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## Muscle Spindles Provide Servo-assistance to Jaw-closing Muscles for Chewing Hard Foods

(Otot Gelendug Memberikan Servo-Bantuan kepada Otot Rahang-Tutup untuk Mengunyah Makanan Keras)

H.M. ZAKIR\*, J. KITAGAWA, A.R. FATHILAH & M.M. BAKRI

### ABSTRACT

*The fundamental pattern of chewing induced by the network of neurons called central pattern generator has been reported to be modified by the information arising from the various oro-facial sensory receptors including muscle spindles of jaw closing muscles. The cell bodies of primary afferent neurons from these muscle spindles lie in mesencephalic trigeminal nucleus (MTN) in the brainstem. The aim of the study was to understand whether muscle spindles from jaw-closing muscles play any role in hard food chewing. Single neuronal discharge of muscle spindle afferents was recorded from the MTN simultaneous with jaw-movement and electromyographic (EMG) activities of the left masseter (jaw-closing) muscle during chewing soft and hard foods (apple and pellet) in awake rabbits. Ten consecutive chewing cycles were taken for analysis. Discharge of nineteen muscle spindles from seven rabbits was successfully recorded. Muscle-spindle discharge was significantly higher during the closing phase of jaw-movement for the hard food chewing than for the soft food. The jaw-closing muscle EMG activity was significantly higher during hard food chewing compared to soft food. The spindle discharge was higher when the masseter muscle activity was greater for chewing hard food. Significant positive ( $r=0.822$ ,  $p<0.001$ ) correlation was found between the difference of muscle activity between apple and pellet and the difference of spindle discharge between apple and pellet. Above findings suggest that the increase of spindle discharge during hard food chewing may play a role for facilitating jaw-closing muscle activities and thereby provides servo-assistance to jaw-closing muscles to compensate the hardness of food.*

*Keywords: Hard food; jaw-closing muscle; muscle spindles; neuronal discharge*

### ABSTRAK

*Pola asas pengunyahan yang diarahkan oleh rangkaian neuron yang dikenali sebagai penjana pola pusat telah dilaporkan boleh mengalami pengubahsuaian daripada maklumat yang diperolehi daripada pelbagai deria orofasial termasuk gelendug otot pada otot penutupan rahang. Jasad sel neuron aferen utama daripada gelendug otot ini, terletak di dalam 'Mesencephalic Trigeminal Nucleus (MTN)' di dalam batang otak. Tujuan kajian ini adalah untuk memahami sama ada gelendug otot dari otot-tutup rahang memainkan sebarang peranan dalam proses mengunyah makanan keras. Pelepasan neuron tunggal oleh gelendug otot aferen telah direkodkan dari MTN serentak dengan pergerakan rahang dan aktiviti elektromiogram (EMG) bahagian kiri otot maseter (rahang-tutup) arnab sewaktu mengunyah makanan lembut dan keras (epal dan pellet). Dalam kajian ini, sepuluh kitar pengunyahan secara berturutan telah dipilih untuk dianalisis. Pelepasan 19 gelendug otot dari 7 ekor arnab telah berjaya direkodkan. Gelendug otot dilepaskan paling tinggi sewaktu fasa pergerakan rahang tutup untuk mengunyah makanan keras berbanding makanan lembut. Aktiviti EMG otot penutupan rahang adalah lebih tinggi dan signifikan semasa pengunyahan makanan keras berbanding dengan makanan lembut. Pelepasan gelendug otot adalah lebih tinggi semasa aktiviti otot maseter tinggi bagi jenis makanan keras. Keputusan yg diperolehi menunjukkan korelasi positif yang signifikan ( $r=0.822$ ,  $p<0.001$ ) bagi aktiviti otot dan perbezaan pelepasan gelendug antara epal dan pelet. Hasil kajian menunjukkan pelepasan gelendug sewaktu pengunyahan makanan keras mungkin memainkan peranan untuk membantu aktiviti otot rahang-tutup dan seterusnya menyediakan servo-bantuan kepada otot rahang-tutup untuk mengkompensasi makanan yang berjenis keras.*

*Kata kunci: Makanan keras; otot gelendug; otot rahang-tutup; pengeluaran neuron*

### INTRODUCTION

The fundamental pattern of chewing is induced by an assembled neuronal network termed as central pattern generator (CPG) (Lund 1991; Yamada et al. 2005). The output of this CPG has been found to be modified by the input from oro-facial sensory receptors including muscle spindles (Lund & Kolta 2006; Yamada et al. 2005). Muscle

spindles are the sensory receptors widely known as stretch receptor which detects the stretch of the muscle (Dessem & Taylor 1989). They have been observed to be activated during chewing (Taylor et al. 1981; Zakir et al. 2010).

Every day we chew different hardness of foods. Jaw closing muscles work hard to compensate the load on jaws (Agrawal et al. 1998). During processing these

foods, continuous changes of sensory information from sensory receptors modify the jaw movement and jaw-closing muscle activities (Agrawal et al. 1998; Hiiemae et al. 1996). Muscle spindles of jaw closing muscles are reported to provide hardness related sensory information (Hidaka et al. 1999). In most of the previous studies muscle spindle neuronal discharge was recorded when animal was chewing one kind of food or non-reducible test objects or lapping of liquid (Hidaka et al. 1999; Masuda et al. 1997; Taylor et al. 1981). However, natural foods are reducible and during natural chewing, the volume, hardness and size of the food particles vary between successive chewing cycles when the food is being triturated and hence food resistance varies from cycle to cycle (Agrawal et al. 1998; Hiiemae et al. 1996; Horio & Kawamura 1989). Besides that anaesthesia have been reported to altered spindle discharge. Therefore, recording of neuronal discharge from muscle spindle afferents during natural chewing of different hardness of edible foods is necessary to understand the role of muscle spindles during hard food chewing. In our recent study we have shown that spindle discharge is modulated during natural chewing of different hardness of foods made of gelatin in rabbits (Zakir et al. 2010). Gelatin made food has a gummy consistency and is not an ordinary food for animals. In this study, we use apple and pellet as soft and hard test foods, respectively, which are more natural for rabbits.

The objective of our study was to understand the role of muscle spindles of jaw-closing muscles during natural chewing of hard foods.

#### MATERIALS AND METHODS

This study involves seven male rabbits (Japanese white, 2-3 kg). The study protocol was reviewed and approved by the Niigata University Intramural Animal Care and the Veterinary Science Committee. All measures were taken to reduce suffering of animals during the experiment. The details experimental procedure was narrated in previous published study (Zakir et al. 2010). In brief, the animal was trained to take test foods. Apple and pellet were used as test foods. They were prepared as same size and shape (cylindrical shape, 15 mm long and 3.5 mm in diameter). The test foods were delivered to the rabbits using a custom made syringe and piston. Spindle discharge was recorded for both test foods from the same units of muscle spindle. The test foods were delivered at 5 min interval so that the spindle discharge can be recorded for both test foods from the same units. Surgeries were performed using proper aseptic precautions to implant head cap so that animal head was able to be fixed stereotaxically to the apparatus (Semi-Chronic Head Holder, SA-8, Narishige, Tokyo, Japan) during recording. Craniotomy was carried out and a metal chamber was fixed with acrylic to cover the exposed area. Teflon-coated stainless-steel wire electrodes were implanted on the left masseter muscle to record EMG. During surgery, the animal was anesthetized with sodium pentobarbital (initial dose: 35-40 mg/kg) administered

through the marginal ear vein. A supplemental dose of the same drug was administered to maintain the depth of the anesthesia at such a level that the withdrawal reflex was not evoked by paw pinching. Lidocaine (2%) was injected into the skin to minimize surgical pain before the incisions were made. A magnetic jaw-tracking device was implanted to record jaw movement (Yamada et al. 1988). Animal was allowed to be recovered after surgery. Surgical dressing was applied on the incision area during recovery period. Penicillin G potassium (20000 units, i.m.; Eli Lilly, Indianapolis, IN, USA) was injected intramuscularly after surgery to prevent infection. Aseptic dressing was applied on the craniotomy area every day and the rabbit remained healthy during all experimental sessions. Single unit neuronal discharge was recorded from left MTN with custom made glass coated elgiloy microelectrode (0.3-0.8 M  $\Omega$  at 1 kHz). It was driven by a micromanipulator at an angle of 30° rostral to the vertical through the exposed area to reach MTN. The single neuronal discharge was identified as muscle spindle afferents on the basis of the following criteria: The units showed discharges in relationship with small passive jaw opening movements but did not respond to the tactile stimulation of the lips, nose, anterior teeth, vibrissae, nor skin on the face and the peak/maximum instantaneous frequency of the unit discharge during chewing was >100 Hz. In addition, gentle probing was done to the left masseter muscle to identify a muscle origin of neuronal discharge (Zakir et al. 2010).

The unit discharge and EMG activities were amplified and the signals were digitized with the Cambridge Electronics power 1401 data acquisition system and Spike2 analysis package (Cambridge Electronic Design Ltd., Cambridge, UK). The data was stored in the computer memory for future analysis by Spike2 analysis package. A chewing cycle was divided into the closing (CL) and opening (OP) phases depending on the vertical jaw movements. Ten consecutive chewing cycles from the early rhythmic chewing period were taken for analysis. The mean frequency of the neuronal discharge for each phase and the area of rectified EMG of left masseter muscle were calculated for apple and pellet. Statistical analysis was performed with paired *t*-test to compare neuronal discharges and EMG activities between apple and pellet. Pearson correlation test was carried out to see relation between the difference of muscle activity between apple and pellet and the difference of spindle discharge between apple and pellet.

Electrical lesions were made with anodal current (10  $\mu$ A for 10 s) at 1-2 locations where units were recorded before sacrificing the animal. Sections of brainstem (sagittal sections at 50 mm) were done and stained with cresyl violet to identify the lesion sites. All lesion sites were in the MTN.

#### RESULTS

Neuronal discharge of nineteen muscle spindle units were successfully recorded and incorporated for analysis. The range of the peak/maximum instantaneous frequency of

unit discharge was variable (165-519 Hz). Fifteen out of nineteen units were responding to gentle probing of left masseter muscle indicating they are originating from masseter muscle.

Figure 1 shows an example of a muscle spindle unit discharge during chewing apple and pellet. The unit showed discharge during closing and opening phase for both apple and pellet chewing. During closing phase discharge was higher for pellet chewing.

The mean frequency of discharge for ten consecutive cycles during chewing was calculated for all the units (Figure 2(a)). Spindle unit discharge was significantly higher for pellet than for apple ( $p < 0.001$ ) in the jaw closing (CL) phase when masseter muscle was active. Masseter muscle activity was higher during pellet chewing than during apple chewing (Figure 2(b) ( $p < 0.001$ )). Pearson correlation test showed a significant positive correlation ( $r = 0.822$ ,  $p = < 0.001$ ) between the difference of muscle activity between apple and pellet and the difference of spindle discharge between apple and pellet (Figure 2(c)).

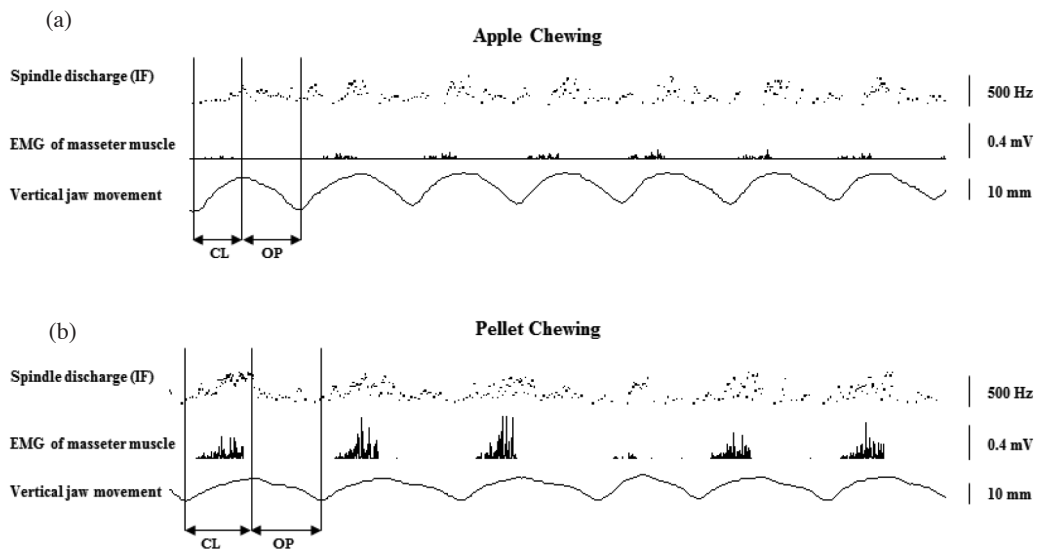
#### DISCUSSION

In the present study neuronal discharge from muscle spindle afferents was correlated with jaw movements and jaw-closing muscle activities during natural chewing of soft (apple) and hard (pellet) foods. It was observed that spindle discharge increased during hard food chewing. Increased spindle discharge was significantly correlated with increased jaw-closing muscle activities when compared between soft and hard food.

Increased spindle discharge along with increased jaw-closing muscle activities in hard food chewing suggests

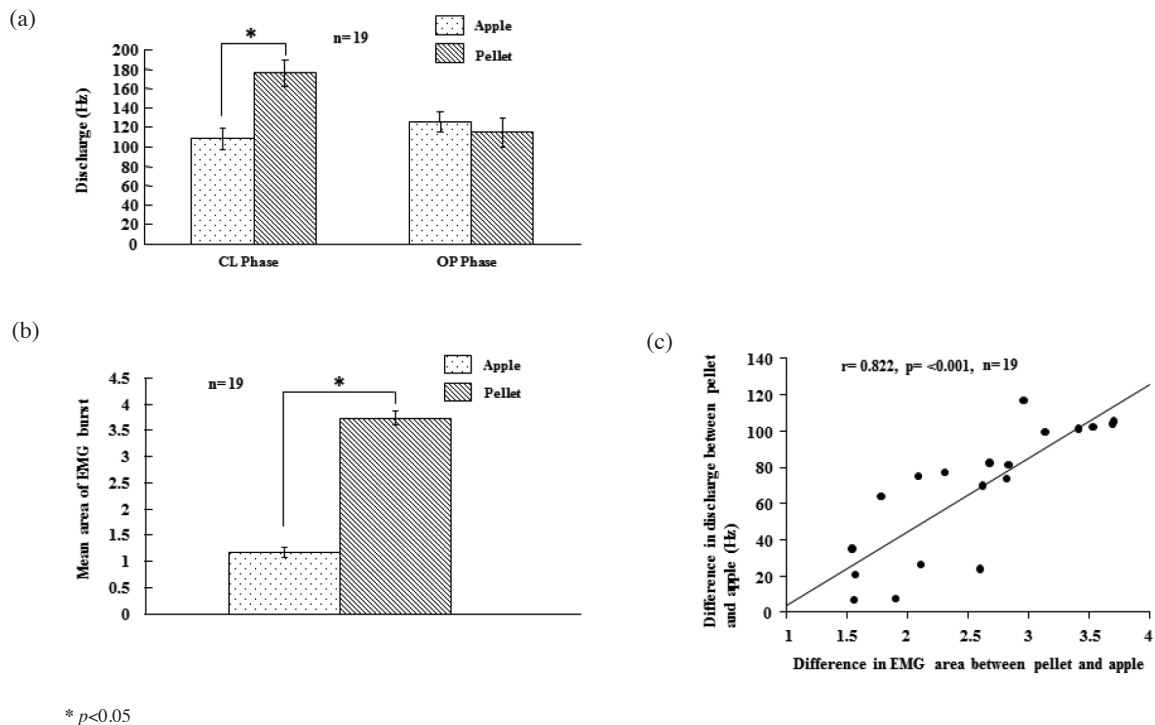
that muscle spindles play a role to increase EMG activity to compensate the load with food. The role of muscle spindle to increase jaw-closing muscle activity was also evident in anesthetized rabbit during rhythmic chewing movement induced by electrical stimulation in the cerebral cortex (Hidaka et al. 1999). Muscle spindle discharge recorded in awake monkey (Matsunami & Kubota 1972) and freely eating rat (Yamamoto et al. 1989) also supports this view. In addition, connection of axon collaterals from muscle spindle afferents to the trigeminal motor nucleus observed in previous study (Appenteng et al. 1978) suggests that spindle discharge influences the trigeminal motor neuronal activity. Existence of synaptic connection from jaw-closing muscle spindle afferents to trigeminal motoneurons has also been demonstrated in electrophysiological study (Nozaki et al. 1985). These previous studies including ours indicate that inputs from the muscle spindles facilitate the motoneurons of jaw-closing muscles during chewing.

Significant positive correlation of increased spindle discharge with increased muscle activity in hard food chewing also suggests the activation of fusimotor ( $\gamma$ -motor neuron) neurons along with ( $\alpha$ ) alpha motor neurons, i.e., underlying  $\alpha$ - $\gamma$  co-activation. In a previous study, where fusimotor neurons were directly recorded in anesthetized animals, it has been found that one subset of fusimotor neuron (presumably static) was activated during reflex closing of the jaw when the corresponding muscle was contracting (Gottlieb et al. 1983). Fusimotor neuron activation in closing phase prevents the unloading of the afferent units when muscle is being shortened (Appenteng et al. 1980; Gottlieb et al. 1983). Along with previous studies our findings also suggest that during natural chewing of hard food, activation of fusimotor neuron is



IF: Instantaneous frequency; CL: Closing of the jaw; OP: Opening of the jaw

FIGURE 1. Spindle afferent discharge recorded from the left mesencephalic trigeminal nucleus simultaneous with vertical jaw movement and electromyographic (EMG) activities of the left masseter (jaw-closing) muscle during chewing apple (a) and pellet (b) in awake rabbits



\*  $p < 0.05$

FIGURE 2. (a) Spindle discharge was significantly higher during pellet chewing, (b) Electromyographic (EMG) activities of the left masseter (jaw-closing) muscle was also significantly higher during pellet chewing and (c) Significant positive correlation was found between the difference of muscle activity between apple and pellet and the difference of spindle discharge between apple and pellet

powerful to prevent unloading of the spindle units during contraction of the corresponding muscles. Activation of fusimotor neuron along with alpha motor neuron increases the muscle spindle discharge during closing phase.

#### CONCLUSION

In conclusion, the increase of muscle spindle discharge with increased muscle activity in hard food chewing suggests that muscle spindles may provide servo-assistance to jaw-closing muscles for chewing hard foods.

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- H.M. Zakir\*, A.R. Fathilah & M.M. Bakri  
Dept. of Oral Biology & Biomedical Sciences  
Faculty of Dentistry, University of Malaya  
50603 Kuala Lumpur  
Malaysia
- J. Kitagawa  
Division of Oral Physiology, Dept. of Oral Biological Science  
Graduate Sch. of Med. and Dent. Sci.  
Niigata University  
Japan
- \*Corresponding author; email: zakir@um.edu.my
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