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Simultaneous Smelling an Incense Outdoor and Putting the Hands Together Activate Specific Brain Areas

Mitsuo Tonoike and Takuto Hayashi

Abstract

Mirror neurons are involved in imitation of habitual behaviors. To increase understanding of the theory of mirror neurons and the default mode network, brain activation was explored in 11 healthy adult volunteers who did or did not have a habit of putting their hands together as if praying. Magnetoencephalography (MEG) data were recorded while the participants simultaneously smelled an odor in two kinds of incenses outdoor and/or while they moved to putting their hands together. A magnetoencephalographic contour map of the recorded findings was drawn and an estimated current dipole (ECD) was set. Regardless of a habit of putting their hands together or not, the inner lobe of the frontal area, anterior area in the temporal lobe, and F5 language area in the left frontal lobe and so on were specifically activated. We used cortisol value as an index of the stress state measured in every state (before and after smelling two different incenses outdoor). These experiments suggest that simultaneous smelling an incense outdoor and the behavior of putting their hands together increased the activity of these specific areas in the human brain due to mutual interactions and enhanced interactions.

Keywords: incense outdoor, putting the hands together, habit/no habit, MEG, F5 language area, mirror neuron, default mode network

1. Introduction

In the olfactory neural processing in humans, evoked magnetic fields by odorant synchronized with respiration and sniffing odors are found in orbito-frontal cortex (OFC) and inferior temporal lobe [1–6]. On the other hand, mirror neurons in the brain are known to activate the inner prefrontal lobe and F5 area which have the function of imitation of behavior in daily life [7–9]. Therefore, mirror neurons are considered to have the function for imitation of habit [10–12]. Super mirror neurons are concerned with determination of values, recognition of oneself and others, and reward from one's work. The inner default mode network controls the fundamental activity of daily movements and the resting state of the human brain [13–15]. Because this default mode network is strongly related to super mirror neurons, discrimination of oneself from others and the determination of social

cognition are considered important in human daily life [16, 17]. The purpose of this study is to clarify that simultaneous smelling an incense outdoor and putting the hands together activate the human brain and to show where specific areas are activated.

2. Materials and methods

2.1 Incense sticks

In this MEG experiment, two types of incense sticks (A: SEIUN-Violet Smokeless, and B: MAINICHI-Kou Sandalwood), which are produced by Nippon Kodo Co. Ltd. in Japan, were used as odors.

2.2 Subjects

Eleven Japanese volunteer subjects (six males, five females) between the ages of 22 and 58 years (mean age 41 ± 11 years) without significant smell loss or a neurologic history participated. All subjects were right-handed and were given the informed consent in accordance with guidelines set by the ethical committee on human studies in both Aino University and the Kansai center in AIST in Japan.

2.2.1 Preparation of subjects

All subjects used non-magnetic clothes, and answered no problem for the questionnaire to exclude metal artifacts. Before the MEG experiments, an individual subject was shown the essence of instructions and possible debriefing for the experiments.

All subjects were given informed consent in accordance with the acceptance for measuring MEG and individual anatomical MRI for each individual brain structure to the experiments.

Participants were requested in seated during MEG experiments, and the head of the participant was positioned in the MEG helmet under the gantry of MEG system in the magnetically shielded room.

Ten of these volunteers (except for one male) were separated into two groups, the A-group, which included individuals with a habit of putting their hands together in their daily life (similar to praying), and the B-group, which included individuals who are not in the habit of putting their hands together or who do not pray.

One person was not included in either group, because he had experience putting his hands together and sometimes prayed. In this MEG experiment, he did not use a burning incense stick and instead directly sniffed his hands, which were painted with a liquid odorant containing the same ingredients as the incense stick.

2.3 Experimental design

2.3.1 MEG system

This MEG system is Neuro-magnetometer with 122 channel DC-SQUID sensors, whole-cortex type system (Neuromag-122™, Electa Co. Ltd., made in Finland).

SQUID sensor is planar DC-SQUID type. Inner helmet of the head, at the 62 points which were selected around the whole head two the first derivative DC-SQUID sensors were located individually (so, the number of total sensors are $122 = 62 \times 2$). This system's version of the acquisition software is

Neuromag-Aquis122-Ver.3. Sampling frequency was used Max 600 Hz, with an analog pass-band filter of 0.01–200 Hz for acquisition filters.

As the location of the head relative to MEG sensors differ across participants, projection onto a common source space would address this issue through well-established techniques for spatial normalization [18], although realignment of the data could also be done in sensor space [19–21].

2.3.2 MRI system

This MRI system is 0.4T Hitachi open type MRI system (AIRIS-Light MRI system: permanent magnetic type, made in Hitachi Co. Ltd. in Japan).

1. *EOG/ECG/EMG*: EOG/ECG/EMG were measured to test for subject's seating state on the chair in MEG system before the experiments, however these data were not used in MEG experiments because no artifacts and no noise for MEG data.
2. *Head shape system*: this MEG system used Head Position Indicator (HPI) for the digital value of the own head shape for individual subjects.
3. *Head movements*: head movements of MEG were recorded continuously by using advanced HPI system, and the head movement compensation algorithm was applied [22]. The difference of between head positions before and after the run of MEG was recorrected.
4. *Position of participants*: participants were in seated in MEG experiments, and the head of the participant was positioned in the MEG helmet under the gantry of MEG system in the magnetic shielded room.
5. *External stimulation and recording devices*: this MEG system has photodiode devices to determine visual stimulus onset with respect to MEG trigger, and MEG has delays of a few msec. MEG data were corrected for these delays.
6. *Coregistration*: this MEG system has the following coregistration procedure. Anatomical MRIs were used individually to apply to individual own MEG data only by oneself. The method section is described for the preprocessing of the MEG study as the following, and the order of these preprocessing steps were carried out.
7. *Bad MEG sensors*: in this MEG system there are sometimes a few bad MEG sensors. This MEG system has tuning program for all 122 sensor's tune, and after tuning processing a few bad sensors were found, and a few bad sensors were excluded during acquisition or analysis. The signals of bad sensors were interpolated to the signal estimation by using signal estimation software.
8. *Filtering processing*: in this MEG experiments we applied the following filtering. We used the digital band-pass filtering (0.3–40 Hz) the second order forward butterworth filtering with the windows algorithms.
9. *IAC algorithms*: ICA program was applied to input data of MEG. The number of components was five for the estimation. Criteria of ICA estimation on the total five components for selecting are determined to 85% to all components of data.

10. *Trials and segments*: trials and segments were anyways applied to reject under the criteria when the external bigger noises mix the income to the MEG data and the subject's unforecasted artifacts of movements.

In this MEG experiment, each subject's head was placed in a helmet with whole-cortex type SQUID sensors (Neuromag-122™, Electa Co. Ltd.). Three-dimensional orthogonal coordinates were determined in the helmet of the neuromagnetometer. Experiments were performed in the Kansai Center in Ikeda city, National Institute of Advanced Industrial Science and Technology (AIST) in Japan.

An incense outdoor was freely presented to the subject by means of a burning incense stick on a holder that was naturally held in front of the subject while seated in a chair in a magnetically shielded room.

2.3.3 Experiments of the stress state using subject's saliva

In these experiments, magnetoencephalography (MEG) was performed, and the cortisol value in the subject's saliva was measured in every state (before and after smelling two different incense outdoors (A and B)).

2.4 MEG experiments for four mode state

MEG response data were measured at the following four mode states, (1): control mode, (2): simple mode of putting the hands together, (3): smelling mode with putting the hands together, (4): only smelling mode. MEG data were added with 100 times averaging with the random sampling method. The subject pushed an optical sensor button with his or her own thumb.

1. In the control mode, the subject sat quietly and naturally in a chair with his or her eyes open and freely pushed the button of the optical fiber sensor at random times with the right thumb in synchronization with active inspiration (i.e., sniffing with the nose) of his or her own respiration rate, and the average MEG brain waves were obtained from raw data collected about 100 times in the control state [23].
2. For the next mode, the simple mode for the behavior of putting the hands together was performed as the experimental task, 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 simple mode of putting the hands together, the subject held the optical sensor between the hands and pushed the button with the right thumb at random times while putting the hands together.

By using the above two modes, we tried to measure the subject's own singular characteristic active area on the control state and to obtain the brain area activated by putting the hand together 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. In the next mode that included smelling and putting the hands together, we measured the MEG response of both brain activities: smelling the odor in synchronization with active inspiration (i.e., sniffing and smelling the incense odor) and the behavior of putting the hands together [6].
4. In the last smelling mode, when the subject smelled only the incense odor without putting the hands together, the averaged MEG response was measured by adding the raw MEG data collected about 100 times by pushing the optical sensor button.

Both the control mode and simple mode of putting the hands together were recorded in the absence of the burning incense odor. After one incense odor was tested, the room air including the odor in the magnetically shielded room was exchanged completely with fresh air by using a large fan for about 10 minutes.

2.5 MEG and data analysis

For the purpose of observing brain activity with greater accuracy, we used a whole-head 122-sensor neuromagnetometer (a DC-SQUID device of the first order differential planar type, by Neuromag, Finland). With an attached digital band filter capable of passing only measurements in the bandwidths of 0.3–40 Hz, only valid readings were collected at an actual sampling rate of 400 Hz and converted into digital values. To observe brain functions in several experimental modes, we used a whole-head type DC-SQUID, which allowed us to detect cortical current directly and to monitor brain activities [24]. This detection method is called MEG. The analog readings detected in this manner of the brain magnetic field were digitized at a sampling rate of 400 Hz with an A/D converter, downloaded, and stored in a PC.

2.5.1 122-channel neuromagnetometer of the Planar Type Gradiometer

The 122-channel neuromagnetometer of the Planar Type Gradiometer can calculate the first derivative of the magnetic vector field B_z through individual SQUID sensors installed on the helmet, or it can calculate $\{(\partial B_z/\partial x)_i, (\partial B_z/\partial y)_i\}$ about SQUID sensor i . Its dimension is $fT/cm \sqrt{Hz}$. The x- and y-axes represent the directions of longitude and latitude, respectively. A total of 122 sensor elements on the helmet were paired with the x- and y-axes, and each pair was assigned to measure one part of the head surface. A total of 61 sets (122 data points total) of magnetic field data can be detected, recorded at a particular interval (j), and calculated using the formula $\{(\partial B_z/\partial x)_{i,j}, (\partial B_z/\partial y)_{i,j}, (i = 1, 2, \dots, t; j = 1, 2, \dots, t)\}$.

The advantage of planar gradiometer is the ability to manufacture them using standard thin-film techniques developed for the semiconductor this can reduce manufacturing costs and increase the precision with which the coils can be made since slight imperfections in the size or orientation of the two loops can reduce their ability to perfectly reject the zero-order field.

2.5.2 Signal Space Separation (SSS) system

Signal processing method for noise reduction to this MEG system is Signal Space Separation (SSS) which reduces environmental noise [25]. This method mathematically decomposes the magnetic field recorded from a spherically distributed array of sensors into a series expansion composed of internal and external terms that represent the proportion of the measured fields arising from inside and outside the sphere, respectively. The measured signal is reconstructed using only the internal terms to discard the environmental noise [19, 20].

2.5.3 Source reconstruction

In general, we use the volume conductor model of the subject's head (e.g., Sphere model, BEM, FEM) individually and the lead fields algorithms for magnetic fields [26]. Normalization procedure was also used for spatial normalization after source localization by using SPM-12 of MRI software. The coordinates of subject's brain are linked to individual subject's brain structures using the source of the lookup table (e.g., FSL atlas).

2.5.4 Dipole fitting

The solutions obtained with dipole fitting approaches depend heavily on the choice that is made by the researcher. Therefore, this choice must be selected in with no intention. The reported solution for dipoles was chosen over a few alternative models. And the minimum current estimation method was used in our dipole fitting to MEG [27]. For example, the choices have to be made about the number of dipoles, time windows (single latency, multiple latencies), exact dipole models (moving, rotating, fixed dipole) for this process are shown as the following [28–32] and the fitting of the best cost function for the stability of solution [28, 33].

2.5.5 Single current dipole tracing method (single sphere model)

The single current dipole tracing method is a common technique for estimating a single source of magnetic field distribution that emerges on the head surface (on the outer surface of the helmet). Given the hypothesis that the brain magnetic field is not distorted, we surmised that the influence of the distribution current (the so-called “volume current”) is balanced by spatial symmetry and that the first order approximation of reading values is not affected, based on Biot-Savart’s law. If these presumptions are valid, an equivalent current dipole, as displayed in three-dimensional vectors, should emerge in the brains.

A critical step in the use of the single sphere model is the choice of the sphere center. The flow of volume currents would be most influenced by the boundary with the largest change in conductivity, the highly resistive inner skull surface is thought to be the optimal choice for defining the sphere surface. A best-fit sphere superimposed on an individual’s structural MRI scan and obtained from performing a least-squares minimization. We can achieve a relatively good fit of a sphere to the superior and lateral aspects of the inner skull, suggesting that a single sphere model is well justified for modeling sources in the central and lateral portions of the brain.

Still, for more nonspherical portions of intracranial space, such as near the inferior frontal and temporal regions, large deviations from sphericity can introduce errors into solutions [34–36]. The distortion of volume currents should be taken in consideration. A variant of the spherical head model that is widely used in clinical MEG applications is the model of local or overlapping spheres. Instead of using a single sphere model, spheres of different curvature are fit to the various areas of the skull underlying each MEG sensor. The individual sphere centers are then used in the forward model to better model local distortions in the volume currents based on the assumption that the local curvature influences the volume currents for nearby sensors more than for distant sensors.

The current dipole can be estimated by solving the inverse problem of the magnetic field distribution as projected on the head surface. For estimation, we first drew a magnetic field contour map in reference to the measured values of $(\partial B_z / \partial z)_{i,j}$ or in reference to the values of $\{(\partial B_z / \partial x)_{i,j}$ and $(\partial B_z / \partial y)_{i,j}\}$ with the inner estimation method. This magnetic field contour map allowed us to estimate a single source by following the least-squares estimation method. Using this method, the signal source can be defined as in the middle position of the extreme and the sink identified on the magnetic field. A single current dipole tracing method relies on the common notion that a higher parameter G value (goodness of fit: GOF) guarantees a higher accuracy in the least-squares estimation, and an estimated single source should therefore be closer to the actual value.

2.5.6 Evaluation method using statistical cost function (GOF)

The statistical cost function measures the goodness of fit (GOF) between the magnetic field predicted by the dipole location and moment and the measured field. Typical statistical cost functions include the percent of variance unexplained (residual variance) or the corresponding chi-square statistic value [37, 38].

Most common approaches for MEG source estimation, and the dominated the field for many decades, is to specify only one or a few equivalent current dipoles (ECDs) to represent the solution. The strength (dipole moment) of ECDs ranges anywhere from 10^{-9} to 10^{-7} Am (or 1–100 nAm). The evoked magnetic responses, which have typical source moments ranging from 10 to 30 nAm, may involve the activation of less than 1 cm^2 of cortex and are therefore reasonably well modeled as a single ECD.

For highly dipolar field patterns with high SNR, such as the early components of sensory responses, ECD solutions can reach a greater than 90% goodness of fit, with good correspondence to the corresponding sensory projection areas of the brain.

2.5.7 Multi-current dipoles tracing method (multi source models)

In general, the single current dipole tracing method is extremely useful if only one single cortical current is observed at a given instance as a result of brain activity. The method is not as valuable, however, if the entire brain is perceptively active and cortical current emerges at multiple points on the head surface. In such a case, use of the multi-current dipoles tracing method may provide a solution, as it presumes the appropriate number of dipoles likely to exist and estimates various current sources that may be occurring in the brain. Using this method, the parameter GOF becomes high only if the presumed number of dipoles is appropriate.

The ECD modeling approach was extended to more complex patterns of the brain activity by adding more dipole sources to the model. One solution is to keep adding dipoles until there is little or no improvement in the goodness of fit (GOF) measure or if the percent of variance obtained reaches a criterion. An alternative is to use an objective measure of signal complexity, such as the number of principal components requested to account for a criterion power.

To further stabilize the solutions, constraints can be applied (fixing the location of one source while allowing additional sources to have free parameters) such that very complex source models can sometimes be attained.

However, if it is not, the resulting estimate in the real clinical MEG is not close to the actual value. Because of the constraints in determining the propriety of the presumed number of dipoles and because of the subsequent, laborious calculations, the multi-current dipoles tracing method is usually deemed relatively unrealistic and impractical to the realistic clinical MEG.

2.5.8 Estimation of the current source by observing the magnetic field distribution

Unlike an experimental observing the spontaneous control state, the task of smelling state and putting the hands together state were designed to activate brain.

As the current dipole method was not originally intended to detect such a spontaneous control state, and because dipoles of the magnetic field are expressed in rather complicated patterns by this method, we traced the variations of the magnetic field distribution by their progress over time, as well as at given intervals.

Drawing a contour map of the recorded findings, we identified extremes (maxima) and sinks (minima) found in pairs respectively on the magnetic field. We then set a virtual current vector in the middle position between each pair of extremes and sinks and traced the variations of the vector over time. Although this method has not been established for signal estimation and can only give approximations, no other available method seems more practical or acceptable for evaluation of the spontaneous control state, where neither the single dipole method nor the multi-dipole method is useful.

We observed a combination of extremes and sinks on the brain magnetic field contour map, vertically upward from the vertex. Extremes and sinks were aligned in such a way that their magnetic fields were tangential to each other. In between, the cortical current ran in the direction of the tangent vector in accordance with Biot-Savart's Law [39, 40].

We calculated the magnetic field contour map at a single time, with all three cortical currents in clear view. The test results were analyzed using the method of contrast between extreme and sink. The pattern recognition analysis of the inverse problem method is also available and more precise; however, this method was too time-consuming considering the number of cortical currents we needed to observe [41]. With respect to our test objective, we prioritized efficiency over numerical precision, which is normally preferred in localizing brain functional foci.

In order to reliable ECD fit, we must have fewer models. Another popular approach that has been used in MEG source modeling is the so-called "Spatiotemporal Dipole Fit" introduced Scherg and Von Cramon [38] in which the time-varying amplitude (time course) of each dipole is used as additional information to constrain the solutions.

2.5.9 Data acquisition, processing and analysis

We traced the cortical current using the first-order differential planar type of DC-SQUID. This device enables us to detect the current source of brain activity directly under its sensor, revealing the maximum of the absolute values. This is the greatest advantage of using the differential planar type device, which has a dimension of $fT/cm \sqrt{Hz}$. When using neuromagnetometers of the axial type, as explained in Section 2.5.2 above (the single current dipole tracing method), we can estimate the current source as defined as the middle position between the minima and maxima of the cranial nerve magnetic field distribution [42].

Neuromagnetometers of the planar type are useful for determining where the current source of brain activity exists by detecting the maximum of absolute magnetic field values. We therefore used these readings to map the distributions of the cranial nerve magnetic field using MATLAB software, illustrating how the magnetic field varies over time [43]. Data acquisition began at the moment of the signal, although the data we actually used began 500 ms after the starting signal. Thus, we sampled the experimental activities of the brains. In the olfactory neural processing in humans, the responses of event related magnetic fields and evoked magnetic fields were obtained within about 250 ms in healthy subjects. In our MEG experiments, subjects sniff an incense odor actively by using his own nose and when starting to sniff he pushes the optical sensor button as a trigger signal. Therefore, to record the more precise changing of MEG we used the sampling interval with every 50 ms. So, measurements MEG responding data were analyzed by every 50 ms. By observing these cranial nerve magnetic field distributions on the surface of the head, we traced and recorded variations in the current source at each particular moment.

3. Results

3.1 Result of signal source estimation of MEG in the brain obtained with the single current dipole tracing method

3.1.1 Advantage of the real-time response of the brain's neural activities by analysis of millisecond-time resolution using the single current dipole tracing method of MEG

This single current dipole tracing method has the advantage of directly obtaining real-time responses of the brain's neural activities. This is different from fMRI and PET methods, which measure metabolism of physiologically active substances. We obtained changing activities of the signal source and estimated the active regions in the brain with analysis using the single current dipole tracing method. In single current dipole tracing method, the first main current dipole is the largest dipole. This current dipole was obtained in the middle position of extreme center and sink center identified on magnetic field. The second and the third current dipoles were smaller and weaker than the first main current dipole. Using this single current dipole tracing method, we can estimate only one current signal source (magnitude, direction, and location) as the most reliable neural activity in the brain.

3.1.2 Mechanism of the real-time estimation method of the active area using the single current dipole tracing method of MEG data

Figure 1 shows the real-time estimation method for obtaining the active area in the subject's brain. **Figure 1(a)** shows an example of a MEG response to random activities such as the control state before putting the hands together as assessed with the single current dipole tracing method. We could not obtain the dipole completely, and thus, we could not identify the generally active area in this control state (with no smelling odor and no putting the hands). **Figure 1(b)** shows contour mapping of MEG response at a control state.

Figure 1(a) shows over head vision, upper is anterior, lower is posterior of the head. Each curves show 122-channel MEG averaging response waves of duration 0.2 s time. A red vertical line shows starting time point for the inspiration of odorless air.

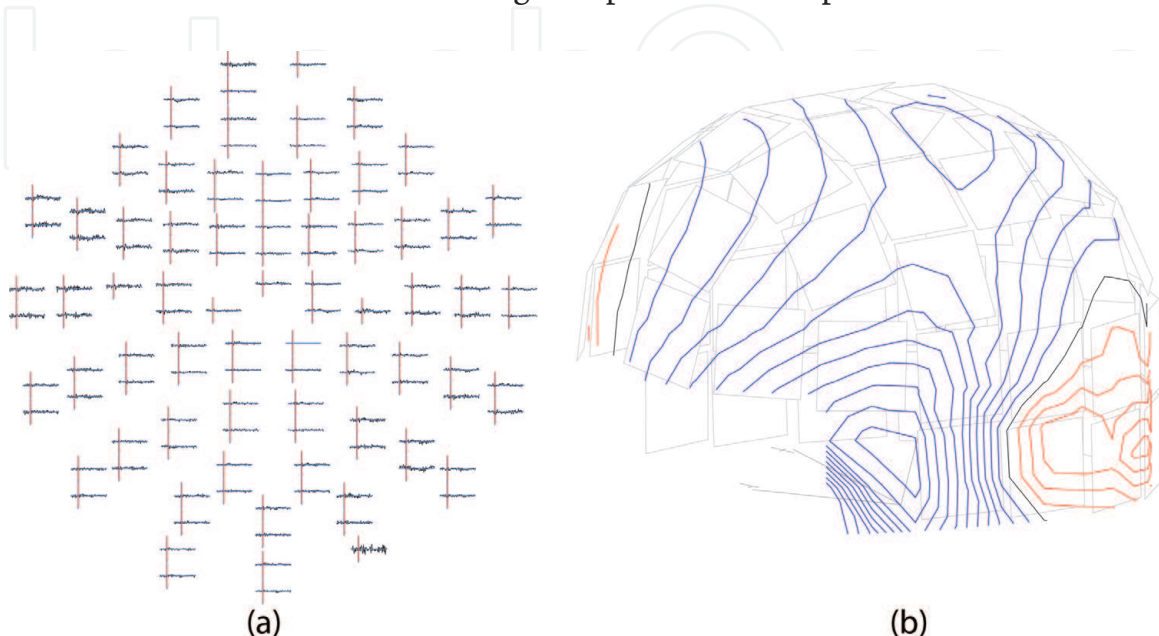


Figure 1.
Real-time active state at a control in our brain.

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

(a) The simple mode of putting the hands together without smelling

3.1.3 The theory of mirror neurons and the default mode network

In this experimental task, the subjects put their hands together or mimicked praying without smelling. We obtained the subject's type as an individual variation for the priority of brain laterality regarding putting the hands together or praying in daily life. **Figure 2** shows an example of the MEG response for the active area obtained with the single current dipole tracing method for this experimental condition. We analyzed estimated active areas continuously using a real-time estimation method. **Figure 2(b)** shows an MEG response on active area of left side brain as a left priority type after only putting the hands together (with no smelling odor). **Figure 2(c)** shows a vector of single current dipole estimated in the brain using 3-D coordinates.

Figure 2(c) shows a vector of single current dipole estimated in the brain using 3-D coordinates after putting the hands together. X-axis is the horizontal line of right to left ear, and Y-axis is the line from nasion toinion, and Z-axis is the upper to lower line of the vertical of the brain.

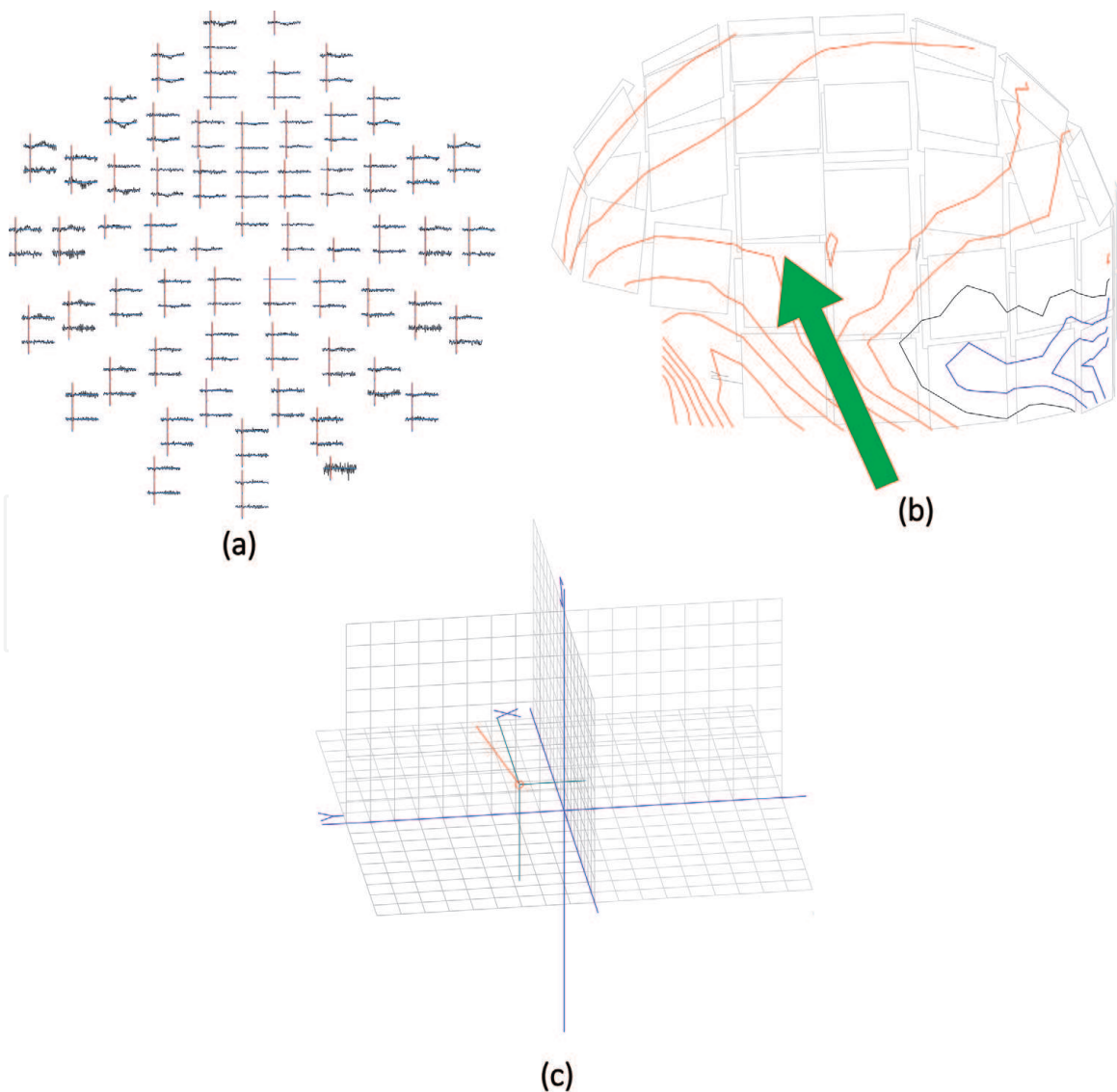


Figure 2.
Real-time estimation of the active area in our brain after only putting the hands together.

Table 1 shows an estimated ECD dipole each subjects for latency tie window (210–1100 ms), priority of the laterality (right or left), activated region, and GOF (statistical goodness of fit, %) for the simple mode of only putting the hands together without smelling.

3.1.3.1 a-1. Right priority brain type

Five of the 11 subjects had the right priority brain type for laterality. Three of these five persons regularly put their hands together in their daily life, and the other two did not.

3.1.3.2 a-2. Left priority brain type

Six of the 11 persons had the left priority brain type for laterality. Two of six subjects regularly put their hands together in their daily life, and the other three did not.

Only one subject of the 11 was not classified in these two groups, and this person (N1) had the left priority brain type estimated in central temporal gyrus (N1: latency 579.0 ms, GOF 32.8%) as shown in the above **Table 1**.

The priorities of brain laterality are considered important for obtaining the characteristic laterality of the active brain in daily life as described below, regardless of putting the hands together and praying or not.

3.1.3.3 A-group: (A1–A5) habit of putting the hands together or praying

As shown in **Table 1**, in the A-group which had the habit of putting the hands together in daily life, the main active areas in the brain were generally estimated to be on the right near the superior regions (A1: latency 309.2 ms, GOF 50.2%; A4: latency 405.6 ms, GOF 47.4%) or the left near central (A5: latency 1065.3 ms, GOF 57.6%) or left caudal regions (A2: latency 613 ms, GOF 47.9%) in the temporal gyrus. The right prefrontal area was activated in only one subject (A3: latency 974 ms, GOF 36.9%).

(a)	A-Group (Habit Group)					B-Group (No-Habit Group)					
Subject	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	N1
Latency (ms)	309.2	613	974	465.6	1065.3	215	236	303.4	419.4	366.3	579
Laterality	R	L	R	R	L	R	L	L	R	L	L
Activated region	superior temporal gyrus	caudal temporal gyrus	prefrontal lobe	superior temporal gyrus	central temporal gyrus	posterior frontal gyrus	central temporal gyrus	posterior frontal gyrus	posterior frontal gyrus	posterior frontal gyrus	central temporal gyrus
GOF (%)	50.2	47.9	36.9	47.4	57.6	28.1	68.6	30.5	19.7	27.3	32.8

Table 1. Results of MEG experiments for the simple modes (a) of only putting the hands together without smelling.

3.1.3.4 B-group: (B1–B5) no habit of putting the hands together or praying

As shown in **Table 1**, in the B-group, which did not have the habit of putting the hands together or praying, the main active areas in the brain were generally estimated to be the right posterior regions (B1: latency 215.0 ms, GOF 28.1%; B4: latency 419.4 ms) in the frontal gyrus and left central region (B2: latency 236.0 ms, GOF 68.6%) and left caudal regions (B3: latency 303.4 ms, GOF 30.5%; B5: latency 366.3 ms, GOF 27.3%) in the frontal gyrus.

(b) Simultaneous smelling an incense outdoor and putting the hands together mode

All 11 subjects were separated into two groups. The A-group had the habit of putting the hands together or praying according to the Japanese traditional conventional style of putting the hands together for a few minutes every day in their daily life. The B-group did not have this habit.

Table 2 shows an estimated ECD dipole for each subjects for latency time (290–1900 ms), priority of the laterality (right or left), and activated region, and GOF (statistical goodness of fit, %) for simultaneous smelling an incense outdoor and putting the hands together mode.

Figure 3 shows that the estimated current dipoles of four subjects were obtained at the F5 language area of the inner region (A5: right priority, latency 981.0 ms, GOF 68.0%; B3: left priority, latency 557.3 ms, GOF 28.3%; B4: left priority, latency 423.9 ms, GOF 58.0%; B5: left priority, latency 328.4 ms, GOF 34.5%) of the frontal gyrus in a simultaneous state of the smelling an incense outdoor and putting the hands together. These responses were presented in two subjects, one is OFC area (A3: right priority, latency 974.1 ms, GOF 33.1%) and another is F5 area (A5: left priority, latency 981.0 ms, GOF 68.0%) in the A-group and four subjects in the B-group after smelling incense odors A and B.

Figure 4 shows that the responses of another two subjects (A2: left priority, latency 627.8 ms, GOF 55.1%; A4: right priority, latency 309.3 ms, GOF 33.7%) in the A-group were obtained at the V1 visual area in the calcarine sulcus in the right or left occipital lobe after smelling incense odors A and B with putting the hands together. These V1 responses were not found in the B-group.

(b)	A-Group (Habit Group)					B-Group (No-Habit Group)					
Subject	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	N1
Latency (ms)	443.5	627.8	974.1	309.3	981	296.1	1851	557.3	423.9	328.4	974.1
Laterality	R	L	R	R	L	R	L	L	L	L	L
Activated region	insula	V1 occipital gyrus	OFC orbito frontal gyrus	V1 occipital gyrus	F5 inner frontal gyrus	insula	insula	F5 inner frontal gyrus	F5 inner frontal gyrus	F5 inner frontal gyrus	OFC orbito frontal gyrus
GOF (%)	62.5	55.1	33.1	33.7	68	36.7	64.6	28.3	58	34.5	67.6

Table 2. Results of MEG experiments for simultaneous smelling an incense outdoor and putting the hands together mode.

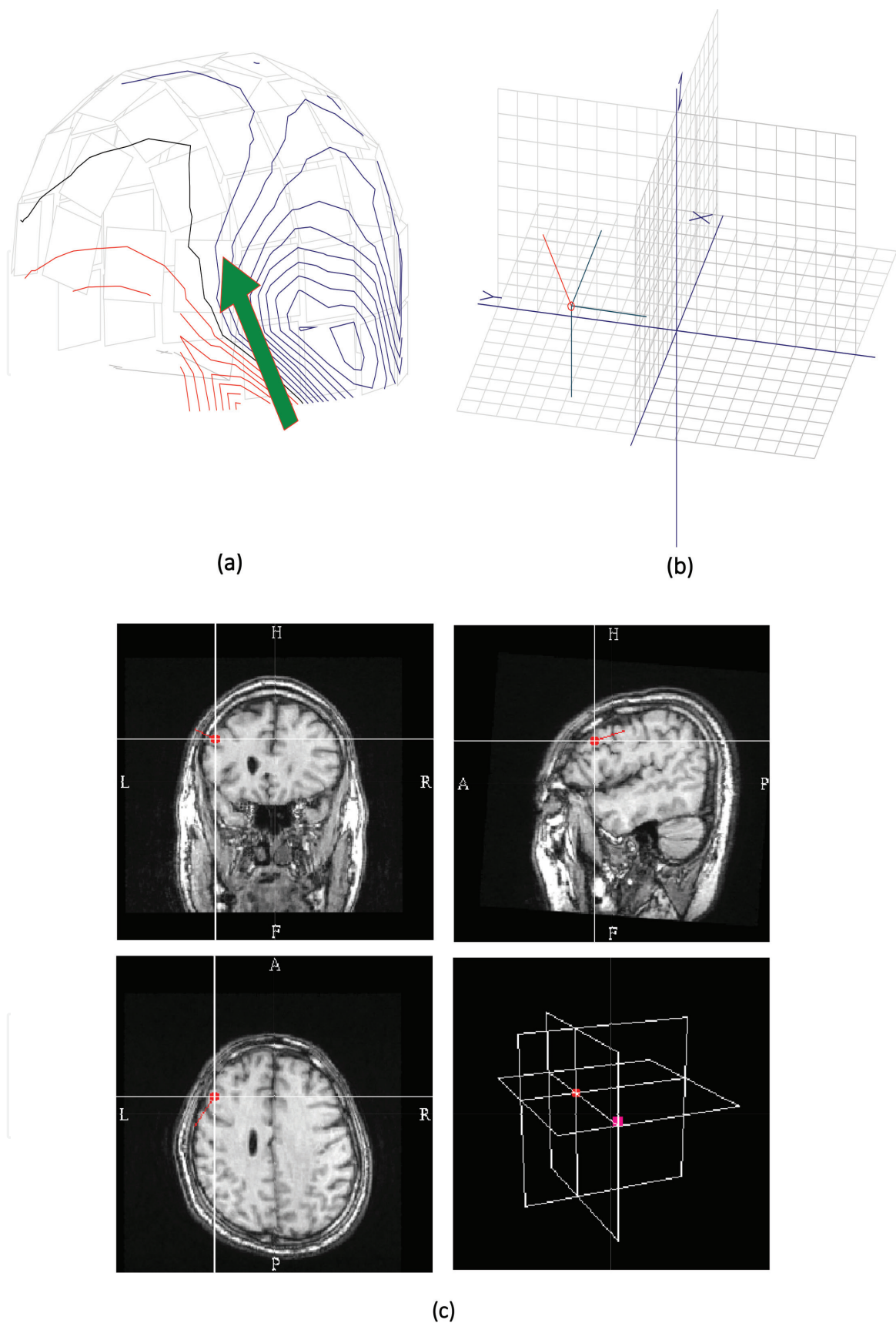


Figure 3.
F5 language area estimated by the simultaneous smelling an incense outdoor and putting the hands together.

Only one in the 11 subjects was classified in neither the A- nor B-group, and this only one subject (N1) was used by coating smell method. He had the left priority brain type. His estimated current dipole was obtained at the OFC orbito-frontal gyrus (**Figure 5**) (N1: left priority, latency 974.1 ms, GOF 67.6%) when he

performed the special activities of directly coating smelling both hands that were coated with the liquid incense odor A and putting his hands together.

As shown in the **Table 2**, another one subject (A1: right priority, latency 443.5 ms, GOF 62.5%) in the A-group with the habit of putting the hands together

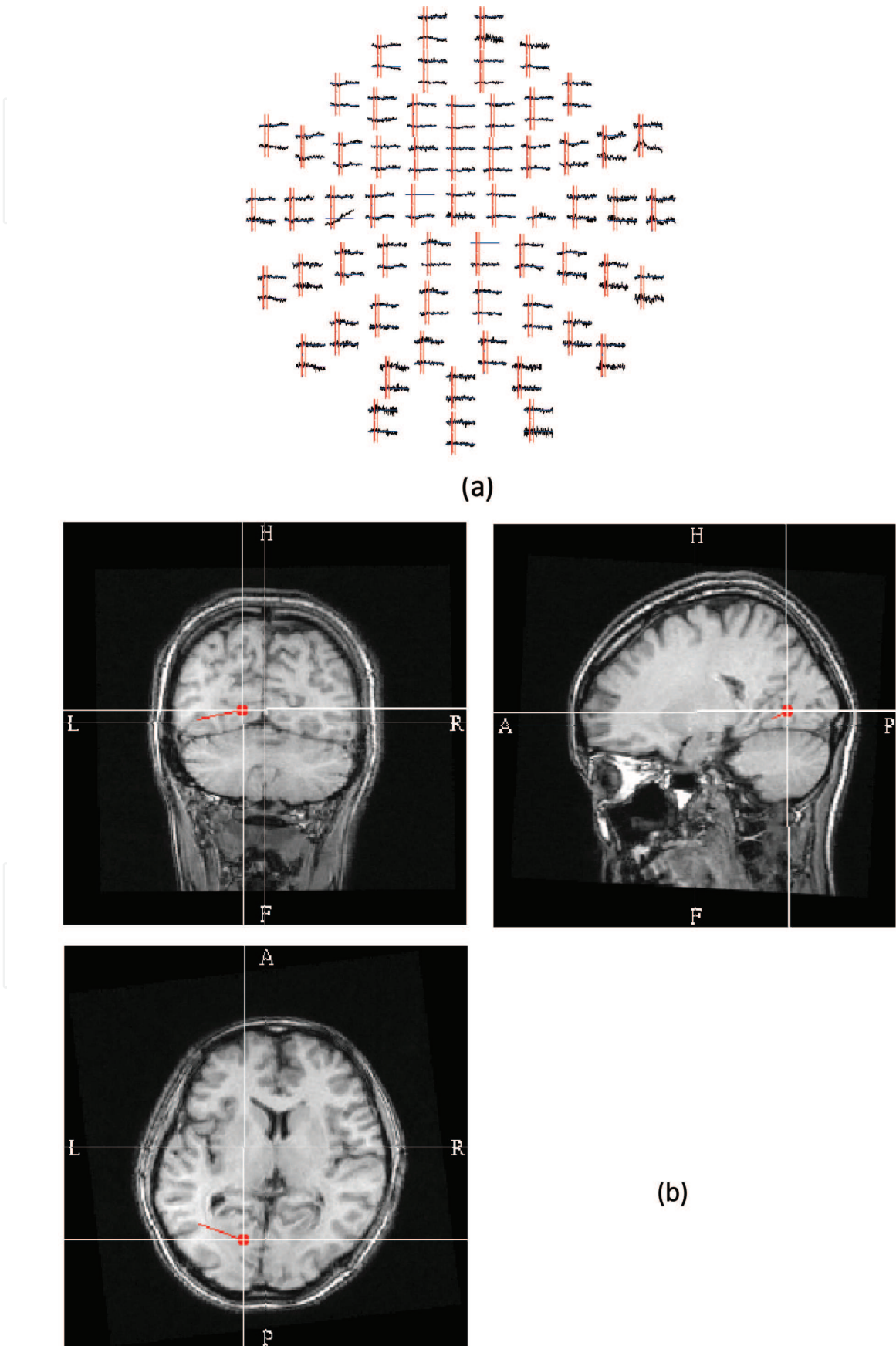


Figure 4.
V1 visual area estimated by the simultaneous smelling an incense outdoor and putting the hands together.

or praying showed the activity in the inner central temporal area in the right insula, and two subjects (B1: right priority, latency 296.1 ms, GOF 36.7%; B2: left priority, latency 1851.0 ms, GOF 64.6%) in the B-group without this habit also showed activity in the inner area in the right and left insula.

Figure 6 shows the estimated current dipoles of three subjects obtained in insula regions in the right and left temporal gyrus in both the groups after simultaneous

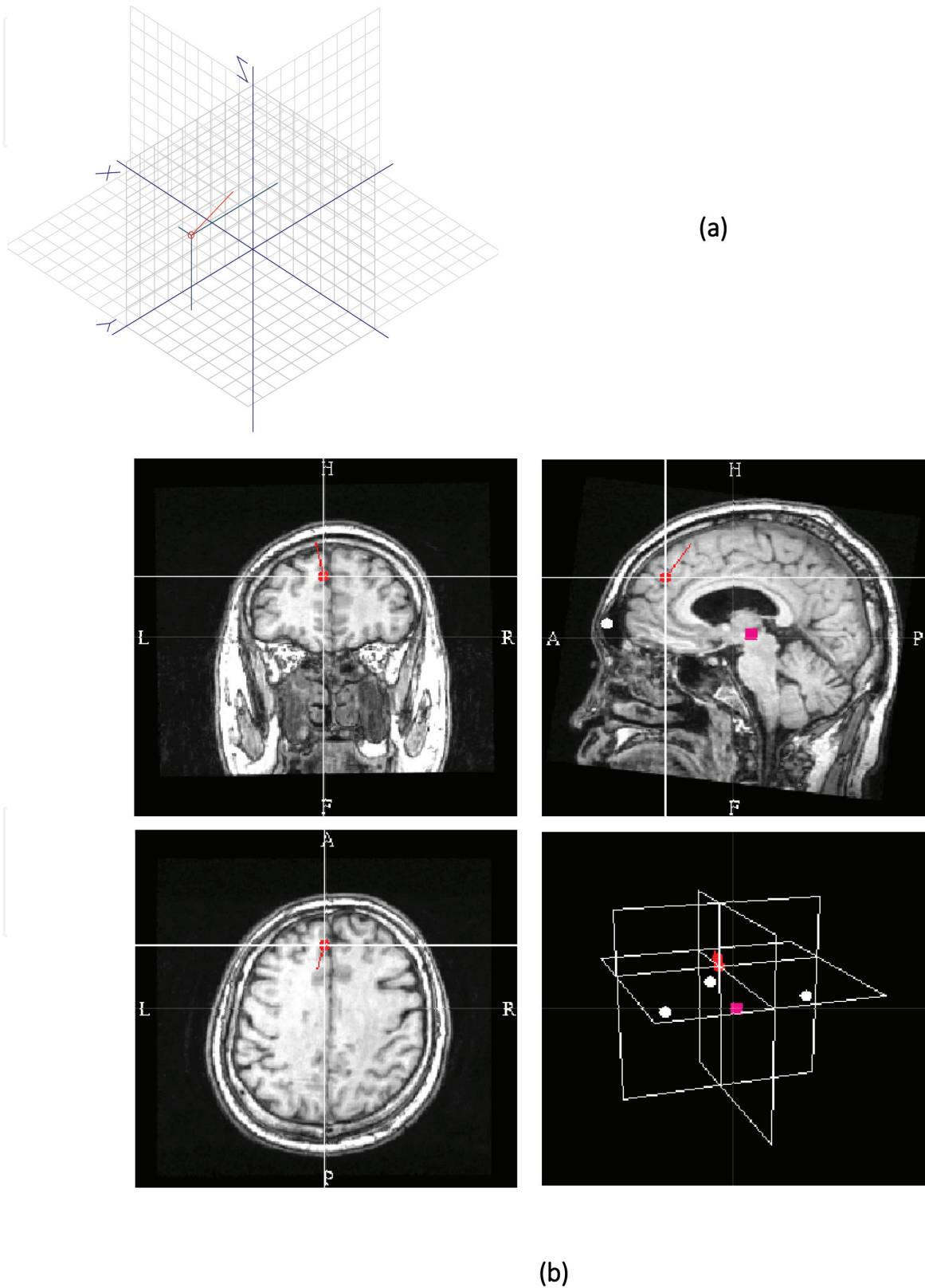


Figure 5. Orbito-frontal area estimated by the coating smell and putting the hands together in only one subject without A and B group.

smelling incense odors A and B outdoor and putting the hands together. In particular, the responses of almost all subjects in the B-group were found in temporal areas very close to the same regions as during the simple mode of only putting the hands together without smelling.

From the above analyses, in this task of simultaneous smelling an incense outdoor and putting the hands together mode, the brains of four subjects were activated in the F5 language area in the left frontal lobe. Two of four subjects had the right priority brain type and did not have the habit of putting the hands together in their daily life. However, their F5 language area in the left frontal lobe was activated after this task when simultaneous smelling an incense odor outdoor and putting their hands together. On the other hand, in two other persons with a habit of putting their hands together or praying in daily life, the right and left calcarine sulci of the V1 visual area in the occipital lobe were activated after the task of simultaneous smelling the odor outdoor and putting their hands together. From these all results, we consider that the F5 language area in the left frontal lobe and V1 visual area in the right and left occipital lobes were activated by the task of simultaneous smelling an incense outdoor and putting their hands together regardless of whether they had the habit of putting their hands together in their daily life. These phenomena are considered to be guided by the activation of mirror neurons and the default mode neural network's function.

(c) The mode of smelling only and not putting the hands together

Table 3 shows an estimated ECD dipole for each subjects for latency time (230–1100 ms), priority of the laterality (right or left), activated region and GOF (statistical goodness of fit, %) for the mode (c) of smelling an incense outdoor only and not putting the hands together.

3.1.4 One person (N1) not classified in the A- or B-group

3.1.4.1 c-1. Orbito-frontal lobe area

AS shown in the above **Table 3**, only one subject was not classified in either the A- or B-group, and this person (N1: right priority, latency 414.0 ms, GOF 43.0%) had the right priority brain type. His estimated current dipole was also obtained at the left or right orbito-frontal lobe when he performed only the mode of smelling both hands, which were coated with liquid odor A or B, without putting his hands together. In this experiment, he could smell and clearly perceive the odorants on both hands.

3.1.4.2 A-group: habit of putting the hands together or praying

AS shown in **Table 3**, one female subject had the right priority brain type. Her estimated current dipole (A4: right priority, latency 473.0 ms, GOF 35.5%) were obtained in the right insula in the temporal gyrus when she performed the mode of smelling only odor A or B without putting her hands together. Also, the estimated current dipoles of a male subject (A3: left priority, latency 563.2 ms, GOF 53.7%) and another female (A5: left priority, latency 520.2 ms, GOF 58.3%) who had the left priority brain type were obtained at the left amygdala in the olfactory nervous pathway system when they performed the mode of smelling odor B without putting their hands together. Another male subject (A1: right priority, latency 1060.3 ms, GOF 28.9%) was obtained at the posterior frontal gyrus and another female subject (A2: left priority, latency 598.3 ms, GOF 21.9%) was obtained at trigonum olfactorium in the olfactory pathway system in A-group.

They could smell and clearly perceive odor A or B, and therefore, we could obtain their nervous pathway system and active area through olfactory nerve projection regions.

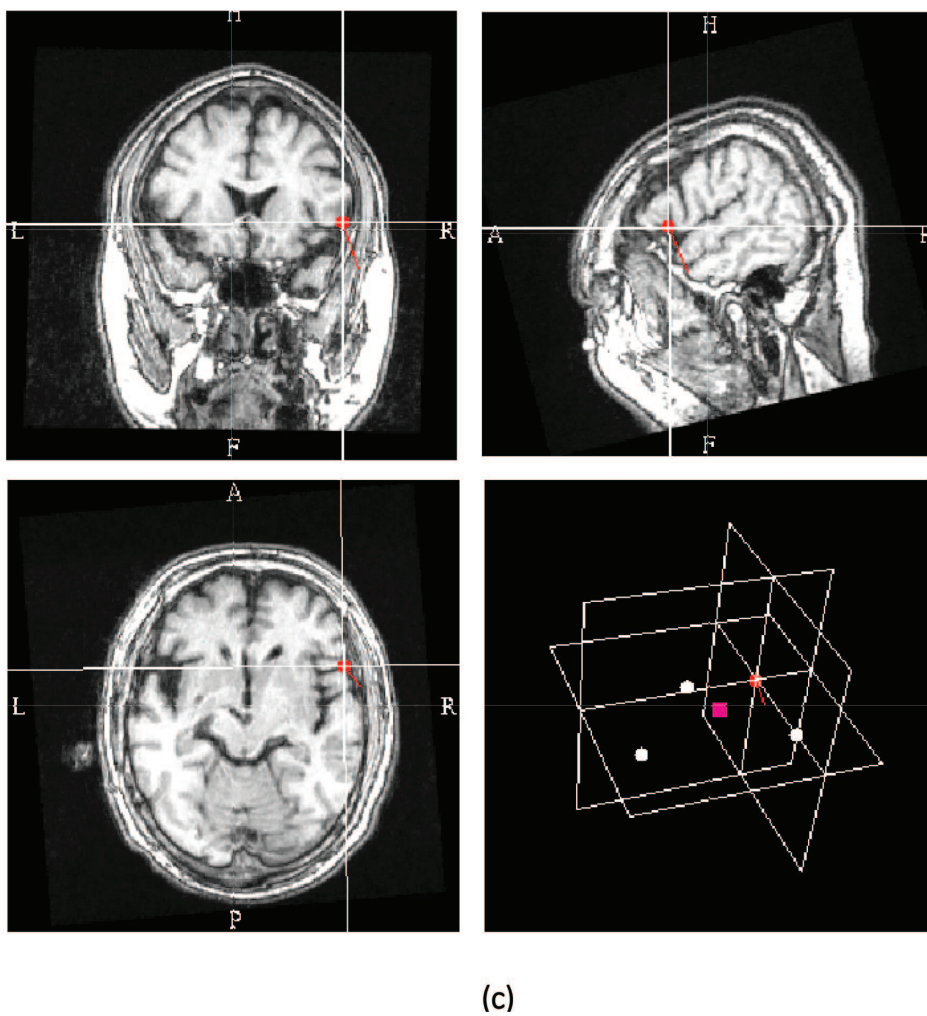
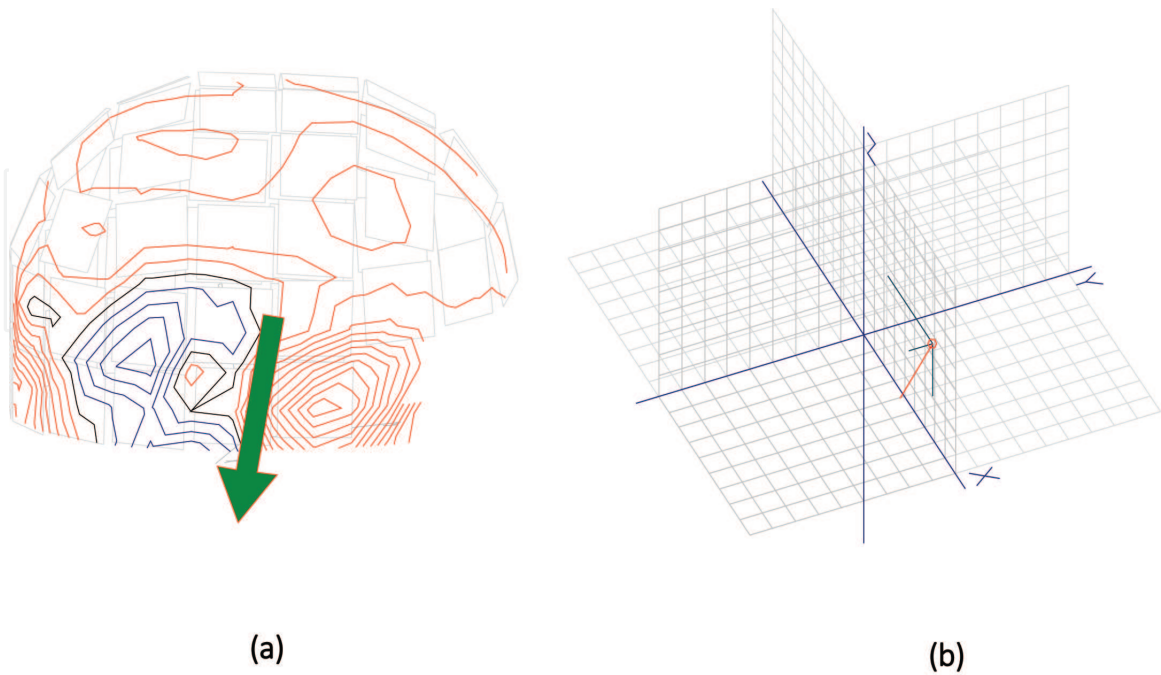


Figure 6. Anterior area in the temporal lobe estimated by simultaneous smelling an incense outdoor and putting the hands together in almost all B group.

(c)	A-Group (Habit Group)					B-Group (No-Habit Group)					
subject	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	N1
Latency (ms)	1060.3	598.3	563.2	473	520.2	502.5	509.7	237.1	252.2	303.4	414
Laterality	R	L	L	R	L	L	L	L	R	L	R
Activated region	posterior frontal gyrus	trigunum olfactorium	amygdala	insula	amygdala	amygdala	insula	insula	insula	amygdala	OFC orbito frontal gyrus
GOF (%)	28.9	21.9	53.7	35.5	58.3	55	38.2	57	35.2	45.3	43

Table 3. Results of MEG experiments for the mode (c) of smelling an incense outdoor only and not putting the hands together.

3.1.4.3 B-group: no habit of putting the hands together or praying

As shown in **Table 3**, two female subjects (B2: left priority, latency 509.7 ms, GOF 38.2; B4: right priority, latency 252.2 ms, GOF 35.2%) and one male subject (B3: left priority, latency 237.1 ms, GOF 57.0%) had the response at insula regions. Their estimated current dipoles were obtained in insula regions at the temporal gyrus when they performed the mode of smelling only odor B without putting their hands together.

On the other hand, other two male subjects had the left priority brain type. Their estimated current dipoles (B1: left priority, latency 502.5 ms, GOF 55.0%; B5: left priority, latency 303.4 ms, GOF 45.3%) were obtained at the left amygdala in the olfactory pathway system when they performed the mode of smelling only odor B without putting their hands together.

Although these subjects did not have the habit of putting the hands together or praying in their daily life, they could smell and clearly perceive odors A and B. Therefore, we could obtain the responses of their olfactory nervous pathway system and active areas through olfactory nerve projection regions.

3.2 Results of statistical analysis of the cortisol level in the saliva of each of the 11 subjects

1. Cortisol value before smelling the odor and MEG experiments
2. Cortisol value after smelling incense A
3. Cortisol value after smelling incense B

The cortisol value ($\mu\text{g/dL}$) is an index of the state of stress.

Table 4 shows the result of statistical analysis of each value, and the mean and standard deviation of the cortisol value were calculated for all 11 subjects, and for ten subjects, five subjects in the A-group and another five subjects in the B-group.

Next, statistical t-tests were performed to compare the cortisol values of each condition in all 10 subjects and each five subjects classified in the A- or B-group, respectively.

1. No significant difference was found among the mean cortisol value of the conditions 1: before smelling the odor, 2: after smelling incense odor A, and 3: after smelling incense odor B for all 10 subjects (see **Figure 7**).
2. The average cortisol value tended to decrease in the order of 1: before smelling the odor, 2: after smelling incense odor A, and 3: after smelling incense odor B in all 10 subjects and the five subjects in the A-group (see **Figure 8**).
3. A significant difference ($p < 0.078$) was found between the mean cortisol value of the condition after smelling incense odor A (2) and after smelling incense odor B (3) for the five subjects in the B-group (see **Figure 9**).
4. The average cortisol value tended to decrease in the order of (1) after smelling incense odor A (2), before smelling the odor, and (3) after smelling incense odor B for the five subjects in the B-group.
5. A different tendency in the average cortisol value was observed between the A-group and B-group. In particular, an effect of stress was observed for smelling incense odor A.
6. All subjects perceived and smelled incense odor B, which had no effect regarding stress.
7. For individual subjects, the cortisol value tended to decrease in the order of 1: before smelling the incense odor, 2: after smelling incense odor A, 3: after smelling incense odor B in five subjects in the A-group.
8. For individual subjects, the cortisol value tended to decrease in the order of 1: after smelling incense odor A, 2: before smelling the odor, 3: after smelling incense odor B in three subjects in the B-group.
9. For individual subjects, especially in the one subject who was different from the other subjects in the B-group whose cortisol value tended to decrease, the cortisol value tended to decrease in the order of 1: before smelling the odor, 2: after smelling incense odor A, 3: after smelling incense odor B, similar to the A-group.

3.3 Relation between the impression of the subject about the incense outdoor and stress measured by the cortisol value

3.3.1 A-group: habit of putting their hands together or praying

Almost all subjects in the A-group, except for one female, felt that incense odor B was more familiar than incense odor A in daily life. However, both incense odors were pleasant for all subjects in the A-group according to psychological inquiries. In these cases, the cortisol value for almost all subjects except this female decreased in the order of 1: before smelling the odor, 2: after smelling incense odor A, and 3: after smelling incense odor B. In other words, almost all subjects except this female reported a decrease in stress in the order 1: odor B, 2: odor A. 3: no odor.

Cortisol value ($\mu\text{g/dL}$)	A-group (habit group)					B-group (no-habit group)					N1	Average	Standard deviation
	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5			
Before experiment	0.34	0.12	0.1	0.15	0.13	0.08	0.16	0.15	0.34	0.07	0.09	0.16	0.177
After incense A	0.32	0.07	0.08	0.1	0.1	0.12	0.16	0.25	0.15	0.23	0.06	0.15	0.184
After incense B	0.26	0.07	0.07	0.07	0.07	0.07	0.16	0.12	0.09	0.16	0.03	0.11	0.163
Average	0.31	0.09	0.08	0.11	0.1	0.09	0.16	0.17	0.19	0.15	0.06		
Standard deviation	0.057	0.035	0.021	0.057	0.042	0.007	0	0.021	0.177	0.064	0.042		

Table 4.
Results of cortisol value ($\mu\text{g/dL}$) in the saliva for each 11 subjects.

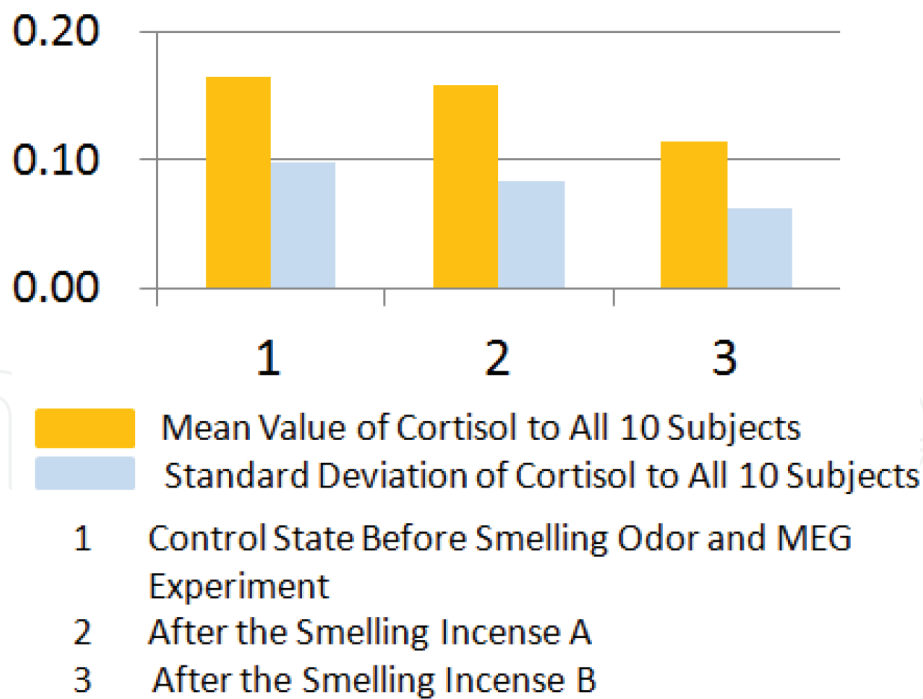


Figure 7.
 Statistical analysis of cortisol value for all 10 subjects.

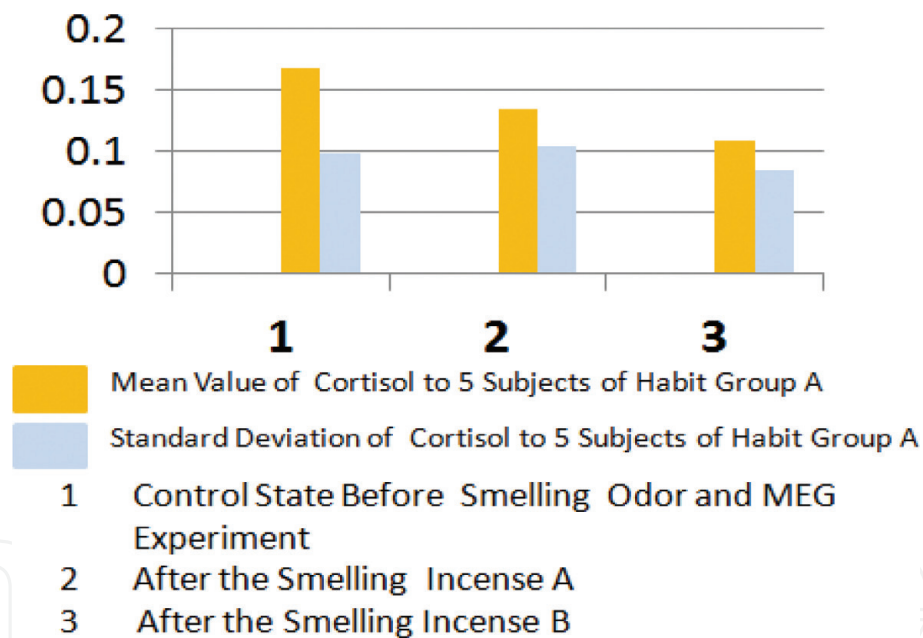


Figure 8.
 Statistical analysis of cortisol value for habit group A.

Only one female subject was different from the other subjects in the A-group. She liked incense odor A more than incense odor B. Therefore, she felt not more stress from incense odor A than incense odor B. Her cortisol value decreased in the order 1: no odor, 2: odor A, odor B.

3.3.2 B-group: no habit of putting the hands together or praying

Almost all subjects in the B-group reported feeling more stress for incense odor A than incense odor B, because incense odor B was considered more familiar in their daily life. In contrast, almost all B-group subjects felt stress for unfamiliar odor A more than the state of no odor before smelling. Their cortisol value decreased in the order 1: incense odor A, 2: no odor, 3: incense odor B.

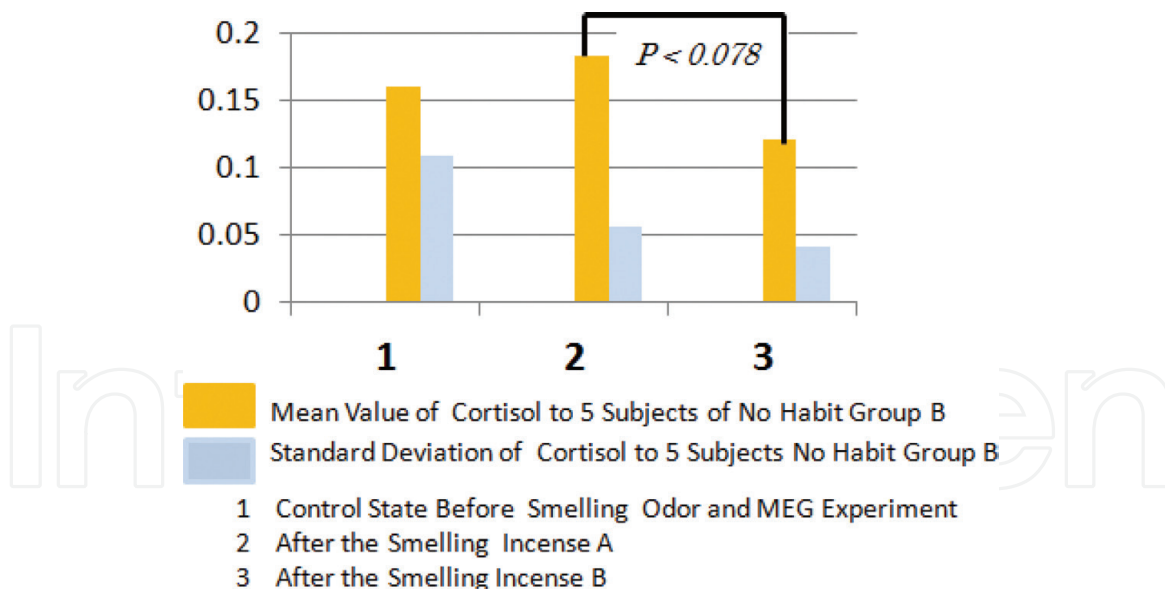


Figure 9.
Statistical analysis of cortisol value for no habit group B.

From these analyses about the relationship between the impression of the odor and the measured cortisol value, the nature of the state of stress was different in the A-group and B-group.

3.4 Summary of results

3.4.1 *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 using the methods of (1) Control mode in section 2.4 as the obtained **Figure 1(a)** and **(b)** showed the distinct and objective activities of our brain on the control state of non-motor system's activity clinically. However, in the MEG experiments of only putting the hands together without smelling using methods of (2) Simple mode of putting the hands together in section 2.4 as shown at (a) in **Figure 2(a)–(c)** we obtained the MEG local estimated signal response areas for the distinct mirror neuron activity. In our MEG experimental results for only putting the hands together without smelling an incense outdoor, 11 subject's detailed responses were obtained as **Table 1** in which A-group subjects were obtained in superior and anterior temporal gyrus or central and caudal temporal and frontal gyrus, on the other hand B-group subjects were obtained also the same temporal and frontal areas. These results show that the estimated local activated regions of B-group having the no habit of putting the hands together or praying are almost all resemble to the activated areas in brain of A-group having the habit of putting the hands together in daily lives. These results of the coincidence active areas in A-group and B-group in the behavioral action for putting the hands together show the distinct activities of mirror neurons activities as the imitation in the brain without the simple artifacts of moving error activities in moving neuronal system.

3.4.2 *The simultaneous new specific stronger effects of both the distinct mirror neuron's activity putting the hands together and the activities of smelling an incense outdoor at the same time*

Our MEG experiments of the above results using the methods of (3) Smelling mode with putting the hands together in section 2.4 as the obtained (b) in **Figure 3(a–c)**

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 an incense outdoor. In this simultaneous status mode of our MEG experiments, this specific active area in **Figure 3** were shown in distinct F5 language areas of the inner regions of the left frontal lobe or orbito-frontal gyrus (OFC) clinically. These responses were presented in two subjects in A-group and four subjects in B-group. These specific results show 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. The specific responses of another two subjects in A-group showed the simultaneous other new specific stronger effects of both the mirror neuron's activities putting the hands together and the activities of smelling an incense outdoor at the same time in V1 visual areas in the calcarine sulcus in occipital lobe clinically as the another distinct active areas as shown in **Figure 4(a)** and **(b)**. Only one person of 11 subjects in neither A- nor B-group who used by the direct coating strong smell over the hands showed the specific simultaneous activities in the orbito-frontal lobe as shown in **Figure 5(a)** and **(b)**. And the simultaneous specific activities in the brain both the putting the hands together and smelling an incense outdoor at the same time of other five subjects were obtained in anterior and posterior areas in the temporal lobes as shown in **Figure 6(a)–(c)**. These detailed MEG response data are shown in **Table 2** for simultaneous smelling an incense outdoor and putting the hands together and these results show the specific new strong effects of simultaneous responses in the relation of both the mirror neuron activities and olfactory effects at the same time.

3.4.3 The mode of smelling an incense outdoor only without putting the hands together (olfactory response with non-mirror neuron activity)

The detailed responses of our MEG experiments of the above results in the mode of smelling an incense outdoor only without putting the hands together (non-mirror neuron activity) were shown in **Table 3** with almost all subject's data. From these clinical and objective MEG measurements and analysis we obtained the distinct olfactory activities clearly such as the frontal and temporal regions in the olfactory nervous projection areas and olfactory nervous pathways nevertheless A- and B-group.

4. Discussions

4.1 The inverse problem: source estimation models

We used dipole models for the source estimation of the recorded MEG signals. The simpler spherical model for the head is adequate for MEG source modeling in most cases. In addition, MEG benefits from very precise knowledge of the real sensor geometry, including registration of sensors to the head.

However, source modeling in MEG remains a challenging mathematical problem, especially for more complex configurations of neuronal sources associated with higher cognitive function.

As a realistic clinical tool to for the spatio-temporal localization of the evoked brain activity by simultaneous smelling an incense outdoor and putting the hands together.

A variety of methods have been applied to the MEG source estimation problem to overcome the limitations. Using the individual's MRI scan of every subjects, template can provide good approximations for realistic head modeling. For example, finite element models (FEMs) could be applied to drastic changes in tissue conductivity and can be modeled more accurately in future [44].

4.2 Mirror neurons and the default mode network

The concept of mirror neurons was described by Marco Iacoboni. These neurons are located in the F5 inner area of the prefrontal lobe [7]. In general, the motion of putting the hands together and mimicking behavior are considered to activate the mirror neuron mechanism [8, 45, 46] and the default mode network in the human brain [9, 47–49]. These neural effects are considered to increase activity in the central areas of the temporal lobe and the caudal area of the frontal lobe according to the imitation principal [50–54].

The theory of these mirror neurons revealed the principal of imitation of behavior. Although these F5 areas in the left side of the human brain are in the same areas as Broca's language regions, F5 areas of both sides of the brain function to mimic motion and behavior. From anatomical research, F5 areas are connected to pre-motor areas and supplemental areas in movement regions in the brain.

Mirror neurons are thus considered to function for imitation of the habit of putting the hands together or praying, which is also performed with both hands by almost all elderly Japanese people in their daily life.

Super mirror neurons are concerned with determination of values, recognition of oneself and others, and reward from one's work. The inner default mode network controls the fundamental activity of daily movements and the resting state of the human brain. Because this default mode network is strongly related to super mirror neurons, discrimination of oneself from others and the determination of social cognition are considered important in human daily life.

4.3 The meaning of simultaneous smelling an incense outdoor and putting the hands together

Odorants stimulate activity in the olfactory nervous center, orbito-frontal areas, and others in the human brain [1–4]. Neurophysiological experiments in monkeys have shown that the olfactory nervous center and olfactory pathway project to the orbitofrontal cortex [55–57]. In humans, olfactory event-related potentials and magnetic fields evoked by odorant pulses synchronized with respiration are also found in the orbitofrontal area [5, 6, 58, 59].

In this experiment, only one subject was not in the A- or B-group and smelled his hands that were coated with liquid odor. By performing this behavior, he clearly experienced strong A and B odors. We estimated that the areas activated by his sniffing of both the A and B odors were the prefrontal area and the right or left orbito-frontal area.

In habits of daily life, the brain of A-group people after smelling incense odors and putting their hands together or praying was activated at the inner lobe of the frontal area, F5 language area, anterior area in the temporal lobe, orbito-frontal area, and others.

The brain of B-group individuals who did not have the habit of smelling incense odor or putting their hands together or praying in their daily life was also activated at the inner lobe of the frontal area, anterior area in the temporal lobe, and F5 language area in the left frontal lobe, similar to the A-group.

These results suggest that mirror neurons or the super mirror neuron system and the default mode network system in the brain of B-group subjects were activated by both smelling the incense odor and their imitation of putting their hands together, although they did not have the habit of smelling incense odors or putting their hands together or praying in their daily life.

From the above analyses, in the task involving simultaneous smelling an incense outdoor and putting the hands together, four person's brains were activated in the

F5 language area in the left frontal lobe. Two of four subjects had the right priority brain type and no habit of putting their hands together in their daily life. However, their F5 language area in the left frontal lobe was activated after this task.

On the other hand, in two persons with a habit of putting their hands together or praying in their daily life, the right and left calcarine sulci of the V1 visual area in the occipital lobe were activated after the task of simultaneous smelling an incense outdoor and putting the hands together [60–63].

5. Conclusions

This research revealed that simultaneous smelling an incense outdoor and putting hands together increased the activity of specific brain areas, for example inner areas of the prefrontal cortex and F5 regions of the human brain. In our experiments, evoked neuronal activity was recorded by the MEG and the cortisol value in the subject's saliva was measured in every experimental stage. From a few previous researches, it is known that F5 area is activated during observation of certain actions, during action execution etc. and these results show F5 have multimodal and different type of neurons. Moreover, the F5p is also known as a hand-related area that encoded goal-directed actions, not only mimic or autonomic actions. Our results demonstrated that the sources of MEG which are postsynaptic signals synchronized activation of intracellular currents across dendrites of cortical pyramidal neurons link strongly with anatomic position of mirror neurons. Mirror neurons in our experiment case are considered to have the function for imitation of the habit of putting the hands together or praying, which almost all elderly Japanese peoples often practice in their daily life. Super mirror neurons are concerned with determination of values, recognition of oneself and others, and reward from one's work. The inner default mode network controls the fundamental activity of daily movements and the resting state of the human brain. Because this default mode network is strongly related to super mirror neurons, discrimination of oneself from others and the determination of social cognition are considered important in human daily life. From these mirror neuron theories and the above summary of our results (1). We can conclude the distinct activities as follows. From these concerns and the above summary results (2) and (3), it can be considered that the specific regions in the brain such as the F5 language area in the left frontal lobe and the V1 visual area in the right and left occipital lobes were distinctly activated by the simultaneous new stronger effects increased with the task of smelling an odor and putting their hands together regardless of the habit in daily lives. These results show that the sources of MEG strongly link with the anatomic positions of mirror neurons and their types. Especially, these phenomena are considered to be guided by the simultaneous new stronger effects increased by both the olfactory activities of smelling an incense outdoor accompanied with the activation of mirror neurons and the default mode neural network [64–66] for the imitation behavior of putting hands together. From the above results, we consider that the F5 language area in the left frontal lobe and V1 visual area in the right and left occipital lobes and other specific brain areas were activated distinctly by the task of simultaneous smelling an incense outdoor and putting their hands together regardless of whether they had the habit of putting their hands together in their daily life. From our experiments, the cortisol value in saliva for the stress and the specific mirror neuron theories. we conclude that the simultaneous new specific effects both the smelling an incense outdoor and the imitating the behavior of putting the hands together can be considered to increase the activities of these areas in the human brain due to mutual interactions, reciprocal connections, or alternative actions.

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Author details

Mitsuo Tonoike* and Takuto Hayashi
Department of Medical Engineering, Faculty of Health and Science,
Aino University, Ibaraki, Osaka, Japan

*Address all correspondence to: gah00161@nifty.ne.jp

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