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Visual and Auditory Stimuli Associated with Swallowing: An fMRI Study

Takeshi Kawai, Yutaka Watanabe, Morio Tonogi, Gen-yuki Yamane, Shinichi Abe*, Yoshiaki Yamada** and Akiko Callan***

Department of Oral Medicine, Oral and Maxillofacial Surgery, Tokyo Dental College, 5-11-13 Sugano, Ichikawa, Chiba 272-8513, Japan
* Department of Anatomy, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan
** Division of Oral Physiology, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho, Niigata 951-8514, Japan
*** Brain Activity Imaging Center, ATR International, 2-2-2 Hikaridai, Keihanna Science City, Kyoto 619-0288, Japan

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Abstract

We focused on brain areas activated by audiovisual stimuli related to swallowing motions. In this study, three kinds of stimuli related to human swallowing movement (auditory stimuli alone, visual stimuli alone, or audiovisual stimuli) were presented to the subjects, and activated brain areas were measured using fMRI and analyzed. When auditory stimuli alone were presented, the supplementary motor area was activated. When visual stimuli alone were presented, the premotor and primary motor areas of the left and right hemispheres and prefrontal area of the left hemisphere were activated. When audiovisual stimuli were presented, the prefrontal and premotor areas of the left and right hemispheres were activated. Activation of Broca’s area, which would have been characteristic of mirror neuron system activation on presentation of motion images, was not observed; however, activation of brain areas related to swallowing motion programming and performance was verified for auditory, visual and audiovisual stimuli related to swallowing motion. These results suggest that audiovisual stimuli related to swallowing motion could be applied to the treatment of patients with dysphagia.

Key words: Deglutition—Supplementary motor area—Premotor area—Primary motor area—Prefrontal area

Introduction

The objective of the study was to use functional magnetic resonance imaging (fMRI) to investigate brain responses upon simultaneous presentation of images and sounds associated with human swallowing motion. Brain responses have been reported when a
person observe images of oral and upper and lower limb motion\textsuperscript{1,2} and activated brain areas are known to be involved in motion programming and performance\textsuperscript{20}; however, response during swallowing motion observation remains to be fully elucidated. The presentation of swallowing motion-related sounds, in addition to motion images, may be appropriate in such studies, as swallowing motion is associated with a swallowing sound. In the present study, differences between auditory or visual stimuli alone and audiovisual stimuli were investigated.

\section*{Materials and Methods}

This study was conducted at the Advanced Telecommunications Research (ATR) International Institute with the approval of the Internal Review Board of the Tokyo Dental College.

\subsection*{1. Subjects}

Twelve healthy adult volunteers (6 men and 6 women, age: 20 to 28 years) participated in the study. On the day of study, the study objectives and predictable risks were explained to the subjects in writing and informed consent for participation in the study was obtained from each volunteer. A handedness test showed that all subjects were right-handed; all subjects had normal vision and hearing.

\subsection*{2. Study design}

Imaging was conducted using eight kinds of swallowing motion-related audiovisual stimuli: (1) swallowing visual moving image + swallowing sound (SVSS) (Fig. 1; upper images), (2) swallowing visual moving image alone (SV) (Fig. 1; lower images), (3) swallowing visual moving image + control sound (SVCS) (Fig. 2; upper images), (4) still image of the neck (still picture related to swallowing) + swallowing sound (NVSS) (Fig. 2; lower images), (5) still image of the neck (still picture related to swallowing) alone (NV) (Fig. 3; upper images), (6) still image of the neck (still picture related to swallowing) + control sound (NVCS) (Fig. 3; lower images), (7) swallowing sound alone (SS) (Fig. 4; upper images), and (8) control sound alone (CS) (Fig. 4; lower images). The presentation time for each stimulus was 6 seconds. The swallowing sound was set to be emitted at 3.5 seconds after all stimulation had been presented, which was synchronized with lifting of the larynx in the swallowing moving images. Identical timing was used for all auditory stimuli.

The still image of the neck (still picture related to swallowing) and control sound were used as controls for the swallowing moving image and swallowing sound, respectively. The still image of the neck without motion was chosen as a control, because, in tests containing visual stimulus, we planned to determine the brain area activated during observation of motion in the neck; \textit{i.e.}, the swallowing motion. In tests involving an auditory stimulus, the waveforms of human swallowing sounds were shuffled; the shuffled sound with the same volume was used as the control sound.

The 8 kinds of stimuli are shown in Figs. 1–4. Each stimulus was displayed for 6 seconds as 180 sequential photographs (33 millisecond/frame); sequential photographs in which the 180 frames are reduced to approximately one-fifteenth (500 millisecond/frame) are shown in Figs. 1–4. The wave below the visual image shows the waveform of the synchronized auditory stimulus.

fMRI was performed with the subject in the recumbent position. The subject looked at a screen containing the visual stimulus presented using a projection system \textit{via} a mirror fixed on the head coil. Auditory stimuli were given \textit{via} high-performance headphones for fMRI (Hitachi Advanced Systems Corp., Yokohama, Japan) under conditions of reduced fMRI noise (reduced by 20 dB SPL).

The cycle and block designs are shown in Figs. 5 and 6, respectively. The first block consisted of four presentations of 6-second stimuli and the first cycle comprised random presentations of the 8 kinds of stimuli. The subjects underwent tests in 3 sessions (Fig. 6), each of which consisted of two cycles (as shown in Fig. 5). It was thought that subjects would be bored with a still image of the neck

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Each stimulus was displayed for 6 seconds as 180 sequential photographs (33ms/frame); Figures 1-4 show sequential photographs in which 180 frames were reduced to approximately one fifteenth (500ms/frame). Waveform of synchronized auditory stimulus is displayed beneath visual image.

**Fig. 1** Eight kinds of stimuli
Upper image: Swallowing visual moving image with swallowing sound (SVSS).
Lower image: Swallowing visual moving image alone (SV).

**Fig. 2** Eight kinds of stimuli
Upper image: Swallowing visual moving image with control sound (SVCS).
Lower image: Still-neck image (still picture related to swallowing) with swallowing sound (NVSS).

**Fig. 3** Eight kinds of stimuli
Upper image: Still-neck image (still picture related to swallowing) alone (NV).
Lower image: Still-neck image (still picture related to swallowing) with control sound (NVCS).

**Fig. 4** Eight kinds of stimuli
Upper image: Swallowing sound alone (SS). Lower image: Control sound alone (CS).
(still picture related to swallowing) and a still image of a hand for 24 seconds; therefore, four kinds of still-neck images with different positions were presented for 6 seconds each. Cycle duration was 24 seconds (2 cycles), and the total duration of the session was 396 seconds with 2 cycles and 12 seconds of the three dummy scans immediately after the start of the presentation. There was a 5-minute interval between sessions.

3. Device and imaging conditions

T1- and T2-weighted and functional images were obtained using an fMRI unit (Magnex Eclipse 1.5T PD250, Shimadzu-Marconi, Kyoto, Japan) housed at the ATR Institute International Brain Activity Imaging Center. The conditions for gradient echo planar imaging were as follows: repetition pulse (TR), 64×64 pixels; matrix size, 192×192 mm; field of view (FOV), 49 ms; echo time (TE), 90° of flip angle; 40 axial slices; and 3×3-mm plane resolution. Forty slices covering the head were imaged at 123 scans per slice with a voxel size of 2×2×4 mm without slice gap. The first three scans in each session were not used in the analysis because of instability of the signal values immediately after the start of imaging.

Analysis Method

1. Data analysis

We determined fMRI signals that synchronized with stimulus presentation using the blood oxygenation level-dependent (BOLD) effect and analyzed differences between the various auditory and audiovisual stimuli. All images were pre-processed using the Statistical Parametric Mapping 2 (SPM2) software. In SPM2, changes associated with interactions
of brain areas were not considered; based on the assumption that each brain area functions independently, local results were examined statistically for all pixels. In the present study, 369 functional images were obtained using fMRI; consequently, the head position tended to move during the long imaging period. Any slip in head position was corrected using three-dimensional translation and rotation in SPM2, with brain imaging data adjusted three-dimensionally to fit the initial data. Data were then normalized in accordance with the standard Montreal Neurological Institute (MNI) brain, and sliced again. The averaged image was constructed using the image in the corrected position, and T2 and T1 structural images were adjusted and normalized based on this position. Anatomically normalized brain functional images were smoothed to meet the condition of a Gaussian random field. Changes associated with activation generally occurred over several pixels; therefore, smoothing made it easy to detect signal changes due to activation. Smoothing was conducted using a Gaussian filter (full width at half maximum (FWHM) = 6×6×8 mm).

In this procedure, signal values and counts had a more normal distribution and could be more effectively used in a statistical model. Low-frequency signals that occurred during imaging were deleted using scaling. The structural image was normalized for each subject and the typical or averaged image was used.

In fixed effect analysis, signal correlation was performed using a boxcar functional model with a blood flow response function to detect brain area that displayed statistically significant signal changes due to targeted activation. Contrast images were constructed for each subject and a sample t-test (a group analysis tool) was performed for these images to detect areas that displayed significant differences sequentially or between sessions. In examining brain activity across the subjects, a sample t-test was conducted between brain-activity distributions calculated for each subject, and areas with common significant brain activity were identified.

As few activated areas were found at p<0.001 without correction of the significance level for multiple comparisons, the threshold was increased to p<0.05; however, BOLD signals were extremely strong and activated areas could not be extracted, probably largely due to the increased significance level. On the basis of these results and to maintain study reliability, the data were analyzed at p<0.005. In analyzing the plural data using a random-effects model at p<0.005, z values of activated areas corresponded to 2.58 and higher, and areas showing this value were considered to be activated; these data are summarized in Table 1. The coordinate axes of the results table (Table 1) were analyzed using SPM2, expressed as MNI coordinates, and converted into Talairach coordinates using MatLab. The Brodmann area and anatomical position were then estimated from the obtained coordinates in accordance with the Co-Planar Stereotaxic Atlas of the Human Brain of Talairach and Tournoux.

Results

1. Analysis of activated brain areas in whole brain

To investigate the effects of swallowing motion related to auditory and/or visual stimuli, areas activated by control sounds and images were subtracted from areas activated by swallowing sounds, swallowing visual moving images, and swallowing visual moving images with swallowing sounds; the remaining areas were considered to have undergone activation. Table 1 shows the x-y-z coordinates of the standard brain coordinate system of Talairach and Tournoux, the Brodmann cyto-architectural area (Brodmann area), and the anatomical position and peak z value of activated areas with swallowing related auditory stimulus alone (SS-CS), swallowing related visual stimulus alone (SV-NV), and swallowing related audiovisual stimuli (SVSS-NVCS). The threshold for significance was determined to be p<0.005 when the significance level was not corrected for multiple comparison (z > 2.58). fMRI signals that synchronized
with stimulus presentation were determined based on the BOLD effect; we then investigated the activation of areas related to swallowing motion. Areas activated by swallowing related auditory stimulus alone (Fig. 7), swallowing related visual stimulus alone (Fig. 8), and swallowing related audiovisual stimuli (Fig. 9) are shown as axial images; whiter areas in the images indicate higher z values during stimulus presentation.

2. Presentation of swallowing related auditory stimulus alone

As shown in Fig. 7, two areas were activated by swallowing related auditory stimulus alone: the superior temporal gyrus (Brodmann area 42) of the left hemisphere, in which the primary auditory area is localized, and the middle frontal gyrus (Brodmann area 6) of the right hemisphere, in which the supplementary motor area is localized.

3. Presentation of swallowing related visual stimulus alone

As shown in Fig. 8, four areas were activated by swallowing related visual stimulus alone: the middle frontal gyrus (Brodmann area 6) of the right and left hemispheres, in which the premotor area is localized; the right and left hemispheres (Brodmann area 4), in which the primary somatomotor area is localized; the middle frontal gyrus (Brodmann area 9) of the left hemisphere, in which the frontal association area is localized; and the lateral occipital gyrus (Brodmann area 19) of the right hemisphere, in which the secondary visual area is localized.

4. Presentation of swallowing related audiovisual stimuli

As shown in Fig. 9, four areas were activated by swallowing related audiovisual stimuli: the superior frontal gyrus (Brodmann area 9) of the right and left hemispheres, in which the
Fig. 7  Brain area activated by auditory stimulus alone (SS-CS)
a: MNI coordinates = +6, z value = 3.29; activation occurred in superior temporal gyrus (Brodmann area 42; primary auditory area) of left hemisphere (arrow). b: MNI coordinates = +48, z value = 2.78; activation occurred in middle frontal gyrus (Brodmann area 6; supplementary motor area) of right hemisphere (arrow).

Fig. 8  Brain area activated by visual stimulus alone (SV-NV)
a: MNI coordinates = +57, z value = 3.10; activation occurred in middle frontal gyrus (Brodmann area 6; premotor area) of left hemisphere (arrow). b: MNI coordinates = +66, z value = 3.08; activation occurred in precentral gyrus (Brodmann area 4; premotor area) of right hemisphere (arrow). c: MNI coordinates = +42, z value = 2.83; activation occurred in middle frontal gyrus (Brodmann area 9; prefrontal area) of left hemisphere (arrow). d: MNI coordinates = +54, z value = 2.74; activation occurred in middle frontal gyrus (Brodmann area 6; premotor area) of right hemisphere (arrow). e: MNI coordinates = +63, z value = 2.71; activation occurred in precentral gyrus (Brodmann area 4; premotor area) of left hemisphere (arrow).
the frontal association area is localized; the middle frontal gyrus (Brodmann area 6) of the left hemisphere and the precentral gyrus (Brodmann area 6) of the right hemisphere, in which the premotor area is localized; and the lateral occipital and orbital and inferior temporal gyri of the right hemisphere and the lateral occipital and inferior temporal gyri (Brodmann area 19) of the left hemisphere, in which the secondary visual area is localized.

**Discussion**

1. **Study method**

Previous fMRI studies on the localization of human brain functions have been performed for finger movement and for mouth opening and closing; however, localization of brain function associated with swallowing-related imaging stimuli has yet to be examined. This may be partly because of difficulties that arise from the long stimulus presentation time. Prior to swallowing, the food is processed in the mouth. The swallowing motion consists of three stages: an oral stage (moving the food into the posterior region of the mouth using the tongue), a pharyngeal stage (from induction of the swallowing reflex to the passage of food through the pharynx), and an esophageal stage (moving the food to the stomach by esophageal peristalsis). Therefore, the
stimulus presentation time is long and a subject’s habituation to the stimulus decreases the BOLD signals.

To reduce this problem, we devised a stimulus presentation method in which four kinds of still images of the neck with different neck positions were presented for 6 seconds each (Fig. 1); however, we cannot state with certainty that the long stimulus-presentation time had no effect on the results. Rasmus et al. proposed that a single-trial paradigm (tracking a chronological change in the brain due to activation by a single stimulus) is better than a block-trial paradigm (repeated imaging and rest) because slight changes in hemodynamic factors induced by a short stimulus can be compared with EEG (electroencephalogram) and MEG (magnetic encephalography) results and artifacts can be deleted. We considered using a single-trial paradigm in the present study, but ultimately decided against it because a short stimulus (i.e., a swallowing reflex only) was not recognized as a swallowing motion by the subjects.

2. Activated brain areas

1) Supplementary motor area

The supplementary motor area was activated by the swallowing related auditory stimulus alone (SS-CS). Penfield and Boldrey first named this region of the inner side of the frontal lobe as the supplementary motor area (SMA). The SMA belongs to Brodmann area 6 and is not specifically defined; however, it is generally considered to be the medial surface of the cerebral hemisphere anterior to the primary motor area (Brodmann area 4), i.e., the medial surface of the superior frontal gyrus (medial part of Brodmann area 6). The SMA is involved in comprehensive motion rather than simple motion, and it is especially associated with the beginning of motion and involved in the control of sequential motions. It has also been hypothesized that the SMA is involved in motion in response to external stimuli or in spontaneous motions. This proposal is supported by the results of several studies; however, it remains unknown as to whether this area performs any or all of these functions, as a previous study reported that movement disorders did not occur following somatic damage to this location, although awkward motions were observed.

Recent studies in monkey have led to a new hypothesis in which the SMA, which had previously been considered to be a single area, was divided into two areas. This hypothesis is becoming widely accepted, and in this context Tanji renamed the caudal SMA as the SMA proper and the rostral SMA as the pre-SMA. The SMA proper projects directly into the motor area and is associated with automatic motions associated with high-order learning, while the pre-SMA receives input from the prefrontal area and cingulate gyrus. There are anatomical differences between the two areas of the SMA. Activation was observed in the SMA proper with auditory stimulus alone, suggesting that stimulation of the SMA proper by an auditory stimulus enhances further activation of related motor areas and is involved in the performance of a series of swallowing motions. In the SMA, the pre-SMA receives input signals from the cingulate gyrus and the prefrontal area. In addition, Watanabe et al. reported that the islet is closely associated with preparation of swallowing motions and that the cingulate gyrus is involved in the actual performance of swallowing; further studies using new methods will be required to confirm these results.

2) Premotor area

The premotor area was markedly activated with presentation of visual stimulus alone (SV-NV) or audiovisual stimuli (SVSS-NVCS). This area, similar to the SMA, belongs to Brodmann area 6 and is on the lateral surface of the frontal lobe just anterior to the primary motor area (Brodmann area 4), forming the extrapyramidal cortical center. Fewer fibers extend to the spinal cord directly from the premotor and supplementary motor areas compared with the motor areas. Instead, fibers extend from the premotor and supplementary motor areas to the red nucleus, reticular formation, and tegmentum, which are transfer points, and run down to the spinal cord as the rubrospinal tract. Most of these
fibers are ipsilaterally dominant and differ from the corticospinal tract of the motor areas; they are mainly involved in motions of the truncal muscles and muscles near the trunk\(^{10}\). The premotor area extends output fibers to the striatum, as well as to the SMA, and regulates motion\(^5,19\); it is thought to be associated with memorization of previously experienced motor activities (motor engram: motor behavioral pattern) and to be involved in the performance of complex motions\(^{23}\).

In addition, the premotor area is strongly associated with area 7, which is a higher visual center, and some fibers input directly from the somatosensory area into the motor area; however, many fibers also input into the motor area through the premotor area via the frontal and occipital association areas\(^4\). Therefore, the motor area is not activated by an auditory stimulus alone but is activated by vision-dominant input such as visual stimulus alone or audiovisual stimuli. Our results generally correspond to those of previous studies; furthermore, the present study suggests that observation of the swallowing motion connects the motor area with the sensory area.

3) Primary motor area

The primary motor area was activated with the visual stimulus alone (SV-NV); this area belongs to Brodmann area 4 and is on the lateral surface of Brodmann area 6. The cerebral cortex is divided into motor, sensory, and association areas; the primary motor area is located in the forehead and consists of three parts: the primary motor and premotor and supplementary motor areas. The prefrontal area is part of the cerebral association area and is also closely associated with motion\(^{12}\).

Penfield et al. reported that electric stimulus of the primary motor area induces motion in many parts of the body\(^{14,15}\), especially in the jaw, oral cavity, and face. Vogt and Vogt\(^{29}\) confirmed in C. cercopithecus monkeys that electrical stimulation of the cerebral cortex induces chewing motion; they showed that this site corresponds cytoarchitecturally to the lateral part of Brodmann area 6\(^{11,29}\). In addition to studies in monkey, similar studies have been conducted in cat, rabbit, and rat\(^{12}\).

These responses to stimulation of the cerebral cortex induce rhythmic coordination of the lower jaw and tongue that is similar to the overall chewing motion of animals; therefore, this brain region has been named the cortical masticatory area\(^7\). Takahashi et al.\(^{25}\) confirmed in humans that the cerebral cortex is involved in voluntary jaw motion (human voluntary bilateral-symmetric mouth-opening and closing movements) by using a motor readiness potential that reflects the cerebral programming process prior to voluntary motion.

Wildgruber et al.\(^{30}\) conducted a study in which healthy subjects were given the task of turning the tongue vertically while shutting the mouth (tongue movement task) and determined brain activation using fMRI; the results showed activation in the lower part of the bilateral precentral gyrus in all 10 subjects in the study. In the same study, subjects were given the task of reciting the names of months one after another without intonation or pronunciation (aprosodic speech task) and of humming a popular Christmas song, “O Christmas Tree” (syllable singing task); in these tasks, the precentral gyri of the left and right hemispheres were activated, respectively.

The primary motor area evokes a certain motion and is also involved in motion preparation\(^3,22\). In our study, the visual stimulus activated the bilateral precentral gyrus, i.e., the subjects imaged the motion as if they had actually made the motion; consequently, the result was similar to that of the tongue movement task of Wildgruber et al.\(^{32}\). These results suggest that visual information associated with swallowing is closely associated with imaging and performance of a swallowing motion.

4) Prefrontal area

With presentation of a visual stimulus alone (SV-NV), the prefrontal area of the left hemisphere was activated; with presentation of an audiovisual stimuli (SVSS-NVCS), the bilateral prefrontal areas were markedly activated. The prefrontal area is a large region that includes Brodmann areas 8–13 and 44–46\(^9\). In the present study, area 9 was activated both by visual stimulus alone and by audiovisual stimuli. The prefrontal area interconnects
most of the cerebral cortex, excluding the primary motor area and the primary somatosensory area\textsuperscript{16}. The function of the prefrontal area has been classified by Rosenkilde\textsuperscript{21} as follows: the major groove of the prefrontal area controls short-term memory (mainly spatial position), delayed response, and delayed alternation; the superior bulbous part is a motor sensor; the inferior bulbous part is associated with delayed conditioning discrimination, reversal learning, GO/NO-GO learning tasks, delayed matching-to-sample, and delayed alternation; the arcuate groove controls visual discrimination learning and visual caution; and the medial orbital frontal area is associated with emotion, motivation, and learning-set formation. Brodmann area 9 belongs to the superior bulbous part of the prefrontal area, is involved in kinesthesia, and recently has been confirmed to have vision-related neuronal activity\textsuperscript{8}. In addition, Takahashi et al.\textsuperscript{26} found that the prefrontal area is involved in the start of motion, and Matsunami and Naito\textsuperscript{8} confirmed that the prefrontal area is involved in the regulation of motion and performance. Hence, this region is associated with the design of a series of motions in advance, the building of a procedure of motion (programming) and performance of this procedure, and evaluation of the result of the motion or performance. In general, regular (stereotypical) motion and behavior in daily life are not disturbed by breakdown of the prefrontal area, but complex behavior cannot be performed under such circumstances. In studies in which two pictures were presented to monkeys taught to press different buttons if the pictures were the same or different, activation of the prefrontal area reflected pattern-specific sensory information, while activation of the premotor area reflected motor information; this suggests that visual information is converted to a behavioral set and then to motor information via the prefrontal area to the premotor area\textsuperscript{30}. In the present study, a visual stimulus alone and audiovisual stimuli activated the unilateral and bilateral prefrontal areas, respectively. These results suggest that observation of the swallowing motion is closely associated with a series of swallowing motions and that the addition of auditory information to the visual stimulus increases visual input and enhances activation of the prefrontal area.

5) Other activated areas

With presentation of the auditory stimulus alone, the primary auditory area (Brodmann area 42) was activated, while with the visual stimulus alone or audiovisual stimuli the visual area (Brodmann area 19) was activated. A more extensive area was activated with the audiovisual stimuli than with the visual stimulus alone. However, it has been reported that visual information is dominant in activation of the motor area, relative to auditory information\textsuperscript{9}; we found that a visual stimulus alone was sufficient to activate the bilateral primary motor areas, whereas audiovisual stimuli did not, which suggests that addition of auditory information to a visual stimulus has an effect on visual input.

**Conclusion**

In the present study, presentation of swallowing motion-related visual and/or auditory stimuli led to activation of the supplementary motor, premotor, and primary motor areas, which are involved in motion programming and performance. The visual and auditory information also showed a statistically significant activation of the superior prefrontal area. Our data also indicate that a swallowing motion-related auditory or visual stimulus alone is involved in the preparation, imaging, and performance of a swallowing motion. Furthermore, presentation of audiovisual stimuli may regulate the order of random motions correctly, as if a puzzle is being constructed, and have some role in programming a series of smooth swallowing motions.

Based on the results of the current study, we intend to establish appropriate swallowing motion-related visual and auditory stimuli as a new rehabilitation method for patients with dysphagia, with the goal of applying the method clinically.
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Reprint requests to:
Dr. Takeshi Kawai
Department of Oral Medicine,
Oral and Maxillofacial Surgery,
Tokyo Dental College,
5-11-13 Sugano, Ichikawa,
Chiba 272-8513, Japan
E-mail:tks-kawai@yellow.plala.or.jp