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Magnetoencephalographic Study of Cortical Activity Evoked by Electrogustatory Stimuli

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Abstract

Electrogustometry is a convenient method to examine taste acuity in clinical situations. Some basic properties of neural activity in the cerebral cortex in response to electrogustatory stimulation were revealed by measuring magnetoencephalography (MEG) signals with a whole-cortex-type system in response to varying intensities of anodal DC currents focally applied to the tongue surface in human subjects. Independent component analysis was used to eliminate stimulus artifacts in MEG signals. Electrogustatory stimulation with intensities of induced electric taste evoked responses bilaterally, mainly in the opercular–insular cortex with a mean onset latency of ~350 ms, while subthreshold electrogustatory stimulation induced modest responses in the cortex. Stronger stimulation induced a tingling sensation and elicited large transient responses in both the opercular–insular and somatic sensory cortices. This is the first description of the basic properties of human MEG responses to electrogustatory stimulation.

Key words: electric taste, human, magnetoencephalography, taste

Introduction

Magnetoencephalography (MEG) is a non-invasive functional brain imaging technique that has good temporal resolution compared to functional magnetic resonance imaging (fMRI) or positron emission tomography (PET). It can give a good estimation of the localization of the activities with more precision than electroencephalography (EEG), and has been used as a tool to analyze cortical taste responses (Kobayakawa *et al.*, 1996, 1999; Murayama *et al.*, 1996).

To obtain a good summation of gustatory evoked brain responses that can be attained by a sophisticated taste-delivery system, one of the requirements in an MEG study is to apply repetitive taste stimulation with a well-synchronized onset timing (Kobayakawa *et al.*, 1999). By applying an anodal DC current to the tongue, which is known to elicit a unique taste called 'electric taste' (Bujas, 1971; Frank and Smith, 1991), this requirement can be easily fulfilled due to the rectangular pulses that result from this electrogustatory stimulation. Further, while usually mouth rinsing must follow stimulation with taste solutions, in this situation it is not necessary, which leads to shorter experimental times. Electrogustatory stimulation also has the advantage of stimulating a confined area of the tongue. Because of these advantages, various electrogustometric analyses have been developed and extensively used by clinicians to evaluate taste disorders using electrogustometers (Tomita and Ikeda, 2002).

However, with the exception of the classical EEG studies (Plattig, 1969, 1971; Plattig and Kobal, 1977; Schaupp, 1971) and the recent fMRI study by Barry *et al.* (Barry *et al.*, 2001), the response characteristics of the human brain to electrogustatory stimuli have not been well analyzed. The present study aimed to use MEG to reveal some basic response characteristics of cortical neurons to electrogustatory stimulation, and also to address the question of whether projections of gustatory information to the human cortex are ipsilateral or contralateral to the side of the tongue stimulated.

Materials and methods

Subjects

Twelve healthy right-handed subjects (six females and six males, aged 21–40 years) participated in this study. All were fully informed of the nature of the experiments, and agreed to be subjects. The experiment was conducted in accordance with the revised version of the Helsinki Declaration and was approved by the Osaka University Ethical Committee.

Stimuli

A commercially available electrogustometer (TR-05, Rion Co., Tokyo, Japan) was used to deliver anodal DC currents with varying strengths and durations. Two circular (7 mm in diameter) anode electrodes plates (Ag–$AgCl_2$) were placed on the edge of the anterior area of the tongue and a similar cathode electrode was taped to the tip of the chin. The electrodes were connected by a twisted-pair of copper wires to the electrogustometer. The anodes were securely placed on the left and right lateral edges, 2 cm from the midline (Tomita and Ikeda, 2002), with a special chamber made of silicon rubber. Currents were applied to the anodes of either side. After a pilot study, we decided on a stimulus duration of 200 ms and an interstimulus interval of 20 s. Current strength varied among subjects due to differences in sensitivity to the anodal currents applied. Therefore, prior to the MEG recording session, subjects were asked to verbally express what they had perceived after the delivery of various current strengths ranging from 5 μA (minimum) to 210 μA (maximum) by decibel steps (Tomita and Ikeda, 2002). A consistently detectable range of stimulus strength was chosen for each subject.

Recording

Each subject was comfortably seated on a non-magnetic chair in a magnetically shielded room. The onset of electrical stimulation provided a trigger signal for MEG averaging. The subjects were instructed not to change their head position, to keep their eyes open and fixate on a point in front of them.

Each subject participated in three sessions per day with intermissions: (i) a weak current eliciting an unidentified sensation; (ii) a moderate current eliciting taste sensation; and (iii) a strong current eliciting a somatosensory tingling sensation (or irritation). Each subject received 60 trials of electric stimulation per session, and data were averaged on-line. Trials containing eye blink artifacts were rejected from the averaging process.

Brain magnetic fields were recorded with a whole-cortex, 122-channel SQUID system (Neuromag-122^{TM}, Neuromag Ltd, Helsinki, Finland). The MEG sensor positions with respect to each subject's head were determined by measuring the magnetic fields generated by three marker coils located on the scalp, whose locations in relation to three landmark points at nasion and two preauricular points were determined before the experiments using a three-dimensional digitizer (Polhemus, Inc., Colchester, VT). Stimulus related epochs of 1100 ms, including a 100 ms pre-stimulus baseline, were recorded with a pass-band of 0.03–100 Hz and a sampling rate of 400 Hz.

Data processing

Stimulus-related artifacts in the MEG signals were removed by independent component analysis (ICA) (Iwaki *et al.*, 2003). We adopted an ICA technique based on an information maximization (infomax) approach (Makeig *et al.*, 1997), which minimizes mutual information among the output independent components by maximizing the joint entropy of the output of a simple neural network that is an ensemble of 'sphered' (zero-mean) input vectors, linearly transformed and sigmoidally compressed (Makeig *et al.*, 1997). Infomax ICA was applied to the 60 daily trials of each subject measured by 122 magnetometer channels producing 122 temporally independent components, and the trials for each component were averaged separately. Independent components obviously representing the stimulus-related artifacts were excluded, and the remaining components were projected onto the original MEG signal space to reconstruct the artifact-free MEG signals. After averaging the data, source estimation was carried out using single equivalent current dipole (ECD) analysis (Imada *et al.*, 2001). In the single ECD analysis, all the MEG sensors were divided into 34 overlapping local sensor groups having 14–20 sensors each. The single equivalent current dipole was estimated for each local sensor group with a time interval of 2.5 ms, and only dipoles that continuously (>10 ms) and simultaneously satisfied the criteria that (i) the GOF values were >80% and (ii) the confidence volume was <2000 mm^3 were selected for further analysis. Estimated ECDs were superimposed on MR images.

Results

Figure 1 shows intensity–perception profiles for seven subjects when different strengths of anodal DC currents were applied to the right side of the tongue surface. Although the strength and range of current varied among subjects, they generally felt no sensation or unidentified sensations (or weak tingling) (<25 μA), taste sensations (25–50 μA) and finally tingling sensations (somatosensory but not a taste sensation) (>50 μA) as the strength of electric current increased. Unidentified sensations occurred before (n = 2) or after (n = 2) taste sensations, but were not reported by three subjects.

Figure 2 shows averaged MEG wave forms obtained from one of 122 channels in a subject undergoing electrogustatory stimulation with a strength of 32 μA and duration of 200 ms. As shown in Figure 2A, the original MEG signal contains a large artificial deflection corresponding to the

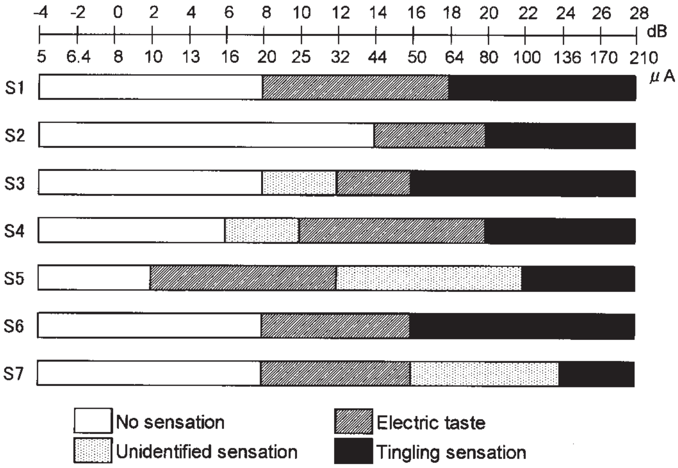

Figure 1 Intensity-perception to electrical stimulation of the right side of the tongue of seven subjects.

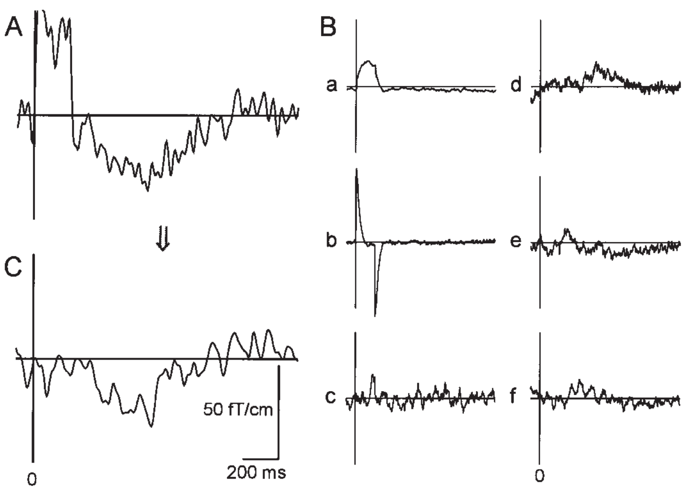

Figure 2 Example of stimulus-related elimination of artifacts by an independent component analysis. Raw MEG recording **(A)** in response to electrogustatory stimulation was separated into six components **(B)**. After elimination of the artifact components (a and b), an artifact-free MEG record **(C)** is obtained.

current delivery period. Figure 2B shows six typical components decomposed by ICA. Components a and b are stimulus-related noise components which should be disregarded. Figure 2C shows the MEG waveform after reduction of these noise components.

Figure 3 shows three superimposed traces from 24 channels overlying the left temporal region in a subject. Each trace represents MEG averaged over 60 trials at one channel. At 4 μA stimulation, this subject felt no particular sensation and showed small, transient responses (Figure 3A). At 32 μA, the subject perceived electric taste and showed prolonged responses during and after the course of analysis (Figure 3B). At 80 μA, the subject felt a tingling sensation without any taste sensation (~230 ms) and showed large, but brief (~400 ms) responses with a shorter onset latency than that for the taste response.

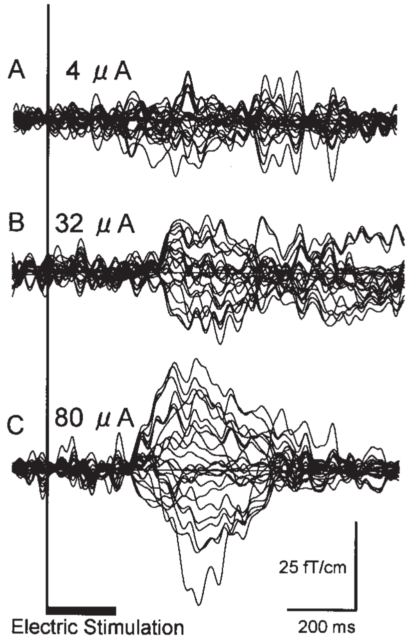

Figure 3 Superimposed traces of MEG signals from 24 channels overlying the temporal lobe to three strengths of electrical stimulation of the tongue. The currents applied were 4 μA **(A)**, 32 μA **(B)**, 80 μA **(C)**.

When the first ECD after onset of stimulation was estimated in the cerebral cortex using a single-dipole model and was superimposed on the subject's MRI, the source was detected in the anterior insular and adjoining frontal operculum known as the 'primary taste area' (PTA) (Kinomura *et al.*, 1994; Kobayakawa *et al.*, 1996; Murayama *et al.*, 1996; Cerf *et al.*, 1998; Small *et al.*, 1999). Figure 4 shows locations of ECDs in the PTA in the left hemisphere when the right side of the tongue was stimulated (32 μA). Group analysis suggested that the areas commonly activated across subjects by electric taste stimuli included a considerable anteroposterior part of the insular–operculum (from the frontal to parietal operculum), and the superior part of the insular rather than the interior part.

MEG responses to electrical stimulation of the tongue were also observed in cortical areas other than the PTA, such as the pre- and post-central gyri, superior temporal gyrus, cuneus, angular gyrus, parahippocampal gyrus and supramarginal gyrus. The cortical areas activated differed depending on current intensity. Figure 5 shows the cumulative numbers of estimated ECDs in the bilateral opercular–insular region, frontal, parietal, temporal and occipital lobes counted every 100 ms after onset of stimulation in seven subjects. At a current intensity inducing electric taste, the most prominent responses were detected in the opercular–insular cortex: ECDs were observed not only in the first 500 ms but also in the second 500 ms with more frequent occurrences (Figure 5A), which corresponds to the

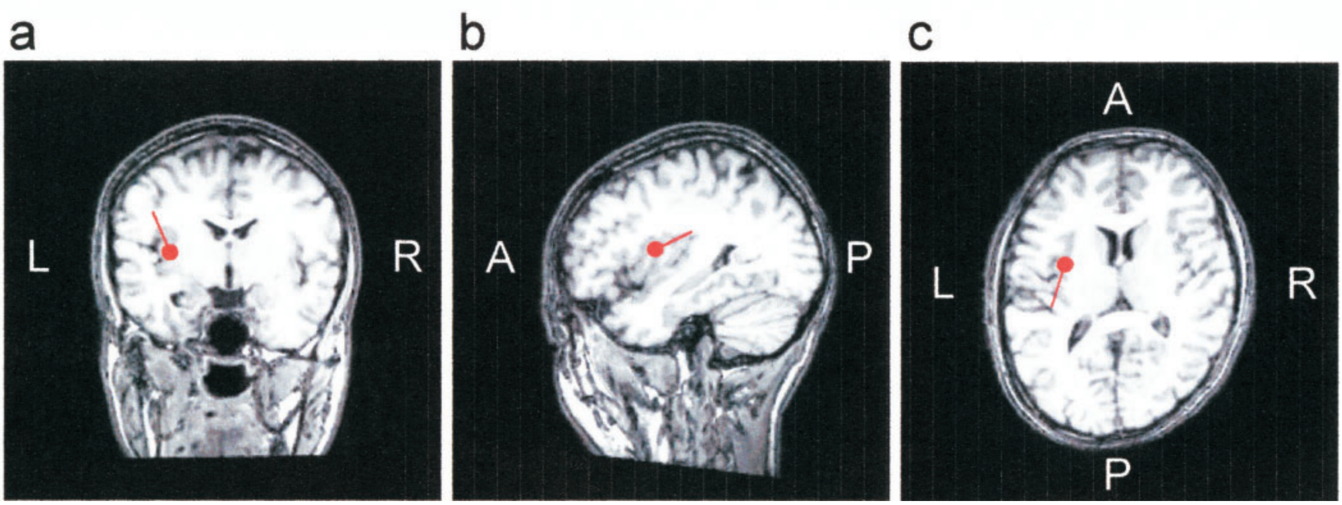

Figure 4 ECDs estimated on a subject's MRIs in response to electrogustatory stimulation (32 μA) applied to the tongue. The ECD is located in the frontal opercular–insular cortex (or primary taste area). **(a)** A coronal section; **(b)** a left sagittal section; **(c)** a horizontal section; L, left; R, right; A, anterior; P, posterior.

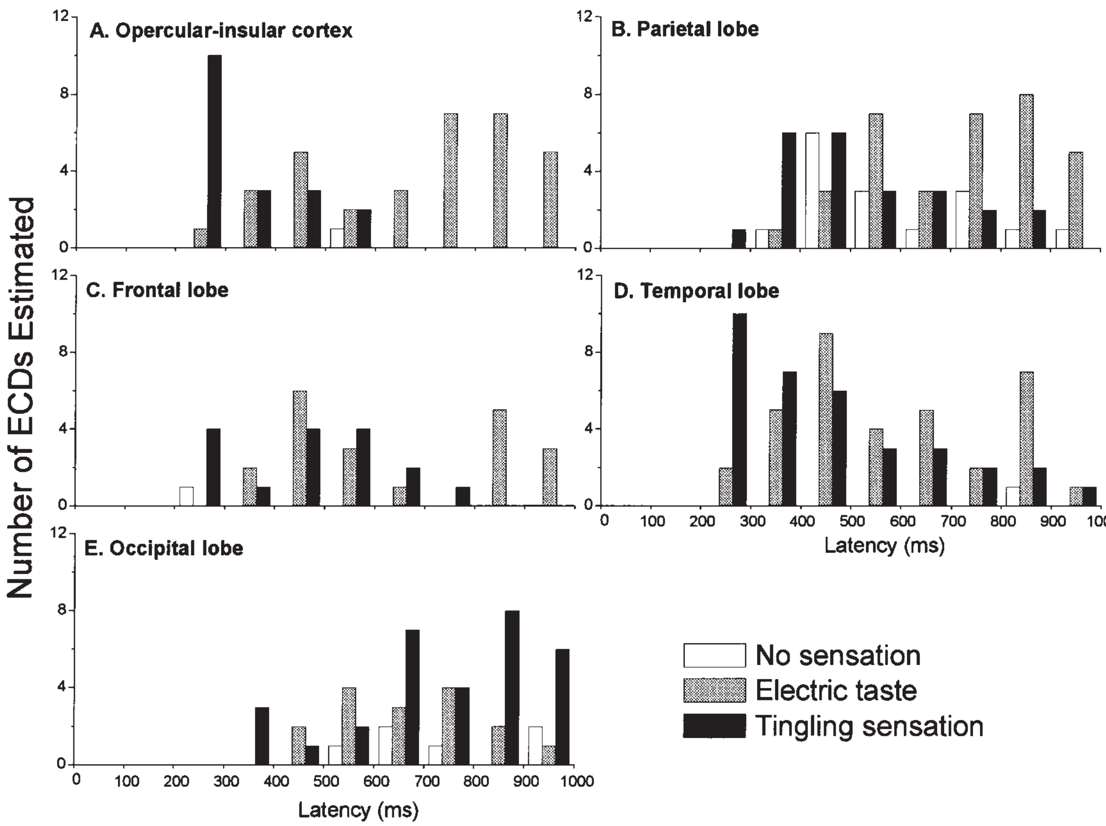

Figure 5 Cumulative numbers of estimated ECDs to three strengths of electrical stimulation applied to the tip of the tongue. The ECDs were separately counted for the opercular–insular region **(A)**, parietal **(B)**, frontal **(C)**, temporal **(D)** and occipital **(E)** lobes.

prolonged MEG taste responses (see Figure 3B). Estimated ECDs to strong currents inducing irritation were observed in both the opercular–insular region (Figure 5A), the interior part of the post-central gyrus that is found in the parietal lobe (Figure 5B), and the superior temporal gyrus that is found in the temporal lobe (Figure 5D). The ECDs were found most frequently within 200–300 ms after onset of stimulation, and the number of ECDs decreased quickly

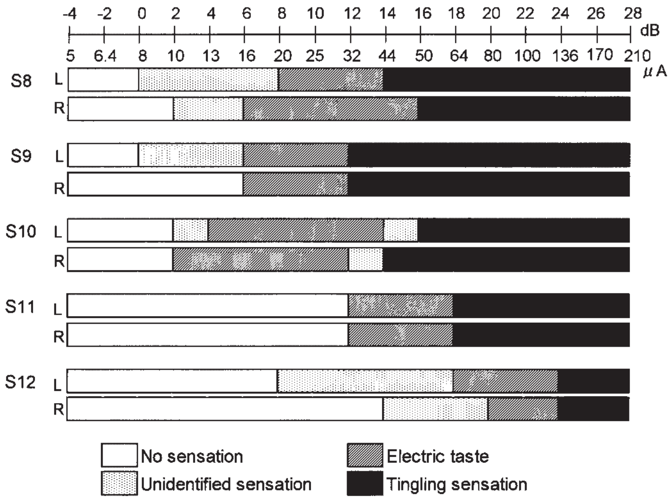

Figure 6 Intensity–perception profiles to electrical stimulation of either the right or left sides of the tongue of five subjects.

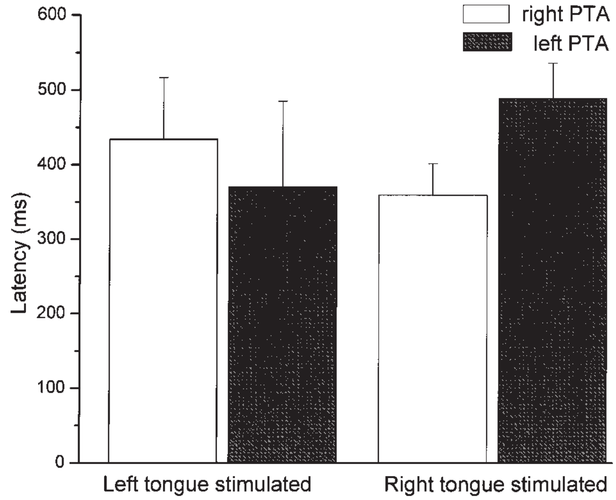

Figure 7 Mean latency of the first detection of ECDs in the primary taste area (PTA) to electrogustatory stimulation applied to the left or right sides of the tongue. The latencies in the left PTA to stimulation applied to the left (n = 5) or right (n = 5) sides of the tongue and those in the right PTA to left (n = 4) or right (n = 4) tongue stimulation statistically significant different from each other.

corresponding to the large, transient MEG responses to strong stimulation (see Figure 3C). ECDs were also detected in the frontal (Figure 5C) and occipital (Figure 5E) lobes.

To examine ipsilateral or contralateral dominance of the projections from the tongue, electrogustatory stimulation was applied separately to the left or right sides of the anterior area of the tongue, and the latency of the MEG response was measured. Figure 6 shows intensity–perception profiles for six subjects when different strengths of anodal DC currents were applied to either the left or right sides of the tongue. Similar to Figure 1, although the strength and range of current varied among subjects, they generally felt no sensation, weak tingling, a taste sensation and finally a tingling sensation as the strength of the electric current increased. The intensity–perception profiles are very similar for right and left stimulations in each subject.

Figure 7 shows the latencies of the first detection of ECDs in the left or right opercular–insular region (or PTA) to electrogustatory stimulation of the left or right areas of the tongue. The mean latencies (~350 ms) of the ipsilateral responses, i.e. responses in the right PTA to right side stimulation or in the left PTA to left side stimulation, tended to be shorter than those of contralateral responses. However, differences among the four latencies were not statistically significant [one-way ANOVA, $F(1,12) = 1.34$, $P = 0.27$].

Discussion

Electrogustometry is widely used by clinicians to examine taste acuity (Krarup, 1958; Fons and Osterhammel, 1966; Nakazato *et al.*, 2002; Tamita and Ikeda, 2002). Anodal DC currents are delivered to focal regions of the dorsal area of the tongue to elicit a unique and complex taste called 'electric taste' that consists mainly of sour and metallic tastes (Bujas, 1971). In the present study, a commercially available TR-05 electrogustometer, originally developed by Tomita (Tomita and Ikeda, 2002), was used. This equipment provides a quantitative evaluation of taste in decibels ranging from –8 dB (3 μA) to 34 dB (400 μA). Thus, electrogustometry has the following advantages (Tomita and Ikeda, 2002): (i) the range of measurement can be kept constant; (ii) quantitative control of the intensity of the stimulation is possible; and (iii) only a short period of time is required for testing.

A disadvantage of using electrogustometry is the elicitation of unfamiliar unique tastes, which are often difficult for subjects to verbally characterize (Bujas, 1971). Another drawback is the induction of large stimulus artifacts, especially when recording neural activities of the brain with EEG (Plattig, 1969) or MEG (present study). Artifacts interfere with recordings of evoked brain activity corresponding to electric stimuli. In the present study, however, independent component analysis successfully eliminated artifact components from the stimulus-elicited MEG signals.

Threshold current intensities for eliciting taste sensation varied considerably among subjects. This might have been due to the difficulty of identifying the electric taste even after familiarization to it through pre-experimental practices. Consequently, in the present study, we had to deliver different current intensities to different subjects to elicit this electric taste. Generally, subjects felt three types of sensations depending on the strength of the current delivered: a weak tingling during weak stimulation (<25 μA), followed by electric taste during mild stimulation (25–50 μA) and tingling or irritation without any taste sensation during strong stimulation (>50 μA). These results suggest that strong electric currents stimulate trigeminal afferents (Frank and Smith, 1991; Murphy *et al.*, 1995), and that somatosensory information may interfere with that via taste afferents.

In agreement with the results of an fMRI study on electrogustatory-evoked cortical activity (Barry *et al.*, 2001), activation was seen in many areas of the brain, but most prominently in the opercular–insular region known as the PTA (Kinomura *et al.*, 1994; Kobayakawa *et al.*, 1996; Murayama *et al.*, 1996; Cerf *et al.*, 1998; Small *et al.*, 1999): numbers of estimated ECDs were most frequently counted in this region. More precisely, the ECDs were observed within 200–1000 ms after onset of electrogustatory stimulation, with the number of ECDs more frequently observed in the second half of the 1 s analysis time than in the first half, indicating a long-lasting activation of neurons in this region to electrogustatory stimulation. Although estimated ECDs to strong currents were also observed in this region, the differences are that the ECDs were found most frequently within 200–300 ms after onset of stimulation, the number of ECDs decreased quickly and were not observed in the second half of the 1 s analysis time (see Figure 3). These results indicate that the strong tingling comes first and disappears quickly, whereas electric taste comes slowly and lasts longer. The present MEG study suggests that electric taste stimulation (25–50 μA) is not a complex stimulus, but has an exclusively gustatory component since the elicited responses were seen mostly in the PTA. On the other hand, stronger stimulation (>50 μA) eliciting a tingling sensation may be a complex stimulus, which activates both the post-central gyrus [somatosensory area (Pardo *et al.*, 1997)] and the opercular–insular cortex. However, elicited activities in both regions were transient, but not as long-lasting as exhibited in electrogustatory MEG responses. There is a possibility that concurrent strong somatosensory inputs interfere with taste-elicited responses in the cortex.

Electric stimulation can be applied focally and specifically on the left or right sides using a small electrode; a 7 mm diameter electrode in the present study. This method may address the issue of whether taste information projects from the tongue to the cortex ipsilaterally or contralaterally. Judging from the latency of the initial response in the opercular–insular region, the results here suggest that taste information is conveyed bilaterally. This is consistent with the report of Genow *et al.* (Genow *et al.*, 1998) showing that latency and amplitude of taste-evoked human EEG recordings were identical in both hemispheres with unilateral stimulation. Barry *et al.* (Barry *et al.*, 2001) found in an fMRI study that electrogustatory stimulation induced responses exclusively in the right hemisphere in right-handed subjects. The results of the present study together with Barry *et al.*'s data, suggest that taste information may extend to the cortex almost bilaterally symmetrically, but the information may predominantly be processed in the right side.

Recording of MEG responses to taste stimulation using chemical solutions can be challenging because a stimulus delivery system that allows repetitive stimulation with a quick rise time of onset to induce synchronized neural activity for a good summation of evoked responses is required. From this aspect, electrogustatory stimulation has an advantage since electrical stimulation can trigger synchronized neural activity in the brain. Further, it does not require rinsing after each stimulation, which can contribute to subjects' fatigue and increase the number of stimulus applications for obtaining better responses. MEG responses to electrogustatory stimulation are eminently suitable for objective evaluations of taste acuity in clinical situations, and the present results should provide fundamental and useful data on which to base analyses of brain activities induced by electrogustatory stimulation.

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