Chapter

Smelling "Zuko": Incense Rubbing into the Hands and Smelling the Hands Activates Specific Brain Regions

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Abstract

The purpose of this study is to clarify the effects of the smelling "Zuko", incense rubbing into hands and putting the hands for the human brain. From our previous studies on the smelling an incense odor with putting the hands together, the activities of "Zuko" incense are also considered to be promoted as the imitation of habitual behaviors by mirror neurons and the default mode network in our brain. In this experiment, the brain activation was measured in 10 healthy adult volunteers who did or did not have a habit of putting their hands together and magnetoencephalography (MEG) data were recorded while the participants smelled "Zuko" incense and putting their hands together. The peak response of MEG P300m for the "auditory odd-ball paradigm" was also measured for a rare auditory pulse stimulation and was more enforced by the smelling "Zuko" incense. We used alpha-amylase value as an index of the stress state measured in the state before and after smelling "Zuko" and MEG experiments. From these results it can be considered that smelling "Zuko" promote the excitation of the higher activities to human brain and make changing the specific brain areas such as OFC, F5 and V1.

Keywords: "Zuko" rubbing into the hands, OFC, P300m, alpha-amylase, MEG, spatiotemporal dipole fit, time-varying analysis

1. Introduction

In general, incense can be classified mainly into two groups, one of which is a kind of smelling the odor of stick by burning it and another is smelling the odor of "Zuko" rubbing into the hands. There are two major types of incense in Japan, one of which is direct burning incense of sticks and another is not burning and called "Zuko," smelling incense rubbed into the hands by using small pieces of powders [1, 2].

In Japanese smelling "Zuko" rubbed into the hands has been recently often applied to the usage as a kind of smelling incense in daily life. The special meaning of "Zuko" is known as directly smelling the hands with rubbed incense and cleaning the self by the hands with cleaning his/her emotion at the same time.

In these experiments, we are trying to study how "Zuko" has the effects to the human brain.

It is known that olfactory neuronal processing was found in the orbitofrontal cortex (OFC) in the human brain from the previous studies [3–5]. In the recent

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researches, sniffing and smelling were important function of the "active olfaction" [6, 7]. On the other hand, imitation of smelling hands and the behavior of putting the hands together were investigated as an activation of mirror neurons and an operation of the default-mode network [8–12].

In the analysis of brain activity, fMRI, PET, EEG, and MEG are usually applied. In general fMRI and PET are suitable to measure the metabolism of physiological activities but not suitable to measure the real-time changes of neuronal activities. On the other hand, it is known that the advantage of MEG is suitable to obtain the real-time changes of the presiding neural activities in the brain by millisecond time resolution [13, 14]. MEG method is more excellent than EEG method because no distortion of an electro-resistance in the brain was found. So, we applied MEG experiments to this study for the estimation of signal source in the brain.

In our MEG experiments, we used to trace the cortical current by the first-order differential planar type of DC-SQUID sensors. This MEG sensor system has the greatest advantage of using the differential planar type of device.

The determination of a current source is very precise and useful because the current source exists in the maximum of absolute magnetic field values [15]. This estimated main current source was the largest dipole, and the second and the third current dipoles were smaller and weaker than the first main dipole.

To improve up fittingness of the estimation, we applied "spatiotemporal dipole fit theory" introduced by Scherag et al. [16] in which the time-varying amplitude of each dipole was applied at every 50 ms intervals. For the estimation of the signal source, we applied time-varying analysis method to obtain the most suitable MEG dipole which is called equivalent current dipole. From this time-varying analysis, we obtained the most suitable single dipole at every 50 ms in real-time analysis continuously.

The event-related responses (ERPs) in the human brain were studied as an inner mental state or the various psychological factors having an inner origin in the brain, for example, using measuring brain waves and so on. A P300 response peak in brain waves was researched as a response of "cognitive function" by using "oddball paradigm" experiment [17–19]. This P300m response (the magnetic P300 response peak is called as P300m in the MEG experiment) was investigated for the olfactory cognitive function, too. From these reasons, we can study P300m response to test the cognitive ability of olfaction [20].

The alpha-amylase value in the saliva is known as a kind of marker and an index of stress states in human [21, 22]. So we can have alpha-amylase in the saliva to test the stress state for the response of olfactory function in human before or after smelling "Zuko" incense.

The purpose of this study is to clarify that smelling "Zuko" incense rubbing into the hands and putting the hands together more activate the human brain than smelling incense odors using sticks burned such as the responses obtained from our previous study and to show how specific areas in the brain are activated.

2. Materials and methods

2.1 Materials

In this MEG experiment, "Zuko" incense rubbing the powder into the hands is used.

These materials were produced especially by Nippon Kodo Co. Ltd. in Japan as follows. This new material is called as a code name "Nou-Katsu-Gassho-Ko" (in Japanese) including the following three basic materials:

1. Agarwood (*Aquilaria agallocha*, *Aquilaria agallocha* Roxburgh)

2. Sandalwood (*Santalum album*, *Santalum album* L)

3. Benzoin (*Styrax tonkinensis*, *Styrax tonkinensis* Craib et Hortwick)

2.2 Subjects

From the previous 11 Japanese volunteer subjects, in this analysis 10 subjects (5 males, 5 females) between the ages of 22 and 58 years (mean age 41 ± 11 years) were chosen. These subjects were tested by using the previous two types of incense sticks for the effects of the simultaneous smelling incense odor and putting the hands together. However, for only one subject N1 in the previous 11 subjects, it was tested how his brain showed the response to smelling a "Zuko" incense into the hands and putting the hands together by using the analyses of MEG and MRI experiment [23].

On the other hand, in this experiment new other 10 subjects (5 males, 5 females) between the ages of 31 and 73 years (mean age 54.1 ± 7.8 years) who were selected with higher ages than the above subjects participated. They were tested by using "Zuko," an incense which were rubbed into their hands for the effects of simultaneous smelling."

All subjects had no significant smell loss, and they were given the informed consent perfectly by the ethical committee on human studies under Helsinki treaty in both AIST and Aino University in Japan.

2.3 Methods of MEG experiments and algorithms of source estimation

2.3.1 Signal source estimation using the theory of "spatiotemporal dipole fit"

In this study, we applied to the signal source an estimation by using "spatiotemporal dipole fit" theory [16]. We obtained the value of an estimated current dipole continuously using a unit time step by step at every 50 ms, in turn. From these time-varying analyses, the most suitable dipole was obtained at the most reliable time for MEG data in the experiment. This "time-varying analysis" is the method using time-varying covariate (also called time-dependent covariate) in statistics, particularly in survival analyses. It reflects the phenomenon that a covariate is not necessarily constant through the whole study to get the suitable higher goodness of fit (GOF) for the estimation [24].

In this study, we were selecting the most suitable dipole from these dipoles estimated in time varying at every 50 ms. In these single dipoles for this time-varying estimation method, the most reliable ECD was of course obtained as a very higher goodness of fit (GOF) more than 80% by using the above time-varying analysis. These ECDs were fitted using iterative algorithms which estimated the source parameters in order to explain the MEG data as accurate as possible [25, 26]. A smoothing spline is also used to propose a novel model for the time-varying quantile of the univariate time series using a state-space approach. A correlation is further incorporated between the dependent variable and its one-step-ahead quantile. Using a Bayesian approach, an efficient Markov chain Monte Carlo algorithm is described where we use the multi-move sampler, which generates simultaneously latent time-varying quantiles [27].

In our source reconstruction analysis, three main components were characterized. The first was related to the definition of the solution space, and the second was reconstructed by the information of the physical and geometrical characteristics of the head. The third was treated by modeling the propagation of the source electromagnetic fields through various tissues in the brain [28, 29]. In these inverse operations, a forward model was used according to some criterion, a unique source distribution to get the unique inverse solution.

2.3.2 Data acquisition

Our planar type DC-SQUD system is useful for the determination of the current dipole of brain activity source where it exists at the maximum of absolute magnetic field value. Data acquisition was applied after starting the signal during the time of 500 ms by using MATLAB software. In our MEG experiment, the subjects sniff an incense odor by using his own nose, and when he starts to sniff, he pushed the optical sensor button as a trigger. To record time-varying MEG amplitude value, we used a sampling interval every 50 ms.

2.3.3 ICA algorithms

This independent component analysis (ICA) program [30] was applied to our input data of MEG experiments. ICA is one method of blind source separation and a computational method for separating a multivariate signal into additive subcomponents. In ICA algorithms, if the subcomponents are non-Gaussian signals, they are statistically independent from each other. The number of components was five for the estimation. The criteria of ICA estimation on the total five components for selecting are determined to 85% (independent rate) to all other components (non-Gaussian components) of data.

As a general definition of ICA algorithms, the MEG data are represented by the observed random vector: x = $(x_1,\ldots,x_m)T$ and the hidden components as the random vector s = $(s_1,\ldots,s_n)^T$. The task is to transform the observed data x, using a linear static transformation W as $s = Wx$ into an observable vector of maximally independent components s measured by some function $F(S_1,\ldots S_n)$ of independence.

2.3.4 MRI system

This MRI system is a 0.4 T Hitachi open type MRI system (AIRIS-Light MRI system, permanent magnetic type, made in Hitachi Co. Ltd. in Japan). These experiments were performed in the Kansai center in Ikeda city, National Institute of Advanced Industrial Science and Technology (AIST) in Japan.

2.3.5 Measurements of the stress state using alpha-amylase activity by sipping the subject's saliva

In general, the stress state in human is evaluated using salivary alpha-amylase activity (sAA) to evaluate the change of the autonomic nervous function by sipping the subject's saliva [31–33]. We used a sheet of polyethylene terephthalate as a chip to collect the subject's saliva by putting it under the subject's tongue during about 30 s. After the collection of the subject's saliva, this chip was soon inserted in an enzyme analyzer (NIPRO Co. Ltd.: type T-110-N in Japan) to detect salivary alpha-amylase activity (sAA) during about 60 s.

In these experiments, magnetoencephalography (MEG) was performed, and for the experiments of the stress state using the alpha-amylase activity by sipping, the subject's saliva was measured at the second times (the first measure, before experiments of MEG, and the second measure, after smelling "Zuko" incense rubbing into the hands and measuring the response of the brain using MEG experiments).

2.4 An MEG experiment for the previous smelling "Zuko" incense and putting the hands together for one subject in the previous 11 subjects

For only one subject N1 in the previous 11 subjects, it was tested how his brain showed the response to smelling "Zuko" incense into the hands and putting the hands together by using the analyses of MEG and MRI experiment [23].

2.5 MEG experiments for five mode states

MEG response data were measured at the following five mode states, (1) control state, (2) cognitive testing mode using "auditory oddball paradigm" without smelling "Zuko" incense rubbing into the hands, (3) smelling "Zuko" incense into the hands mode without putting the hands together, (4) the mode of smelling "Zuko" incense rubbing into the hands and putting the hands together, and (5) cognitive testing mode using "auditory oddball paradigm" with smelling "Zuko" incense into the hands.

1. MEG experiments for control state

Control state was measured under no smelling "Zuko" and no putting the hand together.

2. For the next mode, cognitive testing mode using "auditory oddball paradigm" without smelling "Zuko" incense rubbing into the hands, regardless of whether the subject did or did not have the habit of putting his or her hands together or praying in daily life.

During this cognitive testing mode using "auditory oddball paradigm" without smelling "Zuko" incense rubbing into the hands, the subject held the optical sensor by a hand and pushed the button with the right thumb quickly when he/she caught a rare tone. The averaged MEG response was measured by adding the raw MEG data collected about 100 times by pushing the optical sensor button. By using the above mode, we tried to measure the subject's cognitive ability on the peak of the so-called P300m of cognitive MEG response and own singular characteristic active area for cognition, and we have examined to compare how the brain activity is different for the habit and no habit behavior of putting the hand together in daily life.

3. Next, in the mode of smelling "Zuko" incense into the hands without putting the hands together, the subject rubbed "Zuko" incense into his/her both hands in advance at the preparation room. In this MEG experiment, he/she smelled "Zuko" incense of his/her one hand and at random time pushed the optical sensor button with his/her thumb by another hand. We measured the MEG response of "Zuko" incense into the hand and obtained the active brain area and have examined to compare how the brain activity is different for the habit and no habit behavior of putting the hand together in daily life. The averaged MEG response was measured by adding the raw MEG data collected about 100 times by pushing the optical sensor button.

4. In the next mode that included smelling "Zuko" incense into the hands and putting the hands together, we measured the MEG response and active brain area of both brain activities: smelling "Zuko" incense into the hands in synchronization with active inspiration (i.e., sniffing and smelling "Zuko" incense odor) and the behavior of putting the hands together [30]. In this

MEG experiment, when the subject smelled "Zuko" incense with putting both hands, he/she pushed the optical sensor button by his/her thumb with holding the optical fiber among both hands, and also we have examined to compare how the brain activity is different for the habit and no habit behavior of putting the hand together in daily life.

5. This cognitive testing mode is "auditory oddball paradigm" with smelling "Zuko" incense rubbing into the hands.

During this cognitive testing mode using "auditory oddball paradigm" with smelling.

"Zuko" incense rubbing into the hands, the subject smelled "Zuko" incense into one hand and held the optical sensor by another hand and pushed the button with the right thumb quickly when he/she caught a rare tone. The averaged MEG response was measured by adding the raw MEG data collected about 100 times by pushing the optical sensor button. By using the above mode, we tried to measure the subject's cognitive ability on the peak of the so-called P300m of cognitive MEG response at the state of smelling "Zuko" incense into the hand and own singular characteristic active area for the cognition with smelling "Zuko" incense, and we have examined to compare how the brain activity is different for the habit and no habit behavior of putting the hand together in daily life.

3. Results

3.1 Result of signal source estimation of control state mode with no smelling and no putting the hands in the MEG experiments

Figure 1 shows the result of real-time MEG wave forms in the brain measured at a control state.

Figure 1a shows MEG response waves of all 122ch in the whole head regions with no smelling and no putting the hands together. The figure shows all MEG

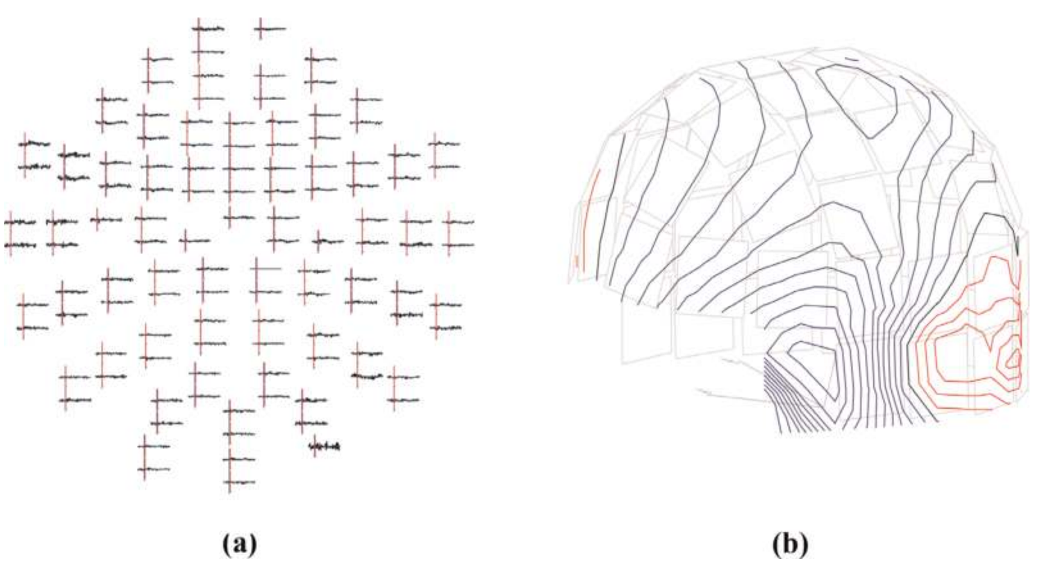

Figure 1.
An example of real-time active state at a control in our brain. (a) Over view of 122ch MEG wave forms on the whole head at a control state. (b) Contour map of equivalent magnetic fields on the head at a control state.

waves from overview. The red vertical line shows the starting time. We can find no remarkable wave peaks in over all around the brain regions.

Figure 1b shows the contour mapping of real-time MEG response at a control state. We could not almost obtain a constricted dipole completely, and then we could not find out the active brain area generally in this control state.

From this result of a control state with no smelling and no putting hands together, we can find no remarkable wave peaks in all around brain regions and no active brain areas in this control state.

3.1.1 The result of the previous MEG and MRI experiment for one subject N1 to smelling "Zuko" incense into the hand and putting the hands together

Only one in the previous 11 subjects was classified in neither the A- nor B-group, and this only one subject (N1) was used by "Zuko" incense into the hand-coating smell method [23]. He had the left priority brain type. His estimated current dipole in his brain was drawn in MRI map coordinates. The X-axis is the horizontal line of the right to left ear, the Y-axis is the line from nasion to inion, and the Z-axis is the upper to lower line of the vertical line of the brain. In this case, we found a vector at the right OFC in the brain.

In our system, MEG data were superimposed to the MRI system on the head data which was obtained from the same subject, and the estimated source was drawn into the brain of MRI imaging as a vector (showed as green vector in MRI **Figure 2b**) of the estimated current dipole(ECD) [34, 35].

Figure 2a shows a vector of single current dipole estimated in the brain using a 3-D model.

Figure 2b shows MRI images and an estimated dipole (red line) in the brain mapping,

In four panels in **Figure 2b**, the upper left panel shows the plane imaging in the coronal plane section, the upper right panel shows the plane imaging in the sagittal plane section, the left lower panel shows the plane imaging in the horizontal plane section, and the right lower panel shows the mapping method by 3-D axis in the brain in the MRI imaging system.

The red line shows a vector of single current dipole estimated in the brain by MRI imaging.

3.1.1.1 P300m mode of MEG response for an "auditory oddball paradigm"

P300m MEG peak of the cognitive response for a rare auditory stimulation without smelling. In this experimental task on an "auditory oddball paradigm," a subject concentrates his attention to the rare auditory pulse stimulation which was given without smelling. The subject must push he optic fiber button quickly when he caught the rare auditory tone. In this "auditory oddball paradigm," two kinds of auditory pulse stimuli were used (1ch, rare stimulation, 1 kHz tone burst, 2ch, frequent stimulation, 2 kHz tone burst). Two auditory pulse stimuli were given to the subject in the duration of 300 millisecond pulse tone burst at random intervals which were controlled at the rates of 1:3 for rare stimuli (1 kHz tone): frequent stimuli (2 kHz tone). We obtained P300 peak response of the subject's type as an individual variation for the priority of brain laterality regarding for "auditory odd-ball paradigm" without smelling.

Figure 3 shows an example of P300m MEG response to an "auditory oddball paradigm" for subject B2 without smelling "Zuko" incense into the hands. The active area in the brain was obtained with the single current dipole tracing method for this MEG experimental condition. We analyzed the estimated active areas

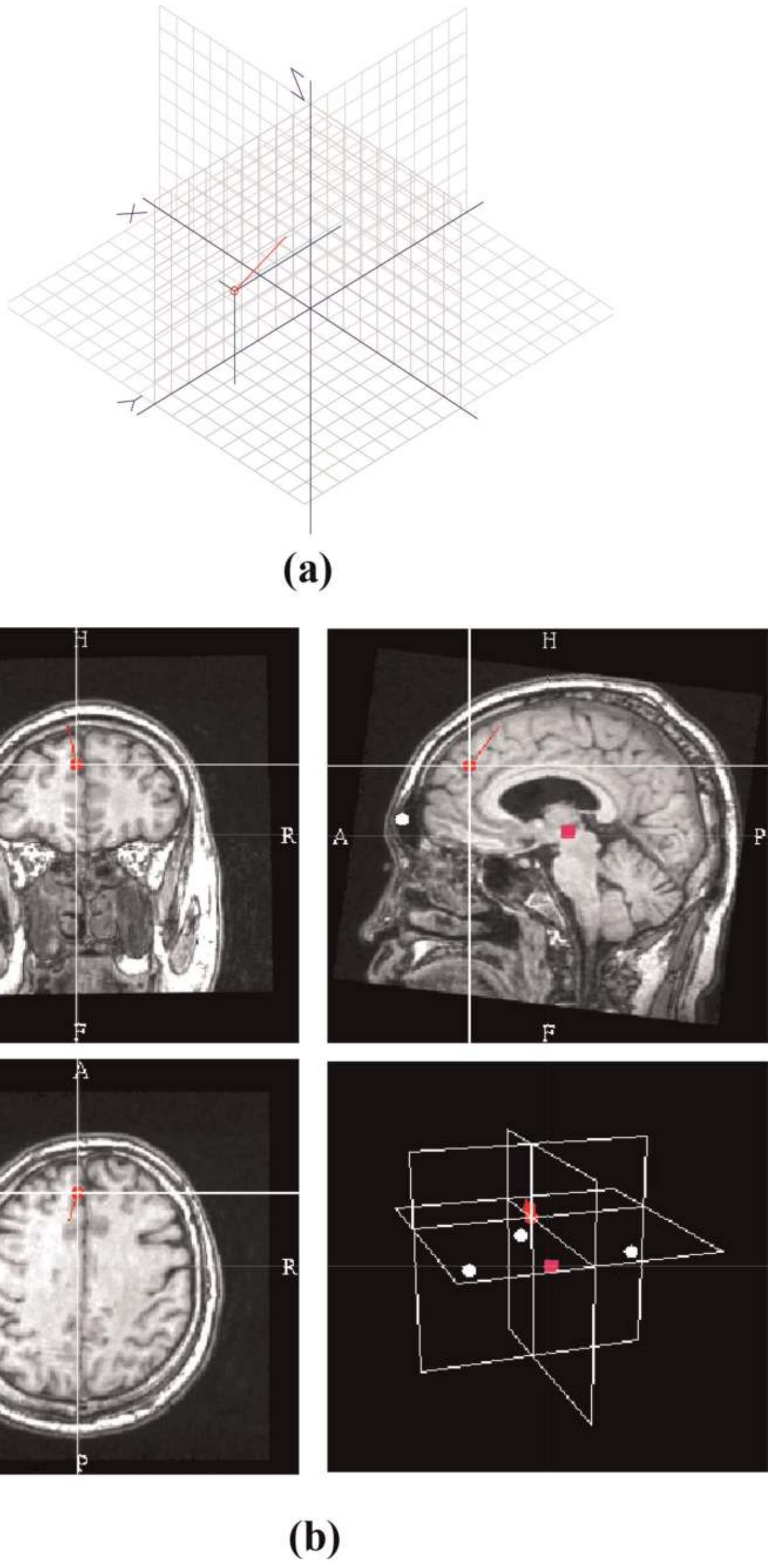

Figure 2.
Orbitofrontal area estimated by coating "Zuko" incense and putting the hands together in only one subject N1 in 11 subjects. (a) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates in subject N1. (b) MRI imagings and an estimated dipole (red line) in each fault brain images.

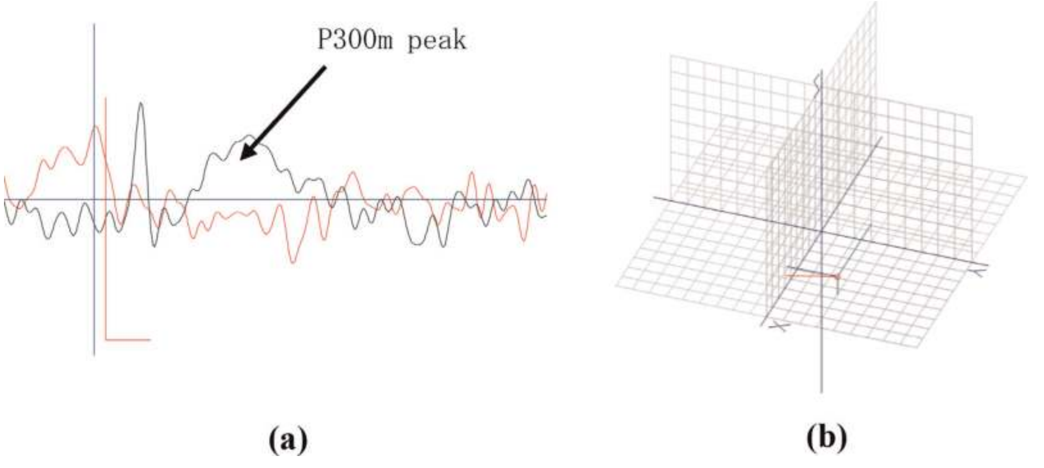

Figure 3.
An example of P300m MEG response for an "auditory oddball paradigm" in subject B2. (a) An example of P300m MEG wave (black wave line) in subject B2. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

continuously using a real-time estimation method. **Figure 3a** shows an example of P300m of MEG wave (black wave line) response to "auditory oddball paradigm" for subject B2 without smelling "Zuko" incense into the hands. **Figure 3b** shows a vector of single current dipole estimated in the brain using 3-D coordinates. In this case, we obtained that active area was at the right anterior temporal area in the brain when he recognized a rare tone at the "auditory oddball paradigm" without smelling "Zuko" incense into the hands.

Figure 3a shows that the value of the maximum peak height of P300m was 26.4 fT/cm and the size of the peak area was S = 14.3 $(fT/cm)^2$ as the latency time of P300m at T = 344.2 ms.

Figure 4 shows another male example of P300m MEG response to an "auditory oddball paradigm" for subject B1 without smelling "Zuko" incense into the hands.

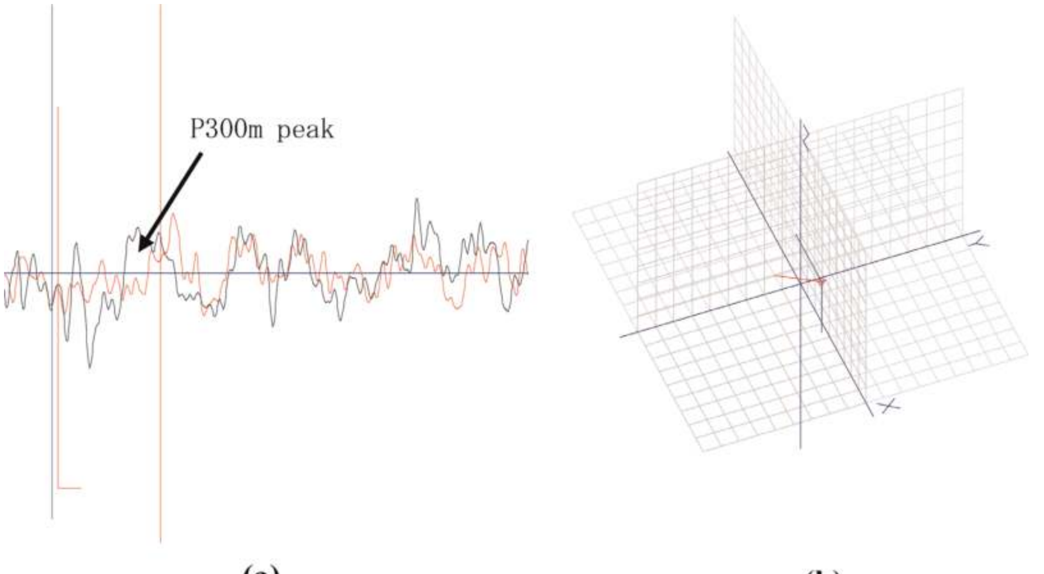

Figure 4.
An example of P300m MEG response for an "auditory oddball paradigm" in subject B1. (a) An example of P300m MEG wave (black wave line) in subject B1. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

In this case, this male subject B1 had the characteristic no habit of putting the hands in daily life. **Figure 4a** shows an example of P300m of MEG wave (black wave line) response to "auditory oddball paradigm" for subject B1 without smelling "Zuko" incense into the hands. **Figure 4b** a vector of single current dipole estimated in the brain using 3-D coordinates. In this case, we obtained that active area was right anterior temporal area in the brain when he recognized a rare tone at the "auditory oddball paradigm" without smelling "Zuko" incense into the hands.

Figure 4a shows that a value of the maximum cognitive peak height of P300m was 11.8 fT/cm and a size of the peak area was $S = 7.5\ (fT/cm)^2$ as the latency time of P300m at T = 364.2 ms. **Figure 4b** shows that a vector of single current dipole was estimated in the right superior temporal cortex of brain.

3.1.1.2 The mode of smelling "Zuko" incense rubbing into the hands without putting the hands

Figure 5 shows an example result of MEG response for the mode of smelling "Zuko" incense rubbing into the hands without putting the hands together. In this case, this male subject A5 had the characteristic habit of putting the hands in daily life. **Figure 5a** shows the result of response of a vector by single current dipole estimation method for smelling "Zuko" incense. **Figure 5b** shows also a vector of single current dipole estimated in the brain using 3-D coordinates. From these estimations we obtained the result of which a right inner frontal gyrus was activated by smelling "Zuko" incense without putting the hands together.

Figure 5a shows the contour mapping of real-time MEG responses in the state of smelling "Zuko" incense rubbing into the hands without putting the hands together. The red curves show equivalent positive magnetic fields, and the blue curves show equivalent negative magnetic fields on the subject's head surface. A green arrow shows an estimated single current dipole obtained from these contour mapping by the computer.

Figure 6 shows another example of MEG response or the mode of smelling "Zuko" incense rubbing into the hands without putting the hands together. In this case, a female subject B5 had the characteristic of no habit of putting the hands in daily life. **Figure 6a** shows the result of the response of a vector by single current dipole estimation method for smelling "Zuko" incense. **Figure 6b** shows also a

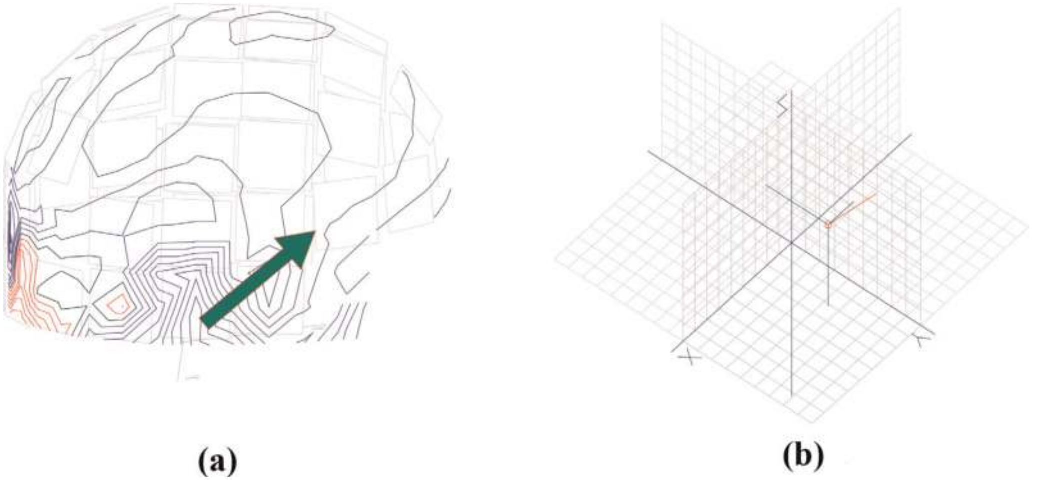

Figure 5.
An example of MEG responses to smelling "Zuko" incense rubbing into the hands without putting the hands together in subject A5. (a) Contour map of equivalent magnetic fields and an estimated current dipole (green arrow) on the head for the smelling "Zuko" incense without putting the hand in subject A5. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

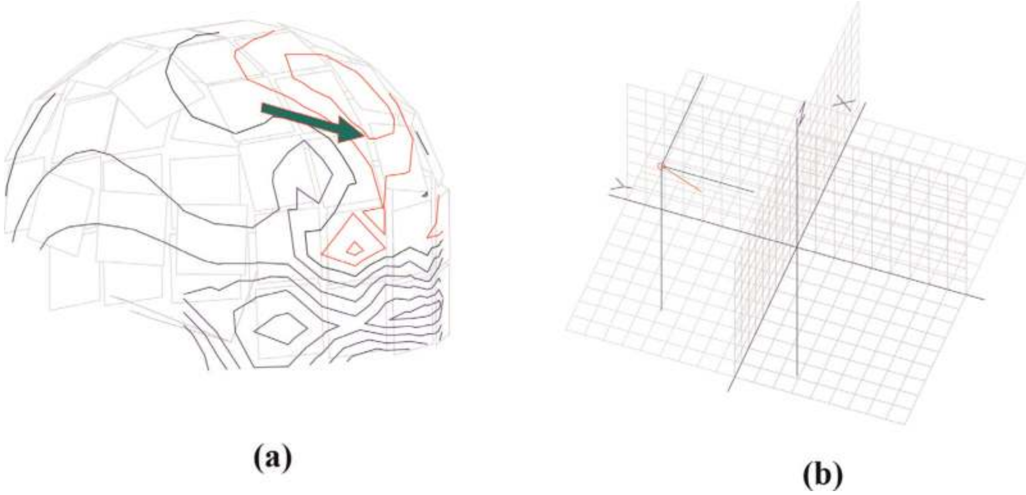

Figure 6.
An example of MEG responses to smelling "Zuko" incense rubbing into the hands without putting the hands together in subject B5. (a) Contour map of equivalent magnetic fields and an estimated current dipole (green arrow) on the head for the smelling "Zuko" incense without putting the hand in subject B5. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

vector of single current dipole estimated in the brain using 3-D coordinates. From these estimations we obtained the result of which a left inner frontal gyrus (F5 language area) was activated by smelling "Zuko" incense without putting the hands together.

Figure 6a shows the contour mapping of real-time MEG responses in the state of smelling "Zuko" incense rubbing into the hands without putting the hands together. The red curves show equivalent positive magnetic fields, and the blue curves show equivalent negative magnetic fields on the subject's head surface. A green arrow shows an estimated single current dipole obtained from these contour mapping by the computer.

Figure 7 shows another example of MEG response for the mode of smelling "Zuko" incense rubbing into the hands without putting the hands together. In this

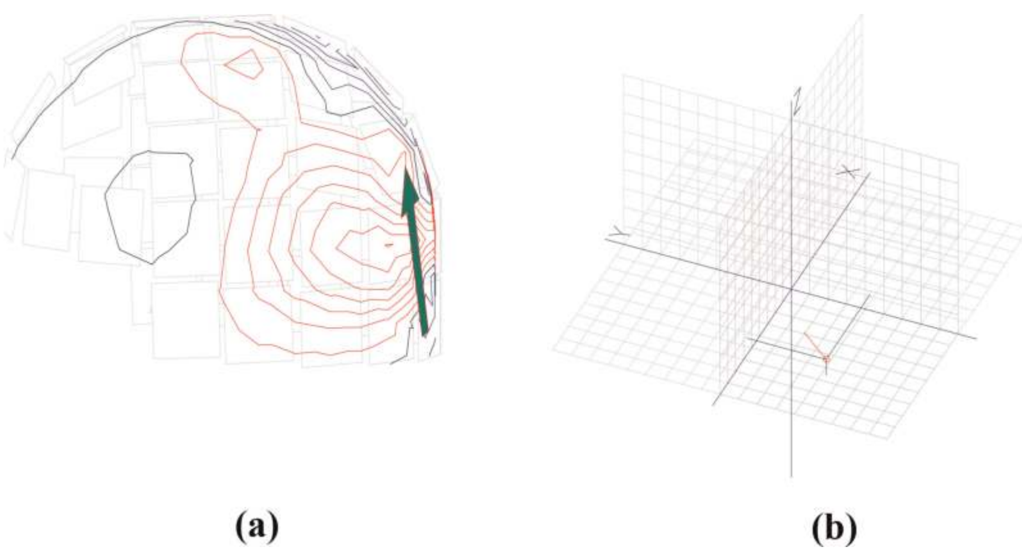

Figure 7.
An example of MEG responses to smelling "Zuko" incense rubbing into the hands without putting the hands together in subject A3. (a) Contour map of equivalent magnetic fields and an estimated current dipole (green arrow) on the head for the smelling "Zuko" incense without putting the hand in subject A3. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

case, another female subject A3 had the characteristic habit of putting the hands in daily life. **Figure 7a** shows also the result of response of a vector by single current dipole estimation method for smelling "Zuko" incense. **Figure 7b** shows also a vector of single current dipole estimated in the brain using 3-D coordinates. From these estimations we obtained the result of which a left occipital gyrus (V1 visual region) was especially activated by smelling "Zuko" incense without putting the hands together. This subject A3 had especial remember impressions of visual image for this "Zuko" incense because of her smelling "Zuko" incense with putting the hands in usual daily life.

Figure 7a shows the contour mapping of real-time MEG responses in the state of smelling "Zuko" incense rubbing into the hands without putting the hands together. The red curves show equivalent positive magnetic fields, and the blue curves show equivalent negative magnetic fields on the subject's head surface. The green arrow shows an estimated single current dipole obtained from these contour mapping by the computer.

3.1.1.3 The mode of smelling "Zuko" incense rubbing into the hands and putting the hands together

Figure 8 shows an example of MEG response for the mode of smelling "Zuko" incense rubbing into the hands with putting the hands together. In this case, a female subject A3 had the characteristic habit of putting the hands in daily life. **Figure 8a** shows also the result of response of a vector by single current dipole estimation method for smelling "Zuko" incense. **Figure 8b** shows also a vector of single current dipole estimated in the brain using 3-D coordinates.

Figure 8a shows the contour mapping of real-time MEG responses in the state of smelling "Zuko" incense rubbing into the hands with putting the hands together. The red curves show equivalent positive magnetic fields, and the blue curves show equivalent negative magnetic fields on the subject's head surface. A green arrow shows an estimated single current dipole obtained from these contour mappings by the computer.

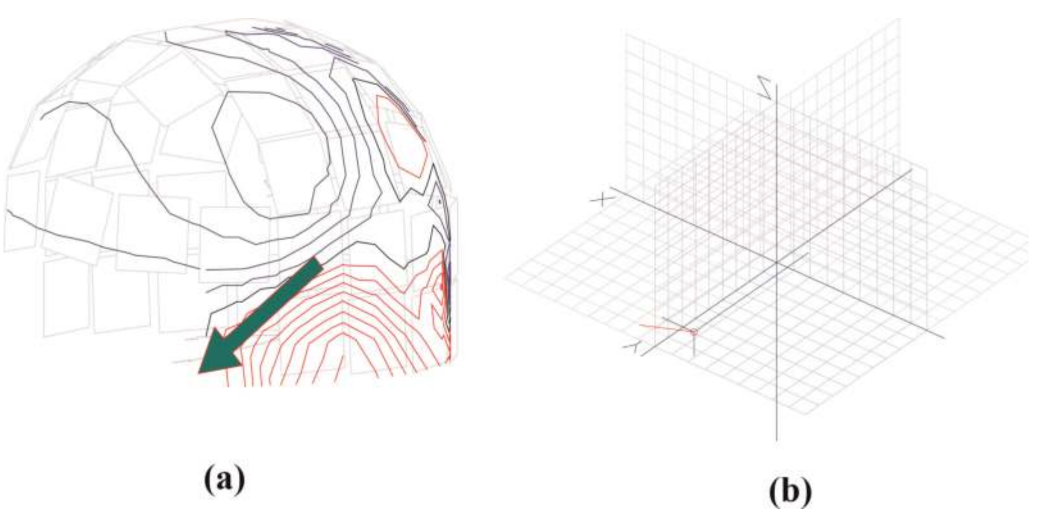

Figure 8.
An example of MEG responses to smelling "Zuko" incense rubbing into the hands and putting the hands together in subject A3. (a) Contour map of equivalent magnetic fields and an estimated current dipole (green arrow) on the head for the smelling "Zuko" incense with putting the hand in subject A3. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

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From these estimations of **Figure 8**, we obtained the result of which a left orbitofrontal cortex (OFC) was activated by smelling "Zuko" incense with putting the hands together.

Next, **Figure 9** shows also another example of a different female subject B5 who had no habit with putting the hands in daily life. **Figure 9a** shows the result of response of a vector by single current dipole estimation method for smelling "Zuko" incense with putting the hands together. **Figure 9b** shows also a vector of single current dipole estimated in the brain using 3-D coordinates.

In this case, a left inner frontal area (F5 language region) was activated. She told that she had the especial impressions for the recall of a few words of the language on the praying when she smelled "Zuko" incense with putting the hands during this MEG experiment.

Figure 9a shows the contour mapping of real-time MEG responses in the state of smelling "Zuko" incense rubbing into the hands with putting the hands together. The red curves show equivalent positive magnetic fields, and the blue curves show equivalent negative magnetic fields on the subject's head surface. A green arrow shows an estimated single current dipole obtained from these contour mapping by the computer.

Figure 10 shows the result of another example of MEG response for the mode of smelling "Zuko" incense rubbing into the hands with putting the hands together. In this case, a female subject A4 who had the habit of putting the hands in daily life hold the especial strong impressions of visual image of praying when she smelled "Zuko" incense with putting the hands during the MEG experiments.

Figure 10a shows the result of response of a vector by single current dipole estimation method for smelling "Zuko" incense with putting the hands together. **Figure 10b** shows also a vector of single current dipole estimated in the brain using 3-D coordinates. From these above estimations, a left occipital area (V1 visual region) in the brain was activated.

Figure 10a shows the contour mapping of real-time MEG responses in the state of smelling "Zuko" incense rubbing into the hands with putting the hands together. The red curves show equivalent positive magnetic fields, and the blue curves show

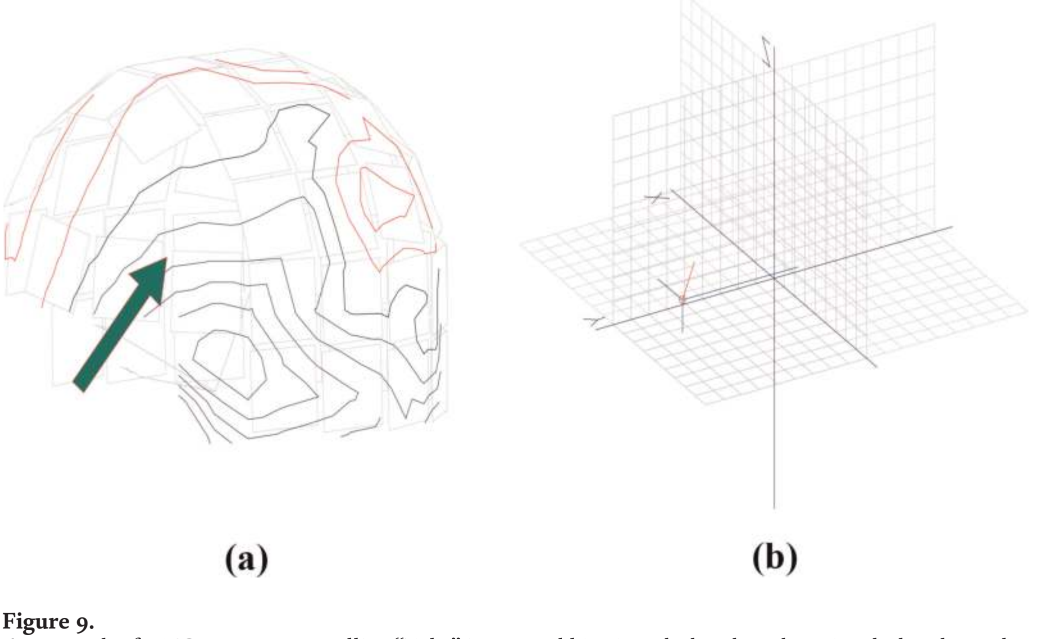
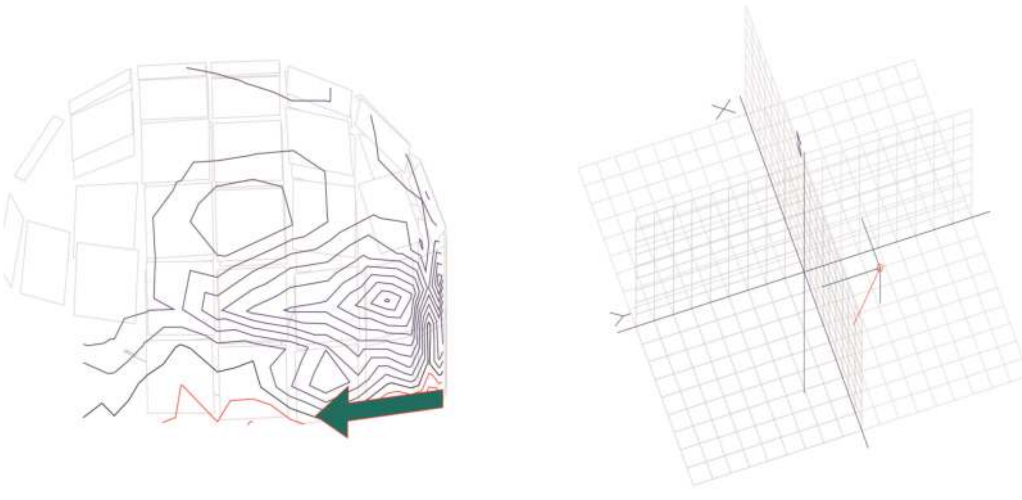


Figure 9.
An example of MEG responses to smelling "Zuko" incense rubbing into the hands and putting the hands together in subject B5. (a) Contour map of equivalent magnetic fields and an estimated current dipole (green arrow) on the head for the smelling "Zuko" incense with putting the hand in subject B5. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.



(a) (b)

Figure 10.
An example of MEG responses to smelling "Zuko" incense rubbing into the hands and putting the hands together in subject A4. (a) Contour map of equivalent magnetic fields and an estimated current dipole (green arrow) on the head for the smelling "Zuko" incense with putting the hand in subject A4. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

equivalent negative magnetic fields on the subject's head surface. A green arrow shows an estimated single current dipole obtained from these contour mapping by the computer.

3.1.1.4 The response for an "auditory oddball paradigm" with smelling "Zuko" incense rubbing into the hands

P300m peak of the cognitive response for a rare auditory stimulation with smelling "Zuko" incense rubbing into the hands. In this experimental task on an "auditory oddball paradigm," a subject concentrates his attention to the rare auditory pulse stimulation which was given without smelling. A subject must push the optic fiber button quickly when he caught the rare auditory tone.

In this "auditory oddball paradigm," two kinds of auditory pulse stimuli were used (1ch, rare stimulation, 1 kHz tone burst, 2ch, frequent stimulation, 2 kHz tone burst). Two auditory pulse stimuli were given to the subject in the duration of 300 ms pulse tone burst at random intervals which were controlled at the rate of 1:3 for rare stimuli (1 kHz tone): frequent stimuli (2 kHz tone).

We obtained a P300 peak response of the subject's type as an individual variation for the priority of brain laterality regarding for "auditory oddball paradigm" with smelling "Zuko" incense rubbing into the hands.

Figure 11 shows an example of P300m MEG response to an "auditory oddball paradigm" for a subject B2 with smelling "Zuko" incense into the hands. The active area in the brain was obtained with the single current dipole tracing method for this MEG experimental condition. We analyzed the estimated active areas continuously using a real-time estimation method. **Figure 11a** shows an example of P300m of MEG wave (black wave line) response to "auditory oddball paradigm" for subject B2 with smelling "Zuko" incense into the hands.

Figure 11b shows a vector of single current dipole estimated in the brain using 3-D coordinates. In this case, we obtained that active area was at the right anterior temporal area in the brain when he recognized a rare tone at the "auditory oddball paradigm" without smelling "Zuko" incense into the hands.

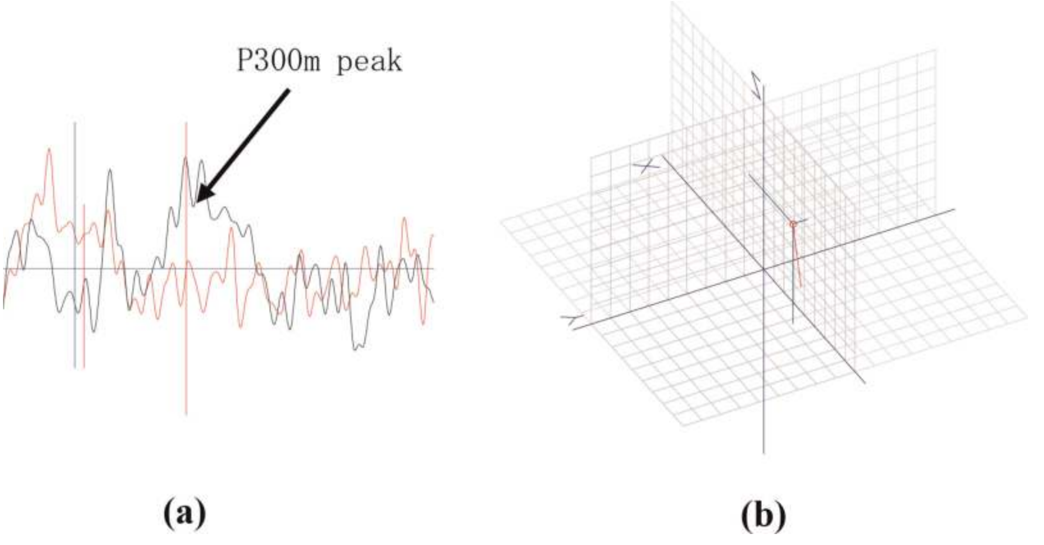


Figure 11.
An example of P300m MEG response for an "auditory oddball paradigm" with smelling "Zuko" incense in subject B2. (a) An example of P300m MEG wave (black wave line) response for an "auditory oddball paradigm" with smelling "Zuko" incense in subject B2. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

Figure 11a shows that the value of the maximum peak height of P300m was 60.4 fT/cm and the size of the peak area was S = 32.0 $(fT/cm)^2$ as the latency time of P300m at T = 310.5 ms. **Figure 11b** shows that an active area of the cognitive P300m peak of "auditory oddball paradigm" with smelling "Zuko" incense into the hands was at the left superior temporal area in the brain.

Figure 12 shows also another example of P300m MEG response to an "auditory oddball paradigm" for a male subject B1 with smelling "Zuko" incense into the hands. The active area in the brain was obtained with the single current dipole tracing method for this MEG experimental condition. We analyzed the estimated active areas continuously using a real-time estimation method. **Figure 12a** shows an

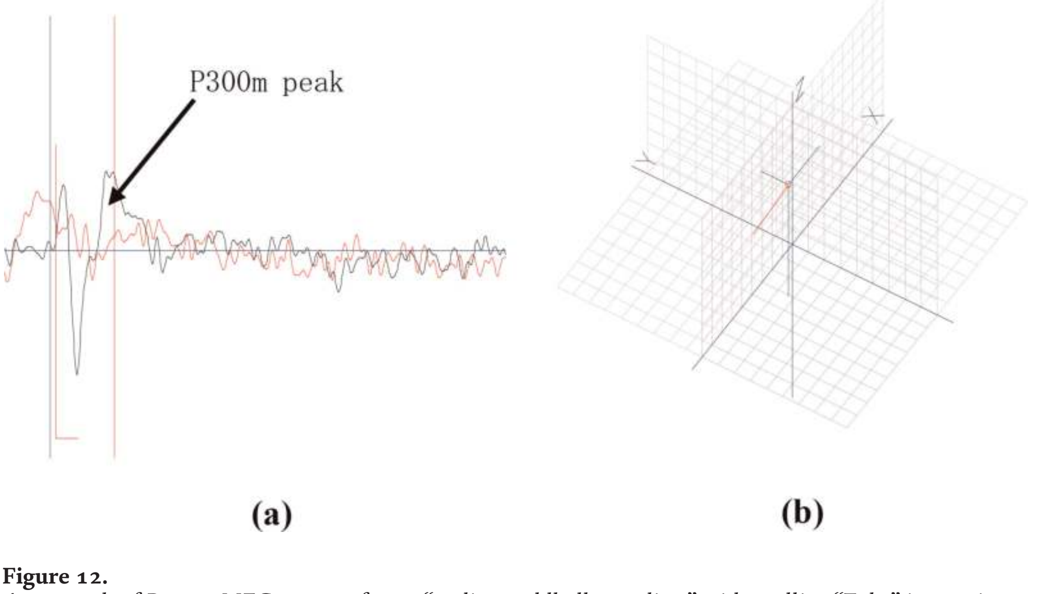


Figure 12.
An example of P300m MEG response for an "auditory oddball paradigm" with smelling "Zuko" incense in subject B1. (a) An example of P300m MEG wave (black wave line) response for an "auditory oddball paradigm" with smelling "Zuko" incense in subject B1. (b) A vector of single current dipole (red line) estimated in the brain using a 3-D coordinates.

example of P300m of MEG wave (black wave line) response to "auditory oddball paradigm" for subject B1 with smelling "Zuko" incense into the hands. **Figure 12b** shows a vector of single current dipole estimated in the brain using 3-D coordinates. In this case, we obtained that active area was at the left posterior temporal area in the brain when he recognized a rare tone at the "auditory oddball paradigm" with smelling "Zuko" incense into the hands.

From these estimations of **Figure 12**, it was obtained that the results of a cognitive P300m peak height in the brain was 26.0 ft./cm and the active wave area value of the P300 peak was S = 15.0 $(fT/cm)^2$ at the time T = 281.4 ms when a rare tone was recognized at "auditory oddball paradigm" with smelling "Zuko" incense in the MEG experiment. And it was also obtained that the cognitive P300 peak was found in left posterior temporal area.

3.1.2 The comparison of a P300m peak of MEG response with nonsmelling and smelling "Zuko" incense

From the example of a female subject B2, a cognitive P300m peak without smelling "Zuko" incense at the oddball paradigm of MEG experiment was shown in the following results of **Figure 3a**. And also a P300m peak of the same female subject B2 with smelling "Zuko" incense was shown in the following results of **Figure 11a**.

An example result of the comparison of female subject B2:

	Latency time	**Peak height**	**S = active wave area**
Without smelling	344.2 ms	26.4 fT/cm	14.3 $(fT/cm)^2$
With smelling	310.5 ms	60.4 fT/cm	32.0 $(fT/cm)^2$

From another example of a male subject B1, a cognitive P300m peak without smelling "Zuko" incense at the oddball paradigm of MEG experiment was shown as the following results of **Figure 4a**. And also a P300m peak of the same male subject B1 with smelling "Zuko" incense was shown as the following results of **Figure 12a**.

An example result of the comparison of male subject B1:

	Latency time	**Peak height**	**S = active wave area**
Without smelling	364.2 ms	11.8 fT/cm	7.5 $(fT/cm)^2$
With smelling	281.4 ms	26.0 fT/cm	15.0 $(fT/cm)^2$

3.2 Results of the statistical analysis of the alpha-amylase value

Table 1 shows the results of statistical analysis of the alpha-amylase value in the saliva of 10 subjects.

A significant difference ($P < 0.079$) was found between the mean alpha-amylase value of the condition before smelling "Zuko" incense and after smelling "Zuko" incense for female subjects (see **Figure 13**).

Figure 14 shows that it has no significant for the statistical alpha-amylase value T-tests of the comparison with habit and no habit with the hands in daily life. From these tests, we could not find a significant difference for habit/no habit with putting hands in daily life and could not also find the difference before smelling and after smelling "Zuko" incense.

Alpha-amylase value (µg/dL)	A-group (habit group)							B-group (no habit)						
Subject	**A1**	**A2**	**A3**	**A4**	**A5**	**Average**	**Standard deviation**	**B1**	**B2**	**B3**	**B4**	**B5**	**Average**	**Standard deviation**
Before experiment	4	22	31	10	3	14	12.14	6	15	28	3	3	11	10.7
After experiment	3	5	72	19	3	20.4	29.61	2	23	3	35	9	14.4	12.15
Average	3.5	13.5	36	14.5	3	17.2	21.07	4	19	15.5	19	6	12.7	11.93

(2)-1 Alpha-amylase value before smelling "Zuko" and MEG experiments.
(2)-2 Alpha-amylase value after smelling "Zuko" and MEG experiments.
The alpha-amylase value (µg/dL) is an index of the state of stress.
A-group is the subject having the habit of putting the hands in daily life.
B-group is the subject having no habit of putting the hands in daily life.

Table 1.
Results of alpha-amylase (µg/dL) in the saliva for each 10 subjects.

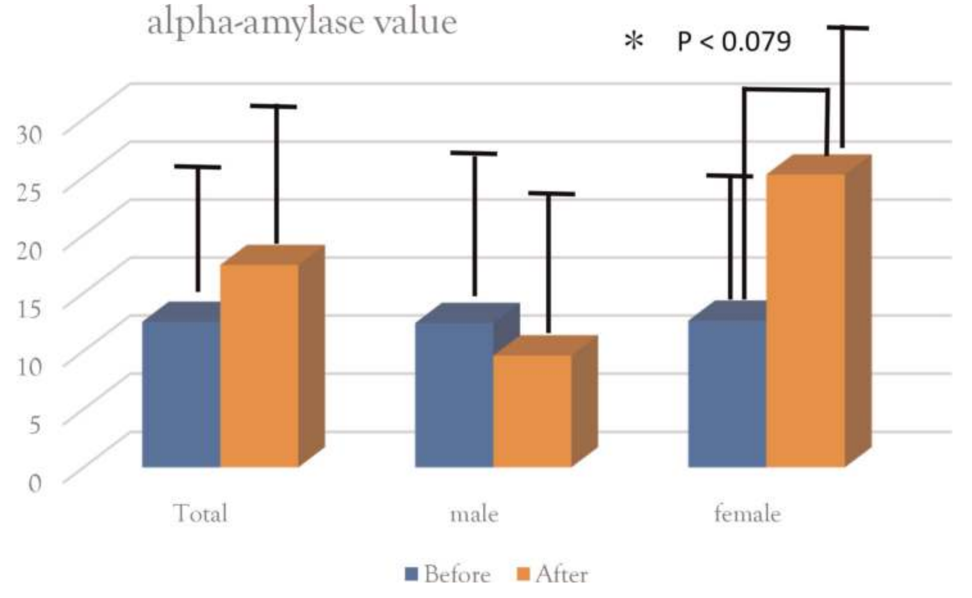


Figure 13.
Results of the comparison for sex difference of alpha-amylase value. P value shows statistical T-tests.

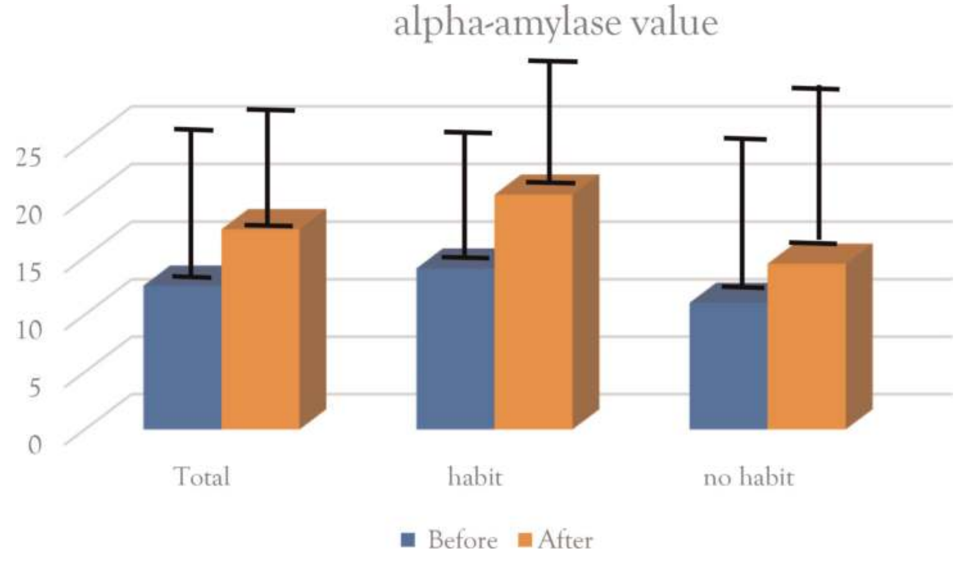


Figure 14.
Results of the statistical comparison with habit and no habit with putting the hands for alpha-amylase value.

From the individual analysis of alpha-amylase value in **Table 1**, we can find that almost all females have more stress after the experiment of smelling "Zuko" incense with putting hands and measuring MEG than before this experiment. And especially the value of subjects A3, A4, B2, and B4 were larger after the experiment than before it. So, we can find that subjects A1, A2, A5, B1, and B2 were almost no stress after the experiment than before it.

3.3 The comparison between the response of only smelling "Zuko" incense and the response of smelling "Zuko" with putting the hands together

A few typical examples of the response of only smelling "Zuko" incense were shown in 3.1.2.2 as in **Figures 5–7**.

	A-Group (Habit Group)					B-Group (No-Habit Group)				
Subject	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Smelling "Zuko" only (laterality)	Right	Left	Left	Left	Right	Right	Right	Right	Right	Left
Smelling "Zuko" only (Activated Region)	inner temporal cortex	lateral temporal cortex	occipital cortex (visual area V1)	innner temporal cortex	inner frontal gyrus (F5 language area)	frontia region of temporal cortex	lateral temporal cortex	OFC (orbito frontal cortex)	inner frontal gyrus	inner frontal gyrus (F5 language area)
Smelling "Zuko" with putting the hands together (laterality)	Left	Right	Left	Left	Left	Right	Left	Right	Right	Left
Smelling "Zuko" with putting the hands together (Activated Region)	inner temporal cortex	lateral temporal cortex	OFC (orbito frontal cortex)	occipital cortex (visual area V1)	innner temporal cortex	inner temporal cortex	occipital cortex (visual area V1)	inner temporal cortex	lateral temporal cortex	inner frontal gyrus (F5 language area)

Table 2.
Summaries of active areas in the brain for the response of only smelling "Zuko" incense and for the response of smelling "Zuko" incense with putting the hands together.

A few typical examples of the response of smelling "Zuko" incense with putting the hands together were also shown in 3.1.3.3 as in **Figures 8–10**.

These results are summarized in **Table 2**.

3.4 The summaries of active areas in the brain for the changing from only smelling "Zuko" incense to smelling "Zuko" incense with putting the hands together

From the results **Table 2** in Section 3.3, we can summarize the active areas in the brain for changing from only smelling "Zuko" incense to smelling "Zuko" incense with putting the hands together.

Figure 15 shows the summaries of active areas in the brain for these changes.

In **Figure 15**, we draw two experimental states, for example, yellow symbol color showed the state of the habituation of putting the hands together, and blue symbol color showed the state of the no habituation of putting the hands together.

In **Figure 15**, active brain areas for the response of smelling only "Zuko" incense showed almost temporal regions in the case of habituation of putting the hands together except for two subjects A3 and A5. The response of subject A3 was shown at the left visual V1 area, and the response of subject A5 was shown at the right inner frontal area. However, active brain areas in the case of smelling only "Zuko" incense but no habituation of putting the hands together also showed almost temporal regions except for one subject B3. The response of subject B3 was only shown at the right orbitofrontal cortex.

On the contrary, active brain areas of smelling "Zuko" incense with putting the hands together showed larger change and different responses from smelling only "Zuko" incense.

In **Figure 15**, we showed these larger changing regions to the square symbols from the ellipse symbols using arrows. In the case of habituation of putting the hands, subject A3 showed larger change to the left orbitofrontal cortex from the left visual V1 area, and another subject A4 showed larger change to the left visual V1 area from the inner temporal area. These results are considerable to suggest that subject A3 and A4 may be activated especially at the orbitofrontal cortex and left visual V1 area, respectively.

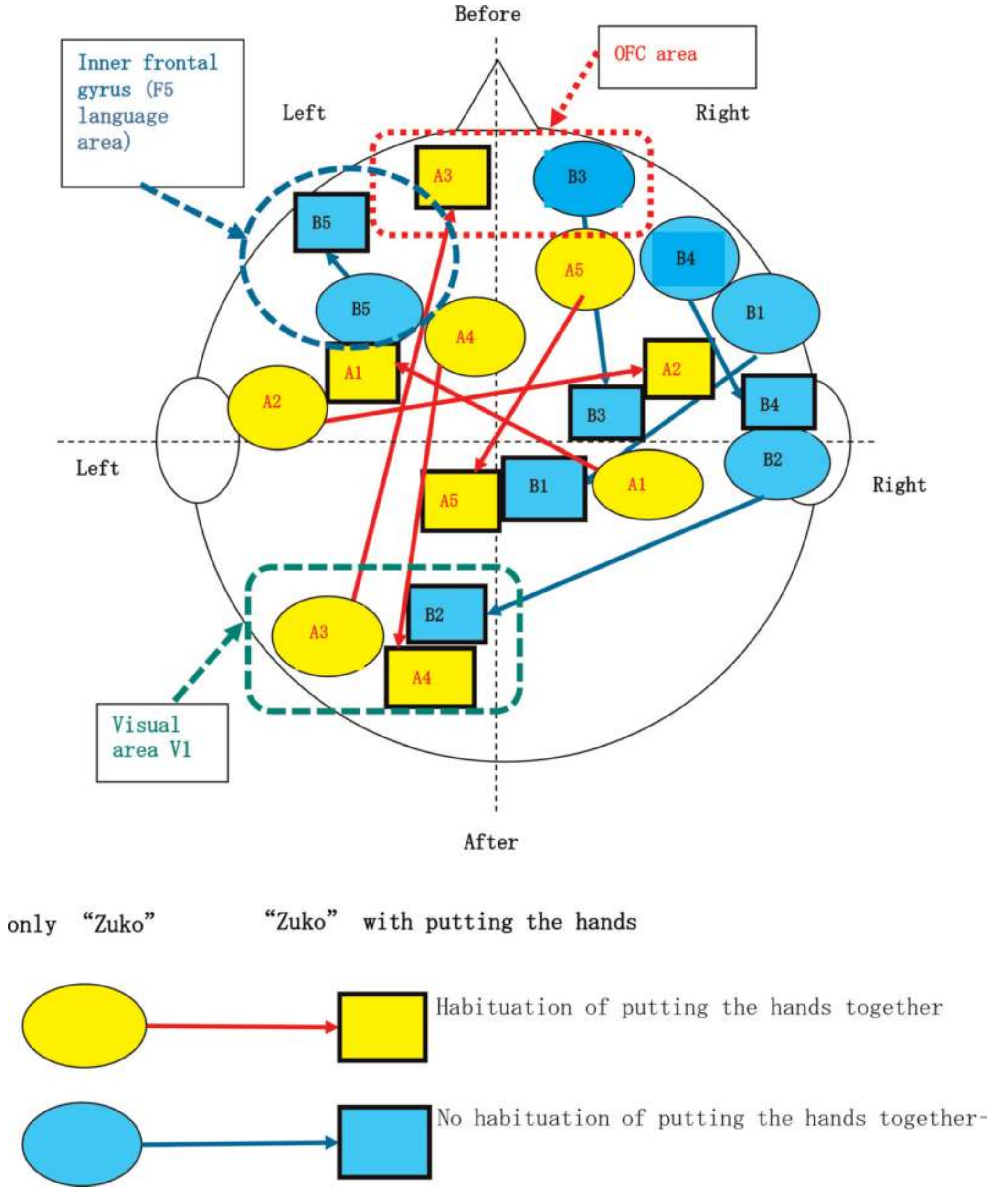


Figure 15.
The summaries of active areas in the brain for changing from smelling "Zuko" incense to smelling "Zuko" incense with putting the hands together.

As the same larger changing, in the case of no habituation of putting the hands the subject B2 showed the larger change to the left visual V1 area from right outer temporal area, the subject B5 the change to the left frontal gyrus F5 area (language area) from inner frontal area. These results are considerable to suggest that subject B2 and B5 may be activated especially at the visual V1 area and the left F5 language area, respectively, although they have no habituation of putting hands together in daily lives.

These larger change results on the brain active areas show that smelling "Zuko," rubbing into the hands, and putting the hands together activate specific brain areas more than smelling only "Zuko".

From **Figure 15**, we can find that smelling "Zuko" incense activates a few special areas, for example, orbitofrontal cortex, left inner frontal gyrus (F5 language area), occipital cortex of visual area V1, and so on.

And also we can find that smelling "Zuko" incense with putting the hands together more activates the same few special areas, for example, OFC area, left inner frontal gyrus (F5 language area), occipital cortex of visual area V1, and so on.

From these results of the comparison among the above two mode states, we can obtain large changes from control state to the state of smelling "Zuko" incense and also larger changes from the state of only smelling "Zuko" incense to smelling "Zuko" incense with putting the hands together. In these changing active areas in the brain, the common special changes was the change of laterality in the brain. **Figure 15** shows large changes for literalities in the brain with the change of active areas too. These results can make sure that Smelling "Zuko" incense activates a few special regions in the brain and raises up a few complex larger changes for activation areas in the brain.

3.5 Summary of results

3.5.1 The specific and distinct mirror neuron activities without the error activity on the hand motor system by putting the hands together

Our MEG experiments of the above results were shown by using the results of 3.1.1 and 3.1.2 in the previous paper [23].

From the previous paper [23], the response areas were obtained in superior and anterior temporal gyrus or central and caudal temporal and frontal gyrus.

Our previous MEG experiments [23] showed the distinct and objective activities of our brain on the state of simultaneous responses of putting the hands together and at the same time smelling incense odor. In this simultaneous status mode of our MEG experiments, these specific active areas were especially shown in distinct F5 language areas of the inner regions of the left frontal lobe or orbitofrontal gyrus clinically. These specific results showed the simultaneous new distinct stronger effects of both the mirror neuronal activities as the imitation without the artifacts of the simple moving error activities and olfactory activated effects.

These results show the specific new stronger effects of simultaneous responses in relation of both the mirror neuron activities and olfactory effects at the same time.

3.5.2 The mode of smelling "Zuko" incense only without putting the hands together (olfactory sensing response and visual imaging response)

The detailed responses of our MEG experiments of the above results in the mode of smelling "Zuko" incense only without putting the hands together (olfactory and visual activities) were shown in **Figures 5–7** in Section 3.1.2.2 and **Table 2** in Section 3.3. From these analyses we mainly obtained the active areas in the brain such as inner frontal gyrus, left F5 language area and left occipital gyrus (V1 visual region), and so on.

3.5.3 The mode of smelling "Zuko" incense rubbing into the hands and putting the hands together

The detailed responses of our MEG experiments of the above results in the mode of smelling "Zuko" incense rubbing into the hands and putting the hands together were shown in **Figures 8–10** in Section 3.1.2.3 and **Table 2** in Section 3.3 with almost all the subject's data. From these clinical and objective MEG measurements and analysis, we obtained the distinct olfactory active areas clearly such as the OFC frontal regions and left inner frontal region F5 (language area) and occipital regions V1 (visual area) for a mirror neuron activity in the brain, nevertheless habit (A-group) and no habit (B-group) of putting the hands in daily life. The larger active effects for smelling "Zuko" incense rubbing into the hands and putting the hands were obtained more than the previous smelling incense odor activity with putting the hands from the results of summaries in **Figure 15** in Section 3.4.

4. Discussions

4.1 A possibility for improving cognitive ability by smelling "Zuko" incense from the results of P300m responses of "auditory oddball paradigm"

We try to discuss a possibility for improving cognitive ability by smelling "Zuko" incense from the results of the following examples of P300m responses of "auditory oddball paradigm" MEG experiments.

4.1.1 The comparison of a P300m peak of MEG response with nonsmelling and smelling "Zuko" incense

Though the typical examples are the results of P300m MEG responses of only two subjects, we can find out the results as follows:

1. As a cognitive P300m response, it was shown that the result with smelling "Zuko" had shorter latency time than without smelling "Zuko."

2. As a cognitive P300m response, it was shown that the result with smelling "Zuko" had bigger peak height than without smelling "Zuko."

3. As a cognitive P300m response, it was shown that the result with smelling "Zuko" had larger active wave area S than without smelling "Zuko."

From these typical results of P300m responses, it can be considered that a possibility of the improving cognitive ability of P300 peak was shown by smelling "Zuko" incense using "auditory oddball paradigm."

4.2 Effects of stress on the comparison with before and after smelling "Zuko" incense by measuring alpha-amylase value in saliva

We can find out few effects of stress for smelling "Zuko" incense by measuring alpha-amylase value in saliva from the results of **Table 1**, **Figures 13** and **14**.

From these results of alpha-amylase value in saliva, it was shown that the following discussions were obtained:

1. This report shows the results of the statistical comparison with habit and no habit with putting the hands by using alpha-amylase value in saliva.

2. We could not find out a significant difference among the sex in 10 Japanese subjects as the statistical value of alpha-amylase in saliva.

3. We could not find out a significant difference among the habit and no habit with smelling "Zuko" incense and putting hands together in daily life.

4. In only female subjects, we found out a significant difference ($P < 0.079$) in T-tests among before and after smelling "Zuko" incense rubbing into the hands.

5. Alpha-amylase value was almost all shown as a characteristic index of stress which was more increasing up after smelling "Zuko" incense than before smelling regardless of male/female and habit/no habit with putting the hands in daily life.

From these results of measuring alpha-amylase value in saliva, it can be considered that something stress on smelling "Zuko" incense was usually given to the subject.

4.3 The meaning of smelling "Zuko" incense rubbing into the hands with putting the hands together

The meaning of smelling "Zuko" rubbing into the hands with putting the hands is as follows.

In habits of daily life, the brain of A-group peoples after smelling "Zuko" incense rubbing into the hands and putting their hands together or praying was activated at the orbitofrontal area, inner lobe of the frontal area, anterior and posterior areas in the temporal cortex, left visual area V1 in occipital cortex, and others. The brain of B-group individuals who did not has the habit of smelling incense odor or putting their hands together or praying in their daily life was also activated at the orbitofrontal cortex, inner lobe of the frontal area, left F5 language area, left visual area V1 in the occipital cortex, and anterior and posterior temporal cortex and larger changed.

Figure 15 shows that one subject's brain was activated at the F5 language area in the left inner frontal cortex. The brain for two of three subjects was activated at the right inner frontal cortex regardless of whether they have a habit of putting their hands together in their daily life or not. On the other hand, the brain for three subjects smelling "Zuko" incense with and without putting their hands together was also activated at the left calcarine sulci of the V1 visual area in the occipital cortex [36–39]. This result means the subject had a something sense of visual imaging by smelling "Zuko" incense with putting the hands together.

4.4 Smelling "Zuko" incense and putting the hands together showed larger changes than smelling incense odor with putting the hands together

We already have the results of the previous MEG experiments for smelling incense odor with putting the hands together as shown in our paper published in IntechOpen [23]. From these results of previous MEG experiments, it was obtained that smelling incense odor with putting the hands activates few specific brain areas.

On the other hand, in these MEG experiments, it was obtained that the results of smelling "Zuko" incense into the hands and putting the hands promote to excite few same specific brain areas as shown in **Figure 15** in Section 3.4.

From the results of **Tables 1** and **2**, we can find out that subject B5 obtained in orbitofrontal cortex OFC, subject A5, and B4 obtained in inner frontal area, subject A3, A4, and B2 obtained in occipital cortex V1 as an estimation active area in the brain clearly show almost all larger value ofα-amylase after smelling "Zuko" and putting the hands and MEG experiments before these experiments. These results are considered to show something stress by smelling "Zuko" incense and putting the hands for the response of subject A3, A4, B2, B3, B4, and B5 which showed large activity in a few special areas in the brain.

From the comparison with these two experimental results and analysis, we can obtain that smelling "Zuko" incense and putting the hands together showed clearly larger changes than smelling incense odor with putting the hands together. We can find larger changes in the brain, for example, from the right area to the left area for subject A5, B1, and B2 and from the left area to right area for subject A2 as shown in **Figure 15**. And we can also find out other larger changes in the brain in the same laterality, for example, from the occipital area to the prefrontal area for subject A3 and from the inferior parietal area to the occipital area for subject A4 at the left side in the brain.

These above results of larger changes show that the usage of "Zuko" incense rubbing into the hands promote to activate the brain more than smelling incense odor.

5. Conclusions

This research revealed that smelling "Zuko" incense rubbing into the hands and putting the hands together promoted to excite a few specific brain areas, for example, the orbitofrontal cortex of olfactory area, inner areas of the prefrontal cortex, left language F5 regions, occipital regions of left imaging area V1, and so on in the human brain.

A P300 response peak which was known as a kind of ERP responses in brain waves was researched as a response of "cognitive function" by using "oddball paradigm" experiment.

In this MEG experiments, P300m response using "auditory oddball paradigm" was measured both before smelling "Zuko" incense and after smelling "Zuko" incense.

In our experiments, evoked neuronal activity was recorded by the MEG and the alpha-amylase value in the subject's saliva was also measured in the stage before and after smelling "Zuko" incense and measuring the response of MEG in the brain.

From the summary of results in 3.5.1-3.5.3 in Section 3.5, we can conclude the distinct activities as follows. Both smelling "Zuko" incense only without putting the hands and smelling "Zuko" incense into the hands with putting the hands promoted to activate mainly a few specific brain areas such as the OFC frontal regions, left inner frontal region F5 language area, left inner occipital regions V1 visual areas, and so on.

As a cognitive P300m response, it was shown that the result with smelling "Zuko" had shorter latency time; the bigger the peak height, the larger the active wave area S than without smelling "Zuko." From these typical results of P300m responses, it can be considered that a possibility of improving cognitive ability of P300 peak was shown by smelling "Zuko" incense using "auditory oddball paradigm."

From these typical results of P300m responses, it can be considered that a possibility of improving cognitive ability of P300 peak was shown by smelling "Zuko" incense using "auditory oddball paradigm."

An alpha-amylase value was shown as a characteristic index of stress which was more increasing up after smelling "Zuko" incense than before smelling although male/female and habit/no habit with putting the hands in daily life. From these results of measuring alpha-amylase value in saliva, it can be considered that something stress on smelling "Zuko" incense was usually given to the subject.

From these results of measuring alpha-amylase value in saliva, it can be considered that something stress on smelling "Zuko" incense was usually given to the subject.

From an individual analysis, we can also find out that subject B5 obtained in orbitofrontal cortex, subject A5 and B4 obtained in the inner frontal area, subject A3, A4, and B2 obtained in the occipital cortex V1 as an estimation active area in the brain clearly show almost all larger value of alpha-amylase after smelling "Zuko" and putting the hands and MEG experiments before these experiments. These results are considered to show something stress by smelling "Zuko" incense and putting the hands for the response of subjects A3, A4, B2, B3, B4, and B5 which showed large activity in a few special areas in the brain.

From the above results, we consider that the F5 language area in the left frontal cortex and V1 visual area were promoted to activate by smelling "Zuko" incense rubbing into the hands with putting the hands together.

Alpha-amylase showed something stress like for smelling "Zuko" incense with putting the hands, and the specific mirror neuron and default-mode network showed activity of the special areas in the brain.

We concluded that new specific effects both in smelling "Zuko" incense into the hands and imitating the behavior of putting the hands together can be considered to promote excitation of the higher activities and to make larger changes in the brain activities dynamically in a few specific regions in the human brain.

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