perceived [38]. During the course of this study we developed a nonadaptive protocol for the delivery of the stimuli that was specifically designed to avoid the issues of adaptation/habituation. The loss of perception of a sensory stimulus is a central process and is referred to as habituation. Peripheral reductions in response are also observed under circumstances of continuous stimulation, although not always so marked, and these are referred to as adaptation [38]. Sensory systems are much better at detecting changes in stimulus input [39] and we therefore designed a stimulus protocol that changed with a non-sinusoidal, triangular wave pattern (Fig.1). We chose a cycle frequency of 60s on the basis of the timescale of olfactory adaptation/habituation observed in Jacob et al. [33].

To study the effects of our integrated sensory stimulation we chose a physiological test parameter, frontal alphawave asymmetry, and a psychometric paradigm – the POMS test. There is substantial support for the suggestion that resting frontal EEG alphawave activity acts as a trait marker for depression (for review, see [36]). Our participants demonstrated a range of positive and negative values for FA. In the first stage of the study we showed that the pleasant smell stimulus and the combined light+smell stimulus caused a significant reduction in frontal asymmetry in the negative FA group (Fig.2). This would be in the direction of positive affect, in other words towards positive FA - the normal, non-depressed, non-anxious state. Although the combined light+smell stimulus produced a greater effect than light and smell stimuli individually, which would suggest a synergistic effect, this did not reach statistical significance. However, it is worth mentioning that a power calculation suggested a sample size of 2,269 subjects would be necessary to obtain 80% likelihood of detecting a significant difference. The effect of the light-only stimulus, while small and not significantly different from control, reduced the negative asymmetry in 9 out of 15 cases. A previous study by Dong and Jacob [32] differentiated the effects of light and three different smell stimuli and used blood pressure, heart rate to monitor physiological response and the POMS test to measure psychological effect. The authors showed that the smell component of the dual stimulus had the greater impact on blood pressure and heart rate. Light treatment on its own had no effect on blood pressure but did significantly reduce heart rate. However, neither stimulus modality on its own had much effect on the POMS mood factors with the exception that Dejection-Depression was reduced only by exposure to lemon odour treatment [32].

The Stage 1 protocol utilized a 90s stimulus, the limit
Figure 6. Effect of light+smell stimulus on POMS. The subjects have been divided into negative (n=32) and positive (n=32) frontal asymmetry (FA) and the scores for each group are presented for each POMS characteristic. Statistical significance (paired t-test) is given in Table 2.

Table 2. Mean POMS scores

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Mean POMS scores and significance (p) of the effect of 15 min light-smell treatment on the two subject groups, negative and positive frontal alphawave asymmetry, (-)FA (n=32) and (+)FA (n=32).
for adaptation/habituation in the olfactory system [33]. However, we wanted to investigate the effects of long-term stimulus and therefore, for Stage 2, we devised a cyclical stimulus delivery programme (triangular wave) with a periodicity of 60s (see Fig.1). This allowed us to give much longer stimuli without adaptation/habituation. Recovery from adaptation/habituation occurs during the 30s gap between stimulus phases. Using this longer, nonadaptive stimulus protocol we found that frontal asymmetry of the negative FA group significantly decreased (Fig.3) and that this was sustained at least for the duration of the experiment. In future trials it would be interesting to determine how long this effect lasted. There was no significant effect on the positive FA group (red bars, Fig.3). In the negative FA group the significant difference of the light-smell treatment resulted from an increase in alpha power in the right hemisphere (F8, red line Fig.4b) and a decrease in alpha power in the left hemisphere (F7, blue line Fig.4b). The increase in alphawave activity in the right hemisphere for the negative FA group would correlate with a decrease in negative affect (withdrawal behaviour) and the decrease in alphawave activity in the left hemisphere would indicate an increase in positive affect (approach behaviour). These results suggest that the change in alphawave activity in the direction of positive affect was particularly pronounced in those people who are either susceptible to, or suffering from, depression and/or anxiety.

In this study we have used EEG frontal alpha asymmetry (FA) as our marker for physiological state. EEG has been used to investigate positive/negative mood states, depression and anxiety. There is evidence of greater right frontal hemisphere activation associated with a negative affect and aversive behaviour, whereas greater left activation is associated with a tendency for approach behaviour and positive affect [34,35,40-43]. Using this as an individual differences measure, frontal EEG asymmetry has been shown to predict depression [19,31, 44], ability to regulate emotion [45], general well-being [46] and the stress response [42]. In addition to being associated with trait differences, asymmetries have been noted following manipulations of positive or negative feedback [47]. The frontal alpha asymmetry findings of this study correlated with the results from the POMS test which showed that light+smell stimulation reduced total mood disturbance and had a positive impact on the mood dimensions anger-hostility (A/H), confusion-bewilderment (C/B), depression-dejection (D/D), and vigour-activity (V/A) in the whole cohort of 64 subjects and, when the cohort was divided according to sign of FA, the negative FA group had significant reductions in all of the above four mood dimensions and, in addition, tension-anxiety (T/A). The light-smell stimulation had a greater impact on those in the negative FA group although it is clear from figure 4 that both groups were affected. Interestingly, for the anger-hostility (A/H), depression-dejection (D/D) and tension-anxiety (T/A), the positive FA group scored lower before treatment and changed less following treatment (Fig.6).

In a control study involving a visual task in which the participants sat in the same chair as for the light+smell stimulus experiment there was no significant change in any of the POMS mood dimensions.

Depression and anxiety represent serious challenges to individuals and healthcare systems and the burden is predicted to get worse. The ability to rebalance negative frontal alpha asymmetry could have implications for the treatment of these and related disorders, for example in obsessive-compulsive disorder (OCD) which exhibits trait-like altered frontal EEG asymmetry [48] and post-traumatic stress disorder, a disorder comprising anxiety and dysphoria symptoms, which similarly exhibits frontal alpha asymmetry [49].

It has been estimated that Common Mental Disorders affect 1 in 6 British adults every week with over half of these having a mixed anxiety and depressive disorder [50]. In the USA, in any given one-year period, 13 million to 14 million people (which equates to approximately 6.6% if the US population) experience depression [51]; globally, more than 350 million people of all ages suffer from the illness [52] and the annual incidence in UK is 36 per 1000 [53]. This contributes to substantial financial burden on healthcare systems, with the total cost of services for depression in England in 2007 estimated to be £1.7 billion, while lost employment as a consequence of suffering depression increased this total to £7.5 billion. By 2026, these figures are projected to be £3 billion and £12.2 billion, respectively [54]. In view of this it is important that new ways are developed to combat this growing problem.

This study has demonstrated that a new integrated method of sensory stimulation involving light and smell produces significant positive physiological and psychological benefits. It raises the question of whether such a stimulus protocol could exert longer-term positive effects and therefore might form the basis of a treatment for anxiety and depression.

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Conflict of interest: Authors state no conflict of interest.
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