# A Method for EEG Fluctuation Processing II
## -Application to Pesticide Difference Analysis in Vegetable Ingestion-

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**Summary**
Electroencephalogram (EEG) tests are frequently used for studying psychological influences, transforming original voltage fluctuation with the Fast Fourier Transformation (FFT) or directly deciphering it. Especially, in the FFT method, the power (μV) of α and β waves are analyzed using various sub-methods to evaluate the brain relaxing or stress states since the internal mechanisms of the brain are still unclear, but it is known that these waves have characteristics depending on the brain state. In our previous work, we measured and analyzed EEG fluctuations when eating komatsuna, a green leafy vegetable also known as Japanese mustard spinach, which is usually grown with three kinds (types) of fertilizers (organic, chemical, and a combination of the two) at two densities (×1 and ×2). Ten examinees (five pairs) ate komatsuna, and we measured their brain activity using EEG. We applied analysis of variance (ANOVA) and relational voltage graphs (basic and detailed analyses) that use relational values derived from the initial values to format data. We generated formatted data from the EEG data, using a specialized program we developed in previous research. In the present study, using the same method, we also measured and analyzed EEG fluctuations while examinees ate komatsuna grown with six different pesticides (in other words, insect control techniques): no pesticides, with insect bites, three chemical pesticides (diazinon, affirm, and agrothrin) and a blend of these three pesticides. From the results, there were significant differences in the α waves depending on the pesticides used. Furthermore, some interactions between defined factors including the kinds of pesticides showed significant differences. However, there were no significant differences in the β waves. Thus, these results indicate that pesticides may not influence the stress state of the human brain but may influence the relaxing state.

***Key words:***
*ANOVA, SOC, EEG, vegetable ingestion, pesticide, komatsuna, algorithm, analysis program*

## 1. Introduction

Electroencephalogram (EEG) is a test for measuring the electrical activity of the brain and is frequently used in many fields. Since the EEG test has a long history, it does not require complex measurement environments and is less expensive compared to other related tests. Since EEG responses indicate fluctuations in the electrical activity of the human brain (in other words, psychological influences), there have been many studies that have measured and analyzed the effects of sensory inputs on EEG activity, such as hearing, smell, vision, and taste [1-6]. Wave groups, such as θ, α, β, and δ defined by representative values between frequency bands, are commonly used. More specifically, in EEG research, α and β waves are frequently used to evaluate the effects of relaxation. For instance, these waves are used to evaluate mental states while working [7-8]. We also use these two waves (including sub-α waves) for evaluating brain relaxing and stress states.

There has been no research on EEG measurement and analysis after vegetable ingestion, except our previous work. Researches on EEG analysis while drinking milk or alcohol are similar to our work [9-10]. However, our work is different because we focus on observing EEG while chewing solid foods. We used the original composite methods, which are ANOVA using statistical software and fluctuation graphs. The former assays significant variances of defined factors and their interactions, and the latter compares defined levels in such factors (basic analysis). Furthermore, fluctuation graphs for interactions having significances of ANOVA represent the levels of factors related to these interactions (detailed analysis). In our previous work, we first conducted two preliminary experiments [11]. We mainly described the method to generate formatted data and its automation algorithm and implemented a system for these experiments. We then conducted a full experiment and analyzed the data using this system [12]. We also analyzed the data using a new method that represents detailed graphs. In this work, we conducted and analyzed a full experiment with enough examinees, using the methods we used in the previous experiment. Thus, many parts of this paper are common to the previous paper since the measurement and analysis processes are basically the same.

We measured and analyzed EEG fluctuation in komatsuna ingestion. Our komatsuna was grown using six kinds (types) of pesticides (in other words, insect control techniques): no pesticides (controlled), with insect bites (punching hole into leaves), three chemical pesticides (diazinon, affirm, and agrothrin) and a blend of these three pesticides. The cultivation of the green vegetable komatsuna, also known as Japanese mustard spinach, has

Manuscript received November 5, 2010
Manuscript revised November 20, 2010

existed since the Edo Period (1603-1868) in Komatsugawa, Edogawa Ward, Tokyo, Japan (current geographic name). The komatsuna vegetable was allegedly named after the prefectures where it was originally cultivated, although now this vegetable is cultivated in Tokyo as well as neighboring prefectures. An example of a komatsuna is shown in Fig. 1. A bunch of komatsuna is weighed using an electric scale, as also shown in this photo.

From the analysis results, there were significant differences in the α waves when eating komatsuna grown with the different pesticides. Furthermore, some interactions between defined factors, including the kind of pesticide, showed significant differences. However, there was no significant difference in the β waves. These results indicate that pesticides may not influence the stress state of the human brain and may influence the relaxing state.

Section 2 explains the preparation of our experiment, including the measurement device and overview of ANOVA theory. Section 3 describes the experiment plan, including our cooking method, experiment schedule, and measurement procedure. We present the results from basic analysis in Section 4, and also present the results of detailed analysis in Section 5. Finally, Section 6 concludes the experiment and analysis results.

Fig. 1 Weighting komatsuna (green leafy vegetables)

## 2. Preparation

### 2.1 EEG Measurements

**1) Simple EEG measurement device:** As introduced in the previous section, we used a simple EEG measurement device called the "Brain Builder Unit" for EEG measurement [13]. A photograph of this device, including the electrodes, is shown in Fig. 2. The two electrodes of the headband contact the skin on the forehead, and the other electrode is an electric wire that clips onto the left ear lobe. The device is connected to a PC with the Windows operating system (The requirement is Windows 98 and later: We used Windows XP). EEG measurement software called Mind Sensor II controls the Brain Builder Unit [13]. The software communicates with the measurement device via a serial port and captures and writes out the EEG data. The system measures EEG data of the right and left brain separately, and the measured data series is written to an ".fft" file that has an internal CSV format. Figure 3 shows the data visualization function of the software, which displays a real-time electric pattern during the EEG capturing process. These patterns are of two kinds: one is the raw voltage fluctuation (unit: μV), and the other is the spectrum that indicates each power of frequency using Fast Fourier Transformation (FFT).

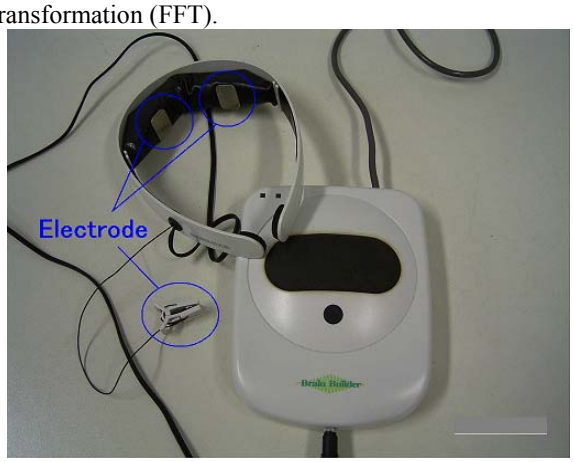


Fig. 2 The Brain Builder Unit with three electrodes

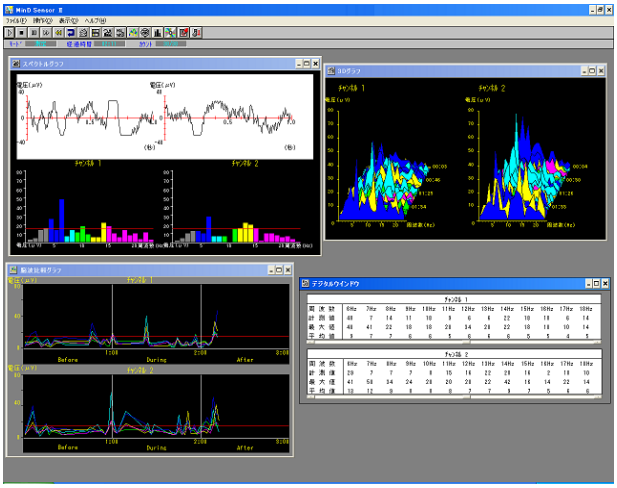

Fig. 3 Display of Mind Sensor II (in Japanese)

**2) Definitions of EEG frequency groups:** The categorized spectrum defined in an EEG frequency grouping is generally used in EEG analysis instead of the raw electrical fluctuation or its spectrum, even though definitions of EEG frequency groupings vary according to the researcher. For example, some EEG books [14-15] introduce their own definitions for EEG frequency groupings. We used the definitions from the manual of the Mind Sensor II software since these definitions are confined to our research and are valid. These EEG frequency groupings are listed in Table. 1.

The δ wave group (hereafter, a wave group is called a "wave") appears in deep sleep, but we could not use this wave since the EEG measurement device mixes electrical noise. We also could not use the θ wave since the examinees were awake. The area of the α wave is separated into three groupings (α-type waves), since these frequencies are the most important for examining relaxation effects. In addition, fluctuations in these frequencies are active only in awake humans. The β wave appears in the attention state, and we compared it with α-type waves. Although the general boundary of the β wave is considered to be 40 Hz, our definition is 23 Hz due to the limitation of the measurement device.

Table 1 EEG types and corresponding mental states

| EEG type | | frequency | mental state | |
|---|---|---|---|---|
| δ | | 1 ~ 3Hz | deep sleep | |
| θ | | 4 ~ 6Hz | light sleep, meditation | |
| α | slow α | 7 ~ 8Hz | relaxing with depressed consciousness | relaxing, creativity uplifting |
| | mid α | 9 ~ 11Hz | relaxing with concentration | |
| | fast α | 12 ~ 14Hz | concentrating with stress | |
| β | | 15 ~ (23Hz) | attention, concentration | |

## 2.2 ANOVA Software for Variance Analysis

For the ANOVA calculations, captured EEG data in the ".fft" file is processed and input into software called JUSE-QCAS Version 7 [16]. This software supports many statistical operations, including the ANOVA function. Figure 4 is a snapshot of the data-editing mode of this software. In this mode, the user can input data to cells that accept integer values, real values, and characters as text labels. The system recognizes the columns of these cells as two kinds of variables: "quality" and "quantity". The quality variables include the "levels" of "factors", and the quantity variables include the analysis data. In Fig. 4, "C3" ~ "C5" are quantity columns and "N6" ~ "N7" are quality columns. The system analyzes quality values (measurement data) according to the factors and allocations of the quantity values (levels of factors). As a result, the software generates a table of ANOVA data (called ANOVA table).

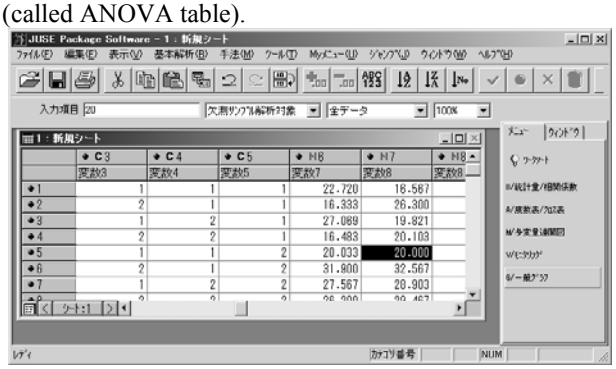


Fig. 4 Display of JUSE-QCAS Version 7 (in Japanese)

## 2.3 Experimental Environments

Our experimental environment and measuring equipment are shown in Fig. 5. The experimental room was enclosed in a tent, as shown in the top left photo. The inside of the tent area is shown in the top right photo and the equipment used for the experiment is shown in the bottom photo. The Brain Builder Unit was connected to the serial port of the PC, and the monitor displayed the functions of the Mind Sensor II software. Lotion was applied to the electrodes of the Brain Builder Unit since they contact skin.

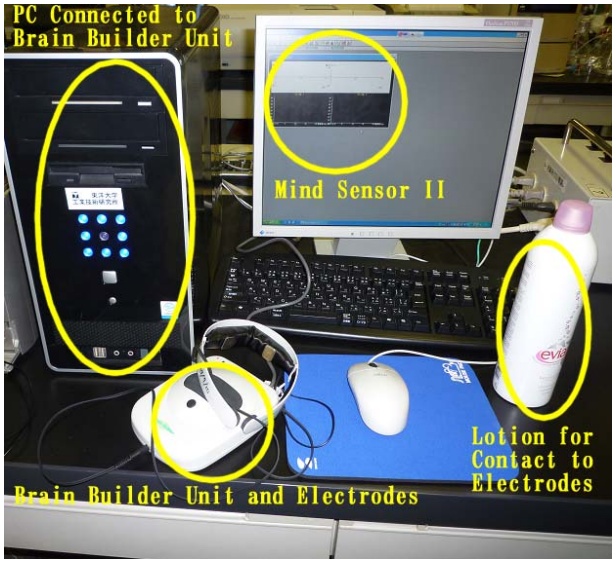


Fig. 5 Experimental equipment for EEG measurements

## 2.4 Basic Concept of ANOVA

The ANOVA statistical analysis method is based on the dependencies of factors related to the movement of measurement values. In this method, independent factors are called "main effects," and factors generated by mixing independent factors are called "interactions." The main factors are denoted as "A", "B" and "C" and the interactions are denoted as "A × B", "B × C", and "A × C". The calculation results are called "p-values" (probability values) and are determined using a function of this method called an "assay." The result of the assay is represented by "**" if the p-value is less than 0.01, or by "*" if the p-value is less than 0.05. The p-value indicates the reliability of the significance. For example, a p-value of 0.5 indicates statistical singular values of 5% that are included in the numerous measured values. In other words, it indicates 95% reliability of the analysis results.

The primary concept of this theory is to express measurement data by the sum of the squares that includes all factors, errors of measurement, and total accidental errors. The formulation is called "structure expression", which is a four-way layout of analysis variance, and is defined in Fig. 6 (we used this expression for our results). The variance meanings of this formulation are as follows: "y" is measurement data, "a", "b", "c", "d" are the level values of factors, "i", "j", "k", "l" are identical suffixes for factors, "m" is an identical suffix for iteration, "ε" is the total accidental error, and "μ" is the error of measurement. The theory of the analysis of variance is explained in other literatures [17-19].

$$y_{ijklm} = \mu + a_i + b_j + (ab)_{ij} + c_k + (ac)_{ik} + (bc)_{jk} + (abc)_{ijk} + d_l + (ad)_{il} + (bd)_{jl} + (abd)_{ijl} + (cd)_{kl} + (acd)_{ikl} + (bcd)_{jkl} + (abcd)_{ijkl} + \varepsilon_{ijklm}$$

Fig. 6 Structure expression of four-way layout

# 3. Experiment Plan

## 3.1 Komatsuna Cooking Method

We prepared komatsuna samples with a precise cooking method, and the examinees ate the cooked komatsuna. In our past experiments "Ex. 1" and "Ex. 2", the cooking method definition had some ambiguities [11]. For instance, the food cutting interval is unclear. For this experiment, a precise cooking method was defined to reduce the errors. We show this method below, which is also used in the previours work [12].

1) Clean 2–2.5 fascicles of komatsuna (one bunch) using tap water, and completely dry them off with paper.
2) Adjust the weight of the bunch to 90 g, cutting and discarding small or bad stems.
3) Add 1g of NaCl to 1ℓ of tap water. Bring the water to a boil using a gas heater.
4) Boil the set of komatsuna for 30 seconds.
5) Squeeze moisture from the boiled komatsuna until they are 90% of their initial weight.
6) Lay the boiled komatsuna onto the cutting board lengthwise, and cut them into 10-cm pieces. This step is shown in Figure 7.
7) Place the cut komatsuna in a plastic container, and mix leaves and stems using a spoon.
8) Affix the corresponding sticker with the encoded kind ("a" ~ "e") onto the plastic container.

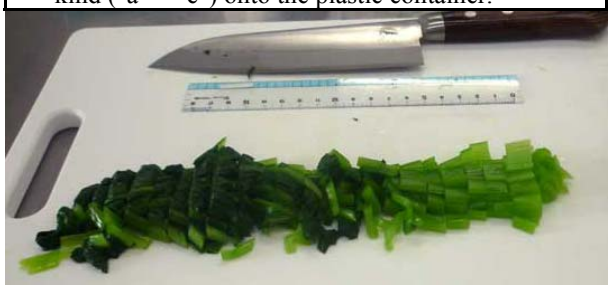

Fig. 7. Boiled komatsuna being cut

## 3.2 Layout of factors and levels for ANOVA

We now explain the measurement condition defined using four factors and their corresponding levels since these condition definitions are needed for ANOVA as described in Section 2.4. The ANOVA method assays significant variances of the "main effects", effects of

individual factors, and "interactions", interactive effects between multiple factors.

The meanings of the factors are "A", the kind of pesticides used on komatsuna (6), "B", the sex of examinees (2), "C", the right or left brain (2), and "D", before, during, or after eating komatsuna phases (3), where the values in parentheses are the number of levels. Table 2 defines the meanings of the factors and levels, and allocates them to labels and numbers for ANOVA. In this table, "controlled" means komatsuna grown with no pesticides, and "insect bitten" means controlled komatsuna with leaves punched with holes to simulate insect bites. Description of "from leaf" means the pesticide is absorbed from the leaf, similarly "from root" means the pesticide is absorbed from the root.

## 3.3 Experiment Order of Examinees

The experiment order was manually shuffled using the random number generation function "RAND" on Excel 2003. This process is required to cancel errors due to historical effect depending on the measurement sequence. Since the historical effect could cause distortion of sense of taste and smell by the previous senses, the shuffle ordering cancels these effects. Table 3 shows the shuffled experiment schedule, which describes the measurement order of examinees. "Start time" indicates the measurement start time for each examinee pair. The experiment for one examinee consisted of two parts, "first half" and "later half". The letters "a" ~ "e" indicate the encoded kinds (pesticides) defined in Table 4. This table also defines the translation rules to rewrite the encoded filenames to make them understandable to avoid any input errors.

Table 2 Four-way layout of the experiment plan for ANOVA

| | factor | | | |
|---|---|---|---|---|
| | A | B | C | D |
| label | pesticide | sex | brain | phase |
| 1 | controlled | male | right | before |
| 2 | bug bitten | female | left | during |
| 3 | diazinon (from root) | | | after |
| 4 | affirm emulsion (from leaf) | | | |
| 5 | agrothlin emulsion + ranman flowable (from leaf) | | | |
| 6 | blend of three pesticides | | | |

Table 3 Experiment plan for the EEG measurements

| | affiliation | name | filename | first half | latter half | start time |
|---|---|---|---|---|---|---|
| pair 1 (female) | 1st grade, Ota lab | | mzaki_x | c f e | a d b | 12:00 |
| pair 1 (male) | 4th grade, Kamijo | | matsu_x | f c b | a e d | |
| pair 2 (female) | 1st grade, Ota lab | | ishi_x | d a e | c f b | 12:35 |
| pair 3 (female) | 1st grade, Ota lab | | fuji_x | c b f | e a d | |
| pair 2 (male) | 4th grade, Kamijo lab (experimenter) | | some_x | e c f | d b a | 13:10 |
| pair 4 (female) | 4th grade, Kamijo | | sakai_x | f e b | d a c | |
| pair 3 (male) | 1st grade, Ota lab | | kuri_x | d c f | a e b | 13:45 |
| pair 4 (male) | 1st grade, Ota lab | | mitou_x | a c d | b e f | |
| pair 5 (male) | 1st grade, Ota lab | | ikeda_x | a e b | c f d | 14:20 |
| pair 5 (female) | 4th grade, Kamijo lab (experimenter) | | taka_x | b c f | e a d | |
| pair 6 (male) | 4th grade, etc. | | saya_x | a d f | e c b | 14:55 |
| pair 6 (female) | 4th grade, Kamijo lab | | sumi_x | e a f | d b c | |

Table 4 Filename translation table

| kind | original name | translated name |
|---|---|---|
| controlled | name_c.fft | name_co.fft |
| insect bitten | name_e.fft | name_ib.fft |
| diazinon | name_a.fft | name_di.fft |
| affirm | name_f.fft | name_af.fft |
| agrothlin | name_b.fft | name_ag.fft |
| blend | name_d.fft | name_bl.fft |

## 3.4 Experiment Condition

The experiment sequence is defined as follows: "Sitting down and being silent (before phase) → eating pieces of komatsuna (during phase) → sitting down and being silent (after phase)". Examinees sit down and be silent during the "before" phase to stabilize their psychological state. The experiments are executed according to the the order defined in Table 3; for instance, "pair 1 female (first half), pair 2 female (first half), pair 1 female (later half), pair 2 female (later half), pair 1 male (first half)...". Both the "examinees" and "experimenters" are blinded so they could not see what kinds of komatsuna are being eaten (double-blind experiment). Details of the experimental conditions are as follows.

- Examinees put on the Brain Builder Unit electrodes.
- All measurements are conducted in the tent-enclosed space, which includes a table for eating the samples. Examinees are instructed to sit down quietly with their eyes open, to avoid α-wave noise.
- The layout of the experiment table is shown in Fig. 8. An edible sample is placed on a sheet of paper resting atop a cup. An alarm timer is located on the corner of the table.
- Six pairs of examinees (12 individuals) participated in the experiment, with one pair comprising a male and female examinee. These pairs are defined in Table 3.
- The experiment sequence is "before → during → after". In the "before" and "after" phases, examinees sit quietly for 20 seconds. In the "during" phase, which lasts 20 seconds, examinees eat a sample of Komatsuna.
- Examinees masticate and swallow a sample of Komatsuna in the "during" phase. They are instructed to masticate more than 10 times before swallowing.

- Examinees are instructed to individually eat the sample since being fed by another person is a rare action that may generate an invalid EEG measurement due to the psychological effect.

Fig. 8. Layout of experimental table

## 4. Basic Analysis and Results

### 4.1 EEG Processing Method for Analyzable data Generation

**1) Manual EEG processing method:** The measurement data were synthesized using our manual EEG processing method (presented in our past research [11]), which is the basic method for our analysis process, and the analyzable data and the basic graph were generated. We manually had processed EEG data files generated from the Brain Builder Unit before the automation method was developed. Table 5 lists the item meanings of EEG data in an ".fft" file on Excel, and the file is renamed to ".csv" before opening. The manual processing algorithm on Excel is as follows.

1. Sort all data by values on columns "A" to split them by brain sides.
2. Insert three row spaces into the bounds between brain sides, and also insert them into the bounds between phases (before, during, after).
3. Sum values on each phase per frequency column, using Excel function "SUM". And, put them into the space cells under data sequences.
4. Select max values on each phase per wave group columns, using Excel function "MAX". And, put them into the space cells under the cells put in the sum calculations.
5. Make a level allocation table including levels of all combinations for ANOVA in a new sheet.
6. Put the max values into the large cells area located in high number of rows outside the data area.
7. Arrange the max data into the new sheet according to the orthogonal table's format.

*The orthogonal table's format is to put data all combinations of data, and it defined in our past work [11].

| cell labels | meanings of data | wave type |
|---|---|---|
| A | right brain = 1, left brain = 2 | |
| B | measurement time from start up (s) | |
| C ~ F | voltages of 0 ~ 3Hz ( $\mu$ V) | noise mainly |
| G ~ I | voltages of 4 ~ 6Hz ( $\mu$ V) | $\theta$ |
| J ~ K | voltages of 7 ~ 8Hz ( $\mu$ V) | slow $\alpha$ |
| L ~ N | voltages of 9 ~ 11Hz ( $\mu$ V) | mid $\alpha$ |
| O ~ Q | voltages of 12 ~ 14Hz ( $\mu$ V) | fast $\alpha$ |
| R ~ Z | voltages of 15 ~ 23Hz ( $\mu$ V) | $\beta$ |
| AA ~ AD | etc. | |

Table 5 Item meanings of EEG data in ".fft" file on Excel

**2) Overview of automation program process:** We devised an automation algorithm for computer processing as a method for reducing complicated manual processing. The algorithm differs greatly from the manual one since automatic processes do not require Excel system. Instead, the algorithm is assumed to be implemented using programs in a development environment. The program implementation of the algorithm is represented in our past paper [11]. The results of our experiment were analyzed using the system described in this section. An overview of this algorithm is described as follows.

#####File Input and ANOVA Data Generation Routine#####
1. Read key inputs on the console window (number of kinds, number of pairs, number of iterations, "before" time, "during" time, "after" time).
2. Read key inputs as a filename and open this file.
3. Add a piece of data into the array, reading the data piece for one column. This process is looped until all data readings from the file have been finished.
4. Average the values of the temporary array when every one phase of "before", "during", and "after" finishes.
5. If the all phases averaging are finished, select the maximum value of the average values per each EEG frequency.
6. Store these data to the data array.
7. Return to 2 if files including unprocessed data exist.
8. Open a new file named "data.csv" by writing mode.
9. Write out the data of the array to the file.
10. Close "data.csv".

###########Graph Data Generation Routine###########
11. Open a new file named "gr.csv" in writing mode.
12. Average the values of the data array per phases of "before", "during", "after".
13. Store these data to the graph array as absolute graph data.
14. Subtract the values of "during" and "after" from the values of "before".
15. Store these data to the graph array as relative graph data.
16. Write out the data of the graph array to the file.
17. Close "gr.csv".

*The memory spaces of the data array and the graph array are allocated on start up (static allocation).

**3) Generating relative data for ANOVA:** The automation program generates absolute data, but the ANOVA for this experiment requires relative data. We generated such data using a manual operation which is described such as below formulation. This means the data are generated by subtracting the "before" value from the value on the "original" sheet. We calculated the relative data on the "relative" sheet, copying by the Excel function "auto fill."

```
=IF($D9=2,original!F9-original!F5,IF($D9=3,origin
al!F9-original!F1,0))
```

## 4.2 Composition of three α waves

The automation program generates formatted and graph data of three α waves (slow, mid, fast) and one β wave for ANOVA and basic graph analysis. However, we focused on two waves, synthesized α waves and β wave, since these waves have the most characteristics for the relaxing and stress states. We calculated the synthesized α wave combining the three waves with the max operation, called "smf α". In other sections, we mention the smf α wave as α wave.

We generated ANOVA data of smf α using the formulation "=MAX(F9,F84,F159)", which means that the datum of the smf α wave is generated using the highest voltage of the data of the three α waves.

The composite graph of the three α waves was calculated using "smf α = max (slow α, mid α, fast α)". This graph represents a comparison of relaxation levels by averaging the values of the three waves. Since this calculation process is simply the average of three waves and not precise, it provides an overview of the difference in pesticides.

## 4.3 The Results of ANOVA

In these experimental results, the p-values of the factors and the interactions of less than 0.2 were added (mixed) to the whole accidental error value (error), using the "auto pooling" function of JUSE-QCAS. The pooling condition is constrained by the relation of each main effect and its dependant interactions, according to the ANOVA theory. Thus, the function determines whether pooling can work and calculates "pooling" automatically. Table 6 lists the ANOVA results that include smf α and β waves in the experiment. The smf α wave indicates the relaxing state of the volunteers as the definition in Table 1. Similarly, the β wave is important for evaluating the active, or stress state, of the examinees.

The ANOVA results of smf α and β waves are listed in Table 6. The result indicates that the smf α fluctuation depends on the combinations of sex, brain side, phase, and kind (type of pesticide). The difference of pesticides may strongly influence the relaxing state of the brain since main effect "A" shows a significance with 99% reliability. Furthermore, interaction "A × C" has a significance of 95% reliability, and interactions "A × B", "A × B × D" also show significances of 99% reliability. Interaction "A × C" indicates EEG fluctuations depending on levels of the pesticide and brain side. Interaction "A × B × D" indicates EEG fluctuations depending on pesticide, sex, and phase. The significance of "A × B" means EEG fluctuations depend on pesticide and sex but not phase, and it also indicates interaction "A × B × D" does not include the effect of "A×D".

However the fluctuations of the β waves show no significances. Since their significances do not include the effects of pesticides, the significances of main effects "C" and "D" are not related to our purpose. For instance, the significance of "C" indicates the difference in EEG fluctuations of the right and left sides of the brain, and the significance of "D" indicates the difference in the "before", "during", and "after" phases.

Table 6 The ANOVA results from the basic analysis

smf α

| Factor | Sum of squares | Freedom degree | Unbiased variance | F0 | Assay | P-value (upside) |
|---|---|---|---|---|---|---|
| A | 129.638 | 5 | 25.928 | 4.069 | ** | 0.009 |
| B | 0.902 | 1 | 0.902 | 0.142 | | 0.71 |
| AB | 153.207 | 5 | 30.641 | 4.809 | ** | 0.004 |
| C | 80.126 | 1 | 80.126 | 12.576 | ** | 0.002 |
| AC | 107.214 | 5 | 21.443 | 3.366 | * | 0.021 |
| BC | 0.199 | 1 | 0.199 | 0.031 | | 0.861 |
| ABC | 69.787 | 5 | 13.957 | 2.191 | | 0.092 |
| D | 6208.646 | 2 | 3104.323 | 487.231 | ** | 0 |
| AD | 134.053 | 10 | 13.405 | 2.104 | | 0.07 |
| BD | 92.931 | 2 | 46.465 | 7.293 | ** | 0.004 |
| ABD | 258.032 | 10 | 25.803 | 4.05 | ** | 0.003 |
| CD | 40.329 | 2 | 20.165 | 3.165 | | 0.062 |
| error | 140.17 | 22 | 6.371 | 0.366 | | 0.997 |
| measurement error | 6259.151 | 360 | 17.387 | | | |
| total | 13674.39 | 431 | | | | |

β

| Factor | Sum of squares | Freedom degree | Unbiased variance | F0 | Assay | P-value (upside) |
|---|---|---|---|---|---|---|
| A | 43.778 | 5 | 8.756 | 1.405 | | 0.247 |
| B | 6.985 | 1 | 6.985 | 1.121 | | 0.297 |
| AB | 56.29 | 5 | 11.258 | 1.806 | | 0.138 |
| C | 28.213 | 1 | 28.213 | 4.526 | * | 0.041 |
| AC | 7.262 | 5 | 1.452 | 0.233 | | 0.945 |
| BC | 2.241 | 1 | 2.241 | 0.359 | | 0.553 |
| ABC | 67.262 | 5 | 13.452 | 2.158 | | 0.082 |
| D | 3598.1 | 2 | 1799.05 | 288.62 | ** | 0 |
| AD | 106.515 | 10 | 10.651 | 1.709 | | 0.119 |
| CD | 24.815 | 2 | 12.407 | 1.991 | | 0.152 |
| error | 211.932 | 34 | 6.233 | 0.712 | | 0.886 |
| measurement error | 3151.479 | 360 | 8.754 | | | |
| total | 7304.872 | 431 | | | | |

### 4.4 The Fluctuation Graphs

We were unable to represent and compare EEG fluctuations depending on the difference in level from the results of ANOVA. The fluctuation graphs represent brain state changes in the waves depending on kinds of pesticide, which are plotted by phase (on the x-axis). Our generation program provides a function to generate graph data with only a fixed pattern, and other factors, except kind and phase are averaged. Thus, the basic results of fluctuation graphs do not reflect the ANOVA results. The purpose of basic analysis is to see effects of kinds; therefore, more details are discussed in the detailed analysis.

The results of fluctuation graphs are shown in Fig. 9, and two types of graphs are represented. The left graph represents fluctuations of β waves, and the right one represents fluctuations of smf α waves. Since the ANOVA results of the β waves show no significances, the values of the β wave graph do not indicate any significant differences. The ANOVA results are prior to graph results, and "no significance" means that the difference in plotted values in a graph is smaller than that for errors.

In the "during" phase in the smf α graph, komatsuna grown with "controlled" and the pesticide "diazinon" showed a similar effect, and the voltages of other pesticides were higher. This indicates that examinees eating komatsuna grown with "insect bitten" and the pesticides "affirm", "agrothrin" and "blend" were in a deeper relaxed state than when eating those grown with two other pesticides. "Insect bitten" had a similar effect to the other three pesticides since the effects of "insect bitten" and "controlled" have obvious differences. The results indicate that punching holes into leaves may generate a pesticide-like effect.

On the "after" phase in the smf α graph, "controlled" and "insect bitten" may have similar effects due to similar voltages. The results indicate that the pesticide-like effect of punching holes into leaves may be none or few, and the reason may be the difference in pesticide components. The effect of the pesticide "diazinon" could be the smallest due to the fact that it had the lowest voltage of the three pesticides (affirm, agrothrin, blend), and the reason for this difference may be that this pesticide is absorbed from the root. The effects of the three pesticides are similar to those of the same pesticides in the "during" phase.

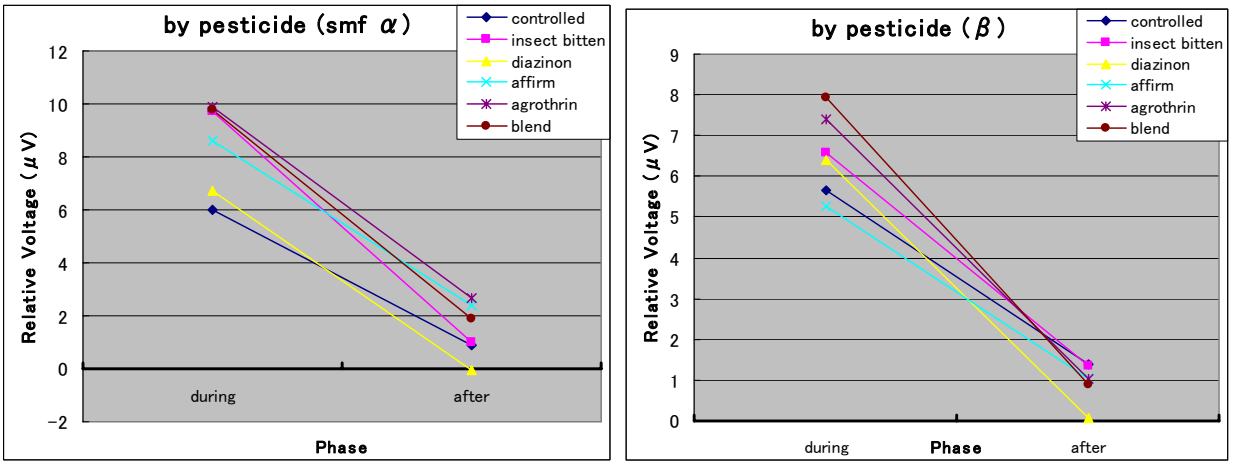


Fig. 9 Fluctuation graphs from basic analysis

## 5. Detailed Analysis

### 5.1 Basic Concept and Methodology

In the previous section, the fluctuation graphs of the basic analysis were only analyzed with the fixed graph layout, which consisted of phase (x axis) and relative voltage (y axis). This fixed graph does not reflect the ANOVA results as in the previous analysis; thus, we introduce the concept of "detailed analysis". This detailed analysis represents the difference in levels depending on interactions using the ANOVA results. Fluctuation graphs of significant interactions are represented (only including kinds). We manually constructed fluctuation graphs using the data of basic analysis, and the formulations below are samples of graph point data.

**["controlled - during" on the graph "smf α, A-D"]**

{=SUM((relative!$A$309:$A$380=1)*(relative!$D$309:$D$380=2)*(relative!$E$309:$J$380)/6)/SUM((relative!$A$309:$A$380=1)*(relative!$D$309:$D$380=2))}

**["diazinon - left after" on the graph "smf α, A-CD"]**

{=SUM((relative!$A$309:$A$380=3)*(relative!$C$309:$C$380=1)*(relative!$D$309:$D$380=2)*(relative!$E$309:$J$380)/6)/SUM((relative!$A$309:$A$380=3)*(relative!$C$309:$C$380=1)*(relative!$D$309:$D$380=2))}

A detailed graph point is calculated, selecting and adding needed values on the "relative" sheet. Unnecessary values are eliminated as zero using equal evaluations (=) and multiple calculations (*), and finally the sum is averaged. These values are more precise than those from basic analysis since the basic analysis generates relative graphs from the absolute graphs.

The calculation image of the detailed analysis is shown in Fig. 10. The blue lines denote selected data by sex "male" and phases, and the red lines denote selected data by sex "female" and phases. The left side of the orthogonal table (the rows of "type", "sex" and "phase") means that data allocation is used in selecting data.

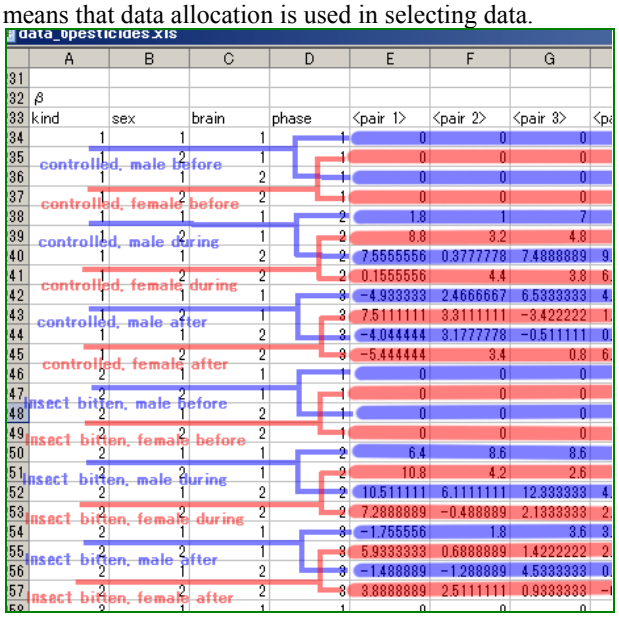


Fig. 10 Calculation of detailed data on Excel 2003

## 5.2 The Detailed Fluctuation Graphs

The fluctuation graphs from detailed analysis results are shown in Fig. 11 and Fig. 12. The graph result of the β wave is invalid since the ANOVA results have no significances related to kinds (pesticides), and it is showed for reference of detailed analysis of β wave. In other words, statistically, it indicates that the difference in pesticide does not influence the stress states of examinees. The variations of the detailed graphs of smf α wave are based on the ANOVA results, and only the graphs for significant interactions related to factor "A" are represented. In fact, Figure 11 and 12 show the combinations of "A, D", "A, B, D", "A, C, D", where "D" is specified as the x-axis.

The "smf α, A-D" graph indicates similar results to the basic analysis, and the difference is only with the calculation method. Since the detailed analysis method is more precise than the basic analysis method, the detailed graph results are more reliable. The pesticide "diazinon" and "controlled" had the most similar voltages in the "during" and "after" phases. Thus, the results indicate the effect of "diazion" on EEG fluctuations may be equal to the effect of the "control". However, in the "A-BD" and "A-CD" graphs, the plots are not similar to each other; the effects of male and female have obvious difference. In common with the basic analysis results, the effect of the pesticide "diazinon" is lowest except for "A-BD, female". The reason for this difference compared to other pesticides (agrothrin, affirm, blend) may be that this pesticide is absorbed from the root, as also mentioned in the basic analysis.

From the general results of the detailed analysis, the fluctuations of smf α indicate that eating komatsuna grown using the pesticides "diazinon", "affirm" and "agrothrin" relax the examinees' brain state more than the controlled komatsuna in the "during" and "after" phases. Furthermore, "insect bitten" may have a similar effect to these pesticides. In the A-BD graph, EEG fluctuations of "male" and "female" represent obvious difference in both "during and "after" phases. Similarly, in the A-CD graph, the EEG fluctuations of "right brain" and "left brain" represent obvious differences. In particular, the graph values of "insect bitten" in "AB-D, male" and "AC-D, left brain" represent the highest voltages in the "after" phase.

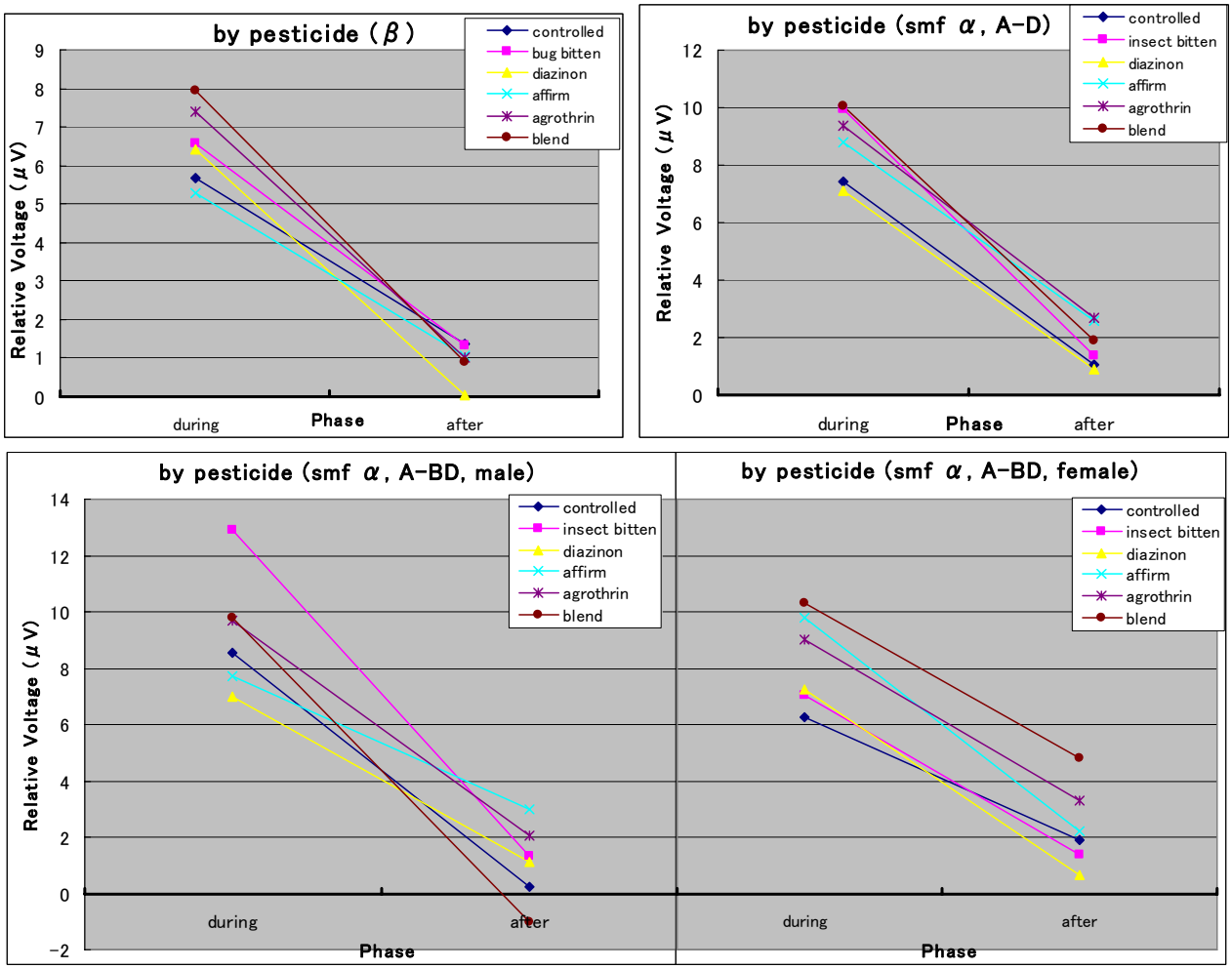


Fig. 11 Fluctuation graphs from detailed analysis (1)

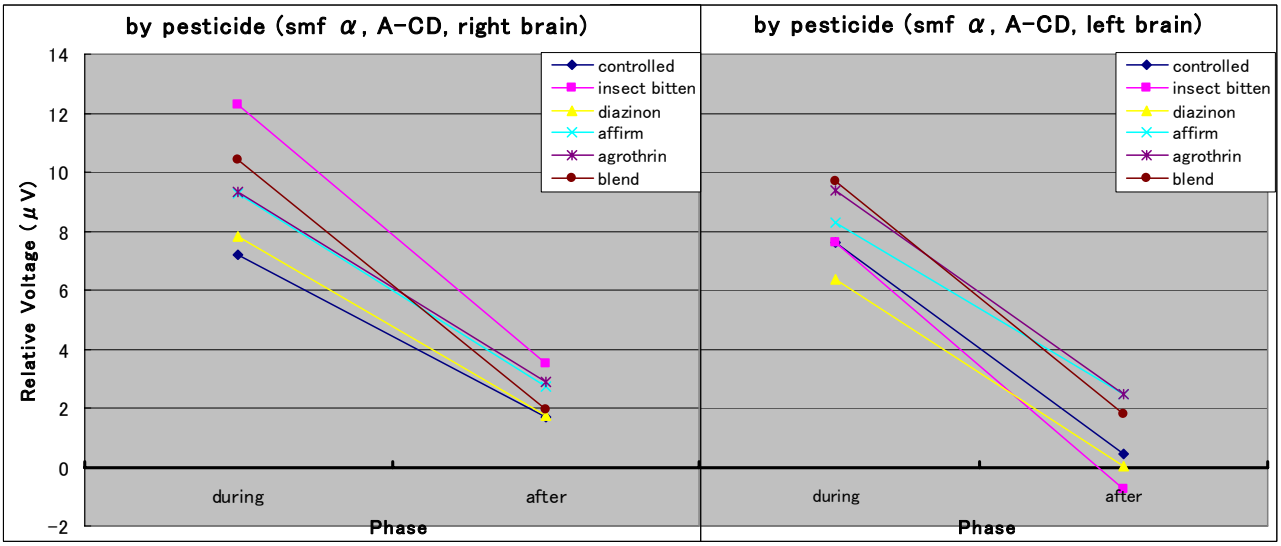


Fig. 12 Fluctuation graphs from detailed analysis (2)

## 6. Conclusion and Future Works

We measured EEG fluctuations during ingestion of komatsuna using the Brain Builder Unit EEG device. We analyzed the fluctuations by ANOVA and fluctuation graphs (basic analysis) using our analysis program, JUSE-QCAS, and Excel 2003. We found that the α wave (as smf α) during ingestion of komatsuna shows significant differences depending on the kinds (pesticides) used to grow them. In particular, eating komatsuna grown using "controlled" (no pesticides) appeared to generate different EEG reactions than eating komatsuna grown with other pesticides since the α waves in the "during" and "after" phase explicitly showed different voltages. We also found that some interactions between the defined factors, including the kinds (pesticides), show significant differences (detailed analysis). Notably, the α waves of males and females show different fluctuations, as indicated by the interactions of ANOVA and by the detailed graphs. EEGs during komatsuna ingestion reflecting the significances of the interactions are also represented in the detailed graphs (including interactions). However, β wave does not show any significant differences. Thus, the results indicate pesticides may not influence the stress state of the human brain and may influence the relaxing state. We conclude that the pesticides techniques used for growing komatsuna may influence the α waves of EEG tests, in our experimental conditions.

For future work, we will design an automation algorithm for the detailed analysis described in Section 5 and develop a program based on this algorithm to reduce workload and expedite analysis. We will then design and develop a more flexible processing system with an easier user interface. For instance, experimental data can be inputted using a simple setting file.

### Acknowledgments

We thank Masako Ota who prepared komatsuna according to our cooking method (Department of Food Life Sciences, Faculty of Life Sciences, Toyo University, an associate professor). We thank Emi Takahashi, Miu Sakai and Yoshio Someya who assisted in our experiment (Faculty of Life Sciences, Toyo University, fourth-year students at the completion of the present study). We also thank the examinees who participated in this study (Department of Food Life Sciences, Faculty of Life Sciences, Toyo University, students) for allowing us to measure their EEGs that were used in the analyses.

## References

[1] G. Neil Martin, "Human electroencephalographic (EEG) response to olfactory stimulation. Two experiments using the aroma of food," Int J Psychophysiol, Vol.30, pp.287-302, 1998.

[2] J. C. Hashida, A. C. de S. Silva, S. Souto and E. José Xavier Costa, "EEG pattern discrimination between salty and sweet taste using adaptive Gabor transform," Neurocomputing, Vol.68, pp.251-257, Oct.2005.

[3] M. Nuki, K. Nagata and H. Kawakami, "The Relations among EEG, Mood, Preference, Personality and Spectrum Power analysis in Listening to Healing music," IPSJ SIG Technical Report, Vol.2004, No.111, pp.35-40, Nov.2004. (in Japanese)

[4] T. Sakurai, M. Nakagawa, "A Study of EEG Dynamics with Photic-Stimulation," IEICE Technical Report, Vol.96, No.569 (NLP96-159), pp.1-8, Mar.1997. (in Japanese).

[5] M. Onoda and T. Noji, "Verification of Effect of Smell Healing by Brain Wave," Proceedings of the IEICE General Conference, Vol.2006 Engineering Sciences, pp.225, Mar.2006. (in Japanese)

[6] Y. Matsuo, "EEG changes by odors of preferable drinks: the effects of coffee and whisky odors on α-wave," The Japanese journal of taste and smell research, Vol.6, No.2, pp.203-210, Aug.1999. (in Japanese)

[7] "A Five-year Study on the Relationships between α-Waves and Business Performance for a Japanese Businessman," Journal of International Society of Life Information Science, Vol.18, No.1, pp.232-241, Mar. 2000.

[8] "Quantifying Mental Relaxation with EEG for use in Computer Games," ICOMP 2006, pp.409-415, June 2006.

[9] J. Sorbel, S. Morzorati, S. O'Connor, T. K. Li and J. C. Christian, "Alcohol effects on the heritability of EEG spectral power," Alcoholism Clinical and Experimental Research, Vol.20 No.9, pp.1523-1527, 1996.

[10] N. W. Grandstaff, "Frequency analysis of EEG during milk drinking," Electroencephalography and Clinical Neurophysiology, Vol.7, No.1, pp.57-65, July 1969.

[11] T. Ajiro, A. Yamanouchi, K. Shimomura, H. Yamamoto and K. Kamijo, "A Method for Structure Analysis of EEG Data -Application to ANOVA in Vegetable Ingestion-," IJCSNS, Vol.9, No.9, pp.70-82, Sep. 2009.

[12] T. Ajiro, K. Shimomura, H. Yamamoto and K. Kamijo, "A Method for EEG Fluctuation Processing -Application to Fertilizer Difference Analysis in Vegetable Ingestion-," IJCSNS, Vol.10, No.10, pp.66-77, Oct. 2010.

[13] "Brain Function Research Center Inc," http://www.alphacom.co.jp/.

[14] T. Okuma, "Rinsho-Nohagaku," Igaku Shoin Co.,Ltd, Tokyo, Nov. 1963. (in Japanese)

[15] A. J. Rowan and E. Tolunsky, "Primer of EEG: With A Mini-Atras," Butterworth-Heinemann, Mar. 2003.

[16] I-JUSE: The Institute of Japanese Union of Scientists & Engineers," http://www.i-juse.co.jp/.

[17] K. Kamijo, K. Maekawa and C. Nakabasami, "Introduction to Informatics by Personal Computer," Kougaku Tosho Co.,Ltd, Tokyo, 1999. (in Japanese)

[18] H. Nakazato, K. Kawasaki, N. Hirakuri and A. Otaki, "A Text for Design of Experiments Method for Quality Control

(revision and new edition)," Union of Japanese Scientists and Engineers, Tokyo, 1993. (in Japanese)
[19] H. Scheffé Henry, "The Analysis of Variance," Wiley-Interscience, New York, Feb. 1950.

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