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A Study Of EEG Changes In The Cerebral Lobes Through Both Scent Stimulation With Cirsium Setidens (Dunn) Nakai Extract And Learning In The Humanities

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Abstract:

In this study, an EEG waveform analysis was conducted by having the subjects take a humanities course while being exposed to the scent of Cirsium setidens (Dunn) Nakai (also known as Gondre in Korea) extract. To determine the antioxidant effect of Gondre extract, DPPH radical scavenging activity was measured. The activity was found to be 4.52±0.06%, 7.78±0.18%, 12.99±0.12%, 19.98±0.12%, and 32.04±0.11% at 100, 300, 500, 700, and 1000 µL, respectively. To determine the antioxidant effect of Gondre extract, a total flavonoid content (TFC) assay was conducted to measure the TFC. The TFC was found to be 2.74±0.48, 6.96±1.17, 8.74±0.93, 9.46±1.00, and 14.14±1.40 at 10, 30, 50, 70, and 100 µL, respectively. For relative delta waves, the activity in most of the cerebral lobes after humanities learning increased sequentially as the measurement was repeated 4 times. However, at electrode T4, the activity decreased in the 4th measurement. This may be considered to be caused by increased sound fatigue, but at electrode T3, the activity showed a sequential increase. For relative theta, the activity

decreased in the 3rd and 4th measurements at electrodes F3, F4, P3, and T4. At electrode Cz, the activity decreased in the 4th measurement for the group with both scent stimulation and humanities learning (i.e., Group A). In particular, at electrode T3, the activity increased sequentially with repeated measurements, whereas at electrode T4, the activity decreased, like that of relative delta waves. At electrodes F3 and F4, for Group A, relative alpha waves tended to be activated again in the 3rd and 4th measurements. At electrodes Cz, P, P, T3, and T4, for Group A, the activity tended to decrease as the measurement was repeated. However, at electrode T4, for Group A, the activity was relatively increased in the 3rd measurement.

Keywords: brainwave, humanities, scent of Cirsium setidens (Dunn) Nakai extract, antioxidation, autonomic nervous system.

1. Introduction

With the job crisis that started in the late 1990s, the 4th Industrial Revolution, and the COVID-19 pandemic, more students are expressing a preference for science and engineering compared to the humanities. Based on KOSIS statistics on the survey results of student distribution by major field from 2015 to 2020, there were 784 people (8.5%) in the humanities and 4,202 people (45.4%) in science and engineering in 2015, whereas in 2020, 762 (8.6%) were in the humanities and 4,512 (52.0%) in science and engineering. This is a 50.1% decrease in students in the humanities, whereas the number in science and engineering increased by 6.6% [1, 2]. In addition, it was reported that the number of science and engineering majors with college degrees or higher was 2.839 million in 2008, and increased by 4.4% to 4.368 million in 2018 [3]. In some cases, students who went to college by choosing their major according to such social preference and their grades had low satisfaction with their major. In addition, college students who are entering adulthood go through new experiences that they did not undergo in adolescence; they face various difficulties including in relationships with parents or between seniors and juniors, and burden of grades and employment, reducing their life

satisfaction [4, 5]. The Korea Federation of Science and Technology Societies (KOFST) conducted a survey on the science and engineering avoidance phenomenon targeting a total of 1,684 students in science and engineering from a total of 41 universities for June–July, 2011. 8.9% and 3.6% of them answered "very high" and "very low" respectively, indicating that students' awareness of avoidance of science and engineering is high [6]. Such complex factors have caused serious stress to science and engineering students [1].

Stress is a combination of physical and mental responses to external or internal stimuli. It can cause various types of physical and mental diseases including diabetes, chronic fatigue, migraines, depression and anxiety, as well as hypertension and indigestion [2, 7]. Stress also induces problems in the brain. Studies using animal models have reported that chronic stress not only induces atrophy of neurons of the hippocampus and prefrontal cortex and brain areas involved in memory, selective attention and executive functions, but also enlarges neurons in the amygdala, a brain region involved in fear and anxiety as well as aggression. Studies also report that exposure to severe stress from childhood affects brain function, as well as normal development, indicating that stress affects the brain [8, 9].

The effects of stress have a direct response in the cerebrum. The frontal lobe (FL) is one of the largest and most developed lobes of the brain, located next to the central sulcus. It affects the physical and mental health of individuals, and plays a role in the functions involved in motivation, planning, social behavior, and language production. The frontal lobes, located in the left and right hemispheres, perform different functions. For the right-hemisphere FL, the lateral surface is expressive language area (Broca's area, Brodmann's area 44 and 45), and damage to this area can lead to aphasia, with difficulty in fluent speech [10]. The left-hemisphere FL is involved in the encoding of new events in episodic memory and the retrieval of information from semantic memory [11]. The temporal lobe (TL) is located near the temple in the outer part of the left brain and plays a role in functions related to understanding language, auditory processing, memory, and emotion, as well as forming new long-term memories [12]. Wernicke's area, located in the left TL, is responsible for language development and comprehension.

Damage to this area results in Wernicke's aphasia, the inability to understand words. The parietal lobe (PL) is also called the lobus parietalis and is known to be located at the rear of the cerebrum. It processes sensory information; it is known that damage to it may disrupt a person's function of reading, writing, and movements [12].

When neurons in the brain are activated, electrical signals are generated, which are called brainwaves. An electroencephalogram (EEG) is a method of measuring and recording these brainwaves as electrical signals [12]. An EEG can be used to evaluate brain function. Brainwaves are divided into alpha waves, beta waves, gamma waves, delta waves, and theta waves according to the characteristics of frequencies in EEG.

Alpha waves are a reference frequency of brainwaves with a frequency range of 8–13 Hz. They occur prominently in the parietal region when a normal adult is awake and relaxing with the eyes closed. Alpha waves are also known as brainwaves that enhance our attention and concentration. Studies suggest that as alpha waves have an inverse correlation with glucose metabolism, the stronger the alpha waves, the lower the glucose metabolism [12, 13]. Stress is associated with a decrease of alpha waves, which increase in a relaxed state [14]. Beta waves are fast brainwaves with a frequency range of 14 Hz or higher. They mainly occur in the FL and are activated when you are nervous, perform tasks, or engage in activities that require concentration. It has been reported that an increase in regional cerebral blood flow was observed in areas with increased beta waves. It is known that an increase in beta waves is observed when you are under stress or have symptoms of anxiety [12, 13]. Gamma waves with a frequency range of 30–40 Hz or higher occur in an excited state, and are activated when you are experiencing a high degree of stress [7, 12]. Delta waves are slow brainwaves with a frequency range of 0.5–3 Hz and are known to occur mainly in the frontal region. Regardless of age, the waves are associated with deep sleep, and are also known to occur when there are abnormalities including epilepsy or brain tumors [12, 14]. Theta waves are slow brainwaves with a frequency range of 4–7 Hz. They are mainly recorded in the occipital and temporal region and appear during a drowsy but still awake period, and in states of meditation [12, 14]. When

learning-related functions are performed in the brain, alpha waves occur in the PL, and beta waves in the FL and TL. However, when you are under stress, the expression of beta waves increases in all regions of the brain, and even gamma waves may appear when you are under severe stress [15].

Since stress causes various physical and mental problems, studies on approaches to reducing stress are continuously being pursued, and the humanities is emerging as a good solution. Humanities is the area of study that puts human values at the center of all areas of human behavior, and encompasses various subjects including history, philosophy, and the arts. Humanities therapy, which uses the humanities to treat people suffering emotionally and mentally, has been actively applied to treatment. A study on the relationship between humanities literacy and stress targeting a total of 306 nurses reported that there was a correlation between these factors [16]. In addition, there are studies that used humanities therapy to solve problems of various people. Of them, a study in which humanities therapy was conducted on North Korean defectors suggested that their stress related to adapting to South Korean culture was alleviated [17].

One of the ways to relieve stress is therapy using aroma. Aromatherapy is a method of treatment using the natural substances extracted from plants rather than chemicals, and is used to heal various mental diseases including depression and sleep disorders. Lavender scent has been reported to be effective not only for sleep disorders, but also for patients with severe depression [18, 19].

In this study, humanities learning and scent stimulation were conducted simultaneously for the subjects to analyze their EEG waveforms. Cirsium setidens (Dunn) Nakai in the family Asteraceae, also called Gondre (Korean thistle), was used for scent stimulation. In Oriental medicine, the aboveground parts or roots of Cirsium setidens (Dunn) Nakai are used. It contains dietary fiber, vitamins, and minerals, as well as flavonoids, triterpene, and coumarin, which promote antioxidant, anticancer, and melanin-production activities [20]. It has many uses: for the treatment of hematemesis, hematuria, leukorrhea, hepatitis and hypertension, and as an ingredient for various

dishes including fritters [21, 27]. In this study, changes in EEG waveforms were examined using Gondre as a scented substance.

2. Experimental Methods

2.1 Fabrication of natural substances and extracts

The leaves of Cirsium setidens (Dunn) Nakai (hereinafter referred to as Gondre) used in this experiment were purchased from Herbmaul (Cheongju, South Korea). 100 g of Gondre leaves was prepared as an extract by being double-boiled for 2 hours together with 1 L of distilled water, and then filtered twice. The yield was 6.35%.

2.2 DPPH radical scavenging test

DPPH radical scavenging activity was measured using a modified Blois method [21]. After adding 0.5 mL of DPPH reagent to a 1000 μL diluted solution in which samples and methanol were diluted for each concentration, they were reacted in a water bath (LabTech, Korea) at a temperature of 25˚C for 20 minutes under light-blocking conditions. Then, the absorbance of samples was measured at 517 nm using an x-ma 1200 spectropotometer (ThermoFisher, USA). As a control, ascorbic acid was purchased and used. It was calculated using the following equation.

DPPH radical scavenging activity (%) = [(control absorbance – sample absorbance)/ control absorbance] ×100

2.3 Total flavonoid content assay

The total flavonoid content (TFC) was measured using an assay modified from that of Kim et al. [22]. 100 μL of solution, in which samples were dissolved in distilled water for each concentration, was mixed with 1 mL of diethylene glycol and 100 μL of 1N sodium hydroxide. After being mixed, they were reacted for 1 hour at 37°C in a water bath (LabTech, Korea), and then measured at 420 nm using an x-ma 1200 spectropotometer (ThermoFisher, USA). Standard calibration curves of 1mg/mL, 2mg/mL, 3mg/mL, 4mg/mL, and 5mg/mL concentrations were

prepared using naringin as a reference material, from which the TFC was converted.

2.4 Subjects

This experiment was conducted with 36 students from a university. They were divided into two groups of 18 each: one group whose EEGs were analyzed after smelling the scent of Gondre extract (i.e., scent stimulation) and then taking humanities lectures (Group A); the other group whose EEGs were analyzed after only taking humanities lectures (Group B). While the scent diffused for about 5 minutes, allowing them to smell it, their EEGs were recorded for 6 minutes. The experiment was conducted twice at the same time for 15 weeks.

2.5 Data analysis

In this study, EEG data repeatedly measured 4 times for each experimental group were analyzed. A multivariate approach was taken under the assumption that data may vary according to 7 electrode locations (Cz, F3, F4, P3, P4, T3, T4). Therefore, the repeated measures MANOVA (RM-MANOVA) was selected as a statistical model. Based on the experimental design, the overall data analysis was performed by dividing into five modules: relative alpha, relative theta, relative beta, relative delta, and concentration. SPSS 26.0 software was used for data analysis.

3. Findings

3.1. DPPH radical scavenging test results

To determine the antioxidant effect of Gondre extract, the DPPH radical scavenging activity was measured. The activity was found to be 4.52±0.06%, 7.78±0.18%, 12.99±0.12%, 19.98±0.12%, and 32.04±0.11% at 100, 300, 500, 700, and 1000 µL, respectively (Figure 1, Table 2).

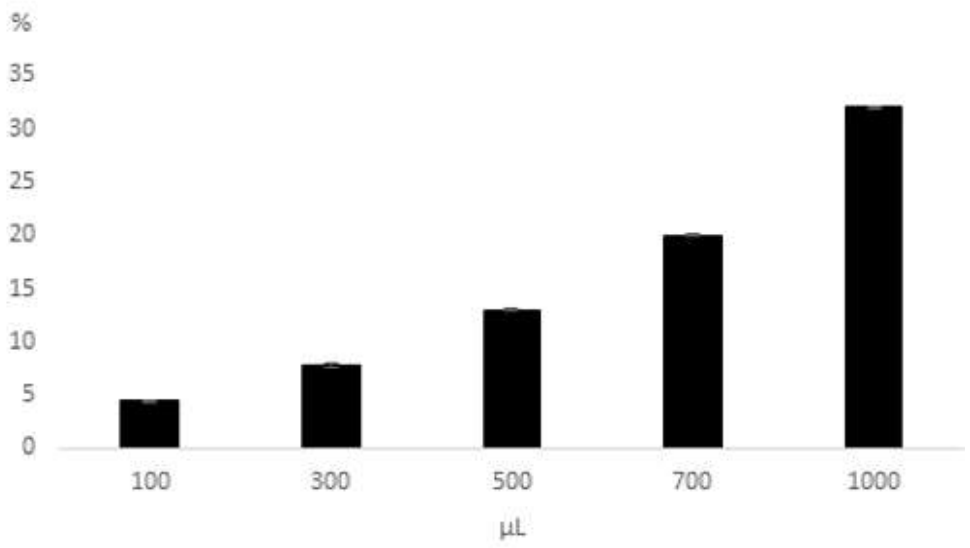


Figure 1. DPPH radical scavenging test

Table 2. DPPH radical scavenging test

µL	100	300	500	700	1000
%	4.52±0.06	7.78±0.18	12.99±0.12	19.98±0.12	32.04±0.11

3. 2. Total flavonoid content assay results

To determine the antioxidant effect of Gondre extract, a TFC content assay was performed. The TFC was measured as 2.74±0.48, 6.96±1.17, 8.74±0.93, 9.46±1.00, and 14.14±1.40 at 10, 30, 50, 70, and 100 µL, respectively (Figure 2, Table 3).

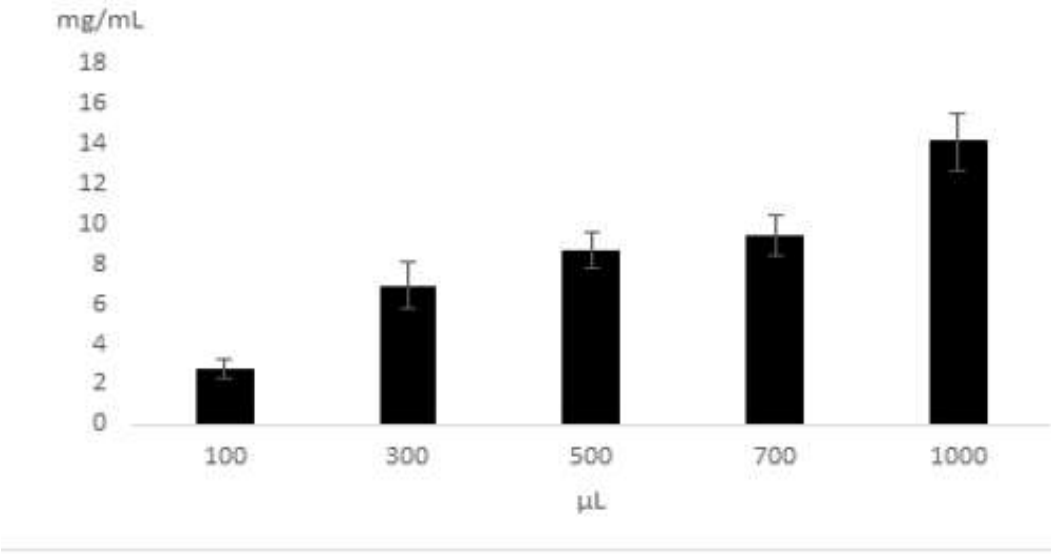


Figure 2. Total flavonoid content

Table 3. Total flavonoid content

µL	10	30	50	70	100
mg/mL	2.74±0.48	6.96±1.17	8.74±0.93	9.46±1.00	14.14±1.40

3. 3. Brainwaves (EEG)

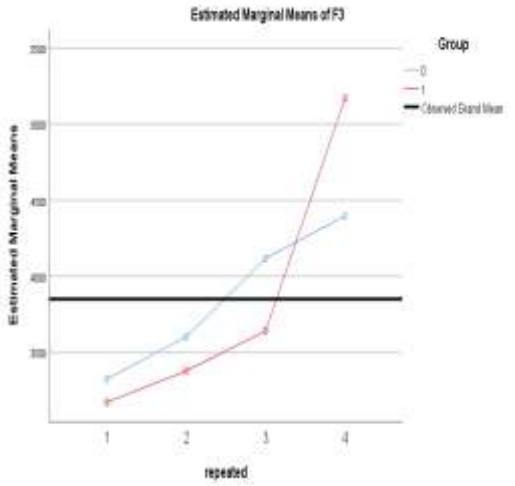
Table 4 encapsulates the findings of omnibus (i.e., multivariate) F tests for EEG relative delta wave: 1) The mean vector difference of seven different points (i.e., F3, F4, Cz, P4, T3, P3, T4) between two groups (i.e., whether receiving scent stimulation with cirsium setidens) was not significant; 2) The variation in outcome variables of repeated measurements was significant ($F(21,14)=6.613$, $p<0.001$); 3) The variability over time across the two groups was not significant, indicating no interaction effect between repeated measurements and the experimental/control groups.

Table 4. Omnibus F tests of between- and within-subject effects for relative delta

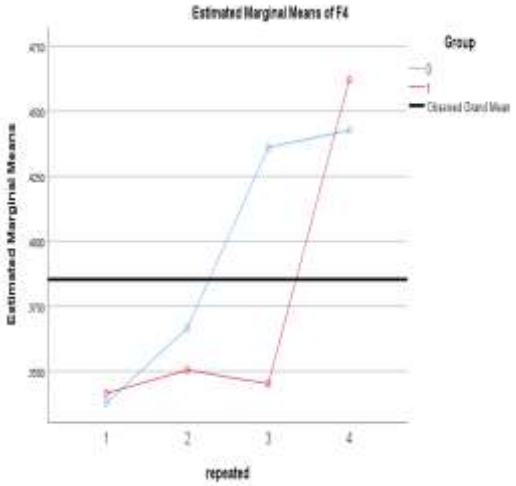
Effect	Value*	F	Hypothesis	Error df	p-value

				df		
Between-subjects (groups)		0.894	0.475	7	28	0.844
Within-subject	repeated	0.092	6.613	21	14	< 0.001
	interaction	0.384	1.070	21	14	0.459

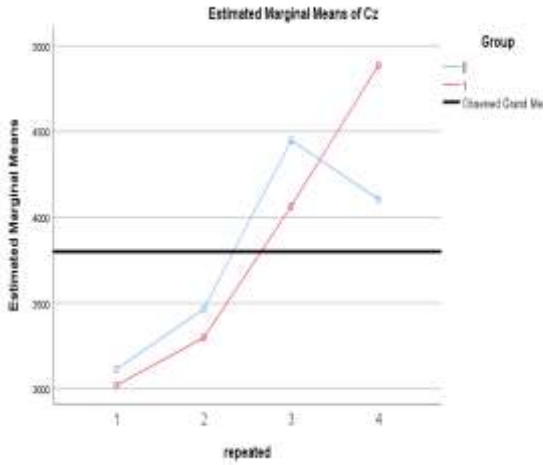
* value: based on Wilk's lambda



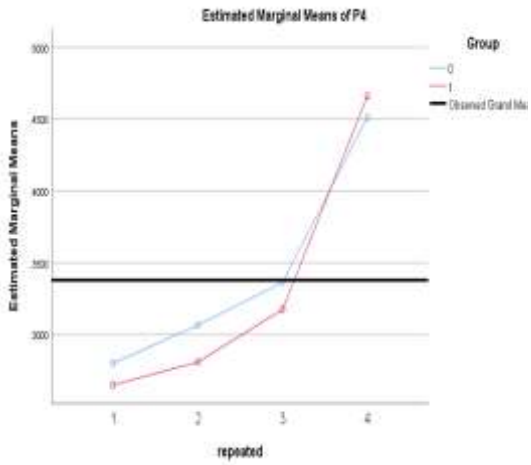
F3



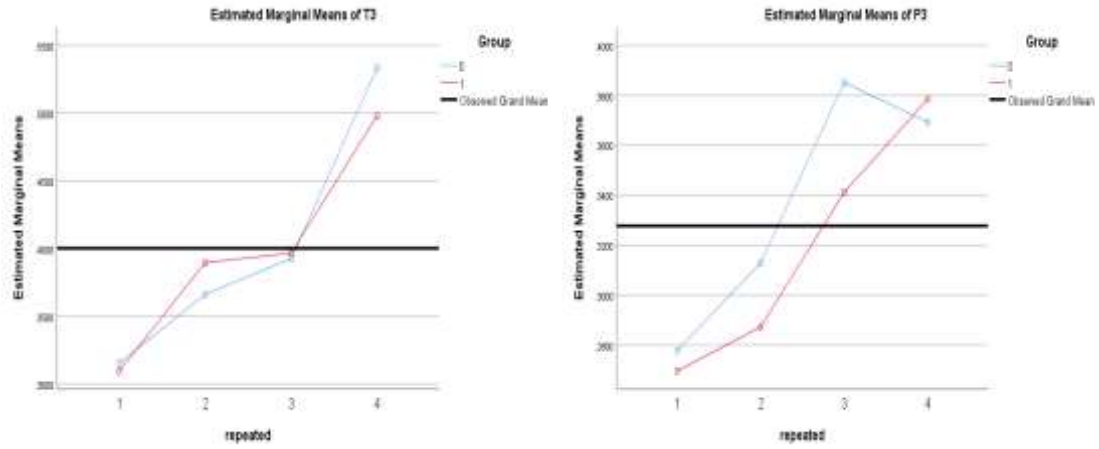
F4



Cz

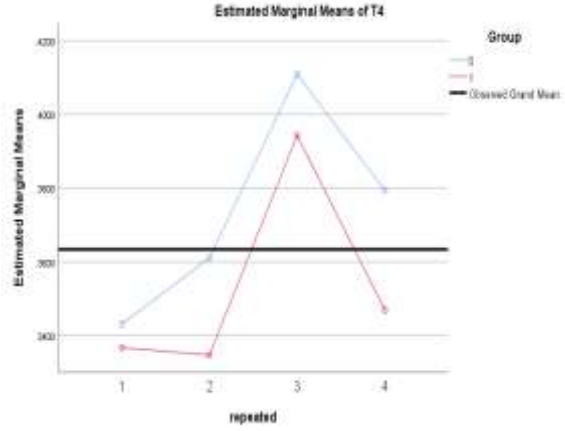


P4



T3

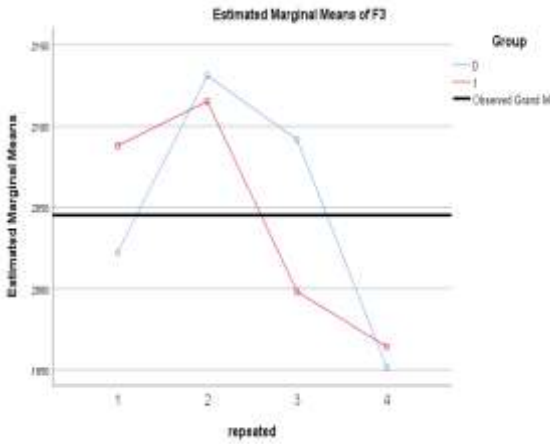
P3



T4

Figure 3. Relative delta

For relative delta, in most of the cerebral lobes, as the EEG was taken 4 times, the activity in the cerebral lobes after humanities lectures learning appeared to sequentially increase. However, at electrode T4, the activity decreased in the 4th measurement. This may be considered to be caused by increased sound fatigue, but the activity was found to increase sequentially at electrode T3.



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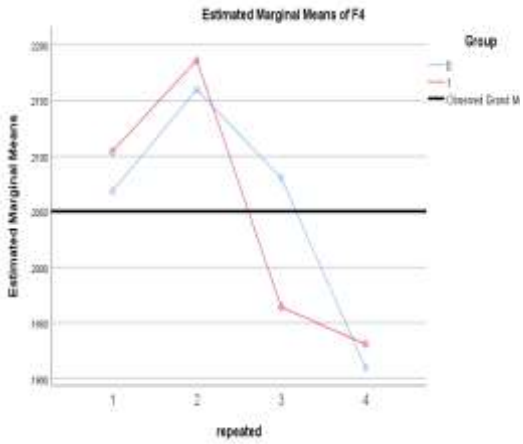
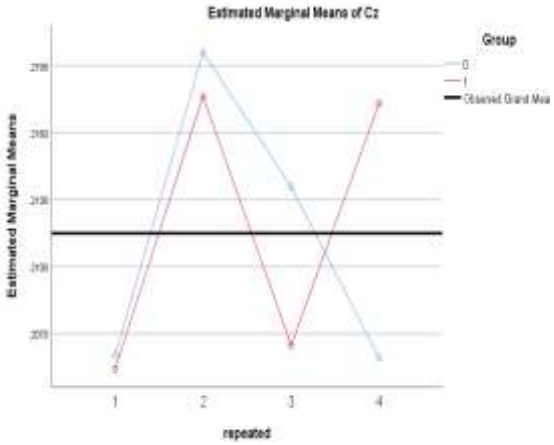
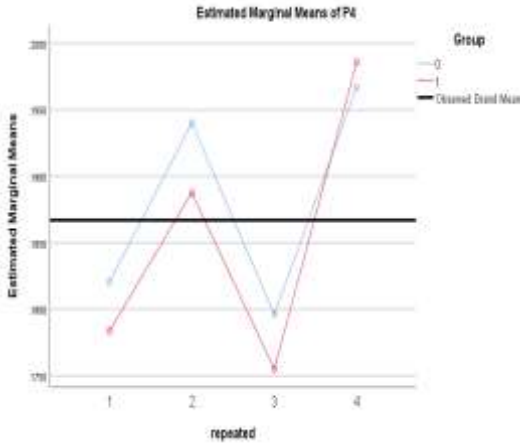


F4



Cz



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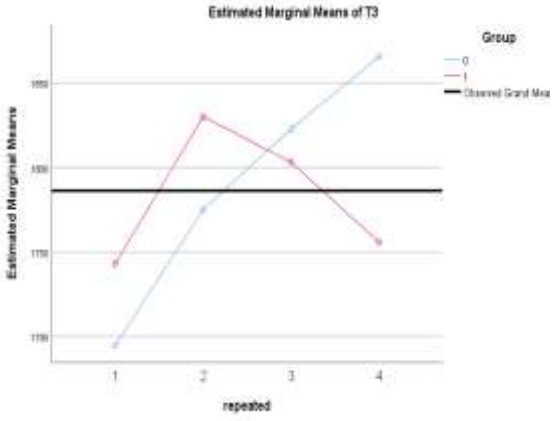

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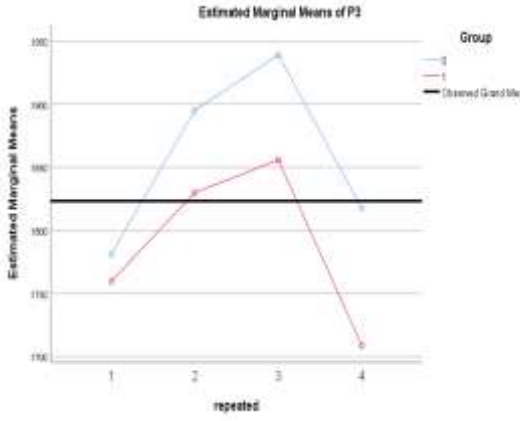

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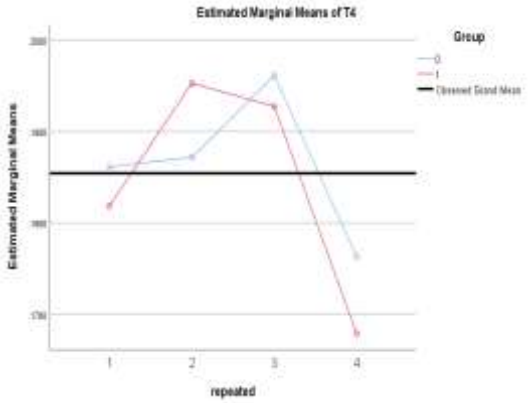
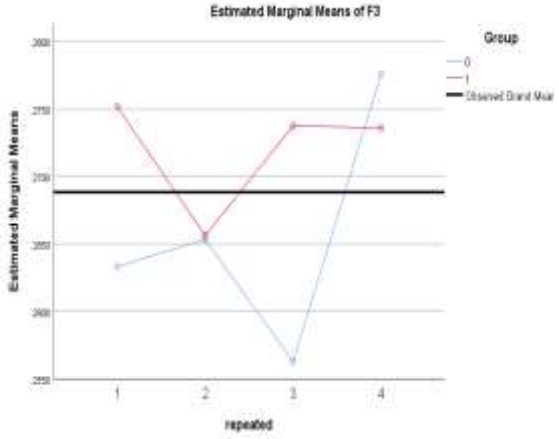


T4

Figure 4. Relative theta

For relative theta, at electrodes F3, F4, P3, and T4, the activity was found to decrease in the 3rd and 4th measurements. At electrode Cz, the activity decreased in the 4th measurement for Group A with both scent stimulation and humanities learning. Notably, the activity at electrode T3 showed a sequential increase according to repeated measurements, while that at electrode T4 showed a decrease like for relative delta.



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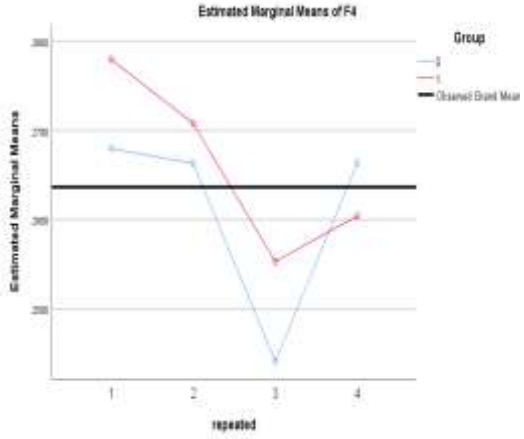
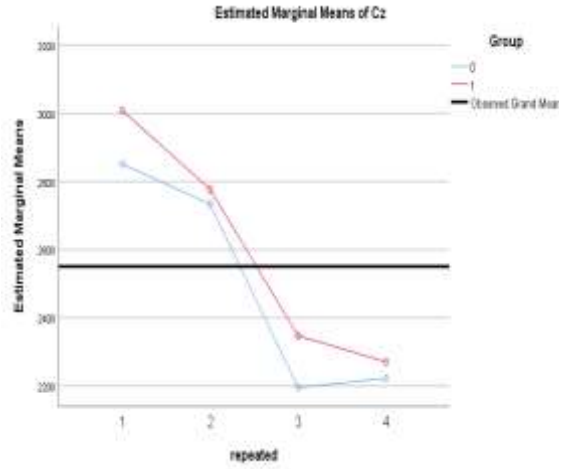
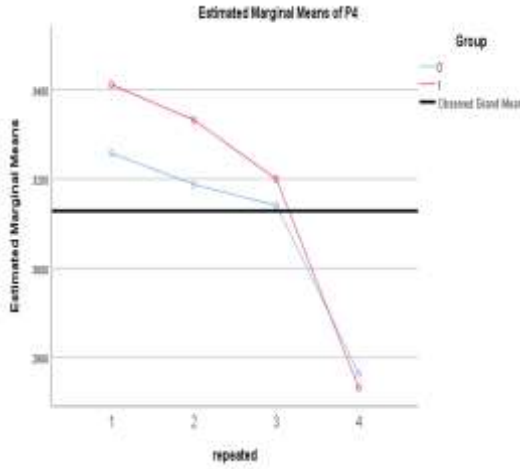


F4



Cz



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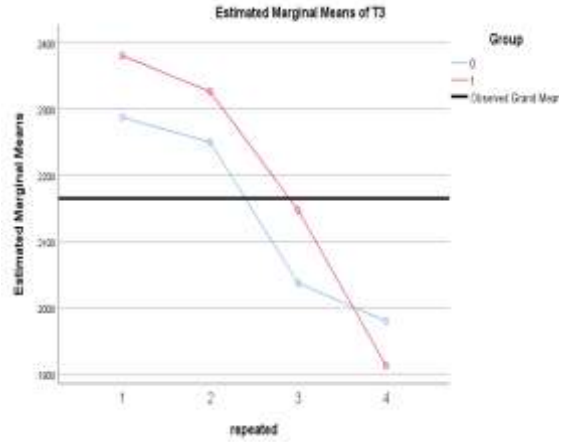

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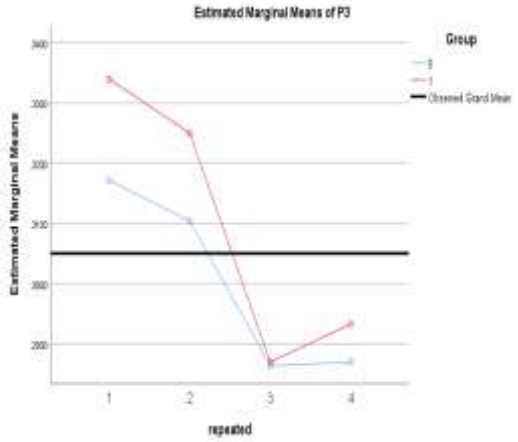

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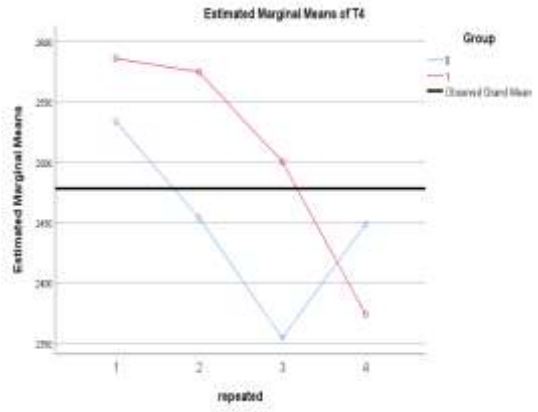

T4

Figure 5. Relative alpha

Relative alpha showed a tendency to be activated again at the 3rd and 4th measurements at electrodes F3 and F4 for Group A.

At electrodes Cz, P3, P4, T3, and T4 for Group A, the activity tended to decrease as repeated measurements were performed. However, at electrode T4 for Group A, the activity was relatively increased in the 3rd measurement.

3.4. Autonomic nervous system (ANS)

The heart rate variability (HRV), a variable that indicates changes in the ANS, showed similarly low activity of the ANS in the 1st and 2nd measurements. In the 3rd measurement, the HRV showed the greatest activity, and in the 4th, a slightly decreased activity.

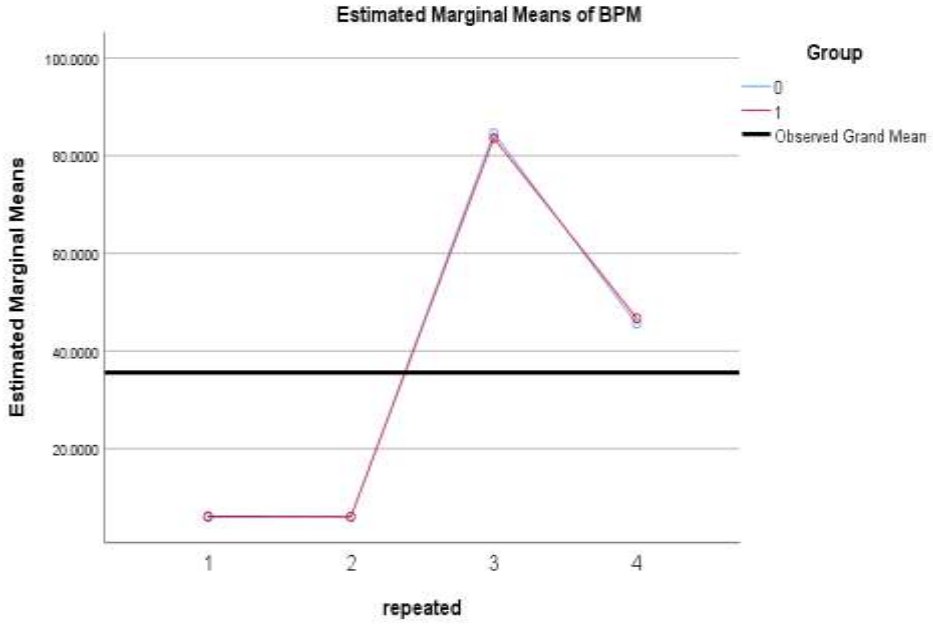

Figure 6. BPM (Beats per minute)

The stress index (SI), another variable that indicates changes in the ANS, also showed a similar tendency to that of the HRV. In the 1st and 2nd measurements, the SI similarly showed a low activity of the ANS. In the 3rd measurement, it showed the greatest activity, and in the 4th, a slightly decreased activity.

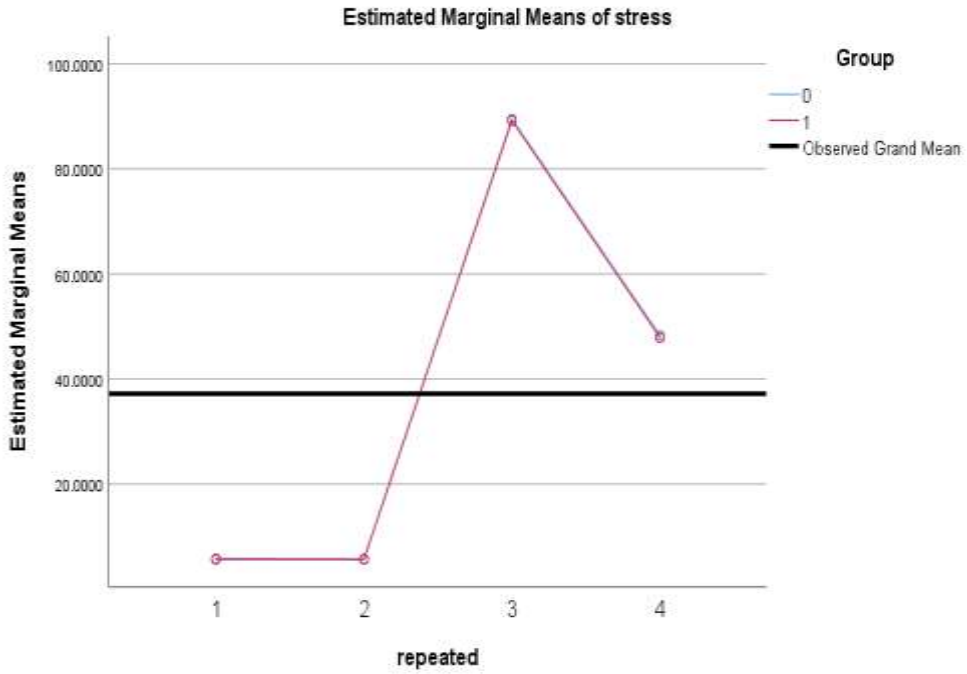

Figure 7. Stress index

4. Discussions

The DPPH radical scavenging ability in Figure 1 showed a concentration-dependent increase. It was reported that Gondre extract was not only effective in hypertension and hemostasis in previous studies, but also had antioxidant effects including anticancer activity on liver cancer cells [23] in various studies. As in previous studies, it seems that the antioxidant effect increased in a concentration-dependent manner.

In Figure 2, flavonoids are contained in almost all parts of Gondre, especially leaves, flowers, stems, and roots. Flavonoids have high antioxidant effects that effectively remove the free radicals that cause cell damage, promoting various physiological activities [24] including anticancer and antibacterial activities. The TFC of Gondre extract was found to increase in a concentration-dependent manner, showing the same results as previous studies.

In Figure 3, an EEG analysis found that for Group A with scent stimulation with Gondre extract and taking humanities lectures, the frequency band was relatively low in the FL (F3, F4) and PL (Cz) areas. A study on neuroblastoma reported that Gondre

extract exhibited a protective effect on brain nerve cells [25]; this finding seems to be similar as it reduces oxidative stress. In follow-up research, we consider that further experiments on the protective effect of Gondre on brain nerve cells are needed.

The brainwave activity at electrode T4 was found to be the opposite to that at the other electrodes. This seems to be because the activity of the TL was affected by the stimulation of sound [26] along with the scent of Gondre extract.

In Figures 4 and 5, the relative theta and relative alpha waves in the cerebral cortex were found to have similar results in the four repeated measurements at electrodes F3 and F4. Excluding the results in the TLs (T3, T4), it seems that scent stimulation with Gondre extract and taking a humanities course had psychological relaxation effects on most of the cerebral lobes by activating theta waves, which are a slow frequency band, and alpha waves, which are a reference frequency; it is expected to have the effect of protecting nerve cells(4), similar to the result of relative delta waves. The results of the TLs (T3, T4) showed a difference between the right TL (T4) and the left TL (T3) for Group A with scent stimulation with Gondre extract. Even if measurements are made under the same conditions in the experiment, it seems that minute changes, including status of the speaker or location of sound in humanities lectures, and subject's condition at the time of measurement, may cause differences between the left and right TLs. Further research on this is considered to be needed.

In Figures 6 and 7, based on repeated measurements of HRV and SI, changes in the activity of the ANS were found in the 3rd measurement, and tended to decrease in the 4th measurement. Although not shown in the results, the relaxed state and the change of the left and right sides were found to be the same, and there was no change in the activity. It seems that scent stimulation or humanities lectures are not sufficient external stimuli to cause changes in the ANS. However, since the small number of participants and the measurement of changes over a short period of time are also considered to be limitations of this experiment, follow-up experiments will be conducted.

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