Modulation of neck muscle activity induced by intra-oral stimulation in humans

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**Highlights**

* The effect of intra-oral electrical stimulation on the dorsal neck muscle activity was investigated in healthy volunteers.

* The electromyographic (EMG) activity was reduced around 50 ms after the painful intra-oral electrical stimulation on average 80 % compared to baseline level in all subjects.

* Local anesthesia of the stimulus site resulted in smaller inhibition of the dorsal neck EMG activities and disappearance of the painful sensation evoked by the stimulation.
Abstract

Objective: To investigate the effect of painful electrical stimuli applied to intra-oral tissues around the teeth on the neck muscle activity in healthy humans.

Methods: Electromyographic (EMG) responses of the dorsal neck muscles evoked by intra-oral electrical stimulation were recorded before and after local anesthesia to the stimulus site in 17 healthy volunteers.

Results: Inhibition of dorsal neck muscle EMG activities on average 80% compared to baseline level was observed with a latency around 50 ms after the electrical stimulation before anesthesia, and the EMG activity inhibition decreased after anesthesia of the intra-oral stimulus site. The perceived intensity of the electrical stimuli as scored on a visual analogue scale (VAS) was 6.1 ± 0.4 cm before anesthesia and 1.5 ± 0.2 cm after anesthesia.

Conclusion: Intra-oral stimulation can inhibit neck muscle activity. This modulation might be attributed mainly to nociceptive afferent nerves however, non-nociceptive fibers could also be responsible.

Significance: Intra-oral information including nociceptive activity can inhibit neck muscle activity. From a clinical viewpoint, the present findings demonstrate the neural connectivity between the trigeminal region and the cervical region raising the possibility that orofacial pain conditions could influence head, neck and shoulder activity.
1. Introduction

In routine dental practice, we sometimes encounter patients suffering from chronic head, neck and shoulder pain that may be related with a dysfunction of the stomatognathic system. Temporomandibular disorders (TMD) have been reported to be accompanied by symptoms such as pain, stiffness, fatigue, and limitations in the muscle function in the head, neck, and shoulder regions (Gelb H and Tarte J, 1975; Sheppard IM and Sheppard SM, 1977; Schroeder S et al., 1991; Luz JGC et al., 1997). It has been suggested that dental treatment may potentially influence head, neck and shoulder symptoms of these TMD patients (Kirveskari and Alanen, 1984; Kirveskari, 1997), but the evidence is still lacking and the topic controversial.

Neural and clinical connections between the stomatognathic system and neck muscles have been reported in many papers (Svensson et al., 2004, 2005; Ge et al., 2004; Wang et al., 2004; Manni et al., 1975; Sumino et al., 1981; Alstermark et al., 1992; Abrahams et al., 1993; Dessem and Luo, 1999; Browne et al., 1993). Some of these reports demonstrate that an electric stimulus to the branches of the trigeminal nerve or a mechanical stimulus to the facial skin modulates neck muscle activity (Manni et al., 1975; Sumino et al., 1981; Alstermark et al., 1992; Abrahams et al., 1993). This response has been called the trigemino-cervical reflex (Lazzaro et al., 1995; Sartucci et al., 1986; Quartarone et al., 2000; Nakashima et al., 1992; Serrao et al., 2003). In addition, neural projections from deep orofacial structures, e.g. masticatory-muscle spindles (Dessem and Luo, 1999), the temporomandibular joint (TMJ) (Yu et al., 1995) and the masseter muscle (Hellström et al., 2000) to the motoneurons or circuits controlling neck muscles were also reported. Other studies report modulation of the neck muscle activity (Svensson et al., 2004), reflexes in the neck muscles (Ge et al., 2004; Wang et al., 2004).
and pain characteristics of the neck region (Svensson et al., 2005) after experimental jaw muscle pain. In contrast to muscle and joint receptors, only few reports in animal studies give specific information about the connections between intra-oral receptors and neck muscle activity (Zeredo et al., 2002), or convergence of tooth pulp, facial and neck afferents on to C1 spinal neurons (Matsumoto et al., 1999; Nishikawa et al., 2004). In humans, only one study (Browne et al., 1993) was found. In this study the authors reported that inhibition of the sternocleidomastoid muscle activity could be evoked by electrical stimulation of the gingiva. Consequently, only sparse evidence has been reported regarding sensory-motor interactions between the intra-oral sensory system and neck muscle activity. The aim of the present study, therefore, was to investigate the effect of painful electrical stimulation of the gingiva on neck muscle activity in humans.

2. Materials and methods

2.1. Participants

Seventeen healthy individuals (7 women, 10 men; aged 23-33, mean 26.2) participated in this study. They were free from any signs or symptoms of craniomandibular, neck and shoulder disorders, periodontal or endodontic disease. None of the subjects took any medication at least one month before participation. This study was approved by the ethics committee of Nagasaki University School of Dentistry (approval no. 0959) and followed the guidelines from the Helsinki Declaration. All subjects gave their informed consent and understood that they were free to withdraw from the experiment at any time.
2.2. Recording and stimulation

A pair of fine wire electrodes 100 µm in diameter (KS211-018; Unique Medical, Japan) was used to record the electromyographic (EMG) activity from the right dorsal neck muscles. The electrodes were inserted into the hair pouch about 3 mm deep and placed 15 mm apart. The electrode-position was vertically in the upper 1/4 part of the dorsal neck between the torus occipitalis and the seventh cervical spine, and horizontally about 2 cm to the right from the midline. To secure the electrode position, retroflex movement of the neck was performed, and stable and appropriate EMG recordings were observed. A reference surface electrode was attached to the right earlobe. The EMG signals were amplified, filtered with bandpass 10 Hz – 5 kHz (Neuropack four mini, Nihon Kohden, Japan), sampled at 2560 Hz, and stored from 50 ms before to 200 ms after the electrical stimulus by use of waveform analysis system (MacLab; ADInstruments, Pty Ltd) for further analysis. Forty sweeps of responses to the electric stimuli were evoked in each condition, i.e. before and after local anesthesia of the stimulus site.

A ball type electrode of 1.5 mm diameter (UL 3010-1; Unique Medical, Japan) was placed on the buccal gingiva of the right upper first premolar as a cathode, and a disc electrode of 8 mm diameter was placed on ipsilateral palatal gingiva as an anode. Both electrodes were fixed by dental resin reinforced with dental wire. That is, a resin frame with a clip-like shape, fixing the two electrodes was made on a die of the upper dental arch of each subject. Then, using the frame, the ball and disc electrodes were fixed to buccal and palatal gingiva, respectively. An electrical square-wave pulse (0.2 ms duration, 0.3 Hz) was delivered by a constant-current stimulator (Neuropack four mini, Nihon Kohden, Japan). To determine the intensity of the electrical stimulation, the sensory threshold (ST) to the electrical stimulation and the tolerance limit as the
upper limit of the intensity were determined for each subject. All stimuli were delivered below the tolerance limit of each subject. Basically, the EMG response was evoked with an intensity of 8 x ST, which was usually perceived as slightly to moderately painful. The perception level was scored in all subjects and the pain level in ten out of 17 subjects, using a 10 cm visual analog scale (VAS, 0 = no sensation, 10 = most pain imaginable) and (VAS, 0 = no pain, 10 = most pain imaginable). During the preliminary test recording, the lowest stimulus intensity needed to obtain a clear reflex response was determined for each subject. The intensity varied between 8 and 15.5 x ST. This stimulus intensity was always within the tolerance limit and used for each subject in the two conditions.

2.3. Experimental protocols

All subjects participated in 2 recording conditions (before and after local anesthesia). The two sessions were carried out during the same experimental day with a 20 min interval. At the start of the experiment, three maximum efforts of 3 sec each were performed to contract the dorsal neck muscles against resistance of the examiner’s hands put on the subject’s occipital region. The maximum voluntary contraction (MVC) using the rectified and integrated EMG was calculated as the maximum value of the 3 efforts. During recording of the responses to the electrical stimuli, the subjects were instructed to contract their dorsal neck with their eyes closed. The rectified and integrated EMG activity of the dorsal neck muscle was displayed on an on-line monitor, and the experimenter instructed the subjects to keep a constant EMG level around 15% MVC. The experimenter used the feedback signal to give the subject verbal instructions. A one-kg weight applied on the subject’s forehead by means of a headgear, made it easier for the
subjects to keep the contraction level of the dorsal neck muscles constant.

2.4. Anesthesia

Infiltration anesthesia around the roots of the right first premolar was performed with a maximum of 1.8 ml of local anesthetic (2% Xylocaine; epinephrine 1:80,000; Fujisawa-AstraZeneca Japan). Five minutes after the anesthesia, stimuli were delivered again. Before restarting the recordings, it was ensured that painful sensations evoked by poking the oral mucosa using a dental probe with a sharp tip around the anesthetized tooth had disappeared.

2.5. Data analysis

Fig. 1 shows a typical EMG responses and the data processing. Every reflex response was A/D converted at 2560 Hz using a waveform analysis system (MacLab; ADInstruments, Pty Ltd) from 50 ms before to 200 ms after the onset of electrical stimulus in order to measure the reflex response. The EMG signals of 40 responses were full-wave rectified and averaged. The integrated and averaged values (µV/ms) for every 10 ms from 10 to 100 ms after stimulation were calculated for each condition. The data from 0 to 10 ms post-stimulus were excluded because of the possibility of stimulus artifact. The integrated and averaged value (µV/ms) in the pre-stimulus interval (-50 to 0 ms) was also calculated. The EMG value for every 10 ms after stimulus was then normalized with respect to the pre-stimulus value. The abbreviation: “n-EMG_{a:b}” in the following text shows the normalized EMG value from “a ms” to “b ms” after the electrical stimulus.
2.6. Statistics

Statistical analyses were performed using IBM SPSS 21.0 software (IBM Corp USA). To analyze the time effect (10 levels) and the effect of anesthesia (2 levels: before and after anesthesia), a two-way repeated measures (RM) ANOVA was performed for the normalized EMG values and followed by post-hoc comparisons with Tukey tests. Paired t-test was used to compare the pre-stimulus EMG activity before and after anesthesia. Even though the number of subjects was low, the effects of gender were also tested. A two-way RM-ANOVA was performed separately for the two conditions that were before- and after-anesthesia. The level of significance was set at $p < 0.05$. Mean values ± SEM are given in the text and figures.

3. Results

3.1. Pre-stimulus EMG activity

The pre-stimulus EMG activities of $8.6 \pm 1.0 \mu V/ms$ (before anesthesia) and $7.6 \pm 0.7 \mu V/ms$ (after anesthesia) were not significantly different (paired t-test: $p > 0.05$). These values corresponded to $11.8 \pm 1.2 \%$ MVC (before anesthesia) and $10.3 \pm 0.9 \%$ MVC (after anesthesia).

3.2. Stimulus intensities

The electrical stimulus intensities used to evoke the dorsal neck muscle responses ranged between 4.8 and 14.4 mA (mean 9.1 mA ± 0.6 SEM). This intensity corresponded to $8 - 15.5 \times$ ST (mean 11.1 ST ± 0.6). The perception levels of the electrical stimuli as scored on the VAS were $6.1 \pm 0.4$ cm (before anesthesia) and $1.5 \pm 0.2$ (after anesthesia). The pain levels (ten
subjects) were 4.8 ± 0.4 cm (before anesthesia) and 0.2 ± 0.1 (after anesthesia) respectively.

3.3. Normalized EMG

In all subjects, the dorsal neck EMG activity decreased by an average of 80% compared to baseline, at a latency around 50 ms after the electrical stimulation before anesthesia, and this EMG inhibition disappeared or decreased after anesthesia of the intra-oral stimulus site. The typical responses from one subject are illustrated in Fig. 1. The interaction between anesthesia and time was significant ($p < 0.05$; Fig 2). The normalized EMG values from 30 to 60 ms after the electrical stimulation (n-EMG$_{30-40}$, n-EMG$_{40-50}$ and n-EMG$_{50-60}$) showed significantly lower values in the before-anesthesia condition compared to after-anesthesia condition ($p < 0.05$). The n-EMG$_{40-50}$ and n-EMG$_{50-60}$ before anesthesia showed a significant reduction compared to the pre-stimulus value ($p < 0.05$). By contrast, the n-EMG$_{70-80}$, n-EMG$_{80-90}$ and n-EMG$_{90-100}$ before anesthesia significantly increased compared to the pre-stimulus value ($p < 0.05$). The n-EMG$_{80-90}$ and n-EMG$_{90-100}$ before anesthesia showed significantly higher values compared to these of after the anesthesia ($p < 0.05$). The n-EMGs after anesthesia did not show any significant changes comparing with the pre-stimulus value ($p > 0.05$) (Fig.2). At the periods from 30 to 60 ms after the stimulation, fourteen out of seventeen subjects showed a reduction of the normalized EMGs to 85% or less of the pre-stimulus value before anesthesia. Such a reduction was still observed in three out of seventeen subjects after anesthesia. However, the degree of the reduction after local anesthesia decreased in all subjects comparing with before anesthesia. There were not any significant gender differences either before ($p > 0.05$) or after anesthesia ($p > 0.05$). Interaction between gender and time also did not show any gender
differences (before anesthesia: $p > 0.05$; after anesthesia: $p > 0.05$). However, the number of subjects was not enough to assess gender differences, therefore interpretation of these results should be made carefully.

4. Discussion

Intra-oral electrical stimulation inhibited the dorsal neck muscle activity. The neck muscle activity was reduced around 50 ms after the stimulation, followed by an increased activity for up to 100 ms after the stimulation. This modulation (inhibition / facilitation) was significantly diminished after local anesthesia of the stimulation site.

4.1. Methodological considerations

The exteroceptive suppression (ES) response in the jaw muscles has been extensively studied as a possible quantitative and qualitative tool for the diagnosis of pain syndromes (De Laat et al., 1998; Cruccu and Deuschl, 2000; Torisu et al., 2007, 2008; Türker, 2007). The ES response is a modulation of the masseter muscle activity induced by stimulation of exteroceptive receptors of the mental, supra- or infraorbital nerves. Moreover, such depressive responses of the masseter muscle activity can be induced by the stimulation of remote sites, i.e., the intra-oral electrical stimulation (Cadden and Newton, 1988). The purpose of the present study was to investigate the effect of the electrical stimulation of the intra-oral tissues around a tooth on neck muscle activity (i.e., exteroceptive receptors for the neck muscles), applying the stimulation and recording techniques previously used for the ES response. However, because visual information influences
the head posture control (Vacherot et al., 2007; Tardieu et al., 2009), all recordings were carried out with the eyes closed condition.

We should also consider whether the present findings are generalized responses or topographically specific. Cadden et al. (1989) demonstrated ES reflexes being evoked in jaw muscles by stimulation of remote areas of the body, i.e., they observed jaw muscle reflexes evoked by stimulation of spinally innervated areas, e.g., the arms and legs. Such findings have obvious parallels with the present study in the separation between the stimulated and responding neural segments. Therefore, the effects of spinal nerve stimulation on neck muscle activity should be investigated in the future.

4.2. Effects of intra-oral stimulation on the neck muscle activity

The relationship between the trigeminal system and the cervical system are frequently described as the “trigemino-cervical reflex” (Manni et al., 1975; Sumino et al., 1981; Lazzaro et al., 1995; Sartucci et al., 1986) which is a short latency excitatory pathway from low threshold sensory afferents in the trigeminal nerve to the neck motoneurons (Alstermark et al., 1992; Abrahams et al., 1993). The reflex response in the neck muscles has been evoked by stimulation of the facial skin, and is considered to be part of head withdrawal response (Abrahams et al., 1993; Lazzaro et al., 1995). In humans, the latency of the trigemino-cervical reflex is 13 -20 ms (Lazzaro et al., 1995; Quatrarone et al., 2000), and this response corresponds to an inhibition period in the neck muscle motor unit activity (Lazzaro et al., 1995). In the present study, however, stimuli to intra-oral tissues modulated the dorsal neck muscle activity in various ways. The response consisted of a reduction of the activity around 50 ms after the stimulus followed
by increased activity from 70 to 100 ms after the stimulus. In view of the different character, the responses observed in the present study may be considered different from the originally described “trigemino-cervical reflex”. On the other hand, previous studies on the trigemino-cervical reflex also reported excitatory responses with a latency of about 40 – 45 ms (Sartucci et al., 1986; Serrao et al., 2003) as well as reduction of the muscle activities (animal study: Manni et al., 1975; human study: Lazzaro et al., 1995). Details about the differences between these two responses should be studied in future studies, for example, including both extra- and intra-oral stimulation.

Fatigue might be involved with the present findings of EMG-reduction. It was reported previously that the ES response in the masseter muscle is enhanced by fatigue (Torisu et al., 2007), and therefore, the effect of fatigue was opposite to the present findings after anesthesia. Consequently, the decrease of the reduction after local anesthesia could not be attributed to fatigue. On the other hand, in the protocol of the present study, the “before-anesthesia session” always preceded the “after-anesthesia session”, therefore, a time or sequence effect can not be dismissed, e.g., habituation. Moreover, a placebo effect (Greene et al., 2009) could also be involved in the present findings. Future studies with both anesthesia and placebo injection including a randomized protocol with separate experimental days should be considered to investigate these issues.

4.3. Possible nerve fibers and receptor-types

The response observed in the present study was rather similar to the ES responses of jaw muscles (Yu et al., 1973; Cadden and Newton, 1988; Cruccu and Deuschl, 2000; Cruccu et al.,
2005). After local anesthesia of the stimulus site, a sensation of slight-tapping around the tooth still remained, while the painful sensation disappeared. Nevertheless, the reflex response was diminished. Moreover, diminished inhibitory responses were still observed after the local anesthesia in some subjects. These findings suggest that the present responses could be mainly attributed to activity in afferent nerves associated with painful input (maybe Aδ-fibers), however, Aβ fibers might also be responsible for these responses. From this point of view, the EMG responses in the present study are different from the trigemino-cervical reflex in which Aβ fibers for tactile sensation are thought to mainly contribute to the pathway, and for which it was suggested that a painful stimulation was not necessary (Lazzaro et al., 1995). From the above considerations we suggest that the pathway of the response observed in the present study is different from that of the trigemino-cervical reflex.

On the other hand, the ES response of jaw muscles consists of an early and a late phase of suppressions (ES1 and ES2, respectively). Several studies have pointed to the likelihood that both ES1 and ES2 can be mediated by Aβ and/or Aδ fibers (Cruccu et al., 1989; Cruccu and Deuschl, 2000). From a view of central pathways, the longer pathways are required for the reflex in the present study compared to the jaw reflexes. Therefore, the present reflex could conceivably be equivalent to the ES1, though the comparison with the present reflex and the ESs of the jaw muscles may be a dubious validity. At any rate the response is too fast to be considered equivalent to the ES2. Moreover, it is unlikely that C fibers could be implicated in the present responses as conduction velocity of C fibers is so slow.

Zeredo et al. (2002) demonstrated that unilateral pressure stimulation applied to the rat’s upper first molars elicited tonic discharges in the splenius muscles on both sides. They
suggested that input from the periodontal mechanoreceptors might play an important role in controlling the motor activity of neck muscles. Matsumoto et al. (1999) and Nishikawa et al. (2004) demonstrated that electrical stimulation of the tooth pulp increased the activity of C₁ spinal neurons whose receptive fields involve the face, neck and jaw. In the present study, electrical stimulation was applied around a premolar. Therefore, nociceptive and non-nociceptive receptors in the intra-oral mucous membrane, periodontal mechano- or nociceptive-receptors, and just possibly, tooth pulp afferents might be involved. The finding that the modulation of the neck muscle activity diminished after local anesthesia, eliminating the painful sensation, suggests that intra-oral nociceptive receptors would mainly contribute to the present reflex. On the other hand, when reduced afferent activity diminishes the magnitude of a reflex, there are at least two explanations: (i) that it is due to the reduced afferent input per se; or (ii) that it is due to the loss of a particular modality of afferent input. Investigations of endodontically treated teeth, edentulous subjects and/or subjects who have received dental implants would provide the condition under the reduced afferent input per se (e.g., dental pulp, periodontal ligament). Future studies, for example including the combination between these subjects and change in modality of afferent input (e.g., induced by local anesthesia) may be useful to clarify these questions about the possible nerve fibers and receptor types.

4.4. The EMG inhibition is followed by an excitatory period

In the present study, a reduction of neck muscle activity was followed by an excitatory period after the intra-oral stimulation. Tonic discharge of the neck motor unit in rats was demonstrated under steady and moderate-intensity pressure stimulation to the periodontal mechanoreceptors
(Zeredo et al., 2002). Also in the masseter muscles in humans, both inhibitory and excitatory responses were observed after mechanical periodontal stimulation (e.g. periodontal-masseteric reflex), and the patterns of this reflex depended on the waveform of the pressure stimulation (Türker et al., 1997). These authors demonstrated that slow rising pressure stimuli induced an excitatory reflex, while fast rising pressure stimuli induced an inhibitory response followed by excitatory response. In the present study, the electrical stimulation also had a “fast rising waveform” and also an inhibitory response followed by excitatory response was observed, similar to the periodontal-masseteric reflex evoked by a fast rising stimulus. However, to begin with, the basic concept of comparing the rise of an electrical stimulus to that of a mechanical one, might be invalid, since the former excites axons, the latter excites receptors, and the relevance of rise time is quite different in the two cases. Therefore, the source of the excitatory period should be studied further in the future studies.

Moreover, the response pattern after mechanical stimulation is a controversial topic. There is the alternative view that regardless of the rate of rise of a mechanical stimulus, inhibitory reflexes are always present (Louca et al., 1996). In the past, the “excitatory” response around 70-100 ms after the stimulation has been attributed to synchronization of motor unit activity (Yemm, 1972; Miles et al., 1987; Louca et al., 1996). In the present study, it is not clear whether the “increased” response is directly produced by the intra-oral stimulation, or that it is a secondary phenomenon induced by the inhibitory response (rebound effect). In this hypothesis, the EMG response would not be the result of a specific synaptic excitation, but rather the consequence of synchronization in the depolarization of the membrane induced by inhibitory response (Miles et al., 1987).
4.5. Relationship between stomatognathic- and head-neck systems

Functional or physiological significance of the coupling between the stomatognathic system and the head-neck system has been frequently reported (Hellström et al., 2000; Zeredo et al., 2002; Eriksson et al., 1998; Torisu et al., 2001 and 2002, Koolstra and Eijden, 2004). Dessem and Luo (1999) observed a neural projection from masticatory-muscle spindle afferents to the cervical spinal cord in rats by combining retrograde and intracellular neural labeling. They suggested that these pathways were involved in the coordination of jaw and neck movement during mastication and biting. The present results demonstrated that intra-oral stimulation around teeth modulates the neck muscle activity. This finding is in line with the report of Browne et al. (1993) that showed inhibition of the sternocleidomastoid (SCM) muscle activity evoked by electrical stimulation of the maxillary gingiva in healthy men. The present findings, therefore, demonstrate additional evidence for the coupling between the stomatognathic and the head-neck systems. Although the neural connection is evident, the exact implications for clinical manifestations of orofacial pain and neck-should problems are not clear.

4.6. Conclusion

In the present study, it is demonstrated that intra-oral nociceptive and non-nociceptive stimulations modulate neck muscle activity. From a clinical viewpoint, the present findings demonstrate the neural connectivity between the trigeminal region and the cervical region raising the possibility that orofacial pain conditions could influence head, neck and shoulder activity. Further studies will be needed to answer this question.
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Figure Legends

Figure 1. An example of the EMG recording from a dorsal neck muscle in one subject. Forty responses are superimposed. Top trace: the raw EMG record. Middle trace: full-wave rectified waveform. Bottom trace: averaged waveform of 40 responses. The left column illustrates the records before anesthesia, and the right column after anesthesia. The open triangle shows the electrical stimulus delivered at t = 0.

Figure 2. Effect of the interaction between anesthesia and time on the normalized EMGs (mean ± SEM). #p < 0.05: Significant difference between the before and after anesthesia conditions. *p < 0.05: Significantly different from pre-stimulus EMG values (Tukey tests).
Before anesthesia

Raw EMGs

Rectified EMGs

Averaged EMGs

After anesthesia

Peristimulus Time (ms)

Fig. 1
Fig. 2

Normalized EMG (%)
Peristimulus Time (ms)

- before
- after

Fig. 2