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Brain waves spectral analysis of human responses to odorous and non-odorous substances: a preliminary study

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Main Article

Salina Husain takes responsibility for the integrity of the content of the paper

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Abstract

Objective. The aim of this study was to identify the potential electrophysiological biomarkers of human responses by comparing the electroencephalogram brain wave changes towards lavender versus normal saline in a healthy human population.

Method. This study included a total of 44 participants without subjective olfactory disturbances. Lavender and normal saline were used as the olfactory stimulant and control. Electroencephalogram was recorded and power spectra were analysed by the spectral analysis for each alpha, beta, delta, theta and gamma bandwidth frequency upon exposure to lavender and normal saline independently.

Results. The oscillatory brain activities in response to the olfactory stimulant indicated that the lavender smell decreased the beta activity in the left frontal (F7 electrode) and central region (C3 electrode) with a reduction in the gamma activity in the right parietal region (P4 electrode) ($p < 0.05$).

Conclusion. Olfactory stimulants result in changes of electrical brain activities in different brain regions, as evidenced by the topographical brain map and spectra analysis of each brain wave.

Introduction

The sense of smell is one of the primary senses of humans and probably the least understood. It serves a variety of purposes in our daily life. Santos *et al*. reported that 45 per cent of hazardous cooking accidents, 25 per cent of spoiled food ingestion, 23 per cent of cases of inability to detect a gas leak and 7 per cent of cases with failure to smell a fire have been attributed to olfactory impairment.[1]

The prevalence of smell impairment ranges from 12.3 to 19 per cent.[2–4] Various olfactory assessments worldwide are classified into psychophysical and electrophysiological tests. The University of Pennsylvania Smell Identification Test and Sniffin' Sticks smell test are psychophysical olfactory tests that are commonly administered. These subjective olfactory assessments are relatively low cost with a short examination time and require patients' compliance with the tests. Therefore, it can be controversial in the medico-legal context.[5,6] On the other hand, electrophysiological tests are objective olfactory evaluations that are independent of the patient's compliance and have high reliability. However, these tests usually consist of complicated system configurations.[5]

So far, several advanced techniques have been used in determining the relationship between brain activities and olfaction, which include functional magnetic resonance imaging, contingent negative variation, near-infrared spectroscopy and electroencephalography. An electroencephalogram (EEG) is an excellent objective tool for analysing the central nervous system, particularly cerebral cortex activity. To date, a number of studies have focused on the effects of EEG brain wave activities upon olfactory stimulation. Electroencephalogram is portable, affordable, non-invasive and easily accessible in most hospitals and has been widely applied in the context of epilepsy, dementia, psychoses and cerebral mass lesions, among others.[7] Measuring the electrical brain activity changes with EEG upon exposure to different olfactory stimuli is one of the objective olfactory assessments that has caught many researchers' attention over the years. However, discrepant findings have been observed in various studies over the years.

For example, a study by Klemm *et al.* (1992; $n = 16$) showed an increased theta activity in the left anterior region of the brain upon exposure to birch tar, jasmine, lavender and lemon.[8] Skorić *et al.* (2015; $n = 16$) found a reduction in the theta activity in the central region of the brain upon exposure to lemon and peppermint.[9] Sayorwan *et al.* (2012; $n = 20$) reported an increase in theta and alpha wave activity upon lavender inhalation.[10] Moreover, Aydemir (2017; $n = 5$) investigated the classification accuracy of EEG bandwidths and found that gamma activity is strongly associated with olfaction upon exposure to odour stimuli (valerian, lotus flower, cheese and rosewater), indicating the highest performance and classification accuracy as confirmed via continuous wavelet transform analysis.[11]

The aim of this study was to assess the effects of olfactory stimulants on electrical brain activity on a large scale using a healthy human population, deriving data from EEG with the objective to identify the potential electrophysiological biomarkers of human responses to odorous and non-odorous substances.

Materials and methods

This study included a total of 44 healthy volunteers (15 males and 29 females, aged 21 to 53 years) with a mean age of 33 years. All participants presented no known allergy or eczema history, history of nasal surgery, chronic rhinosinusitis, allergic rhinitis or neuropsychiatric or neurodegenerative disorder.

Ethical approval for this study was obtained from the Research Ethics Committee of the Universiti Kebangsaan Malaysia Medical Center (code number: JEP-2019-740). The study was conducted in accordance with the Declaration of Helsinki for research on human patients. Informed consent was obtained from each participant before the study.

Cultural adaptation of the Sniffin' Stick smell test

Cultural background tends to affect olfactory performance.[12] Hence, consenting participants were screened for smell impairment with a validated culturally adapted Malaysian version of the Sniffin' Sticks smell identification test with 16 types of odours.[12,13] The tip of each pen with its own odour was placed approximately 2 cm in front of the nostrils for approximately 3 seconds. The participants thereby identified the odorant from a list of four items (including one correct answer and three distractors). A score of 11 out of 16 indicated that the participants had passed the screening test.[14]

Stimuli

In this study, one olfactory stimulant (odorous) and one control (non-odorous) of lavender essential oil and normal saline were used. The lavender essential oil was used to represent a fresh and soothing odour; it is also cost effective, widely accepted by the general population and readily available in the market. Moreover, the concentration of the lavender solution can be easily standardised. By using the lavender essential oil, the results can be easily compared with the other studies as most of the previous studies utilised lavender as one of the olfactory stimulants. Instead of using various olfactory stimulants, we used only the lavender essential oil (odour) to compare it with normal saline (non-odour) to avoid odour-mixture perception or masking one smell with another, which may lead to inaccuracy in the findings.[15] Normal saline, a neutral non-odorous substance, was used as the control. The lavender smell was prepared by diluting one drop of lavender essential oil into 7 cc of normal saline, and a non-odorous substance was prepared with 8 cc of normal saline. Each of the diluted lavender solution and pure normal saline solution was delivered to the participant via independent tubing and a facemask, which was placed 5 cm in front of the nose of the participant through a separate Philips Respironics InnoSpire Essence Nebulizer Compressor System (Princeton, USA) to avoid olfactory contamination. All participants subjectively rated the standardised diluted lavender as soothing and non-pungent before starting the experiment to minimise the possibility of trigeminal stimulation. Olfactory contamination is least anticipated in our study as the experiment was performed in a controlled laboratory with a constant temperature of 24°C. No food or drinks were permitted in the laboratory. Furthermore, all the experiments were performed by a single researcher.

Electroencephalogram recording

The EEG was used to determine the brain's functional response to the olfactory stimulant. The spectrum was used as a specific feature of the brain activity.[16] It decomposed the total energy in the EEG into each frequency band. Thus, it could be used to identify the most dominant oscillations present in the signal.[17]

The participants were then placed in a sound and light-insulated laboratory with its own ventilation system, and the head of the bed was placed at a 45° angle. They were instructed to relax and minimise blinking in order to reduce internal artefacts.[9] Subsequently, they were instructed to keep their eyes open and then closed for a few cycles for calibration before recording. Moreover, they were presented with normal saline (non-odorous substance) and lavender essential oil (odorous) via two separate nebulisers, each for 2 minutes in eye-open and eye-closed conditions for 3 cycles.

The EEG machine used in our centre was the Natus Nicolet One EEG V32 (Natus Medical, Middleton, USA) system with a sampling rate of 2000 Hz and filter setting of Hi Cut 70 Hz Lo Cut 0.5 Hz. Upon delivering the olfactory stimulants, the electrical brain wave activity was recorded with an EEG cap with 19 active electrodes positioned in accordance with the international 10–20 system (Figure 1). Active electrodes, based on silver or silver chloride sensors with integrated circuits were used for noise reduction. Areas under each electrode were cleaned with an abrasive paste to reduce the impedance. Impedance was set below 10 k ohms in this study.

Fast Fourier transform analysis

The spectra were estimated via periodogram analysis using MATLAB 2020a. Fast Fourier transform analysis was performed on each set of EEG data (normal saline and lavender, each in eye-open and eye-closed condition). Moreover, the band-limited power spectra were computed based on the periodogram (squared magnitude of fast Fourier transform of EEG) for five main EEG frequency bands: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz) and gamma (30–100 Hz). The EEG was recorded at 2000 Hz, and their auto-spectra were estimated at each of the classical bandwidths. The band power spectra were then averaged across all participants.

Statistical methods

A paired *t*-test statistical analysis was performed to identify the significant difference in the EEG mean band power differences

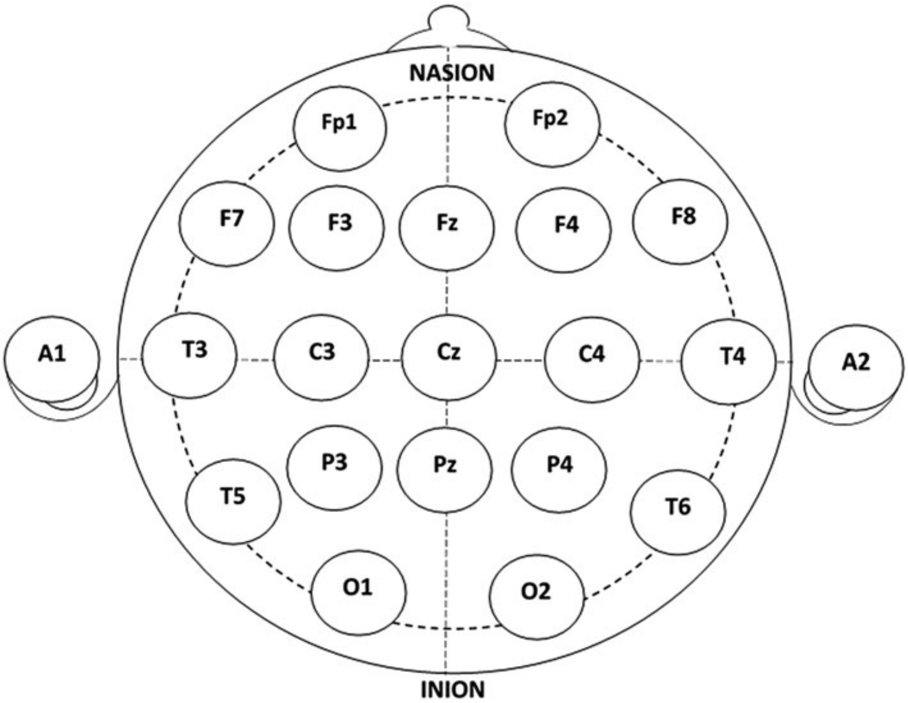

Figure 1. Diagram showing the 10-20 system for placement of electroencephalogram electrodes.

between the eyes-open and eyes-closed conditions when perceiving lavender and normal saline odour independently, with a significant value represented by $p < 0.05$.

Results

The EEG data of 44 participants was reviewed. The mean age ($n = 44$) was 33.07 (7.64) with a male to female ratio of 1:1.93. All participants passed the strict smell identification test. The frequency analysis of EEG was performed by computing the mean band power for five main frequency bands using the MATLAB 2020a software with a customised source code.

The EEGlab toolbox in MATLAB was used to plot the EEG topographical maps. The EEG topographical map was used to visualise brain activation as measured by the EEG spectral power in the five frequency bands. Each electrode was analysed separately for the alpha, beta, delta, theta and gamma wavebands. In the eyes-closed condition, the lavender essential oil

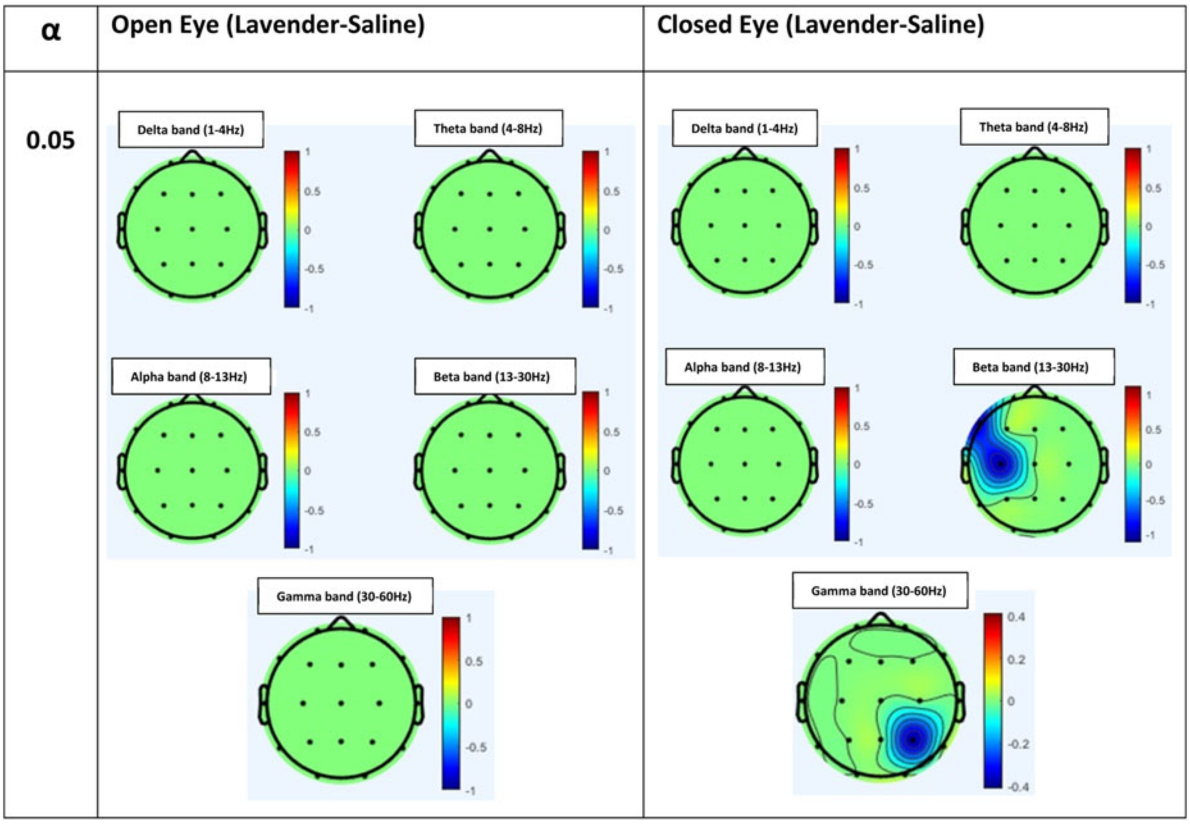

Figure 2. Topographical brain map in eyes open and closed conditions.

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Table 1. Mean band power differences of electroencephalogram spectra between normal saline and lavender stimulant

	Delta		Theta		Alpha		Beta		Gamma	
Channel	*P*-value	Difference in μ	*P*-value	Difference in μ	*P*-value	Difference in μ	*P*-value	Difference in μ	*P*-value	Difference in μ
Fp1	0.2087	−15.6185	0.3275	−1.7980	0.0814	−2.9834	0.5370	−0.8210	0.5917	−0.5202
Fp2	0.2452	−11.1866	0.2752	−1.6484	0.0888	−2.8683	0.6816	−0.4628	0.9829	0.0154
F3	0.9631	0.2092	0.4044	−0.7652	0.1013	−2.6868	0.1923	−0.8827	0.4506	−0.8490
F4	0.6490	−1.1243	0.5946	−0.3863	0.0884	−2.8669	0.1704	−0.8221	0.2240	−0.4635
C3	0.4839	−1.7280	0.8356	−0.1647	0.1525	−1.4697	0.0442*	−1.0640	0.0975	−2.4713
C4	0.6339	−1.1459	0.7965	−0.2289	0.2085	−1.4007	0.0697	−1.0433	0.0539	−0.7675
P3	0.4815	−1.5722	0.7608	−0.2502	0.2272	−3.1381	0.2228	−0.8282	0.6207	−0.2495
P4	0.4609	−1.7274	0.6575	−0.3856	0.1286	−3.7940	0.1345	−0.9170	0.0342*	−0.3936
O1	0.8416	−0.6079	0.8396	−0.3038	0.1564	−13.6939	0.1868	−1.4149	0.3926	−0.2463
O2	0.7097	−1.0458	0.8410	−0.3370	0.2101	−16.2090	0.1307	−1.6403	0.8348	−0.0845
F7	0.7156	−1.1386	0.5286	−0.4680	0.0816	−2.1908	0.0428*	−0.9528	0.6371	−0.3551
F8	0.9048	−0.4247	0.4398	−0.5450	0.1089	−1.9930	0.0801	−0.8685	0.1176	−0.7868
T3	0.4023	−1.8015	0.6319	−0.3358	0.3062	−0.7967	0.2448	−1.8877	0.3122	−1.3033
T4	0.1546	−5.6809	0.3748	−0.6132	0.1906	−0.9620	0.1393	−2.2040	0.1681	−2.0778
T5	0.2340	−3.1685	0.1863	−1.2742	0.0945	−6.6968	0.0543	−1.2353	0.2738	−1.0931
T6	0.3651	−2.1693	0.2423	−1.1766	0.2024	−8.4176	0.0718	−1.1764	0.3171	−0.2806
Fz	0.7958	−0.8144	0.5043	−0.5317	0.1252	−3.2740	0.2419	−0.4583	0.3281	−0.1592
Cz	0.5437	2.5908	0.5515	0.9263	0.2046	−2.4100	0.4126	−0.4094	0.4902	0.4690
Pz	0.8246	0.6734	0.6701	0.5648	0.1935	−3.0889	0.3283	−0.5520	0.3825	−2.4831

*Significant p-value, less than 0.05. For all participants: closed eyes ($\alpha = 0.05$)

resulted in a reduction in the beta wave activity in the left frontal region (F7 electrode) and central region (C3 electrode) as well as a reduction in the gamma activity in the right parietal region (P4 electrode) when compared with normal saline in the topographical brain map (Figure 2). A paired *t*-test showed a significant reduction in beta wave activity at the C3 and F7 electrodes and gamma wave activity at the P4 electrodes ($p < 0.05$) (Table 1). Among the statistically significant band powers of the EEG channels (beta-C3, beta-F7, gamma-P4) in the eyes-closed condition, 31 participants showed a reduction in beta wave activity at C3 (central region), while 32 participants exhibited a reduction in beta wave activity at F7 (left frontal region) and gamma wave activity at P4 (right parietal region). Figure 3 shows the percentage of the participants indicating a statistically significant decrease in the mean band power differences of EEG channels (beta-C3, beta-F7, gamma-P4) between normal saline and lavender in the eyes-closed condition. No significant difference was observed in the EEG mean band power spectra between lavender and normal saline in the eyes-open condition (Table 2).

The participants rated the pleasantness of the normal saline and lavender essential oil using a 5-point Likert scale, in which 5 represents the most pleasant smell, while 1 represents the least pleasant smell. Statistical significance was set to $p = 0.03$ for the paired *t*-test of the subjective pleasantness comparing normal saline and lavender essential oil (Table 3), wherein lavender was rated as the pleasant smell generally.

Discussion

Knowledge on the sense of smell has improved as recent research continues to obtain breakthrough discoveries and determine its relation with the human brain activity. Olfaction has long been discovered to be affected in certain diseases, such as Parkinson's disease,[18] Alzheimer's disease[19] and coronavirus disease 2019 infection.[20] This has laid the foundations for our current research study. However, it is imperative to conduct further research on the sense of smell.

Electroencephalograms are generally analysed based on the frequency, amplitude and shape displayed, which include delta, theta, alpha, beta and gamma waves.[21] Delta waves (0.5–4 Hz) are a slow brain wave generally seen in the deep sleep state.[22] Theta waves (4–8 Hz) are seen in a deeply relaxed state, whereas the alpha wave (8–13 Hz) is seen in wakeful relaxation.[21] Alpha waves are accentuated upon eyes closing and attenuated upon eyes opening.[23] Beta waves (13–30 Hz) are related to consciousness and occupying brain activities. In comparison, gamma waves (30–100 Hz) are fast oscillations with low amplitude observed during brain activities, cognitive functioning and meditation states.[24] In general, EEG brain waves often consist of useful information about the state of our brain.

Klemm *et al.* (1992) studied 16 participants and concluded that theta waves have a special significance towards olfactory stimulants in the left anterior region of the brain.[8] The study by Lorig *et al.* (1988) in nine adults reported a reduction in the theta wave activities in the left posterior region of the brain upon exposure to various olfactory stimulatants.[25] Martin (1998) conducted a study on 21 participants who passed a simple 4-odour screening test and found that a chocolate and rotting meat smell were associated with a significant reduction in theta activity in the central and left temporal regions of the brain.[26] Krbot *et al.* (2014) showed reduced theta activity in the brain's central region upon exposure to lemon and peppermint odours.[9] On the other hand, Sayorwan *et al.* (2012) reported that theta and alpha waves were significantly increased upon lavender inhalation in the central and right posterior regions of the brain in 20 participants.[10] Diego *et al.* assigned 40 participants randomly into the rosemary and lavender group for 3 minutes. However,

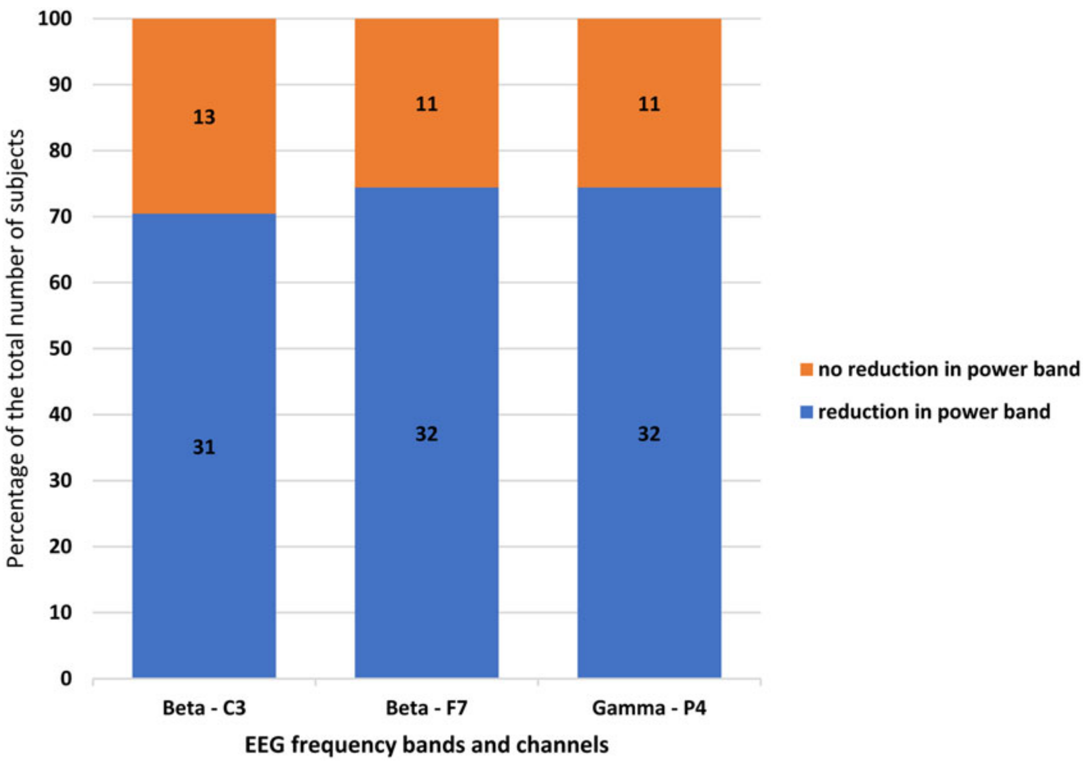

Figure 3. Percentage and number of participants exhibiting reduction of the statistically significant mean band power differences of electroencephalogram channels (beta-C3, beta-F7, gamma-P4) between normal saline and lavender during the closed eyes condition. EEG = electroencephalogram

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Table 2. Mean band power differences of electroencephalogram spectra between normal saline and lavender stimulant

	Delta		Theta		Alpha		Beta		Gamma	
Channel	*P*-value	Difference in μ	*P*-value	Difference in μ	*P*-value	Difference in μ	*P*-value	Difference in μ	*P*-value	Difference in μ
Fp1	0.6502	−78.8669	0.4416	−17.7764	0.4656	−1.1434	0.9698	−0.0769	0.8861	0.2950
Fp2	0.9435	13.5652	0.9580	0.9022	0.8625	0.3231	0.7346	0.8487	0.7097	1.0121
F3	0.6148	3.2226	0.8265	0.1526	0.9136	0.0854	0.5219	0.6841	0.3414	1.0945
F4	0.6437	2.8384	0.8466	−0.1212	0.9105	0.0918	0.6632	0.6596	0.6831	0.6661
C3	0.3341	−5.8188	0.3186	−0.6624	0.7807	−0.1502	0.3529	0.8045	0.0796	1.8339
C4	0.5648	−2.5572	0.4788	−0.3185	0.9272	−0.0490	0.5192	0.3533	0.6782	0.3644
P3	0.5655	−3.8689	0.3300	−0.6651	0.6472	−0.4363	0.5309	0.3367	0.1663	0.4485
P4	0.8506	−1.1725	0.5871	−0.3612	0.7410	−0.3138	0.6427	0.2246	0.5526	0.1533
O1	0.8907	−1.1234	0.3536	−0.9529	0.8403	0.8321	0.6906	0.2681	0.9765	−0.0118
O2	0.6957	−3.6634	0.4809	−0.8187	0.9298	0.4531	0.9125	−0.0862	0.8763	−0.0961
F7	0.3547	21.9126	0.7918	−0.4806	0.7145	0.2144	0.2388	0.9668	0.1762	1.6306
F8	0.3736	−58.4016	0.3250	−4.6315	0.7454	0.1814	0.1780	0.7627	0.3375	0.8079
T3	0.8769	−1.1305	0.7289	0.2954	0.6336	0.2541	0.3375	2.6826	0.2130	3.6300
T4	0.9339	0.5094	0.8782	−0.0971	0.7253	0.1567	0.4781	1.5882	0.5112	1.6381
T5	0.3206	−21.3482	0.3663	−1.0782	0.8160	0.3823	0.8691	0.1151	0.5680	0.4553
T6	0.9577	−0.4020	0.7521	−0.2814	0.8177	0.9439	0.5308	0.3357	0.2008	0.4930
Fz	0.9717	−0.1911	0.8470	−0.1132	0.8350	0.2110	0.5135	0.2200	0.5169	0.1662
Cz	0.3637	−3.9730	0.2873	−0.5094	0.8886	0.1202	0.6222	0.1827	0.4356	0.1592
Pz	0.7421	−1.9909	0.5101	−0.4087	0.6680	−0.3264	0.7105	0.1389	0.4948	0.0954

For all participants: open eyes ($\alpha = 0.05$)

Table 3. The pleasantness of smell between lavender and normal saline

Pleasantness	Mean	Standard deviation	95% of confidence interval of the difference		P-value (2 tailed)
			Lower	Upper	
Normal saline	3.36	0.838			
Lavender	3.73	0.817	−0.030	−0.758	0.035

Table 4. Review of studies of electroencephalogram brainwave changes in response to different olfactory stimulants

Year	Author	Sample size (*n*)	Odour delivery	Duration (minutes)	Material	Electrodes (*n*)	Electroencephalogram wave changes
1988	Lorig & Schwartz[25]	9	Present with vials	1	Spiced apple, eucalyptus, lavender, distilled water	4	Reduction in theta activity in spiced apple
1993	Van Toller *et al.*[34]	15	Smelling strip	–	5-alpha-androstan-3-one, bangalol, white sapphire, linalyl acetate, indole & a mixture of eucalyptus & ammonia	28	Increase in alpha activity
1992	Klemm & Warrenburg[8]	16	Olfactometer	2	Birch tar, jasmine, lavender & lemon	19	Increase in theta activity in birch tar, jasmine, lavender & lemon
1998	Martin[26]	21	Perfume paper strip	–	Synthetic odour: chocolate, spearmint. Real odours: chocolate & rotting meat	19	Reduction in theta activity in chocolate & spearmint
1998	Diego *et al.*[27]	40	Cotton dental swab		Lavender fragrance. Rosemary	4	Lavender: increase in beta activity. Rosemary: reduction in frontal beta and alpha activity
2012	Sayorwan *et al.*[10]	20	Oxygen pump system	–	Lavender. Sweet almond oil	31	Lavender: increase in theta & alpha activity
1995	Brauchli *et al.*[35]	5	Olfactometer	–	Phenylethyl alcohol: rosy smell. Valeric acid	17	Valeric acid: increased alpha2 power in frontal & parietal location
2000	Kline *et al.*[29]	58	Diluted in bottle & sniff in	–	Vanilla (pleasant), valerian (unpleasant), water (neutral)	19	Vanilla: reduction in alpha activity in left frontal indicated left frontal activation towards pleasant smell
2016	Sowndhararajan *et al.*[30]	20	Filter paper	30 seconds	I. helenium	8	Reduction of theta in all regions except left temporal. Reduction of beta activity
2016	Lee[36]	25: experiment. 25: control	Nebuliser	–	Control: 1 drops lavender oil in 20 cc water. Experiment: 1 drop lavender + 1 drop bergamot oil in 20 cc water	8	Theta increase in right prefrontal region in the experimental group. Relatively fast alpha: increase in experimental group but decrease in control group. Relatively slow alpha: increase in experimental compared with control group
2014	Krbot Skorić *et al.*[9]	16	Perfume paper strip	–	Lemon, peppermint, vanilla,	31	Reduction in theta activity in central region
2017	Aydemir[11]	5	–	–	valerian, cheese, rosewater, lotus		Gamma activity
2023	Chow *et al.* (this paper)	44	Nebuliser	2	Lavender, normal saline	19	Reduction of beta activity in the left frontal & central region). Reduction of gamma activity in the right parietal region

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without a prior smell screening test, they observed an increase in frontal beta activity upon exposure to the lavender, while there was a decrease in frontal alpha and beta activity upon exposure to rosemary. This could suggest an increase in drowsiness upon exposure to lavender, while an increase in alertness upon exposure to rosemary as evidenced by EEG brain wave activity changes.[27] Aydemir (2017) conducted a study on five healthy participants upon exposure to odour stimuli (valerian, lotus flower, cheese, rosewater). The brain wave spectra were analysed using the continuous wavelet transform method. This was the only study that showed olfaction being significantly associated with gamma wave activities.[11] However, a limitation of this study is that the sample size was too small with no control participants and screening smell test to confirm that the participants had no smell impairment. Hence, the results might not be representative. Table 4 summarises the concise review of the published studies on the human EEG response towards olfactory stimulation.

Given the discrepant findings among various studies over the years, we conducted a case–control study involving 44 healthy participants who had passed a validated culturally adapted smell screening test with 16 types of odours prior to the EEG experiment using lavender essential oil and normal saline. Our results suggested an association of olfaction with gamma wave activity, which is consistent with one of the latest studies by Aydemir which reported the close association between olfaction and gamma waves.[11] In our study, the participants were administered with lavender essential oil (odorous) and normal saline (non-odorous), respectively, in both the eyes-open and eyes-closed conditions. In the eyes-closed condition, with less visual stimuli and conscious perception, we found a reduction in the beta activity in the left frontal and central regions. This finding is in relation to the fact that the frontal region of the piriform cortex is associated with olfactory processing.[28] Interestingly, our study also found a reduction in the gamma wave activity over the right parietal region. It suggested that the parietal region is involved in processing sensory information and smell is a sensory signal. The gamma and beta waves are high-frequency brain waves in which gamma waves are linked to learning and memory and beta waves are linked to consciousness with a high activity in the brain.[23] We postulate that lavender odour results in the reduction of consciousness as evidenced by the reduction of beta and gamma wave activities in our study.

With regard to the pleasantness of the smell in regard to brain wave activity, our findings on the predominance of the reduction of beta activity in the left frontal and central regions upon exposure to lavender, which was perceived as a pleasant smell, were based on the concept of the left frontal activation in the brain being associated with pleasant smell.[29] Despite the previous studies on smell and EEG, our research findings on olfaction and beta and gamma waves with left frontal activation in the brain are supported by modern studies.[11,29,30] Aydemir suggested a strong association of olfaction with gamma activity.[11] A study by Sowndhararajan reported a reduction in the beta activity in the left prefrontal region upon inula helenium essential oil exposure as observed in the present study.[30] A study by Kline *et al.* reported left frontal activation in the brain upon exposure to a pleasant odour (vanilla) with a reduction in the alpha activity in the left frontal region.[29] Our study similarly showed left frontal activation with a reduction of beta activity upon exposure to lavender odour, which was perceived as a pleasant smell, as evidenced in the EEG topographical map in blue (Figure 2). This has strengthened the finding that pleasant smell does result in left frontal region activation through different brain wave activities upon exposure to different types of smell. Schriever *et al.* ($n = 78$) showed that olfactory-induced EEG power changes upon analysis with time frequency analysis with 75 per cent sensitivity and 89 per cent specificity in the normal population as compared with the population with smell impairment.[31]

We performed the experiment in both the eyes-open and eyes-closed condition and compared the results. This was to eliminate the variables of visual stimuli in the eyes-open condition, which may lead to an increase in consciousness and subsequently affect the accuracy of odour stimulation with regard to brain wave activity. Besides, a number of variables can affect EEG findings on exposure to olfactory stimulants, such as the number of electrodes utilised in each of the studies, giving rise to a variation in results.[26] Of note, much of the ambiguity in these studies can also be attributed to the difference in the duration of the exposure to the olfactory stimulant and also the duration of EEG recordings. Smell adaptation is known to occur after a prolonged and constant exposure to a particular smell, which results in a decrease in sensitivity to the stimulus.[32] It is an important variable in determining the outcome of the EEG activities in response to the odour. Pierce *et al.* observed that the perceived intensity of lavender and vanillin was significantly reduced after 10 minutes and 5 minutes of exposure independently.[33] Hence, we adopted two minutes of lavender exposure, as in similar studies, in order to minimise the possible smell adaptation with the prolonged constant exposure.[8,25,30]

The strength of our study is that our sample size is relatively good, with 44 participants having strictly passed the culturally adapted Malaysian version of the Sniffin' Sticks smell identification screening test, with the aim of excluding participants with any olfactory abnormality prior to their enrolment for the EEG recording. Objective screening of smell is essential to formulate a strict and standardised enrolment in order to generate conclusive results.[13]

- Recent research has identified breakthrough discoveries with regard to sense of smell and its relation with the human brain
- Electroencephalogram with spectral analysis is useful in identifying the most dominant oscillations present in the brain and its functional response to the olfactory stimulant
- This study strengthened the evidence of electrical brain activity changes upon exposure to olfactory stimulant with lavender
- Olfactory stimulant does result in changes of electrical brain activity in different regions of the brain

Although the results of the brain wave changes with regard to olfactory stimulants are encouraging, our study has several limitations: (1) we utilised only one lavender smell to compare it with the neutral normal saline. Responses to different types of smell are lacking. A variety of other smells can be employed in future studies with a strict Sniffin' Sticks screening test; and (2) we only performed the test on a healthy normal population. Diseased or anosmia patients can be further studied with a similar methodology.

Conclusion

Our study concluded that olfactory stimulant results in the changes of the electrical activity in different regions of the brain as evidenced by the EEG topographical map and spectral analysis of each power.

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Data availability statement. A complete table of all results for all participants will be made available in GitHub. The MATLAB (version 2020a) source code will be shared with the permission of the authors upon publication.

Competing interests. None declared

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