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Original Article

Enhancement of Saltiness Perception by Monosodium Glutamate Taste and Soy Sauce Odor: A Near-Infrared Spectroscopy Study

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Abstract

Previous studies have reported that the umami taste of monosodium l-glutamate (MSG) and salty-smelling odors (e.g., soy sauce, bacon, sardines) enhance the perception of saltiness. This study aimed to investigate the neural basis of the enhancement of saltiness in human participants using functional near-infrared spectroscopy (fNIRS). University students who had passed a taste panel test participated in this study. Sodium chloride solutions were presented with or without either 0.10% MSG or the odor of soy sauce. The participants were asked to drink a cup of the stimulus and to evaluate only saltiness intensity in Experiment 1, as well as other sensory qualities in Experiment 2, and temporal brain activity was measured using fNIRS. In Experiment 3, the participants were asked to evaluate saltiness intensity using the time-intensity (TI) method, and the response of the parotid salivary glands was measured using fNIRS. The fNIRS data showed that the added MSG and soy sauce enhanced the hemodynamic response in temporal brain regions, including the frontal operculum, but no effect on the hemodynamic salivary responses was detected. These results indicate that the perceived enhancement of saltiness occurs in the brain region that is involved in central gustatory processing. Furthermore, the results of the sensory evaluations suggest that enhancement of saltiness by the addition of MSG is mainly based on fusion of the salty-like property of MSG and saltiness of NaCl, whereas enhancement by the addition of soy sauce odor is mainly based on modulation of the temporal dynamics of saltiness perception.

Key words: near-infrared spectroscopy, odor-induced taste enhancement, saltiness perception, taste–taste interaction, time-intensity

Introduction

Since high salt intake is associated with several health concerns, strategies to decrease the salt content of the daily diet without decreasing saltiness perception and palatability are desirable. One candidate is taste–taste interaction (for review see Keast and Breslin 2002), in which the perceived intensity of a taste (e.g., sweetness) is modulated by the presence of another taste (e.g., bitterness). Previous studies have shown that the perceived saltiness and palatability of a low salt diet (e.g., clear soup) were enhanced by the addition of monosodium l-glutamate (MSG) (Yamaguchi and Takahashi 1984), the taste of which is predominantly described as umami, but also as salty (Dehan et al. 1994). Another candidate is odor-induced taste enhancement, in which the perceived intensity of a taste (e.g., sweetness) is enhanced by the presence of an odor (e.g., strawberry) that has similar qualities to those of the taste (Frank and Byram 1988; Stevenson et al. 1999; Sakai et al. 2001). Recently, it has been shown

that the perceived saltiness of a sodium chloride (NaCl) solution is enhanced by the addition of a salty-smelling odor, such as the odor of soy sauce, sardines, or bacon (Djordjevic et al. 2004b; Lawrense et al. 2009; Nasri et al. 2011).

The physiological bases of the taste–taste interaction and the odor-induced taste enhancement effects are still unclear. Studies that have addressed this issue include one in which MSG had no detectable enhancing effect on the rat chorda tympani nerve response to NaCl (Yoshii et al. 1986), and one in which odor-induced sweetness enhancement was found even when taste and odor were presented separately, namely, aspartame in the mouth and a vanilla odor in the nose (Sakai et al. 2001). These findings suggest that taste enhancement effects do not occur in the oral periphery but rather occur in the brain.

The present study aimed to examine neural basis of taste enhancement effects using functional near-infrared spectroscopy (fNIRS), a method that enabled us to continuously monitor relative changes in hemoglobin concentrations. In Experiment 1, participants were asked to drink a cup of a salty-tasting solution with or without the addition of MSG or a soy sauce odor and evaluate saltiness intensity. In Experiment 2, participants were asked to evaluate saltiness intensity as well as other sensory qualities of salty-tasting solutions with or without the addition of MSG (Experiment 2A) or a soy sauce odor (Experiment 2B). The taste-evoked hemodynamic response of the temporal brain region, including the frontal operculum (Fop), was recorded using fNIRS. A functional magnetic resonance imaging (fMRI) study previously showed that the Fop responds to a salty tasting stimulus (Ogawa et al. 2005). Another fMRI study showed enhanced activation in the Fop when a salty tasting solution was presented together with a salty-smelling bacon odor, compared to that reported with odorless air and a sweet-smelling strawberry odor (Seo et al. 2013). Compared to fMRI, fNIRS allows participants to taste and evaluate food stimuli in more natural circumstances (e.g., sitting) and this can exclude the potential problems arising from tasting in restricted or unnatural body positions (e.g., lying in a scanner), since the latter pose an additional cognitive load on participants and can distort their responses. It was hypothesized that the response evoked by a salty taste in the temporal brain region, including the Fop, would be enhanced by the addition of MSG or a soy sauce odor.

Although fNIRS is a useful brain imaging technique, it is uncertain whether the taste-evoked response signal from the temporal region of the head is actually derived from the brain. A previous fNIRS study (Sato et al. 2011) indicated that the taste-evoked response signal from a part of the temporal region was also derived from the parotid salivary glands, which respond to taste stimuli (e.g., Froehlich et al. 1987; Hodson and Linden 2006). To clarify this point, in Experiment 3, the taste-evoked hemodynamic response of areas anterior to the ears, which is thought to cover the parotid glands but not the cerebral cortex, was recorded using a fNIRS system designed to measuring these areas. By comparing the brain-fNIRS (Experiments 1 and 2) and the salivary fNIRS (Experiment 3) measurements, it was possible to examine the possibility that the parotid gland response contributes to the result from brain-fNIRS measures.

Material and methods

Participants

Twelve university students (6 men and 6 women; M_{age} = 20.3 years) participated in Experiment 1. A total of twenty university students participated in Experiment 2, consisting of 9 students (3 men and 6 women; mean age (M_{age}) = 21.6 years) in Experiment 2A and 11 students (4 men and 7 women; M_{age} = 21.3 years) in Experiment 2B. Twelve university students (5 men and 7 women; M_{age} = 21.3 years) participated in Experiment 3. All participants were right-handed, healthy, and did not report any olfactory or gustatory disorders. All participants passed the taste panel test in our laboratory, which investigated 1) whether they could differentiate low concentration of the 5 basic tastes solutions (i.e., sweetness, sourness, saltiness, bitterness, and umami) and 2) whether they could discriminate a slight difference of concentration for each of NaCl and MSG solution. Verbal and written explanations about the experiment were given to the participants and written informed consent was obtained. These experiments were conducted according to the Declaration of Helsinki for Research involving Human Subjects, and received approval from the Ethics Committee of the Graduate School of Arts and Letters in Tohoku University, Japan.

Stimuli

Three sets of salty tasting solutions were prepared using water as the solvent. The solutions (10 mL each) were delivered to the participants in paper cups. The first set of solutions consisted of the 4 concentrations of NaCl; 0% (i.e., water), 0.18%, 0.58%, and 0.80%. The second set consisted of these 4 NaCl concentrations, each plus 0.10% MSG. The third set consisted of the 4 NaCl concentrations presented along with the odor of soy sauce. The odor of soy sauce was presented by putting 4 mL (Experiment 1) or 8 mL (Experiments 2 and 3) of soy sauce (Kikkoman, Japan) onto a cotton pad that was attached to the back of the lid of the cup (Figure 1) (Sakai et al. 2011). This method enabled the delivery of the soy sauce odor without taste contamination. Table 1 lists the stimuli presented in each

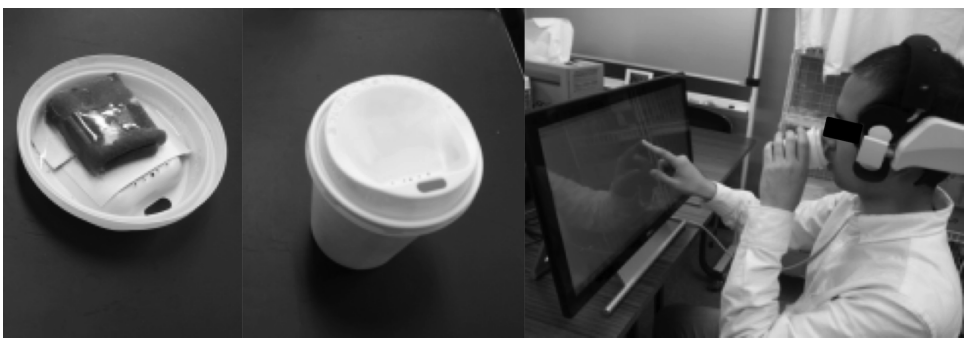

Figure 1. Presentation method of soy sauce odor in the present study and a photographic example of a participant drinking the solution (Experiment 3). The soy sauce was putted into a cotton pad that was attached in the back of a lid of the cup.

Table 1. Presented stimuli in the present study

Addition NaCl	Pure				+MSG				+Odor			
	Water	0.18%	0.58%	0.80%	Water	0.18%	0.58%	0.80%	Water	0.18%	0.58%	0.80%
Experiment 1	○		○[a]	○[a]	○		○		○		○	
Experiment 2A		○	○	○		○	○	○				
Experiment 2B		○	○	○						○	○	○
Experiment 3		○	○	○		○	○	○		○	○	○

[a]The stimuli were presented twice.

experiment: Nine stimuli in Experiment 1, 6 stimuli in Experiments 2A and 2B, and 9 stimuli in Experiment 3.

Sensory evaluation

In Experiments 1 and 2, a visual analogue scale (VAS) was used for the sensory evaluation of the stimuli. Evaluations were carried out using 100 mm horizontal scales. In Experiment 1 participants were asked only to evaluate perceived saltiness intensity. In Experiment 2, on the other hand, participants were asked to evaluate multiple attributes of the stimuli: Perceived intensities of saltiness, umami, sweetness, odor, and irritation, and the subjective evaluation of liking. The scales for perceived intensity were anchored "not at all" and "very strong" at the left and right ends of the scales, respectively, and the scale for liking was anchored "do not like at all" and "like very much" at the left and right ends, respectively. The length (mm) from the left edge of the scale to the mark the participant made was measured and used as the rating. Thus, the ratings had a theoretical range from 0 to 100.

In Experiment 3, a time-intensity (TI) method was used for saltiness evaluation of the stimuli. This enables intensity to be monitored over time and provides more valid information about flavor perception than conventional static methods (Dijksterhuis and Piggott 2001). Evaluations were carried out using a 21.5-inch touch panel display (S2240T, Dell Inc.), and data were collected using FIZZ software (Biosystemes) running on a laptop computer (Dynabook Satellite B254/K, TOSHIBA). Participants were asked to continuously evaluate their perception of saltiness intensity (0–100). The scales used for the TI method were presented on the display as a horizontal scale in which the anchors "not at all" and "very strong" were on the left and right ends of the scale, respectively. The duration of the evaluation was fixed at 80 s, and the data were collected every 50 ms. The participants were trained for this evaluation method by participating in other experiments in our laboratory prior to this experiment.

fNIRS system

In Experiments 1 and 2, a multi-channel NIRS system (FOIRE-3000) was used for measuring hemodynamic brain responses. This system consists of 12 pairs of emitter and detector probes, and reflected lights were detected every 100 ms. The probes were placed over the temporal area of each hemisphere with reference to the international 10–20 system, each consisting of a 4 × 3 array with 6 emitters and 6 detectors, constituting 17 channels (Chs) in each hemisphere (Figure 2A and B). The cortical regions corresponding to each channel were estimated by measuring the 3-dimensional (3D) coordinate data from each probe using a 3D digitizer (FASTRAK, Polhemus) and NIRS-SPM software (Singh et al. 2005; Ye et al. 2009). Then, the estimated channel positions were averaged across all participants and rendered on to the Montreal Neurological Institute (MNI) standard brain (Figure 2C), and the corresponding Brodmann's areas (BA) were estimated.

In Experiment 3, a fNIRS system, which was developed for measuring the response of the parotid glands (WOT-S20, Hitachi High-Technologies Corporation), was used to measure the hemodynamic responses of the parotid glands. This system consisted of 2 pairs of emitter and detector probes. Near-infrared light (705 and 830 nm) was emitted, and the reflected light was detected every 200 ms. The pairs of emitters and detectors were carefully positioned on the right and left areas anterior to the ears (Figure 1).

Using these systems, the relative changes in oxygenated hemoglobin (oxy-Hb), deoxygenated hemoglobin (deoxy-Hb), and total hemoglobin (total-Hb) concentrations were measured. This study focused on the oxy-Hb signal, because it has been suggested that this signal change of brain-fNIRS is more sensitive to changes in regional cerebral blood flow than deoxy-Hb and total-Hb (Hoshi 2003) and that of salivary fNIRS is positively correlated with the taste-evoked salivary secretion volume (Sato et al. 2011).

Procedure

Participants were seated in a comfortable chair in a sound-proofed room at a constant temperature (23–25°C). Participants were given verbal and written instructions for the procedure and then fitted with the fNIRS headset.

In Experiments 1 and 2, there were sequential blocks of 30-s rest periods and 160-s task periods (Figure 3). Participants followed the instructions presented on a computer display. During the rest periods, the participant rinsed their mouth with a cup of mineral water. During the task periods, the participant held and raised the cup to their mouth (5 s) according to the instruction on the display. Then, the participant took a stimulus solution into their mouth (3 s) and swallowed it immediately (2 s). The stimulus onset was determined as the point 2 s after the instruction for swallowing was given. The oxy-Hb signal changes in the temporal brain area were recorded for 70 s; 10-s recording before onset and 60-s recording after onset. After recording ended, the participants started the evaluation of the stimuli using the VAS. These rest-task procedures were repeated for the 9 stimuli in Experiment 1 and for the 6 stimuli in Experiment 2. The presentation order of the stimuli was counter-balanced across participants. A computer (PCG-81411N, Sony) running the PPT2TTL software (WAWON DIGITECH) presented the instructions and triggered the fNIRS system.

Experiment 3 consisted of sequential blocks of rest periods and 85-s task periods (Figure 4). Each participant followed the instructions presented on the touch panel display. During the rest periods, the participant rinsed their mouth with water. The salivary response was carefully monitored by the experimenter using the salivary fNIRS system and rest periods were continued until the signal changes settled (for at least 60 s). During the task periods, the participant held and raised the cup to their mouth for 5 s. Then, the participant took a solution into their mouth and swallowed it immediately. They then started their rating by moving the cursor along the scale according

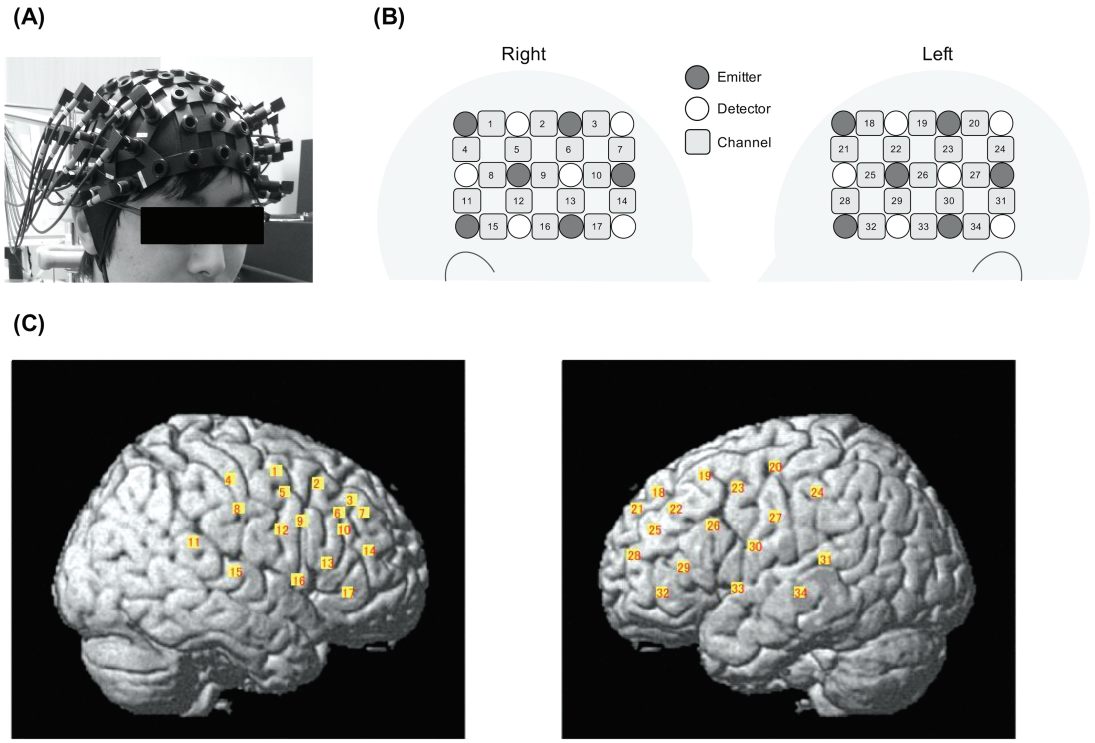

Figure 2. (**A**) Photographic example and (**B**) schematic illustration of the placement of the brain-fNIRS probes. The brain-fNIRS system consisted of 12 emitters, 12 detectors, and 34 channels. (**C**) Estimated channel positions rendered on to the cortical surface of the MNI standard brain by NIRS-SPM software.

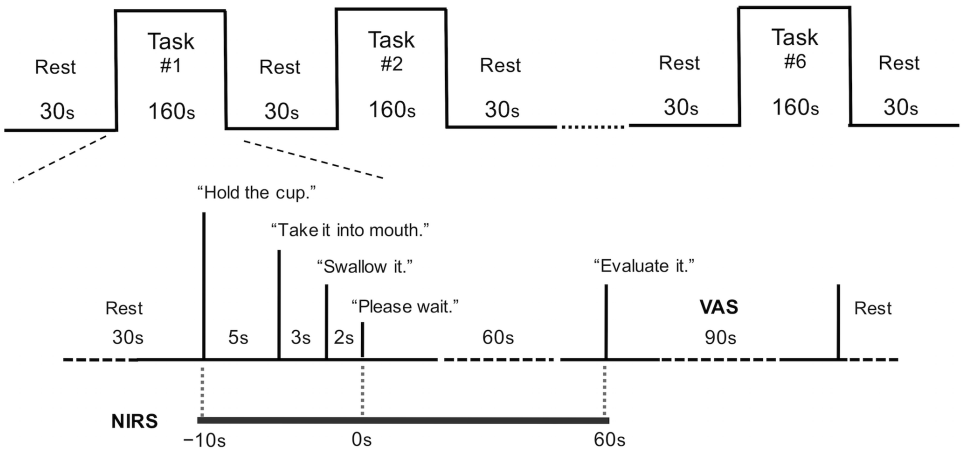

Figure 3. Schematic diagram of rest-task sequences in Experiments 1 and 2. The rest period was followed by the task periods, which consisted of holding the cup and taking and swallowing the stimulus. The hemodynamic response of the temporal brain area was recorded by fNIRS for 10s before and for 60 s after the onset. After recording ended, evaluation with VAS was conducted. These rest-task sequences were repeated for the 9 stimuli in Experiment 1, and for the 6 stimuli in Experiments 2A and 2B, respectively.

to the perceived saltiness intensity with their finger (maximum time 80 s). Oxy-Hb signal changes in the parotid glands were recorded for 90 s, 10 s before the instruction for swallowing appeared on the display and for 80 s after. These rest-task sequences were repeated for all 9 stimuli for each participant. The order of presentation of the stimuli was counter-balanced across participants.

Data analyses

For the obtained sensory evaluation data, the VAS ratings were analyzed in Experiments 1 and 2. In Experiment 3, TI curves for each stimulus were plotted for each participant. Three different parameters were then calculated according to the methods used in a previous study (Dijksterhuis and Piggott 2001): 1) peak intensity, 2) total area under the curve (AUC), and 3) perceived duration (total duration between the time the cursor departed from the left end and arrived at the left end again) (Figure 5A).

For the obtained fNIRS data, the waveforms of the oxy-Hb signal changes for each stimulus were plotted for each participant and high-pass filtered at 0.02 Hz to remove artifacts. Then, the baselines were corrected by zero-degree fitting to make the signal at stimulus onset zero. After that, the mean signal changes during the full width at half maximum (FWHM) of each curve, the width between the points on the *y*-axis which are half the maximum amplitude, were calculated (Figure 5B). Then, the signal changes were standardized as *z*-scores

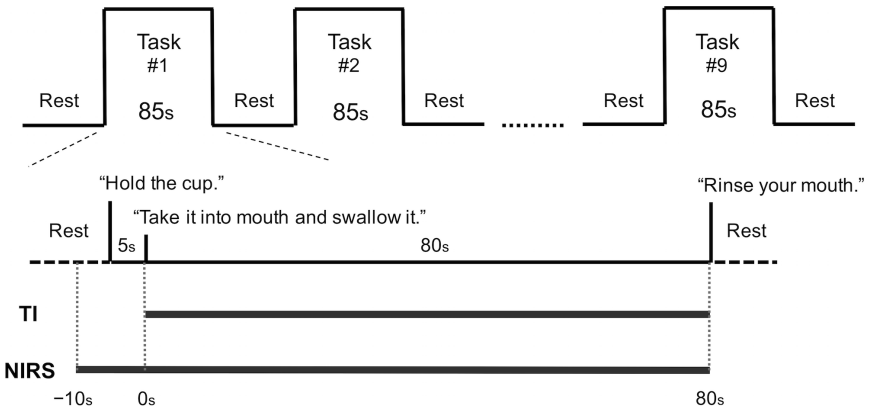

Figure 4. Schematic diagram of rest-task sequences in Experiment 3. The rest periods continued until participants' saliva response settled, at least for 60 s. The rest periods were followed by the task periods, which consisted of holding the cup and taking and swallowing the stimulus. Saltiness intensity was evaluated for 80 s with the TI method after the stimulus onset. At the same time, the hemodynamic response of the parotid glands was recorded by salivary fNIRS for 10s before and for 80s after the onset. These rest-task sequences were repeated for the 9 stimuli.

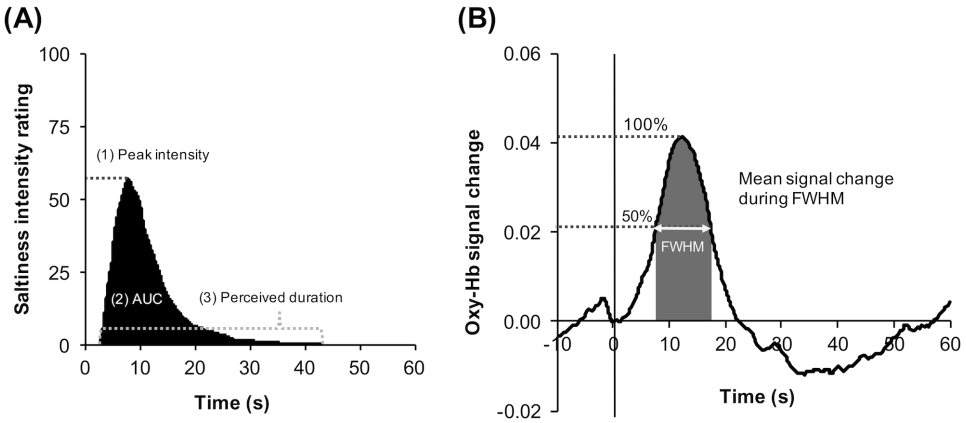

Figure 5. (**A**) Representative example showing TI data calculated in Experiment 3. Peak intensity, area under the curve (AUC), and perceived duration were calculated. (**B**) Representative example showing brain-fNIRS data calculated in Experiment 2. Mean signal change during full width at half maximum (FWHM) of the oxy-Hb signal change curve was calculated and used as the indices of the brain response.

for each participant and used as indices of the response of the brain in Experiments 1 and 2 and of the parotid glands in Experiment 3.

For the standardized sensory evaluation and fNIRS data, a multiple regression analysis was performed in this study. To examine the effects of NaCl concentration and MSG/odor and their interactions, *dummy coding* was applied in the present study (te Grotenhuis et al. 2017). In Experiment 1, NaCl concentrations were represented by a couple of dummy variables ($D_{0.58\%}$ and $D_{0.80\%}$), setting water as a reference: water as $D_{0.58\%}$ (0) and $D_{0.80\%}$ (0), 0.58% NaCl solution as $D_{0.58\%}$ (1) and $D_{0.80\%}$ (0), and 0.80% NaCl solution as $D_{0.58\%}$ (0) and $D_{0.80\%}$ (1). The addition of MSG/odor was also represented using a dummy variable, setting pure NaCl solutions as a reference: pure NaCl solution as D_{MSG} (0) and D_{odor} (0), NaCl solution with MSG added as D_{MSG} (1) and D_{odor} (0), and NaCl solution with odor added as D_{MSG} (0) and D_{odor} (1). Our model included the interaction between NaCl concentration and MSG/odor as follows:

$$Y = a + b_1 D_{0.58\%} + b_2 D_{0.80\%} + b_3 D_{\mathrm{MSG}} + b_4 D_{\mathrm{odor}} + b_5 D_{0.58\%} D_{\mathrm{MSG}} + b_6 D_{0.58\%} D_{\mathrm{odor}} + e$$

where a represents an intercept, b represents regression coefficients of the variables, and e represents an error term.

In Experiment 2, NaCl concentrations were represented by setting 0.18% NaCl solution as a reference: 0.18% NaCl solution as $D_{0.58\%}$ (0) and $D_{0.80\%}$ (0), 0.58% NaCl solution as $D_{0.58\%}$ (1) and $D_{0.80\%}$ (0), 0.80% NaCl solution as $D_{0.58\%}$ (0) and $D_{0.80\%}$ (1). Addition of MSG/odor was represented by setting pure NaCl solutions as a reference: pure NaCl solutions as $D_{\mathrm{MSG/odor}}$ (0), and NaCl solutions plus MSG or the soy sauce odor as $D_{\mathrm{MSG/odor}}$ (1). Our model included the interaction between NaCl concentration and MSG/odor as follows:

$$Y = a + b_1 D_{0.58\%} + b_2 D_{0.80\%} + b_3 D_{\mathrm{MSG/odor}} + b_4 D_{0.58\%} D_{\mathrm{MSG/odor}} + b_5 D_{0.80\%} D_{\mathrm{MSG/odor}} + e$$

In Experiment 3, NaCl concentrations were represented by setting 0.18% NaCl solution as a reference: 0.18% NaCl solution as $D_{0.58\%}$ (0) and $D_{0.80\%}$ (0), 0.58% NaCl solution as $D_{0.58\%}$ (1) and $D_{0.80\%}$ (0), 0.80% NaCl solution as $D_{0.58\%}$ (0) and $D_{0.80\%}$ (1). Addition of MSG/odor was represented by setting pure NaCl solutions as a reference: pure NaCl solution as D_{MSG} (0) and D_{odor} (0), NaCl solution with MSG added as D_{MSG} (1) and D_{odor} (0), NaCl solution with odor added as D_{MSG} (0) and D_{odor} (1). Our model included the interaction between NaCl concentration and MSG/odor as follows:

$$Y = a + b_1 D_{0.58\%} + b_2 D_{0.80\%} + b_3 D_{\mathrm{MSG}} + b_4 D_{\mathrm{odor}} + b_5 D_{0.58\%} D_{\mathrm{MSG}} + b_6 D_{0.80\%} D_{\mathrm{MSG}} + b_7 D_{0.58\%} D_{\mathrm{odor}} + b_8 D_{0.80\%} D_{\mathrm{odor}} + e$$

To investigate the brain region involved in the saltiness enhancement effect, the following steps were taken for the brain-fNIRS data in Experiments 1 and 2. First, if a brain region is involved in the saltiness enhancement effect, the region should represent the intensity of saltiness perception. Thus, Pearson's correlation coefficients between the saltiness intensity rating and the mean signal change in the all channels were calculated, and the channels in which the significant correlation was observed were determined as target channels. Second, the multiple regression analysis for the mean signal change was performed in each target channel. To avoid the problem associated with multiple testing, the resulting regression model *p*-values for the target channels were corrected with the false discovery rate (FDR) method (Benjamini and Hochberg 1995; $p <$ FDR 0.05). Then, the effect of NaCl concentration and MSG/odor on the mean signal change was examined for the target channels in which the multiple regression model itself was significant.

Results

Experiment 1

VAS data

The saltiness intensity rating showed an NaCl concentration-dependent increase and also showed a tendency towards enhancement by the addition of MSG, but not by the addition of the odor (Figure 6A). The multiple regression analysis (Table 2) revealed that there were significant main effects of $D_{0.58\%}$, $D_{0.80\%}$, and D_{MSG}, but no significant main effect of D_{odor} nor significant interactions. This result showed that the saltiness intensity increased as a function of NaCl concentration and enhanced by the addition of MSG, but was not enhanced by the addition of the odor.

Brain-fNIRS data

The mean signal changes for the FWHM of the oxy-Hb signal change curve were calculated. Pearson's correlation coefficients between the

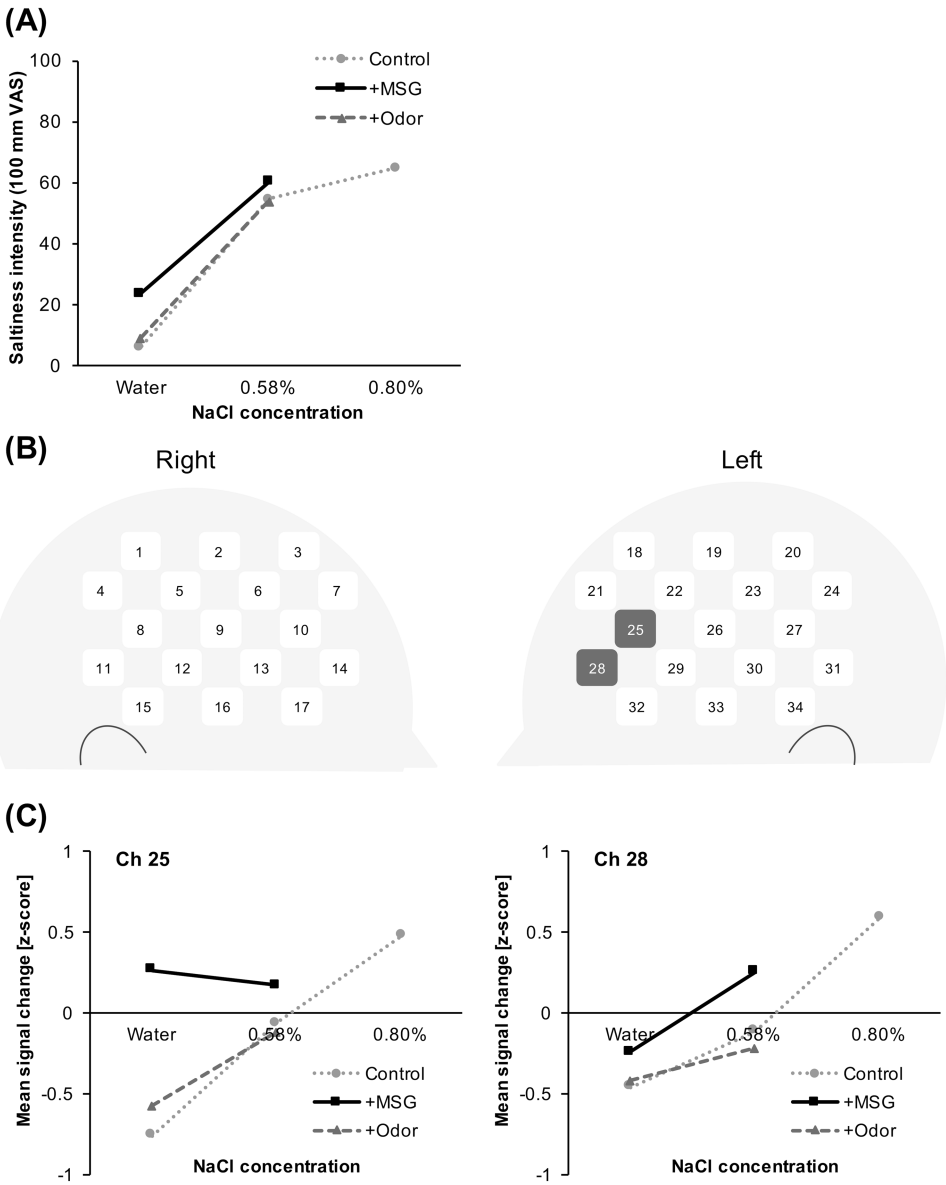

Figure 6. VAS and brain-fNIRS data in Experiment 1. (**A**) Averaged ratings of saltiness intensity for the various concentration of NaCl solutions with or without the addition of MSG or the soy sauce odor. (**B**) The target channels in which the multiple regression model was significant ($P <$ FDR 0.05). (**C**) The mean signal changes of Ch 25 and 28 for the various concentration of NaCl solutions with or without the addition of MSG or the soy sauce odor.

saltiness intensity rating and the mean signal change in the all channels were calculated (Table 3). Significant correlation between the saltiness intensity rating and the mean signal change was observed for Ch 5, 8, 22, 25, 28, 32, and 33. The multiple regression analysis was performed for these target channels, and only the multiple regression models for Ch 25 and 28 were statistically significant (p < FDR 0.05) (Figure 6B; see also Figure 9A).

For Ch 25, which was estimated to include signals from the left pars triangularis (BA45), the response seemed to be NaCl concentration-dependent and was also found to be enhanced by the addition of MSG (Figure 6C). The multiple regression analysis (Table 2) revealed that there were significant main effects of $D_{0.58\%}$, $D_{0.80\%}$, and D_{MSG}, but not that of D_{odor} nor significant interactions. The result indicated that responses from Ch 25 for the 0.58% and 0.80% NaCl solution were higher than for water, and that these responses were enhanced by MSG but not by the odor.

Similar results were found for Ch 28, which also detected signals from the left BA45. However, the multiple regression analysis revealed that there was only a significant main effect of $D_{0.80\%}$, but not that of D_{MSG}, D_{odor} nor significant interactions. The result indicated that the response from Ch 28 for the 0.80% NaCl solution was higher than for water, but that the response was not enhanced by the addition of MSG or the odor.

Experiment 2A

VAS data

The VAS data from Experiment 2A are shown in Figure 7A. The saltiness intensity rating showed an NaCl concentration-dependent increase, and also showed a weak tendency towards enhancement by the addition of MSG. The multiple regression analysis (Table 4) revealed that there were significant main effects of $D_{0.58\%}$ and $D_{0.80\%}$, but not that of D_{MSG} nor significant interactions. This result showed that the saltiness intensity increased as a function of NaCl concentration but the addition of MSG had no detectable effect.

The umami intensity rating was enhanced by the addition of MSG. The multiple regression analysis revealed that there was a significant main effect of D_{MSG}, but not that of $D_{0.58\%}$ and $D_{0.80\%}$ nor significant interactions. This result showed that the intensity of umami was enhanced by the addition of MSG but that the concentration of NaCl had no effect.

There was no difference between the sweetness intensity ratings. The multiple regression analysis revealed that there were no significant main effects nor interactions. This result showed that the sweetness intensity was independent of both NaCl concentration and the addition of MSG.

There was no difference between the odor intensity ratings. The multiple regression analysis revealed that there were no significant main effects nor interactions. This result showed that the odor intensity was independent of both NaCl concentration and the addition of MSG.

The irritation intensity rating showed an NaCl concentration-dependent increase. The multiple regression analysis revealed that there were significant main effects of $D_{0.58\%}$ and $D_{0.80\%}$, but not that of D_{MSG} nor significant interactions. This result showed that the irritation intensity increased as a function of NaCl concentration but that the addition of MSG had no effect.

Table 2. Results of the multiple regression analysis for the VAS and the brain-fNIRS data in Experiment 1

					Mean signal change							
	Saltiness intensity				Ch 25				Ch 28			
	Coef	SE	*t*	*P*	Coef	SE	*t*	*P*	Coef	*SE*	*t*	*P*
Experiment 1												
Main effects												
Intercept (a)	0.01	0.05	0.20		0.02	0.09	0.19		0.00	0.09	−0.02	
0.58% (β1)	0.85	0.06	13.27	***	0.24	0.11	2.12	*	0.18	0.11	1.61	
0.80% (β2)	0.96	0.08	11.57	***	0.55	0.15	3.71	***	0.46	0.15	3.10	**
MSG (β3)	0.23	0.06	3.61	***	0.30	0.11	2.68	**	0.13	0.11	1.17	
Odor (β4)	0.00	0.06	−0.01		0.03	0.11	0.29		−0.01	0.11	−0.11	
Interaction effects												
0.58% × MSG (β5)	−0.11	0.06	−1.69		−0.18	0.11	−1.64		0.03	0.11	0.31	
0.58% × Odor (β6)	−0.03	0.06	−0.50		−0.05	0.11	−0.48		−0.03	0.11	−0.29	

*P < 0.05, **P < 0.01, ***P < 0.001.

Table 3. Pearson's correlation coefficients between the saltiness intensity rating (VAS) and the mean signal change (brain-fNIRS) in Experiments 1 and 2

Saltiness intensity	Mean signal change (Right hemisphere)																
	Ch 1	Ch 2	Ch 3	Ch 4	Ch 5	Ch 6	Ch 7	Ch 8	Ch 9	Ch 10	Ch 11	Ch 12	Ch 13	Ch 14	Ch 15	Ch 16	Ch 17
Exp 1	0.08	0.10	0.09	−0.10	0.19*	0.15	0.03	0.25**	0.16	0.11	0.08	0.08	0.14	0.00	0.14	0.18	0.09
Exp 2A	0.12	0.31*	−0.05	0.01	0.15	0.31*	0.01	0.15	0.25	0.21	−0.19	0.14	0.11	0.07	−0.09	0.16	0.06
Exp 2B	0.05	0.13	0.09	0.11	0.17	0.29*	−0.05	0.12	0.20	0.36**	0.09	0.12	0.15	0.16	0.12	−0.04	0.14
Saltiness intensity	Mean signal change (Left hemisphere)																
	Ch 18	Ch 19	Ch 20	Ch 21	Ch 22	Ch 23	Ch 24	Ch 25	Ch 26	Ch 27	Ch 28	Ch 29	Ch 30	Ch 31	Ch 32	Ch 33	Ch 34
Exp 1	0.05	0.05	0.12	0.03	0.27**	0.17	−0.01	0.25**	0.18	0.16	0.26**	0.17	0.18	0.17	0.20*	0.20*	0.06
Exp 2A	−0.05	0.23	0.18	−0.10	0.10	0.33*	0.10	0.12	0.16	0.29*	0.14	0.07	0.29*	0.37**	0.24	0.26	0.28
Exp 2B	−0.25*	−0.11	0.08	−0.09	−0.03	−0.07	0.22	−0.05	0.03	0.12	0.07	0.04	0.14	0.09	0.13	0.11	0.22

*P < 0.05, **P < 0.01.

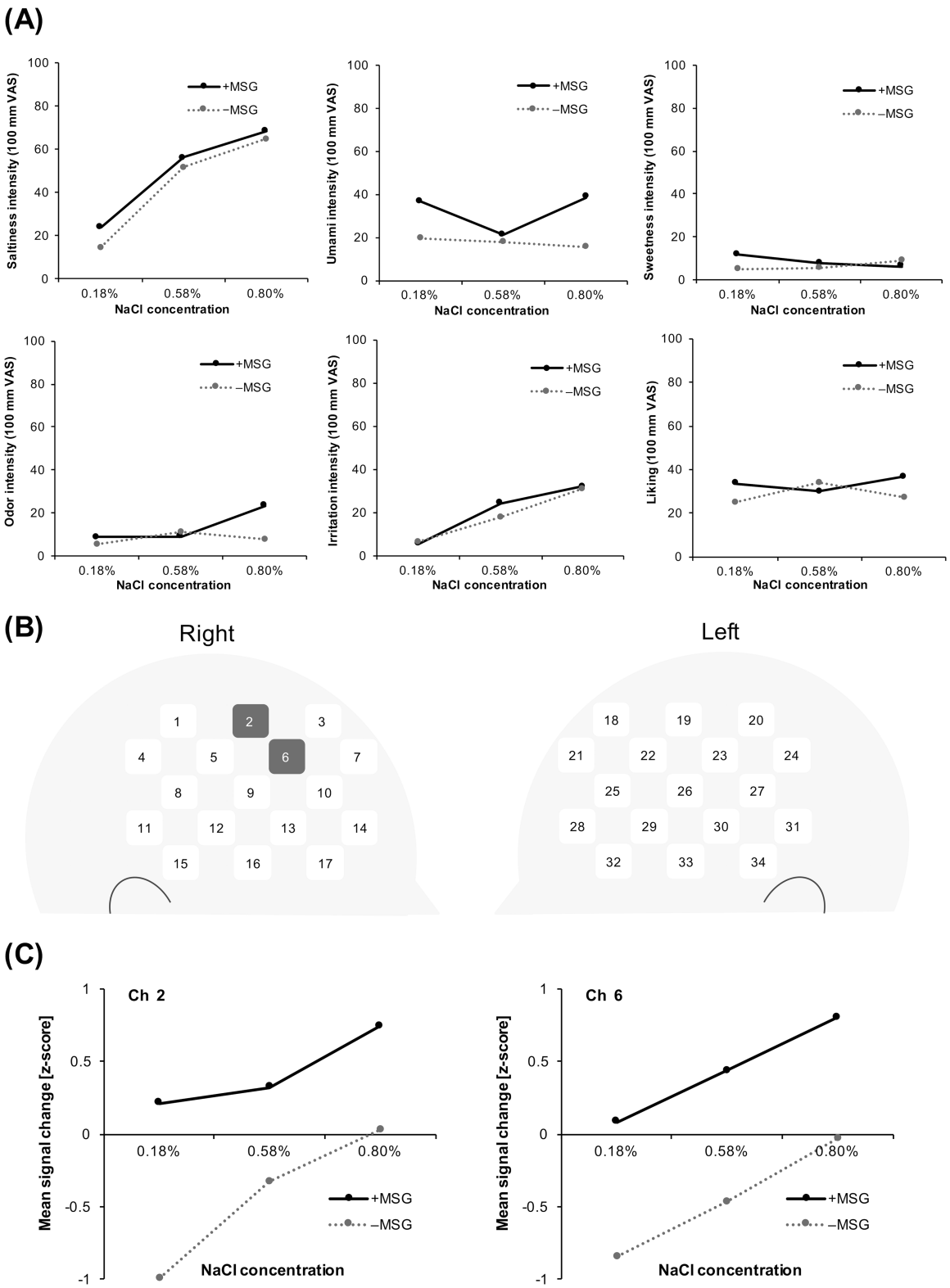

Figure 7. VAS and brain-fNIRS data in Experiment 2A. (**A**) Averaged ratings of saltiness intensity, umami intensity, sweetness intensity, odor intensity, irritation intensity, and liking for the various concentration of NaCl solutions with or without the addition of MSG. (**B**) The target channels in which the multiple regression model was significant ($p <$ FDR 0.05). (**C**) The mean signal changes of Ch 2 and 6 for the various concentration of NaCl solutions with or without the addition of MSG.

There was no difference between the liking ratings. The multiple regression analysis revealed that there were no significant main effects nor interactions. The result showed that liking was independent of both NaCl concentration and MSG.

Brain-fNIRS data

Significant correlations between the saltiness intensity rating and the mean signal change were observed for Ch 2, 6, 23, 27, 30, 31, and 34 (Table 3). The multiple regression analysis was performed for these target channels, and only the multiple regression models for Ch 2 and 6 were statistically significant (p < FDR 0.05) (Figure 7B; see also Figure 9B).

For Ch 2, which was estimated to include signals from the right pars opercularis (BA44), the response seemed to be NaCl concentration-dependent and was also found to be enhanced by the addition of MSG (Figure 7C). The multiple regression analysis (Table 5) revealed that there were significant main effects of $D_{0.80\%}$ and D_{MSG}, but not that of $D_{0.58\%}$ nor significant interactions. Similar results were found for Ch 6, which detected signals from the right BA45 and the right BA44 (Figure 8B). The multiple regression analysis revealed that there were significant main effects of $D_{0.80\%}$ and D_{MSG}, but not that of $D_{0.58\%}$ nor significant interactions. These results indicated that the responses from Ch 2 and Ch 6 for the 0.80% NaCl solution were higher than for the 0.18% NaCl solution, and that the responses were enhanced by the addition of MSG.

Experiment 2B

VAS data

The VAS data from Experiment 2B are shown in Figure 8A. The saltiness intensity rating showed an NaCl concentration-dependent increase, but the addition of the odor did not enhance the rating. The multiple regression analysis (Table 4) revealed that there were significant main effects of $D_{0.58\%}$ and $D_{0.80\%}$, but not that of D_{odor} nor significant interactions. This result showed that the saltiness intensity increased as a function of NaCl concentration, but that it was not enhanced by the addition of the odor.

The umami intensity rating showed a tendency of an NaCl concentration-dependent increase and enhancement by the addition of the odor. The multiple regression analysis revealed that there was a significant main effect of D_{odor}, but not that of $D_{0.58\%}$ and $D_{0.80\%}$ nor significant interactions. This result showed that the intensity of umami was enhanced by the addition of the odor, but that the NaCl concentration had no effect.

The sweetness intensity rating showed a tendency of enhancement by the addition of the odor. The multiple regression analysis revealed that there was a significant main effect of D_{odor}, but not that of $D_{0.58\%}$ and $D_{0.80\%}$ nor significant interactions. This result showed that the sweetness intensity was enhanced by the addition of the odor, but that the NaCl concentration had no effect.

The odor intensity rating was enhanced by the addition of the odor. The multiple regression analysis revealed that there was a significant main effect of D_{odor}, but not that of $D_{0.58\%}$ and $D_{0.80\%}$ nor significant interactions. This result showed that odor intensity was enhanced by the addition of the odor but that NaCl concentration had no effect.

The irritation intensity rating showed a tendency of NaCl concentration-dependent increase. The multiple regression analysis revealed that there were significant main effects of $D_{0.58\%}$ and $D_{0.80\%}$, but not that of D_{odor} nor significant interactions. This result showed that the irritation intensity increased as a function of NaCl concentration but the addition of the odor had no effect.

The liking rating was enhanced by the addition of the odor. The multiple regression analysis revealed that there was a significant main effect of D_{odor}, but not that of $D_{0.58\%}$ and $D_{0.80\%}$ nor significant interactions. This result showed that liking was enhanced by the addition of the odor but the NaCl concentration had no effect.

Brain-fNIRS data

Significant correlation between the saltiness intensity rating and the mean signal change was observed for Ch 6, 10, and 18 (Table 3). The multiple regression analysis was performed for these target channels, and only the multiple regression models for Ch 6 and 10 were statistically significant (p < FDR 0.05) (Figure 8B; see also Figure 9C).

For Ch 6, which detected signals from the right BA44 and BA45, the response seemed to be NaCl concentration-dependent, and was also enhanced by the addition of the odor (Figure 8C). The multiple regression analysis (Table 5) revealed that there were significant main effects of $D_{0.58\%}$ and $D_{0.80\%}$, but not that of D_{odor} nor significant interactions. This indicated that the response recorded by Ch 6 for the 0.58% and 0.80% NaCl solution was higher than that for the 0.18% NaCl solution, but the response was not enhanced by the addition of the odor.

Similar results were found for Ch 10, which detected signal changes from the right BA44 and the right BA45. The multiple regression analysis revealed that there were significant main effects of $D_{0.58\%}$, $D_{0.80\%}$, and D_{odor}, but not significant interactions. This indicated that the responses recorded by Ch 10 for the 0.58% and 0.80% NaCl solutions were higher than for the 0.18% NaCl solution, and that the response was enhanced by the addition of the odor.

Experiment 3

TI data of perceived saltiness intensity

The averaged TI curves (Figure 10A) showed that the saltiness intensity ratings increased as a function of NaCl concentration. The curves also showed that the addition of MSG or the soy sauce odor enhanced the perception of saltiness, which was especially notable at lower NaCl concentrations (i.e., 0.18% and 0.58%).

The peak intensity showed a tendency of enhancement by the addition of MSG or soy sauce odor (Figure 10B). The multiple regression analysis (Table 6) revealed that there were significant main effects of $D_{0.58\%}$, $D_{0.80\%}$, D_{MSG}, and D_{odor}, but no significant interactions. This result showed that the peak intensity of the saltiness intensity curve increased as a function of NaCl concentration, and that it was enhanced by the addition of MSG or the odor.

The AUC was also enhanced by the addition of MSG or the odor. The multiple regression analysis revealed that there were significant main effects of $D_{0.58\%}$, $D_{0.80\%}$, and D_{odor}, but not that of D_{MSG} nor significant interactions. This result showed that the AUC of the saltiness intensity curve increased as a function of NaCl concentration, and that it was enhanced by the addition of the odor.

The perceived duration was also enhanced by the addition of MSG or the odor. The multiple regression analysis revealed that there was a significant interaction between $D_{0.80\%}$ and D_{MSG}, and significant main effects of $D_{0.58\%}$, $D_{0.80\%}$, $D_{MSG,}$ and D_{odor}. This result showed that the perceived duration of saltiness intensity increased as a function of NaCl concentration, and that it was enhanced by the addition of MSG or the odor. However, the effect of MSG with 0.80% NaCl solution $[(a + \beta_2 + \beta_3 + \beta_6) - (a + \beta_2) = \beta_3 + \beta_6 = 0.06]$ was significantly lower than the effect of MSG with 0.18% $[(a + \beta_3) - a = \beta_3 = 0.25]$ and 0.58% $[(a + \beta_1 + \beta_3) - (a + \beta_1) = \beta_3 = 0.25]$ NaCl solutions.

Salivary fNIRS data

The averaged oxy-Hb signal curves (Figure 11A) showed that the hemodynamic response of the parotid glands increased as a function of NaCl concentration. However, the curves showed that the addition of MSG or soy sauce odor did not affect the response.

Table 4. Results of the multiple regression analysis for the VAS data in Experiment 2

	Saltiness intensity				Umami intensity				Sweetness intensity				Odor intensity				Irritation intensity				Liking			
	coef	*SE*	*t*	*p*	coef	*SE*	*t*	*p*	coef	*SE*	*t*	*p*	coef	*SE*	*t*	*p*	coef	*SE*	*t*	*p*	coef	*SE*	*t*	*p*
Experiment 2A																								
Main effects																								
Intercept (a)	0.00	0.09	0.00		0.00	0.13	0.00		0.00	0.14	0.00		0.00	0.13	0.00		0.00	0.12	0.00		0.00	0.14	0.00	
0.58% (β1)	0.62	0.11	5.83	***	−0.19	0.15	−1.23		−0.08	0.16	−0.48		0.08	0.16	0.53		0.34	0.14	2.38	*	0.07	0.16	0.46	
0.80% (β2)	0.85	0.11	7.92	***	−0.02	0.15	−0.15		−0.03	0.16	−0.20		0.25	0.16	1.60		0.58	0.14	4.05	***	0.07	0.16	0.46	
MSG (β3)	0.11	0.09	1.17		0.33	0.13	2.48	*	0.10	0.14	0.71		0.17	0.13	1.30		0.05	0.12	0.39		0.13	0.14	0.91	
Interaction effects																								
0.58% × MSG (β4)	−0.05	0.11	−0.42		−0.14	0.15	−0.94		−0.09	0.16	−0.57		−0.07	0.16	−0.46		0.09	0.14	0.60		−0.17	0.16	−1.02	
0.80% × MSG (β5)	−0.05	0.11	−0.47		0.07	0.15	0.43		−0.20	0.16	−1.22		0.19	0.16	1.20		0.02	0.14	0.17		0.01	0.16	0.07	
Experiment 2B																								
Main effects																								
Intercept (a)	0.00	0.09	0.00		−0.01	0.11	−0.05		0.00	0.12	−0.01		0.00	0.09	−0.01		0.00	0.12	0.00		0.00	0.11	0.00	
0.58% (β1)	0.46	0.11	4.23	***	0.16	0.13	1.19		−0.05	0.14	−0.32		−0.02	0.10	−0.15		0.24	0.14	1.65		0.06	0.13	0.50	
0.80% (β2)	0.78	0.11	7.14	***	0.20	0.13	1.48		−0.16	0.14	−1.17		0.06	0.10	0.61		0.30	0.14	2.13	*	0.06	0.13	0.51	
Odor (β3)	0.00	0.09	0.01		0.36	0.12	3.12	**	0.30	0.12	2.44	*	0.73	0.09	8.28	***	0.04	0.12	0.33		0.52	0.11	4.73	***
Interaction effects																								
0.58% × Odor (β4)	0.05	0.11	0.45		0.01	0.13	0.07		−0.11	0.14	−0.81		−0.04	0.10	−0.36		0.03	0.14	0.22		−0.14	0.13	−1.08	
0.80% × Odor (β5)	0.05	0.11	0.42		0.19	0.13	1.38		−0.08	0.14	−0.54		0.00	0.10	0.01		0.02	0.14	0.16		−0.12	0.13	−0.98	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 5. Results of the multiple regression analysis for the brain–fNIRS data in Experiment 2

	Mean signal change							
	Coef	SE	*t*	*P*	Coef	SE	*t*	*P*
Experiment 2A								
Main effects	Ch 2				Ch 6			
Intercept (a)	0.00	0.11	0.00		0.00	0.12	0.00	
0.58% (β1)	0.20	0.13	1.50		0.19	0.13	1.41	
0.80% (β2)	0.40	0.13	3.02	**	0.40	0.13	2.95	**
MSG (β3)	0.47	0.12	4.06	***	0.48	0.12	4.17	***
Interaction effects								
0.58% × MSG (β4)	−0.14	0.13	−1.07		−0.01	0.14	−0.04	
0.80% × MSG (β5)	−0.13	0.13	−0.95		−0.02	0.14	−0.17	
Experiment 2B								
Main effects	Ch 6				Ch 10			
Intercept (a)	0.00	0.11	0.03		0.00	0.11	0.04	
0.58% (β1)	0.35	0.13	2.66	**	0.29	0.13	2.18	*
0.80% (β2)	0.42	0.13	3.16	**	0.42	0.13	3.19	**
Odor (β3)	0.22	0.11	1.94		0.23	0.11	2.00	*
Interaction effects								
0.58% × Odor (β4)	0.15	0.13	1.13		0.14	0.13	1.03	
0.80% × Odor (β5)	0.02	0.13	0.19		−0.02	0.13	−0.16	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The mean signal changes for the right and left channels were not enhanced by the addition of MSG or odor (Figure 11B). For both channels, the multiple regression analysis (Table 6) revealed that there were significant main effects of $D_{0.58\%}$ and $D_{0.80\%}$, but not that of D_{MSG}, D_{odor}, nor significant interactions. These results showed that the mean signal changes increased as a function of NaCl concentration but that this was not enhanced by the addition of MSG or the odor.

Discussion

The present study aimed to elucidate the neural basis of the saltiness enhancement effects; thus, the taste-evoked responses in the temporal brain region (Experiments 1 and 2) and in the parotid salivary glands (Experiment 3) were recorded using fNIRS. In Experiment 1 both saltiness intensity measured by the single VAS and the responses of the temporal brain regions, which positively correlated with the subjective saltiness intensity and increased as a function of NaCl concentration, were enhanced by the addition of MSG, but not by the addition of the soy sauce odor. In Experiment 2, saltiness intensity measured by the multiple VAS was not enhanced but the responses of the temporal brain regions were enhanced by the addition of MSG or the soy sauce odor. In Experiment 3, saltiness intensity measured by the TI method was enhanced by the addition of MSG and by the soy sauce odor, but the response of the parotid glands was not, even though this increased as a function of NaCl concentration.

Physiological data measured by the brain- and salivary fNIRS

The results from the brain-fNIRS in Experiment 1 showed that the responses detected from several channels (Ch 5, 8, 22, 25, 28, 32, and 33) were significantly correlated with the saltiness intensity rating. The multiple regression analysis among these target channels revealed that the responses from Ch 25 and 28 increased as a function of NaCl concentration. These channels were estimated to detect signals from the pars triangularis (BA45), and this brain region constitutes the Fop, which was previously found to respond to salty taste stimulation (Ogawa et al. 2005). More importantly, the response from Ch 25 was enhanced by the addition of MSG, even the solutions did not contain NaCl (i.e., water with MSG). In contrast, the response was not enhanced by the addition of the soy sauce odor.

In Experiment 2, where the amount of the soy sauce presented was doubled, the brain response was enhanced not only by the addition of MSG but also by the addition of the soy sauce odor. The results from the brain-fNIRS showed that the responses detected from several channels (Ch 2, 6, 23, 27, 30, and 31 in Experiment 2A; Ch 6, 10, and 18 in Experiment 2B) were significantly correlated with the saltiness intensity rating, and the multiple regression analysis revealed that the responses from Ch 2, 6, and 10 increased as a function of NaCl concentration. More importantly, the analysis also revealed that the responses were enhanced by the addition of MSG (Ch 2 and 6 in Experiment 2A) and by the soy sauce odor (Ch 10 in Experiment 2B). These channels were estimated to detect signals from the pars opercularis (BA44) or the BA45, constituting the Fop. These results from Experiments 1 and 2 were consistent with those of previous studies indicating that the multimodal convergence of gustatory and olfactory information can occur in several regions, including the Fop (Small et al. 2004; Seo et al. 2013).

As the previous fNIRS study (Sato et al. 2011) indicated, however, enhanced responses observed in the brain-fNIRS can be derived from the parotid salivary glands, not from the cerebral cortex. To examine this possibility, the taste-evoked response of the parotid salivary glands was also measured in Experiment 3. The results from the salivary fNIRS showed that the response of the parotid glands increased as a function of NaCl concentration. These results are consistent with those of previous studies showing that the salivary secretion from the parotid glands correlates with salty taste stimuli concentration (Froehlich et al. 1987; Hodson and Linden 2006). Since a previous study (Hoshi et al. 2014) had not found NaCl concentration-dependent fNIRS signals from the parotid glands, the results from the present study are the first to show that salivary fNIRS can detect taste reflexes evoked by various concentrations of

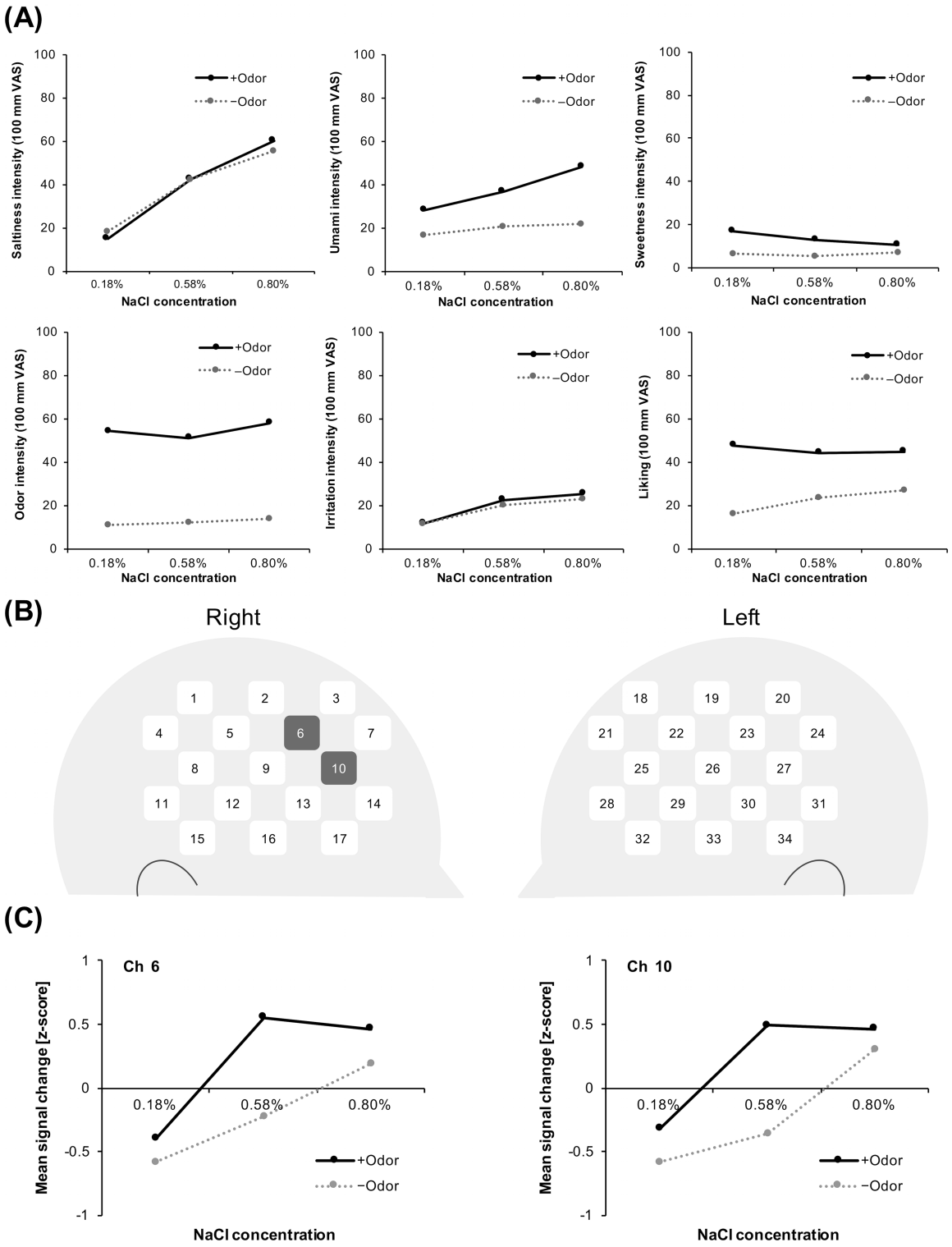

Figure 8. VAS and brain-fNIRS data in Experiment 2B. (**A**) Averaged ratings of saltiness intensity, umami intensity, sweetness intensity, odor intensity, irritation intensity, and liking for the various concentration of NaCl solutions with or without the addition of the soy sauce odor. (**B**) The target channels in which the multiple regression model was significant ($p <$ FDR 0.05). (**C**) The mean signal changes of Ch 6 and 10 for the various concentration of NaCl solutions with or without the addition of the soy sauce odor.

salty taste stimuli. However, in contrast to the result from the brain-fNIRS, the result from the salivary fNIRS showed that neither adding the MSG nor the soy sauce odor to the NaCl solution resulted in enhancement of the hemodynamic response of the parotid glands. These results suggest that the enhanced responses observed in the brain-fNIRS were not derived from the parotid glands.

For the brain-fNIRS, effects of NaCl concentration and of the addition of MSG or the soy sauce odor were found in the left Fop in

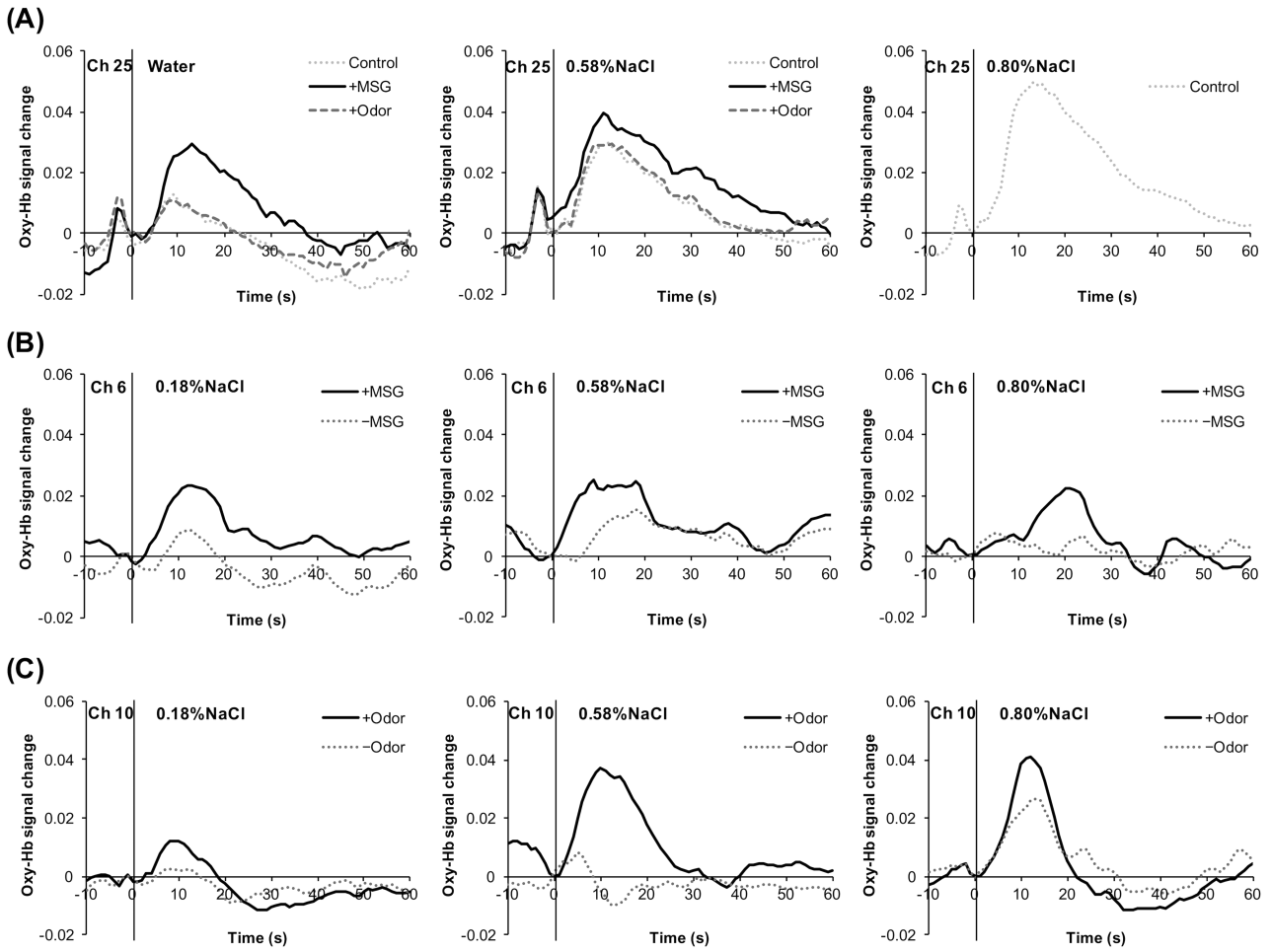

Figure 9. Grand averages of the oxy-Hb signal change curve for the various concentrations of NaCl solution with or without the addition measured by the brain-fNIRS, representative examples from (**A**) Ch 25 in Experiment 1, (**B**) Ch 6 in Experiment 2A, and (**C**) Ch 10 in Experiment 2B.

Experiment 1, whereas in the right Fop in Experiment 2. Although it is difficult to explain the different laterality between the experiments, one possibility is that the stimulus variety made the difference. For instance, more various stimuli were presented in Experiment 1 (i.e., tasteless water, MSG added water, the odor added water, NaCl solution, MSG added NaCl solution, and the odor added NaCl solution) than in Experiments 2A (i.e., NaCl solution and MSG added NaCl solution) and 2B (i.e., NaCl solution and the odor added NaCl solution). Because of the greater stimulus variety in Experiment 1, the participants might have paid more efforts to differentiate the stimuli by mentally verbalizing the quality of stimuli. It has been demonstrated that the verbalization during encoding of stimuli exhibited left lateralized brain activations (e.g., Cabeza and Nyberg 2000). Therefore, the left lateralized activation of the Fop found in Experiment 1 might reflect both the enhanced taste processing and the participants' greater efforts to verbalizing the stimuli. The laterality is beyond the scope of the present study, but it is interesting to investigate the point by well controlled experiments in future.

Sensory evaluation data measured by the VAS and TI method

Compared to the fNIRS data, the sensory evaluation data were more complicated. Umami-induced saltiness enhancement was demonstrated when participants were asked to evaluate only saltiness intensity using the VAS (Experiment 1) or TI method (Experiment 3), but not when they were asked to evaluate saltiness intensity as well as the other sensory qualities using the VAS (Experiment 2). On the other hand, odor-induced saltiness enhancement was demonstrated only when participants were asked to evaluate the saltiness intensity using the TI method (Experiment 3). These differences indicate that umami- and odor-induced saltiness enhancement involve different mechanisms.

The umami-induced saltiness enhancement was evident when participants were required to evaluate a single sensory quality (i.e., saltiness only) in Experiment 1 and 3, but not when they were required to evaluate multiple sensory qualities (e.g., saltiness, umami, and odor intensity) in Experiment 2A. This task-dependent nature of the taste enhancement effect, named the "halo-dumping effect" (Clark and Lawless 1994), has been often reported in previous studies examining taste-taste and/or taste-odor interaction (e.g., Frank et al. 1993; van der Klaauw and Frank 1996). When participants are required to evaluate only a single quality (e.g., sweetness) of a complex flavor mixture (e.g., strawberry-flavored sucrose solution), participants mistakenly combine a rating for a perceived quality (e.g., fruitiness of strawberry odor) with a rating for another quality (e.g., sweetness of sucrose) because of their similarities; sweetness

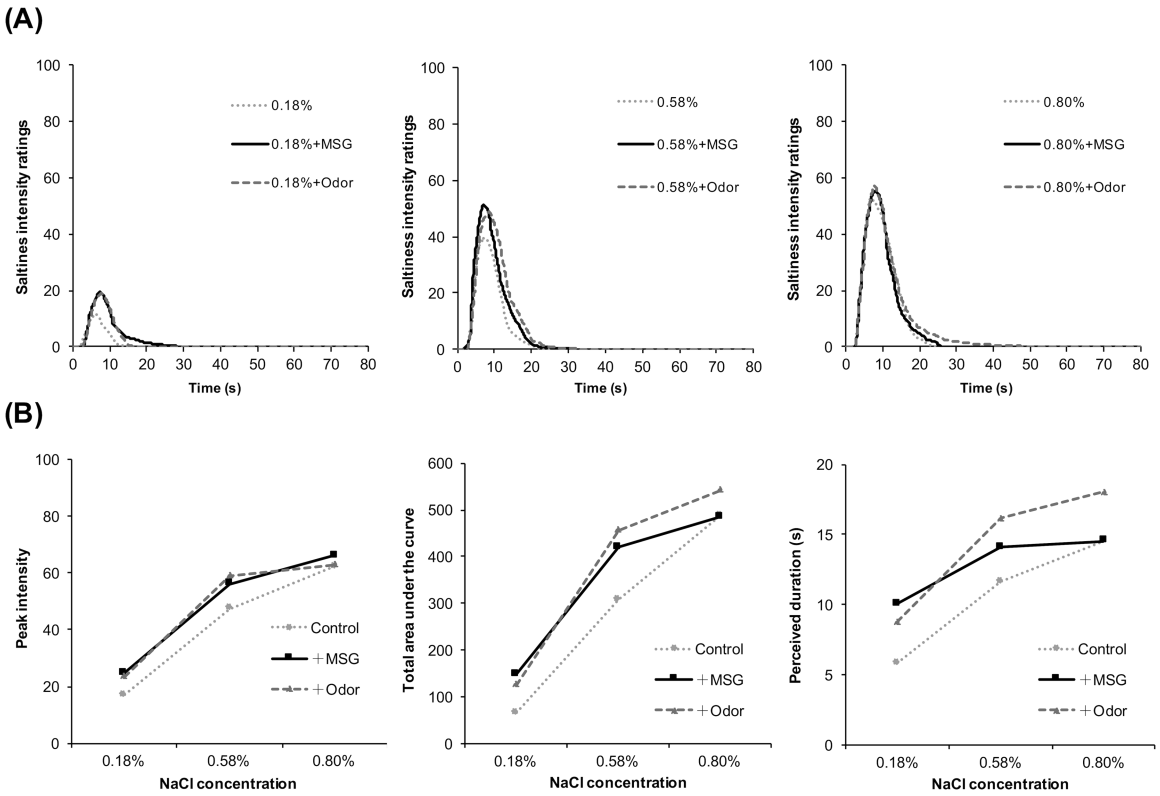

Figure 10. (**A**) The averaged saltiness intensity curves of 0.18%, 0.58%, and 0.80% NaCl solutions with or without addition of MSG or the soy sauce odor in Experiment 3. (**B**) The averaged TI parameters, peak intensity, total area under the curve (AUC), and perceived duration.

intensity rating is enhanced by addition of the sweet-smelling odor. When participants are required to evaluate multiple qualities (e.g., sweetness and fruitiness), on the other hand, they properly evaluate each quality without combining qualities; sweetness intensity rating is not enhanced by addition of the odor.

From this view, it can be suggested that in Experiments 1 and 3 the saltiness intensity rating was enhanced because umami perception of MSG was combined with saltiness perception of NaCl. This possibility was supported by the result of Experiment 2A showing that addition of MSG did not enhance the saltiness intensity rating when the umami intensity rating scale was also presented. In addition, the result of Experiment 1 showed that MSG solution itself was rated as salty, and that MSG solution itself evoked the Fop response. Taken together, these results suggest that the umami-induced saltiness enhancement is based on combining salty-like umami taste property of MSG and saltiness of NaCl into a single flavor percept, which may be represented in the Fop.

Halo-dumping effect was originally taken as evidence that taste enhancement in a single scaling situation is a rating bias or an artifact (Clark and Lawless 1994). This notation seems plausible, but may be problematic. A recent theory of flavor perception suggests that the nature of flavor perception is, namely, *fusion*; sensory signals are effortlessly combined to produce a single flavor percept, but also can be perceived as a collection of elements, depending on attention (Prescott 2012). Under the normal conditions of savory soup consumption, we are typically asked "How salty is it?" and so on. This kind of question, analogous to the single scaling situation, directs people's attention toward the overall flavor percept and prompts to combine flavor qualities that are similar to each other (e.g., saltiness and umami) into a single flavor percept (e.g., salty soup flavor); saltiness intensity is enhanced by the addition of an umami taste substance. On the other hand, if people's attention is directed analytically toward its elements, analogous to the multiple scaling situation, the overall flavor can also be perceived as a collection of elements (e.g., salty taste, umami taste, and so on); saltiness intensity is not enhanced. From this view, both the enhancement in the single scaling and the lack of the enhancement in the multiple scaling are true results, representing modulatory effect of attention on flavor perception. Therefore, contrary to the original interpretation of the halo-dumping effect (Clark and Lawless 1994), it is suggested that the task-dependent nature of taste enhancement effect does not mean the effect is simply an artifact, and does not invalidate the theoretical and the practical importance of research on the mechanism of the taste enhancement effect.

MSG was used as an umami taste substance in the present study, but MSG itself contains a small sodium component. This leads to concerns that the saltiness enhancement by addition of MSG simply reflects the added sodium. This seems unlikely because, if saltiness enhancement is simply based on the sodium component of MSG, saltiness enhancement should have been observed throughout the present study, regardless of the sensory evaluation methods used in the experiment. However, the results showed that saltiness enhancement by addition of MSG was clearly task-dependent and this cannot be explained in terms of the physically added sodium components. Therefore, it is again suggested that the saltiness enhancement by addition of MSG is based on the fusion of the umami taste property of MSG and saltiness of NaCl, which is governed in the brain. To clarify this point further, future research should use an umami taste substance without a sodium component, such as monopotassium glutamate (MPG).

Table 6. Results of the multiple regression analysis for the TI and the salivary fNIRS data in Experiment 3

	Peak intensity				Total area under the curve				Perceived duration				Mean signal change (R)				Mean signal change (L)			
	Coef	SE	*t*	*P*	Coef	SE	*t*	*P*	Coef	SE	*t*	*P*	Coef	SE	*t*	*P*	coef	*SE*	*t*	*P*
Main effects																				
Intercept (*a*)	0.00	0.04	0.00		0.00	0.05	0.00		0.00	0.06	0.00		0.00	0.09	0.00		0.00	0.09	0.00	
0.58% (*β1*)	0.75	0.05	15.25	***	0.70	0.05	12.96	***	0.62	0.07	8.98	***	0.27	0.11	2.53	*	0.23	0.11	2.09	*
0.80% (*β2*)	0.99	0.05	20.01	***	0.96	0.05	17.62	***	0.80	0.07	11.62	***	0.34	0.11	3.15	**	0.29	0.11	2.60	*
MSG (*β3*)	0.13	0.05	2.57	*	0.10	0.05	1.91		0.25	0.07	3.63	***	−0.02	0.11	−0.14		−0.19	0.11	−1.72	
Odor (*β4*)	0.14	0.05	2.78	**	0.18	0.05	3.32	**	0.36	0.07	5.19	***	−0.01	0.11	−0.08		−0.02	0.11	−0.21	
Interaction effects																				
0.58% × MSG (*β5*)	0.00	0.06	0.01		0.01	0.06	0.19		−0.08	0.08	−1.03		0.09	0.13	0.74		0.01	0.13	0.05	
0.80% × MSG (*β6*)	−0.05	0.06	−0.88		−0.10	0.06	−1.66		−0.19	0.08	−2.37	*	−0.08	0.13	−0.62		−0.01	0.13	−0.07	
0.58% × Odor (*β7*)	0.06	0.06	0.99		0.10	0.06	1.56		0.03	0.08	0.43		0.08	0.13	0.66		−0.04	0.13	−0.34	
0.80% × Odor (*β8*)	−0.06	0.06	−0.99		−0.04	0.06	−0.66		−0.08	0.08	−0.95		−0.12	0.13	−0.96		0.01	0.13	0.10	

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

The odor-induced saltiness enhancement was demonstrated neither by the single and multiple VAS measurement in Experiments 1 and 2B, but was only demonstrated by the TI method in Experiment 3, which enables the taste intensity to be monitored over time (Dijksterhuis and Piggott 2001). In addition, the result of Experiment 1 showed that the odor itself (i.e., water added with the soy sauce odor) was not rated as salty, and that the odor itself did not evoke the Fop response. Contrary to the umami-induced saltiness enhancement, these results cannot be simply explained by the fusion of flavor qualities. Rather they indicate that odor-induced saltiness enhancement is based on modulation of the temporal dynamics of the saltiness perception, which is more easily detected by the TI method than by the temporally static VAS.

Of course, the lack of the odor-induced saltiness enhancement in Experiment 1 can be also attributed to the smaller amount of the soy sauce presented (4 mL) than in Experiments 2B and 3 (8 mL), which might have caused the lack of the Fop response by the soy sauce odor in Experiment 1. However, the TI data from Experiment 3 indicates the effect of the soy sauce odor is different from that of MSG. For instance, the addition of the soy sauce odor not only enhanced the peak intensity of the saltiness intensity curve, but also enhanced the AUC of the intensity curve and prolonged the perceived duration of saltiness. More importantly, the effect of adding the soy sauce odor on the perceived duration ($\beta = 0.36$) was greater than on the peak intensity ($\beta = 0.14$). This tendency was also found when MSG was added, but the difference between the effect on the perceived duration ($\beta = 0.25$) and on the peak intensity ($\beta = 0.13$) was smaller than that when the odor was added. Furthermore, the effect of MSG on the perceived duration was limited to the low and middle concentration of NaCl solution, whereas the effect of the soy sauce odor was evident in the all concentrations of NaCl solution.

The odor-induced effect on the temporal dynamics of taste perception might be based on improved detectability of taste substances. For instance, previous studies have demonstrated that sweet-smelling odor increases the detection accuracy of a sweet taste presented in the mouth (Djordjevic et al. 2004a; Prescott 2004). Taken together, it is suggested that addition of the salty-smelling soy sauce odor improves the detectability and prolongs the perceived duration of salty taste substances presented in the mouth, which may be governed in the Fop, and this effect might be well detected by the temporally dynamic TI method than by the temporally static VAS.

The possibility that the odor-induced saltiness enhancement is based on modulation of the temporal dynamics of the saltiness perception was presented for the first time by the present study, because most of the previous studies reporting the odor-induced saltiness enhancement used temporally static intensity ratings (e.g., VAS) (Djordjevic et al. 2004b; Lawrense et al. 2009; Nasri et al. 2011; Seo et al. 2013). To contribute to the development of effective strategies of decreasing salt intake, the possible difference between the umami-induced (i.e., taste–taste interaction) and the odor-induced (i.e., taste–odor interaction) saltiness enhancement should be examined further in future research.

Limitations

The present study has a number of limitations. First, the 2 different kinds of sensory evaluations were used in the present study (i.e., VAS in Experiments 1 and 2, and TI in Experiment 3); it would have been better to use the same sensory evaluation method throughout experiments and this would have allowed a direct comparison between the results from the brain-fNIRS and those from the salivary fNIRS.

Second, the Fop, where enhancement by the addition of MSG or the soy sauce odor was observed, is involved both in taste processing

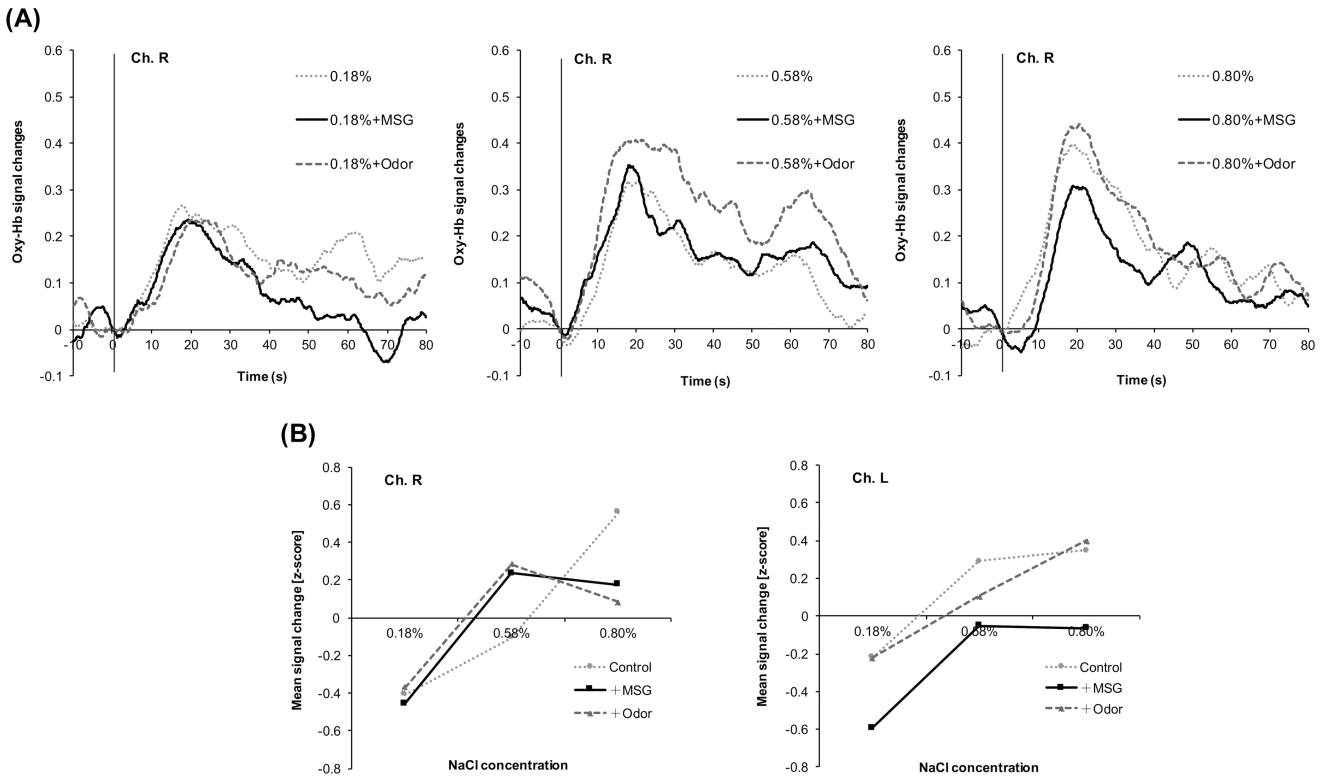

Figure 11. (**A**) The averaged oxy-Hb signal curves of 0.18%, 0.58%, and 0.80% NaCl solutions with or without addition of MSG or the soy sauce odor for the right channel in Experiment 3. Vertical line represents the stimulus onset. (**B**) The averaged mean signal changes during the full widths at half maximum (FWHM) of the curve for the right and the left channels.

and in other cognitive processes (e.g., Okamoto et al. 2006). This suggests that the enhancement effects detected in this brain region simply indicated that the flavor quality became more complicated, and information that participants had to keep in their working memory for later sensory evaluation became higher. To clarify this point, future study should compare the effect of MSG or soy sauce odor, which are congruent with the target salty taste, to the effect of other taste or odor substances incongruent with the target salty taste (e.g., sugar or sweet-smelling odor). If the enhancement effect observed in the present study was simply due to the complexity of the stimuli, the enhancement effect of the Fop would be equally found both by addition of congruent and incongruent substances; otherwise, the enhancement effect would be found only by addition of the congruent substances.

Third, based on the result from the salivary fNIRS showing that neither adding the MSG nor the soy sauce odor to the NaCl solution resulted in enhancement of the hemodynamic response of the parotid glands, it was suggested that the enhanced responses observed in the brain-fNIRS were not derived from the parotid glands. However, since equipment and measurement conditions were different for the brain-fNIRS and the salivary fNIRS experiments, difference of the sensitivity to the response of the parotid glands between these 2 systems can simply explain the results. If the sensitivity to the parotid glands response, for instance, is higher for the brain-fNIRS than the salivary fNIRS, the lack of the enhancement in the salivary fNIRS measurement cannot reject the possibility that the enhanced responses observed in the brain-fNIRS are derived from the parotid glands. Although both the brain-fNIRS (Sato et al. 2011) and the salivary fNIRS (Hoshi et al. 2014) studies reported a strong relationship between the recorded response signals and actual saliva secretion volume evoked by gustatory stimulation, there has been no research which directly compared these 2 fNIRS systems so far. Therefore, the possible sensitivity difference can limit the present findings, but this seems unlikely because the salivary fNIRS probes were located at more preferable positions to measure the hemodynamic response of the parotid glands (i.e., anterior areas to the ears corresponding to the position of the parotid glands), which indicated the sensitivity should be higher for the salivary fNIRS than the brain-fNIRS. To clarify this point and strengthen the present findings, the relationship between the recorded response signals and actual saliva secretion volume should be compared between the brain- and the salivary fNIRS in future research.

Conclusions

In summary, the present study investigated the neural basis of umami- and odor-induced saltiness enhancement effects. The results from fNIRS measures showed that the addition of MSG or the soy sauce odor to NaCl solutions enhanced responses in the Fop, but did not alter responses of the parotid salivary glands. These results indicate that the umami- and odor-induced saltiness enhancement effects occur in the brain region which is involved in the central gustatory processing. Furthermore, the results of the sensory evaluations suggest that umami-induced saltiness enhancement is mainly based on the fusion of the salty-like property of MSG and saltiness of NaCl, whereas that odor-induced saltiness enhancement is mainly based on modulation of the temporal dynamics of the saltiness perception. These findings may contribute to the development of effective strategies to solve salt-related health concerns.

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Conflict of interest

The authors declare to have no conflict of interest.

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