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Theta Brainwave Activity as the Response to Lavender *(Lavendula angustifolia)* Aromatherapy Inhalation of Postgraduate Students with Acasdemic Stress Condition

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Abstract The objective of this study is to determine the activity of Theta brainwave as the response to Lavender aromatherapy inhalation of postgraduate students with academic stress condition. This research involved 10 postgraduate students consisted of 5 males and 5 females who were still pursuing their final research and were recruited based on assigned criteria and filled two modified questionnaires to determine the stress category. The procedure of Lavender aromatherapy inhalation was given intentionally for a period of 16 days of experiments for 15 minutes of each day. Evaluation of brain electrical activity focused on Theta (frequency 4-7Hz) and were recorded by Electroencephalograph (EEG) EMOTIV EPOC from 6 channel electrodes on the scalp (AF3, AF4, T7, T8, P7, P8). Essential oil compound from Lavender was evaluated by using GCMS (Gas Chromatography Mass Spectrometry). Data were analysed by comparing baselines before treatment and two weeks after aromatherapy administration using General Linear Model and Paired t-test statistical procedure. The result revealed that: (1) the main constituents of Lavender essential oil are linalool (20,11%) and linalyl acetate (26,34%), (2) there was no significantly increase in Theta power both in women and men ($p > 0,05$), (3) there was an asymmetry of Theta brainwave pattern at P7 and P8 channel in women. The constituents of Lavender essential oil activate olfactory neuron and modulate inhibitory neurotransmitter to influence EEG pattern.

1. Introduction

Stress influences body homeostasis which can be indicated by the response of neuroendocrine activity[1]. This condition is generally experienced by people in many aspects of life. In academic environment, stress become the important issues to be coped regards to the academic workload as a stressor. One of the example of academic stressor is a research workload which usually experienced by the students pursuing the last academic years[2]. Research workloads or laboratory works in the final research usually need students to give more attention, focus, and motivation because it is one of the

determination factors for students to pass the degree in universities. Some of the activities and workload during final research are the stressor which affect academic performance and cause some stress manifestation[3].

Stress can be observed through physical assessment, perception and behavior[4]. This condition influences neuronal response from the central nerve system and triggers the change of brainwave activity known as EEG (electroencephalograph) pattern in a certain brain regions[5]. Stress and emotion cause asymmetry of brainwave in area which has relation with stress regulation and also gives impact to cognition area[6]. Parts of the limbic system such as amygdala and hippocampus play a role as the centre of emotion regulation during stress[7].

Stress indicates neuron activity which is stimulated by the changes of ion in a neuron membrane causing electrical flow indicated by potential electrical changes and able to conduct an electromagnetic field around the neuron[8]. This electrical flow is observed by EEG and the results will be interpreted as a brainwave frequencies and power spectral in each time.

Regarding to the risk and physical manifestation of stress which impacts both of emotion and cognition, there were some studies to discover some treatments in purpose to reduce stress level such as yoga, acupuncture and meditation[9]. Lately in society, the usage of aromatherapy became more popular. Essential oil from aromatherapy can be one of the stress reliever. Bioactive components from essential oil can promote relaxation effects[10]. Therefore, essential oil is able to reduce the activity of parasympathetic nerve[11].

Essential oil from Lavender *(Lavendula angustifolia)* is defined as one of the stress reliever and promotes relaxation effect. The action mechanism of Lavender essential oil effects central nerve system[12]. The main constituents of lavender are linalool and linalyl acetat modulates GABA (γaminobutiric acid) neurotransmitter in sinaps[13]. GABA is known as major inhibitory neurotransmitter. The modulation of GABA opens Cl^- ion channel and increases Cl^- entrance to the membrane. This Cl^- influx causes internal membrane became more negative as knowns as IPSP (Inhibitory Post Synaptic Potential)[14].

Studies about Lavender administration to reduce stress have been done, but still there were different results about the response from sex differentiation between male and female. Therefore, previous studies have not described clearly yet about Theta brainwave activity (Frequency 4-7Hz) both in males and females regards to its interval dynamics and dominance of brain hemisphere before and after Lavender aromatherapy inhalation. From the research, it can be considered the usage of Lavender aromatherapy as a stress reliever for students pursuing the final research in the last academic to give a good performance.

2. Material and Methods

2.1. Subject

Ten selected participants of equal number of 5 males and 5 females aged 22-27 years old. Participants were postgraduate students pursuing their final research and recruited based on assigned criteria and filled two modified questionnaires Student Stress Scale (SSS) and Mind over Mood Anxiety Inventory (MoMAI) to determine the stress category. None of the subjects had abnormalities affecting smell or a history of smoking and drug addiction. They were requested not to use aromatherapy, massage or another type of relaxation, refrained from consuming alcohol and also have enough sleep to avoid feeling drowsy during the experiment. Women who were menstruating were not included. Subjects were given explanation about the research and informed consent. The study had been approved by the Ethical Review Committee, chaired by Dr. Hasan Sadikin Hospital No. LB.04.01/A05/EC/475/XI/2015.

2.2. Procedure

The length of the research was 16 days including three times of data collection, they were baseline (H0), the end of the first week (H8), and the end of the second week (H16) while the days in between

were used for aromatherapy inhalation. Experiment was conducted in laboratory of medical instrumentation, Department of Physics Engineering Bandung Institute of Technology in between 08.00-10.00 in dark condition with light intensity 1-2 lux. Experiment was conducted as long as 25 minutes for the data collection, with 10 minutes for EEG setting, 15 minutes aromatherapy inhalation while EEG recording.

2.2.1. Student Stress Scale (SSS) and Mind over Mood Anxiety Inventory (MoMAI) questionnaires
Student stress scale was used to evaluate student stress level regarding to academic workload of final research. There were 17 questions with 5 scale in which 1 was Moderate Stress (MS), 2 Stress (S), 3 Mild stress (MLS), 4 No Stress (NS). This questionnaire was adapted from Ros Shannon (2008)[15]. Mind over Mood Anxiety Inventory (MoMAI) questionnaire was used to evaluate the frequency of psychosomatic symptoms as a body response to academic stress. The questionnaire consisted of 18 questions which were modified from Balaji Rao (2013)[16]. There were 5 scales; 1 indicated Never (N), 2 Rarely (R), 3 Sometimes (S), 4 Often (O), 5 Always (A). Scores from SSS and MoMAI questionnaires were calculated and categorized based on criteria from Arikunto (2010)[17] to determine student stress category and frequency of psychosomatic symptoms.

2.2.2. Essential oil administration
Lavender aromatherapy was obtained from Young Living Singapore Company 100% essential oil. It was administered intentionally for 15 minutes each day. 0.25 ml of essential oil was diffused in 50 ml of water by Ultrasonic Ionizer Aromatherapy Diffuser (type YHL668/I, ultrasound frequency 2.5MHz, Nature Co. Ltd, made in Taiwan. There were two diffusers used in this research, each of it was placed at the right and left side of the subject and kept 90 cm away of distance. Before experiment, subjects were asked to inhale Lavender oil to rate the pleasantness of the smell on a five point Likert Scale. Before being used as stimulus, essential oil composition was identified by Gas Chromatography Mass Spectrometry (GCMS).

2.2.3. Electroencephalograph (EEG) recording
EEG EMOTIV EPOC with 128 Hertz sampling rate was used in this research. Electrodes were placed on the scalp at 6 channel AF3, AF4, T7, T8, P7, and P8 which represented frontal, temporal and parietal lobes from two brain hemispheres. EEG was connected wirelessly with Emotiv Testbench Xavier 3.0.041 to start the recording. Data was filtered to Theta Frequency (4-7Hz). Theta brainwave was compared from H0, H8, and H16, and was analysed based on 3 minutes of interval for as long as 15 minutes to evaluate the response of brainwave activity to Lavender aromatherapy given.

2.3. Statistical analysis
The SPSS statistical package was used for data analysis. General linear model of Repeated Measurement and Paired t Test were used to evaluate Theta brainwave activity as the response to Lavender inhalation.

3. Result

3.1. Student stress category and psychosomatic frequency
Based on the analysis of SSS, male postgraduate students experienced different category of stress. From 5 male students, 2 experienced Stress (S), Mild Stress (MLS), and Moderate Stress (MS). From the MoMAI analysis, all male students experienced psychosomatic symptom in frequency of sometimes (S). For the females, 2 students experienced Moderate Stress (MS), 2 Stress (S), 1 Mild Stress (MLS). MoMAI result showed that female students experienced different frequencies of psychosomatic symptom. 2 students experienced Often (O), 2 Sometimes (S), and 1 experienced Rarely (R).

3.2. Essential oil constituents

The constituents of Lavender Essential oil were identified matching their mass spectra and retention time. The main components of Lavender oil are linalool (20.11%) and linalyl acetate (26.34%). Another dominant components are 1-octene-3 acetate (9.43%), lavandulol acetate (4.94%), caryophyllene (4.95%) 1,6,10-dodecatriene-7,11-dimethyl (3.81%), cis-ocimene (3.03%), 1,3,6-octatriene-3,7dimethyl (2.73%).

3.3. Theta (4-7 Hertz) Brainwave activity

3.3.1. Male Theta activity

The analysis of Theta (4-7Hz) activity in male students showed there was no significantly increase of Theta power in general as the response to Lavender aromatherapy administration but specific observation to each channel showed significantly increase of Theta power at AF3, T7 and T8. Data can be seen at **Figure 1.**

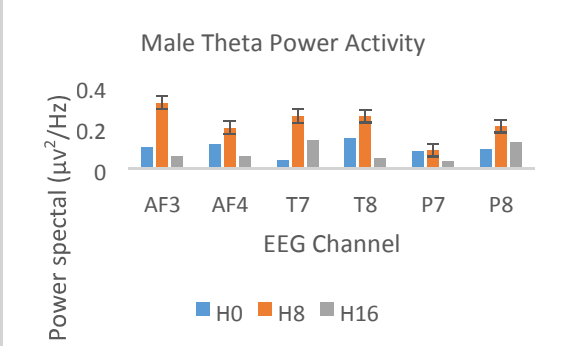

Figure 1. Male Theta power activity before and after aromatherapy inhalation

Based on the data, Lavender aromatherapy increased Theta power after first week of administration (H8) at all channel of EEG, otherwise the power decreased at the second week of administration (H8) in male postgraduate students with academic stress. The pattern of Theta activity is shown in **Figure 2** as a representative from male subjects.

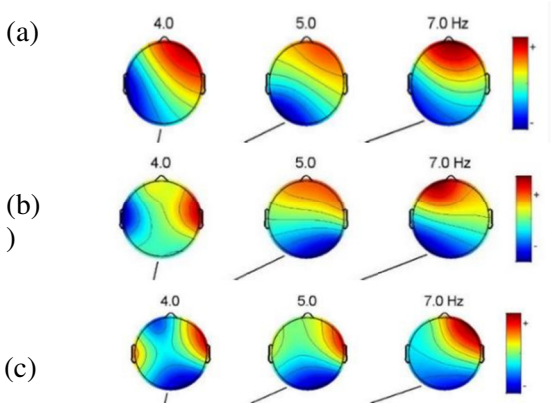

Figure 2. Brain topological map of the distribution of Theta brainwave activity before and after inhalation at male representative (a) H0, (b) H8, (c) H16.

The picture showed about the distribution of Theta brainwave in male students. Before and after aromatherapy administration, Theta was distributed dominantly at frontal and temporal lobes at right hemisphere. Specific observation to Theta activity which was observed based on interval can be seen from **Figure 3.**

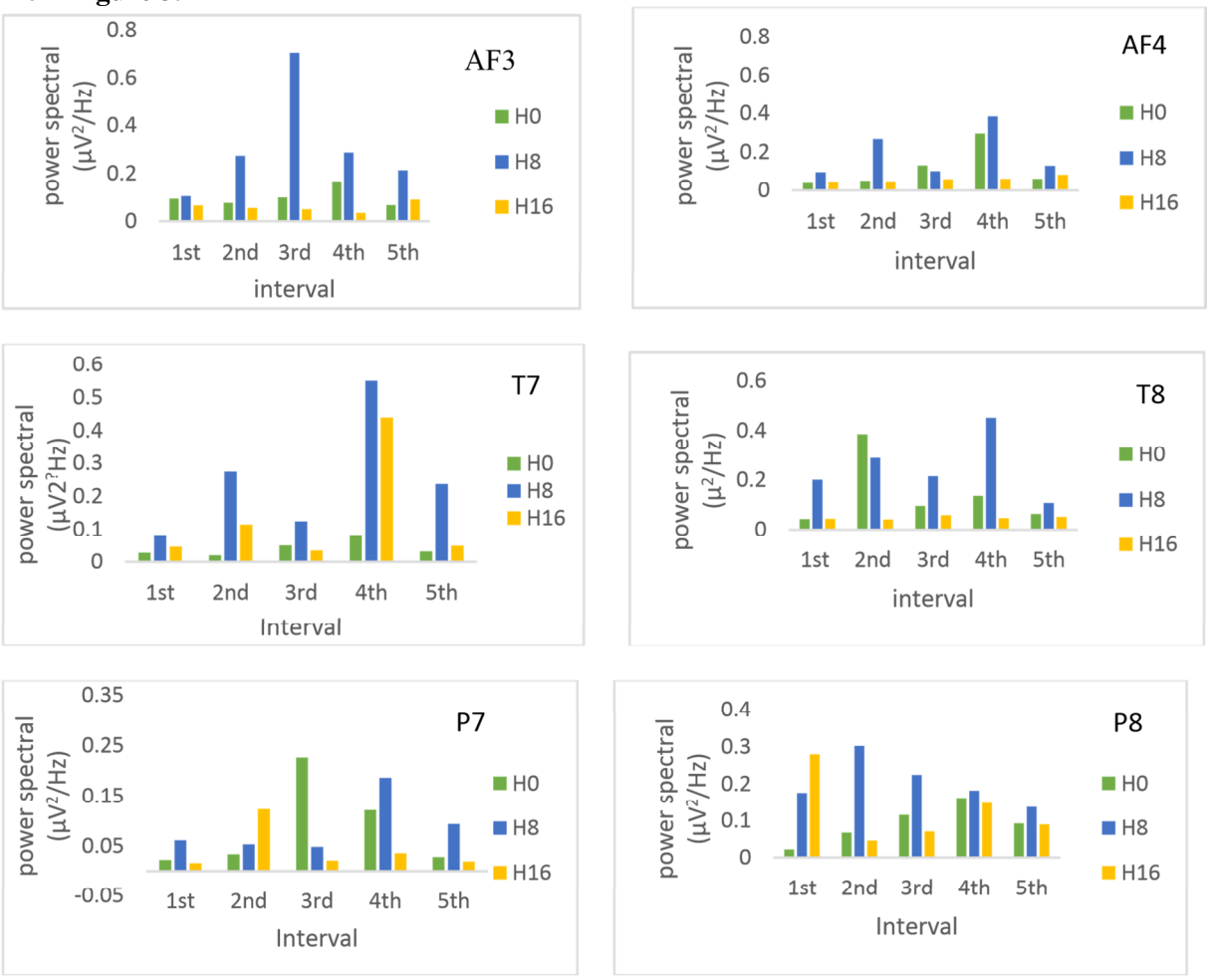

Figure 3. Activity of Theta brainwave based on interval in male subjects

The data showed that Theta brainwave performed a fluctuation in each interval before and after Lavender aromatherapy inhalation in male students. The highest peak of Theta activity tended to occur at the 3rd and 4th interval which means it happened since the 7th minutes. The difference of Theta power distribution between hemispheres can be seen based on statistical data in **Table 2.**

Table 2. Statistical analysis for Theta activity in right and left brain hemispheres before and after Lavender inhalation in male subjects

Comparation between channel	**Observation**			
	H0	**H8**	**H16**	**General**
AF3 and AF4	P = 0,65	P = 0,38	P = 0,86	P = 0,53
T7 and T8	P = 0,19	P = 0,98	P = 0,36	P = 0,18
P7 and nP8	P = 0,86	P = 0,06	P = 0,20	P = 0,28

Observation to the difference of Theta power distribution between right and left hemisphere showed there was no significantly difference between left and right hemisphere from all channel (AF3 and AF4, T7 and T8, P7 and P8) in male (P>0,05). This data indicated that there was no asymmetry pattern of Theta brainwave as the effect of Lavender oil inhalation in male subject with academic stress condition.

3.3.2. Female Theta activity

Analysis of Theta brainwave activity in female subjects showed there was no significantly increase of Theta power generally except at AF3 and P8 channel **(Figure 4)**.

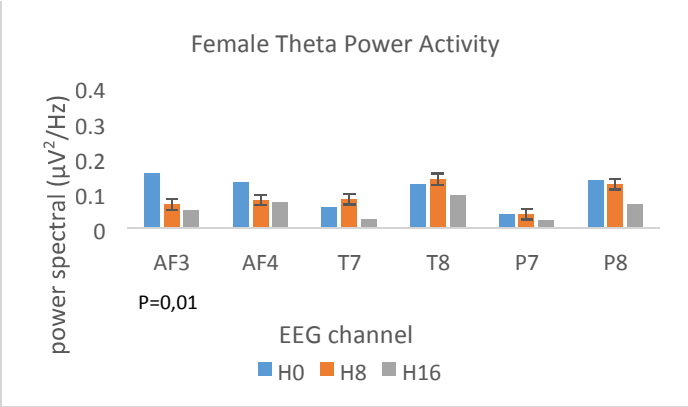

Figure 4. Female Theta power activity before and after aromatherapy inhalation

In female, power spectral of Theta brainwave was higher at baseline condition (H0) than H8 and H16 for AF3 and AF4 channel, meanwhile higher at H8 for T7, T8, P7 and P8 channel and decreased at H16. Activity of Theta brainwave can be described as brain topological mapping. **Figure 5** shows the EEG pattern of Theta brainwave activity before and after Lavender inhalation at one of the representative female subjects.

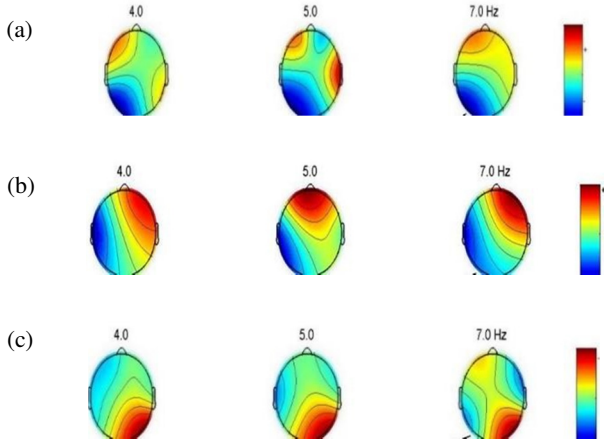

Figure 5. Brain topological map of the distribution of Theta brainwave activity before and after inhalation at female representative. (a) H0, (b) H8, (c) H16

The picture showed about the distribution of Theta brainwave in female students. Before aromatherapy administration (H0) and at the first week (H8) Theta brainwave was distributed at frontal and temporal lobes at the right hemisphere but at the second week (H16) Theta activity distributed at parietal lobes. Based on the EEG pattern, it can be seen that Theta brainwave was distributed dominantly at H8, as indicated by the deeper red colour indicator. Specific observation to Theta activity observed based on interval can be seen from **Figure 6.**

Figure 6. Activity of Theta brainwave based on interval in female subjects

In females, Theta brainwave fluctuated since H0, H8 and H16. At the frontal lobes which was represented by AF3 and AF4, Theta brainwave reached the peak at baseline (H0) during the 1st and 3rd minutes. At temporal (T7 and T8) and parietal lobes (P7 and P8), Theta brainwave reached the peak since H8 at the 3rd interval. Based on analysis of Theta brainwave in both hemisphere, there was no significantly differences of Theta power between AF3 and AF4, T7 and T8, but there was significant difference between P7 and P8 indicating that there was asymmetry of Theta brainwave in parietal lobes in woman which Theta power distributed dominantly in the right hemisphere as the response of aromatherapy administration during stress condition. Statistical data is shown at **Table 4.**

Table 4. Statistical analysis for Theta activity in right and left brain hemisphere before and after Lavender inhalation in female subjects

Comparation between channel	Observation			
	H0	**H8**	**H16**	**General**
AF3 and AF4	P = 0,25	P = 0,36	P = 0,00	P = 0,59
T7 and T8	P = 0,25	P = 0,45	P = 0,00	P = 0,47
P7 and nP8	P = 0,02	P = 0,01	P = 0,00	P = 0,02

4. Discussion

Based on general analysis to Theta power in males and females with academic stress condition regarding to research workload, it can be found that there was a significant increase at male Theta power particularly at AF3, T7 and T8 and female Theta power at AF3 and P8 as a brainwave response to Lavender aromatherapy inhalation. The increase of Theta power began at the first week of aromatherapy administration (H0) but decreased at the second week (H16). This condition was assumed that academic stress which was experienced by the subjects play a role at the activity of Theta power. In the second week of aromatherapy administration, Theta brainwave increased regarding to the odorant stimulus which had been given through Lavender inhalation. This condition indicates that both of male and female subjects were in the relaxation without any cognitive activity such as attention and concentration[18].

In sleep physiology, relaxation activity which was shown by Theta brainwave in the low frequency (47Hz) indicates that both male and female subjects were in the 1st level of sleep (scale 1-4) from Nonrapid Eye Movement (NREM) which has a high amplitude but low frequency as usually said as synchronization [19]. In the NREM condition, neuron in neocortex and thalamus hyperpolarize which was caused by the lack of neuron excitation stimulator in hypothalamus. In the early phase of NREM, VLPO region (Ventroapteral preoptic) in hypothalamus was deactivated [19]. Linalool and linalyl acetate which plays a major role to promote relax, will modulated the activity of GABA (γ-aminobutiricacid) in the VLPO neuron and inhibit excitation neuron at thalamus and cortex.

As the projection from olfactory tract to the cortex area, odorant is projected to anterior perforated to connect with Hypothalamus. The characteristic of pyramidal neuron in this area is glutamatergic[19]. The main constituent of Lavender aromatherapy is linalool and linalyl acetate assumed to trigger glutamin biosynthesis into GABA and exhibit excitation in neuron. Inhibition mechanism was also performed at hippocampus interneuron which is GABAergic. GABA activity shows the modulation of Theta brainwave which stimulates the relax condition of the subjects.

The power of Theta activity increased in H8 but then decreased at H16. This regards to the condition of the subjects in both females and males. At the second week (H16) the subjects were more exposed to academic stressor of research workload such as deadline for 80% result, result seminar and progress report. The result shows that Theta power in male is higher than female. This condition shows that males have more tendency to cope with the stressor than females. According to Donchin (2000)[20], sex differentiation contributes to the strategy of coping with stress and emotion which has a correlation with the Theta spectral power. The Theta activity in hippocampus also indicated that there is a memory forming[19]. This memory to odorant recognition contributes to the decrease of Theta spectral power both in males or females at the second week of aromatherapy administration. The interval analysis shows that the peak of Theta power was in the 3rd and 4th interval or 7 to 12 minutes of inhalation. This regards to research from Jirovetz et al., (1990) that Lavender essential oil was absorbed by the body in 7 minutes.

5. Conclusion

Lavender aromatherapy stimulate calmness and relax condition of subject with academic stress. Aromatherapy inhalation caused significant increase of Theta power spectral at AF3, T7 and T8 in males and at AF3 and P8 in females. There is no asymmetry and dominance of Theta power both in right and left brain hemispheres in males but it causes asymmetry of Theta power at parietal lobe of right hemisphere dominance in females.

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