Illusion caused by vibration of muscle spindles reveals an involvement of muscle spindle inputs in regulating isometric contraction of masseter muscles

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ABSTRACT

Spindle Ia afferents may be differentially involved in voluntary isometric contraction, depending on the pattern of synaptic connections in spindle reflex pathways. We investigated how isometric contraction of masseter muscles is regulated through the activity of their muscle spindles that contain the largest number of intrafusal fibres among skeletal muscle spindles, by examining the effects of vibration of muscle spindles on the voluntary isometric contraction. Subjects were instructed to hold the jaw at resting position by counteracting ramp loads applied on lower molar teeth. In response to the increasing-ramp load, the root mean square (RMS) of masseter EMG activity almost linearly increased under no vibration, while displaying a steep linear increase followed by a slower increase under vibration. The regression line of the Load-RMS relationship was significantly steeper under vibration than under no vibration, suggesting that the subjects overestimated the ramp load and excessively counteracted it as reflected in the emergence of bite pressure. In response to the decreasing-ramp load applied following the increasing one, the RMS hardly decreased under vibration unlike under no vibration, leading to a generation of bite pressure even after the offset of the negative-ramp load until the vibration was ceased. Thus, the subjects overestimated the increasing rate of the load while underestimating the decreasing rate of the load, due to the vibration-induced illusion of jaw opening. These observations suggest that spindle Ia/II inputs play crucial roles both in estimating the load and in controlling the isometric contraction of masseter muscles in the jaw-closed position.

Keywords: EMG activity; muscle spindle; jaw-closing movement
INTRODUCTION

Spindle Ia afferents are shown to be highly active during the voluntary isometric contraction of monkey jaw-closing muscles (Lund et al. 1979) as well as during that of human lumbral muscles (Vallbo 1970, 1971), due to the co-activation of α- and γ-motoneurons (α-MN and γ-MN, respectively). By using local anesthesia, it has been found that during voluntary isometric contractions of leg and hand muscles, the contribution of spindle Ia inputs to the activation of α-MNs is about 30–40% (Gandevia et al. 1990; Macefield et al. 1993).

Isometric contraction of the jaw-closing muscles occurs transiently during the slow-closing phase of masticatory movement induced by electrical stimulation of the motor cortex in anesthetized rabbits (Lavigne et al. 1987; Morimoto et al. 1989). With the progress of the slow-closing phase, the EMG activity of jaw-closing muscles increases (Lund and Olsson 1983). Such increases in the masseter EMG activity and concomitant increases in spindle Ia activities were enhanced depending on the hardness of chewing test strips applied during the artificially induced slow-closing phase in anesthetized rabbits (Hidaka et al. 1997, 1999). This observation suggests the presence of a fusimotor drive that is activated depending on the hardness of chewing strips. Furthermore, the hardness was found to be sensed primarily by muscle spindles, and their resulting activities were likely to be fed back to activate γ-MNs presumably through the cerebellum as demonstrated by lesion experiments (Komuro et al. 1999, 2001) in addition to the direct feedback to α-MNs. When performing isometric contraction of jaw-closing muscles against linearly increasing load, the rate of load increase may correspond to the hardness of the chewing strip applied during the slow-closing phase. Then, it can be hypothesized that the activity of spindle primary endings is involved in both...
the estimation of load changes and the execution of the voluntary isometric contraction.

This possibility can be investigated in human subjects by artificially altering the activity of spindle endings when performing the voluntary isometric muscle contraction. Mechanical vibration applied to muscle tendons elicits kinesthetic illusions as if the muscle were stretched (Eklund 1972; Goodwin et al. 1972). Initially, spindle primary endings were considered to be chiefly responsible for such illusions based on the difference in sensitivity to vibration among spindle primary and secondary endings and Golgi tendon organs (Brown et al. 1967; Cordo et al. 1993). However, a recent study revealed no difference in the sensitivity among the three receptors in active muscles while responses from Ia/II afferents were much stronger than those from Ib afferents (Fallon and Macefield 2007), suggesting that spindle primary and secondary endings are largely responsible for the illusion.

In the present study, effects of vibration of masseter muscles on the isometric contraction were investigated to clarify how the activity of muscle spindles is involved in the regulation of the voluntary isometric contraction of masseter muscles. We demonstrate that the activity of spindle afferents plays a crucial role in regulating the isometric contraction of masseter muscles in the jaw-closed position, presumably through the calibration between the activity of spindle afferents and that of periodontal mechanoreceptor afferents.
METHODS

Subjects. Experiments were performed on 21 subjects (16 males and 5 females; 27.0 ± 1.2 year-old), without any history of neuromuscular disorder or injury to their jaws. Each participant had a normal occlusion and was fully dentate. The study was conducted in accordance with the Helsinki Declaration and was approved by the ethics committee at Osaka University Graduate School of Dentistry. Each subject gave an informed consent prior to involvement in the study.

Custom-designed testing apparatus and the instruction to subjects. A custom-designed testing apparatus was built to measure EMG activity induced during isometric contraction of jaw muscles to counteract a ramp load (Fig. 1A). The motor (1 in Fig. 1A) with worm reduction gear was connected to the lower bite plate (2 in Fig. 1A) through a wire and spring (20 N/cm). Using the motor, the spring was stretched to apply linearly increasing or decreasing ramp load (positive- and negative-ramp load; 100 N/3.8 sec) to subject’s jaw through the lower bite plate. The height of the upper bite plate was adjusted to contact with the upper 1st molars of subjects seated in the upright position, and the lower bite plate was placed on the lower 1st molars of the jaw in the resting position. Subjects were instructed to “hold the jaw in the resting position by adjusting the jaw-closing force just sufficient to counteract the load that increases or decreases in a ramp-like manner”, so that the lower bite plate was kept in contact with the upper bite plate with a minimal contacting force (< 2 N). The subjects were read this instruction before each experiment. With a pressure gauge that was placed between the upper and lower bite plates (3 in Fig. 1A), the contacting force was measured. As soon as the jaw-closing force exceeds that required to counteract the load, it can be detected as the bite pressure (> 2 N).
Experimental setup. Displacement of jaw position was monitored with a CCD laser sensor (LK-500 with LK-2500, Keyence Japan, Osaka, Japan; $d$ in Fig. 1A), and the load amplitude was measured by a load cell (LTZ-50KA, Kyowa Electronic Instruments, Tokyo, Japan; $5$ in Fig. 1A). Muscle activity was recorded by using surface EMG ($6$ in Fig. 1A). The electrodes were placed at electrode intervals of 20 mm on the middle portions of the bilateral masseter muscles and in some experiments on those of digastric muscles. The reference electrode was placed on the oral angle. EMG signals were recorded by an AC amplifier with 0.03 sec time constant (AB-621G, Nihon Kohden Co., Tokyo, Japan). The voltage signals from the CCD laser displacement sensor and EMG were recorded simultaneously at a sampling frequency of 2000 Hz through an A/D converter (PCD320A, Kyowa) and stored on a personal computer. The signals of the electric current from the pressure gauge and load cell were simultaneously recorded through an A/D converter (PCD300A, Kyowa).

Vibration of masseter muscles. In this study, we applied the 80 Hz vibration with 1.0 mm amplitude to superficial masseter tendon (5 mm below zygomatic arch) on both sides with vibrators (Vibro Pet PET-01; IMV Corp., Osaka, Japan; $7$ in Fig. 1A) to activate sensory endings of muscle spindles without tonic vibration reflex (Godaux and Desmedt 1975). The contact pressure was controlled to be less than 200 gram not to cause unpleasantness (Williams and LaPointe 1987).

Experimental protocols. Experiment 1 was performed with or without vibration that was applied 5 sec before the ramp load and was maintained until 4 sec after the onset of the hold load (ramp-and-hold load; Fig. 1Ba). In experiment 2, they were instructed to do the same, while
counteracting the positive- and negative-ramp loads repeated twice (Fig. 1Bb). Vibration was applied 2 sec before the series of ramp loads and was maintained until 2 sec after the offset of the load. Each protocol with or without vibration was attempted 6 times randomly. Twenty-one subjects (16 males and 5 females) participated in the experiment 1, and 8 subjects (6 males and 2 females) participated in the experiment 2.

Data collection and analysis. To compare the EMG activity between subjects or trials, the root mean square (RMS) values of the EMG signals were assessed with a time window of 0.25–0.50 sec, and were plotted against the mid time point of the moving time window. For pooled data analysis except Figs. 8C and 10, EMG RMS values were normalized (NormRMS) to those exerted at a load of 90 N during the 1st positive-ramp in the absence of vibration in each subject.

The Load-RMS relationship was examined for 10–90 N to evaluate the influence of the vibration of muscle spindles on the performance of isometric contraction. Since the stretch reflex occasionally occurred at the onset of the ramp load application (100 N/3.8 sec), the initial response to the ramp load less than 10 N (< 380 ms; arrows in Figs. 3 and 6; Poliatkov and Miles 1994) was not included in this analysis. The piecewise two linear regression analysis was performed as described earlier (Vieth 1989) to approximate the Load-RMS relationship, by using Excel (Microsoft Japan, Tokyo, Japan). The averaged RMS values of EMG were plotted against the load values (Load) ranging 10 and 90 N (bin width, 1 N). The relationship between the Load and RMS values can be described by the two linear regression lines as defined below:

\[
\begin{align*}
\text{RMS} &= a_1 + b_1 \cdot \text{Load}, \quad \text{Load} \leq c \\
\text{RMS} &= a_2 + b_2 \cdot \text{Load}, \quad \text{Load} > c
\end{align*}
\]
where $c$ gives a Load value at the intersection of the two regression lines, $a_1$ and $a_2$ are y-intercepts, and $b_1$ and $b_2$ are coefficients, of the 1st and 2nd regression lines, respectively. The breaking point $(c, a + b \cdot c)$ is on the Load-RMS curve (two-segment “hockey stick” regression). These parameters were estimated by the least-squares method. The sum of the residual sum of squares (SRSS) of each regression line is given as below:

$$SRSS = \sum_{Load \leq c} \{RMS - (a_1 + b_1 \cdot Load)\}^2 + \sum_{Load > c} \{RMS - (a_2 + b_2 \cdot Load)\}^2$$

The optimal values of $a_1$, $b_1$, $a_2$, $b_2$, and $c$ were obtained by finding the best fitting two lines that gives the minimal SRSS.

Numerical data are expressed as the mean ± SD. Statistical significance was assessed using paired Student’s $t$-test between two groups and single Pearson product-moment correlation coefficient. $p < 0.05$ was considered statistically significant.
RESULTS

Illusion of jaw opening. We first examined whether or not the vibration causes an illusion as if the masseter muscles were stretched, i.e., whether or not the subjects felt as if their jaws were open without any actual downward displacement of their jaws. When the vibration was applied to the both sides of the masseter muscles in the resting state, the subjects definitely felt that their jaws were unintendedly or involuntarily open during the vibration, as they reported so after the experiments. However, there was neither jaw opening as monitored with a CCD laser sensor nor any appreciable EMG activity of the digastric muscle, a jaw opener, during the period of vibration (Fig. 2A). Right after this experiment, the degree of the illusory sensation was subjectively evaluated by asking the subjects to open their jaws to the same degree as that they felt during the vibration (left side, Fig. 2B). The amount of illusory jaw opening was 5.1 ± 1.3 mm (n = 11).

Then, the subjects were asked to perform submaximal jaw opening to confirm that the EMG activity of the digastric muscles is properly monitored (right side, Fig. 2B). We next examined whether or not the antagonistic digastric muscles are involved in the performance of isometric contraction of masseter muscles to counteract the positive- and negative-ramp loads together with the masseter muscles in the absence of vibration. The subject counteracted the positive- and negative-ramp loads by activating and deactivating the masseter muscles with a very small tonic activity of the digastric muscles, as revealed by their EMG activities together with no apparent downward displacement of the jaw position (Fig. 2C). Such a small constant tonic activity of the digastric muscles may have served to stiffen the jaw during the ramp load, as the subjects were asked to counteract the load with minimal jaw-closing force, to keep the lower bite plate in contact with the
upper bite plate with a minimal contacting force. Thus, neither loading nor unloading caused any
marked activities of the digastric muscles. Therefore, the EMG recordings from digastric muscles
were not presented hereafter.

Effects of vibration of muscle spindles on isometric contraction generated in response to

the ramp-and-hold load. In response to the ramp-and-hold load applied in the absence of vibration
(Vib⁻), the subject efficiently performed isometric contraction of masseter muscles to keep the jaw at
the resting position as reflected in smooth increases in the right and left masseter EMG activities
without apparent downward displacement of the jaw position (Fig. 3A). However, when the ramp
load was applied during the vibration of the both sides of the masseter muscles (Vib⁺), masseter
EMGs increased more than those seen under Vib⁻, as reflected in the abrupt emergence of an
apparent bite pressure, indicating that masseter muscle was excessively activated to counteract the
downward jaw displacement (Fig. 3B). Regardless of under Vib⁻ or Vib⁺, there was little activity in
digastric muscles in response to the ramp load. To analyze the effects of vibration quantitatively,
the relationship between the amplitude of load (Load) and the RMS of masseter EMG activity was
examined under Vib⁻ and Vib⁺.

Under Vib⁻, masseter RMS increased almost linearly with the ramp load (black curve, Fig.
4A). In contrast, under Vib⁺, RMS displayed an initial steeper increase, which was followed by a
much slower 2nd increase (gray curve, Fig 4A). Such Load-RMS relationships could be
approximated with the two regression lines obtained by the piecewise least-squares method (dotted
and interrupted lines, Fig. 4A). Under Vib⁻, there was no marked difference in the slope, i.e., the
regression coefficient between the two regression lines as if composing of two segments of a single
line (black dotted and interrupted lines, Fig. 4A). In contrast, under Vib+, there was a marked difference in the regression coefficient between the two regression lines, as in an inflected line (gray dotted and interrupted lines, Fig. 4A). The 1st regression coefficient was much larger under Vib+ (gray dotted line) than under Vib− (black dotted line), whereas the 2nd regression coefficient was vice versa (gray and black interrupted lines) (Fig. 4A). The difference between the EMG RMS (ΔRMS; dotted curve) in response to the ramp load under Vib− and that under Vib+ was compared with the bite pressure (solid curve) generated in response to the ramp load under Vib+ (Fig. 4B).

Since any bite pressure was not generated by the ramp load under Vib−, the generated bite pressure can be attributed to the ΔRMS. Although the horizontal axis represents the load amplitude (lower axis, Fig. 4B), it rather represents time (100 N/3.8 sec; upper axis, Fig. 4B) when comparison is made between the ΔRMS and the bite pressure. It is natural that there was a time delay between the ΔRMS and the generation of bite pressure.

Analysis of the pooled data obtained from the 21 subjects (Fig. 5A) revealed that the 1st regression coefficient was significantly (p < 0.001) larger under Vib+ (0.023 ± 0.006 N−1) than under Vib− (0.014 ± 0.005 N−1). This indicates that the vibration caused an overestimation of the linearly increasing load, which resulted in the excessive isometric contraction as reflected in the emergence of bite pressure (Fig. 3B). A Load value at the intersection of the two regression lines was 48 ± 17 N under Vib− and 47 ± 11 N under Vib+. There was no significant difference between these values. The ratio of the 2nd regression coefficient to the 1st one was calculated as a measure of the angle between the 1st and 2nd regression lines, which becomes larger (≤ 1) as the overall linearity in the Load-RMS relationship increases. The coefficient ratio was significantly (p < 0.001) larger under
Vib⁻ (0.54 ± 0.24) than under Vib⁺ (0.14 ± 0.16) (Fig. 5B). These results indicate that under Vib⁺, the excessive counteraction due to the overestimation of the rate of rise of the load was partly alleviated by perceiving the excessive bite pressure through the periodontal mechanoreceptors (PMRs) (see the instruction given to subjects in Methods). Therefore, it is suggested that the activity of spindle endings is involved both in estimation of the linearly increasing load and in performing the isometric contraction.

**Effects of vibration on isometric contractions generated in response to positive- and negative-ramp loads.** In response to the positive- and negative-ramp loads successively repeated twice, masseter EMGs smoothly increased and decreased under Vib⁻ (Fig. 6A). Since neither an apparent downward displacement of the jaw position nor an apparent bite pressure was induced (Fig. 6A), the subject efficiently performed isometric contraction of masseter muscles. In contrast, when the positive- and negative-ramp loads were applied under Vib⁺, masseter EMG activity increased much more than those seen under Vib⁻ (Fig. 6B). Excessive masseter EMG responses and subsequent emergence of bite pressure in response to the positive-ramp load were almost similar to the case shown in Fig. 3B. Surprisingly, this excessive activation did not decrease in response to the negative-ramp load and remained active in spite of terminating load application (filled arrowheads) until the offset of vibration (Fig. 6B). This resulted in generations of a marked bite pressure around the valley between the negative- and positive-ramp loads and before and after the offset of the 2nd negative-ramp load (asterisks, Fig. 6B).

As illustrated in Fig. 7Aa, the Load-RMS relationships obtained during the 1st positive-ramp load under Vib⁻ and Vib⁺ were similar to those seen in Fig. 4A. The vibration markedly increased
the 1st regression coefficient (compare black and gray dotted lines) whereas it decreased the 2nd regression coefficient (compare black and gray interrupted lines) (Fig. 7Aa). In contrast, the Load-RMS relationship obtained during the 1st negative-ramp load under Vib− was quite distinct from that under Vib+, and also distinct from that obtained during the positive-ramp load under Vib+: the RMS of EMG activity did not decrease under Vib+ (gray curve) while decreasing under Vib− (black curve) (Fig. 7Ab). Consequently, the 1st regression coefficient (see Methods) was invariably larger under Vib− than under Vib+. Similar to Fig. 4B, there was a time delay between the ΔRMS and the generation of bite pressure in response to the positive-ramp load (Fig. 7Ba) whereas there was little delay in generating larger bite pressures in response to the negative-ramp load (Fig. 7Bb). This discrepancy between the presence and absence of delay is largely attributable to the calculation method of RMS (time window; see Methods). RMS would precede or follow the real EMG activity depending on whether the EMG activity increases or decreases.

**Overestimation and Underestimation of the Load Amplitude Changes.** Analyses of pooled data obtained from 8 subjects revealed that, during the 1st positive-ramp load, the 1st regression coefficient was significantly \((p < 0.003)\) larger under Vib+ \((0.019 \pm 0.004 \text{ N}^{-1})\) than under Vib− \((0.012 \pm 0.002 \text{ N}^{-1})\) (Fig. 8Aa), whereas the 1st regression coefficient was significantly \((p < 0.002)\) larger under Vib− \((0.014 \pm 0.002 \text{ N}^{-1})\) than under Vib+ \((0.006 \pm 0.004 \text{ N}^{-1})\) during the 1st negative-ramp load (Fig. 8Ba). The ratio of the 2nd regression coefficient to the 1st one obtained from the Load-RMS curves during the positive-ramp load was significantly \((p < 0.003)\) larger under Vib− \((0.65 \pm 0.21)\) than under Vib+ \((0.20 \pm 0.18)\) (Fig. 8Ab). These results indicate that under Vib+, the subjects overestimated the rate of rise of the load while underestimating the rate of decrease of
the load, suggesting a critical involvement of spindle activities in estimating the load. Such an
overestimation and underestimation are not incompatible but can be reconciled by considering the
illusion caused by vibration (see Discussion).

The NormRMS value at zero load represented as the value of y-intercept of the 1st
regression line during the 1st negative-ramp load was significantly \( (p < 0.001) \) larger under Vib\(^+\) (0.55 ± 0.23) than under Vib\(^-\) (0.13 ± 0.13) (Fig. 8Bb). There must be a linear relationship between
the RMS value of y-intercept and the emerged bite pressure (Fig. 7Bb).

**Illusion of muscle spindles causes illusion of mechanoreceptors.** Before applying the
ramp loads, there was no significant difference \( (p > 0.1, n = 8) \) between RMS under Vib\(^+\) (0.073 ± 0.034 μV) and that under Vib\(^-\) (0.050 ± 0.019 μV) (Fig. 8C), indicating that the vibration itself did
not induce significant additional EMG activities. There was also no significant \( (p > 0.5, n = 8) \)
difference between the RMS obtained before (0.050 ± 0.019 μV) and after load (0.056 ± 0.020 μV)
under Vib\(^-\) (Fig. 8C). However, under Vib\(^+\), the RMS obtained after the offset of the 2nd
negative-ramp load (0.38 ± 0.20 μV) displayed significantly \( (p < 0.002, n = 8) \) higher values than
those obtained before the load (0.073 ± 0.034 μV) (Fig. 8C). Thus, due to the high EMG activity, a
marked bite pressure remained even after the offset of the 2nd negative-ramp load until the vibration
was ceased. This is in contrast to the case seen in response to the ramp-and-hold load under Vib\(^+\),
where the excessive bite pressure might have been alleviated presumably through the perception of
bite force by the activity of PMRs. These notions suggest that proprioceptive illusion induced by
vibration causes illusory sensation of bite force when unloading the load (Fig. 9B; see Discussion).
DISCUSSION

Involvement of the spindle activity in the isometric contraction of masseter muscles. In the present study, we addressed the question whether or not the activation of the spindle reflex pathway is involved in the voluntary isometric contraction of jaw-closing muscles. At first, it should be noted that the spindle reflex circuit is quite different between large limb muscles and jaw-closing masseter muscles. In human, the number of intrafusal fibres included in single muscle spindle of masseter muscles was found to be extremely large, up to 36 (Eriksson et al. 1994), which is larger than those of any other muscles. However, the number of synapses made between a single spindle Ia afferent and an α-MN innervating masseter muscles is much smaller (rat, Dessem et al. 1997; cat, Yabuta et al. 1996) than that for limb muscles (cat, Redman and Walmsley 1983a, b).

Thus, it is likely that in limb muscles the spatial summation of Ia-EPSPs would easily activate α-MNs, while in masseter muscles the temporal summation of Ia-EPSPs would be required to activate α-MNs, due to the insufficient spatial summation of Ia-EPSPs as reflected in the difficulty for single electrical stimulation to evoke H-reflex in resting masseter muscles (Fujii and Mitani 1973). Therefore, γ-MNs may play more crucial roles in activating the spindle reflex pathway in isometric contraction of masseter muscles than in that of limb muscles. The spindle reflex pathway in masseter muscles may be activated by the temporal summation of a barrage of Ia-EPSPs that is induced by a burst of impulses in a single Ia afferent innervating the large number of sensory endings.

Although γ-MNs would similarly activate spindle II afferents, these afferents may not be largely and directly involved in the activation of α-MNs innervating masseter muscles due to less extensive and more remote dendritic inputs compared to spindle Ia inputs (Kishimoto et al. 1998).
Origin of illusion. It is reported that the vibration induces the activity of muscle spindle endings, thereby causing an illusion as if the parent muscle were stretched (Eklund 1972; Goodwin et al. 1972). Indeed, in the present study, the subject experienced an illusion as if the jaw were opened when vibration was applied to masseter muscle. Provided that vibration similarly activates spindle primary and secondary endings and Golgi tendon organ in masseter muscles as previously reported in leg muscles (Fallon and Macefield 2007), the illusion of jaw opening would be caused by the activities of all the three kinds of afferents. Then, the illusion caused by vibration may be mediated by the composite population response of these muscle receptor afferents, which reflects the numbers of respective receptors and their responsiveness to vibration. The contribution of Golgi tendon organs may be small because the number of Golgi tendon organs is much smaller than those of muscle spindles as demonstrated in the cat masseter muscles (Lund et al. 1978), and their responsiveness to vibration is much weaker than those of spindle primary and secondary endings despite the similar vibration threshold (Fallon and Macefield 2007). Considering the number of dynamic bag fibers (40–42%), from which spindle II afferents do not arise, among the sampled total number of intrafusal fibers in muscle spindles in human masseter muscles (Eriksson et al. 1994), the number of spindle primary endings on intrafusal fibers can be estimated to be approximately 1.7 times larger than that of spindle secondary endings. Therefore, the illusion caused by vibration may reflect the two population responses of spindle Ia and II afferents (approximately 63 and 37%, respectively), provided that there is no difference in the responsiveness to vibration between spindle primary and secondary endings.

Calibration of spindle Ia/II afferent activity against PMR afferent activity. During the
execution of isometric contraction to counteract the load applied to the lower jaw and to maintain the jaw in the same position, the load is perceived as the bite pressure by the activity of the lower 1st molar PMR afferents (Johnsen and Trulsson 2005; Linden 1978), and the jaw position is perceived as the absolute masseter muscle length by the activities of spindle Ia and II afferents (Boyd 1981). Mesencephalic trigeminal neurons separately innervate PMRs and spindle primary and secondary endings (Corbin 1940; Lazarov 2002; Linden 1978). Provided that the activity of $\gamma$-MNs through spindle Ia impulses is involved in producing a constant percentage (30–40%) of the isometric contraction regardless of its strength as was the case with hand and leg muscles (Gandevia et al. 1990; Macefield et al. 1993), there would be a stereotyped relationship between the activity of spindle Ia afferents and that of PMR afferents in the jaw-closed position, during isometric contraction (solid curves, Fig. 9A and B). Since not only spindle Ia but also spindle II afferents would be activated by $\gamma$-MNs during the isometric contraction, there would also be a positive relationship between the activities of spindle II and PMR afferents, although their synaptic impacts on $\alpha$-MNs is much smaller compared to spindle Ia afferents (Kishimoto et al. 1998). Such a calibration mechanism may exist in the cerebellum as an internal model as postulated previously (Taylor and Elias 1984). This idea is also supported by the findings that cerebellar ablation abolished the facilitation of isometric contraction caused by the activity of $\gamma$-MNs (Hidaka et al. 1997, 1999; Komuro et al. 1999). Thus, the subjects would be able to figure out the muscle tension and the jaw position to counteract the load applied to the lower 1st molars by calibrating the spindle Ia/II afferent activity against the PMR afferent activity in the jaw-closed position, presumably in the cerebellum (Fig. 9C).
Illusory positive shift of “calibration” curve caused by vibration of muscle spindles.

Since vibration steadily induces spindle Ia/II afferent activity to an extent neither inducing EMG activity nor changing the actual jaw position, the subject would have an illusion as if the “calibration” relationship were shifted to the right to positively offset the spindle Ia/II afferent activity elicited by vibration ($S_0$; dotted curves, Fig. 9A and B). This idea is not necessarily incompatible with the report that voluntary isometric contraction enhanced the effect of vibration on inducing spindle Ia afferent activity through the co-activation of the fusimotor system that compensates for mechanical unloading caused by the extrafusal shortening (Burke et al. 1976). This is because the effects of shortening intrafusal fibres by $\gamma$-MNs and those of vibration on stretching of spindle primary and secondary endings are indistinguishable. Then, the spindle Ia/II impulse activity caused by the vibration would be summated with that caused by $\gamma$-MNs. It is therefore likely that the spindle Ia/II afferent activity elicited by vibration is positively offset in the calibration curve.

Illusion reveals a critical involvement of spindle activity in regulating isometric contraction of masseter muscles. As illustrated in Fig. 9A, when the subject perceived a load amplitude as $P_1$ activity of the lower molar PMR afferent, the spindle afferent activity of $S_1$ would be judged to be necessary to counteract the load to maintain the jaw in the same closed position following the calculation based on the calibration curve (solid curve). However, under Vib+, the spindle afferent activity of ($S_0 + S_1$) would be judged to be necessary to counteract the same load ($P_1$) following the calculation based on the illusory relationship (dotted curve), which results in an
excessive counteraction ($P_2$) to the actual load ($P_1$) to be detected as the bite pressure ($P_2 - P_1$) under Vib$^+$ (Fig. 9A). In contrast, when decreasing the counteraction force in response to the load decrease from $P_2$ to a smaller value of $P_1$, the subject would judge that the spindle afferent activity must be decreased from $S_2$ to ($S_0 + S_1$) by referring to the illusory relationship (dotted curve, Fig. 9B), which results in little actual decrease in the counteraction force or EMG activity, leading to the generation of the more bite pressure. Thus, such an illusory positive shift of the calibration curve would give an explanation for the hysteresis between the over-increment and under-decrement of EMG activities in response to the positive- and negative-ramp loads, respectively, under Vib$^+$. Therefore, it is strongly suggested that spindle Ia/II afferent activity is involved in the estimation of rate of rise or decrease of the load as well as in the direct and/or indirect execution of the isometric contraction (Jankowska and Edgley 2010).

**Individual difference in the performance of smooth clenching.** The regression coefficient varied markedly among the subjects, and the effect of vibration also varied from subject to subject (Figs. 5 and 8). To examine the effect of vibration in each subject, we calculated the ratio of the 1st regression coefficient during the 1st positive-ramp load under Vib$^+$ to that under Vib$^-$ obtained in the experiments 1 and 2 as a measure of sensitivity to vibration. When the ratio was plotted against the 1st regression coefficient under Vib$^-$ (Fig. 10), there was a significant negative correlation ($r = -0.63; p < 0.003, n = 21$). This analysis indicates that the subjects who can clench more efficiently and accurately showed larger effects of vibration whereas those who clench less efficiently and accurately showed smaller effects of vibration, suggesting that skillfulness at clenching, i.e., strict regulation of the isometric contraction is dependent on the strictness of the relationship between the
activity of spindle afferents and that of PMRs. If a subject shows a more strict relationship, the sensitivity to vibration would be larger. Such individual difference in performing isometric contraction of masseter muscles may be due to the difference in the experience of biting or eating hard food in younger age, at which the motor learning would be acquired by constructing the calibration curve between the activity of spindle Ia/II afferents and that of PMR afferents presumably in the cerebellum (Fig. 9C).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES


Corbin KB. Observations on the peripheral distribution of fibres arising in the mesencephalic nucleus of the fifth cranial nerve. J Comp Neurol 73: 153-177, 1940.


Kishimoto H, Bae YC, Yoshida A, Moritani M, Takemura M, Nakagawa S, Nagase Y, Wada T, Sessle BJ, and Shigenaga Y. Central distribution of synaptic contacts of primary and secondary jaw muscle


Macefield VG, Gandevia SC, Bigland-Ritchie B, Gorman RB, and Burke D. The firing rates of human motoneurones voluntarily activated in the absence of muscle afferent feedback. *J Physiol* 471: 429-443,


Figure Captions

Fig. 1. Schematic illustrations of the experimental setup and protocols.

A: A custom-designed apparatus to apply the increasing (positive) and decreasing (negative) ramp loads to the lower bite plate (2), which can smoothly slide vertically by a winch (1) through the spring. 3: A pressure gauge cell placed between the upper and lower bite plates to measure bite pressure. 4: A CCD laser displacement sensor to measure the vertical mandibular position. 5: A load cell. 6: Surface bipolar electrodes to measure EMG activities of bilateral masseteric muscles. 7: Vibrators to apply vibration bilaterally to the tendon of the superficial layers of masseteric muscles at the position 5 mm below the zygomatic arches.

Ba: Protocol for Experiment 1, the positive-ramp load increasing 0 to 100 N at the rate of approximately 27 N/sec applied 10 sec after starting the EMG recording. Tendon vibration for 13 sec was applied 5 sec after starting the EMG recording.

Bb: Protocol for Experiment 2, the positive- and negative-ramp loads applied twice 5 sec after starting the EMG recording. Tendon vibration for 19 sec was applied 3 sec after starting the EMG recording.

Fig. 2. Illusion caused by vibration of muscle spindles of masseter muscles.

A: The subjects reported a definite perception of unintended powerful jaw opening in response to vibration. Thick part of the uppermost trace showing the timing and duration of the vibration applied to masseter muscles in the resting state. The 2nd and 3rd traces showing no changes in load and bite pressure (B-P), respectively. The 4th trace showing no changes in mandibular position.
(M-P) monitored with a CCD laser sensor. The 5th and 6th traces show no EMG activity of right and left digastric muscles (R-D and L-D), respectively. The 7th and 8th traces show no EMG activity of right and left masseter muscles (R-M and L-M), respectively.

B: A voluntary jaw opening to mimic the illusion followed by a submaximal jaw opening. The 4th trace shows a small downward displacement of mandibular position by 6 mm followed by a large downward displacement of mandibular position by 38 mm. The 5th and 6th traces show a small and large EMG activities of digastric muscles, seen during a small and large jaw opening, respectively.

C: The 2nd trace showing a positive- and negative-ramp loads. The 5th and 6th traces show small tonic EMG activities of R-D and L-D seen during the ramp loads. The 7th and 8th traces show EMG activities of R-M and L-M, which precisely counteracted the ramp loads.

Fig. 3. Masseteric muscle activity in response to the ramp-and-hold load.

A and B: The uppermost traces show the absence and presence of the tendon vibration: Vib− and Vib+, respectively. The 2nd trace shows the ramp-and-hold load. The 3rd and 4th traces: Sample records of R-M and L-M EMG, respectively. The 5th trace: An averaged trace of RMSs of R-M and L-M EMG. The 6th and 7th traces: B-P and M-P, respectively. Abbreviated titles for respective sample traces are the same as those applied in Fig. 2. Arrows indicate the stretch reflex that occurred at the onset of the ramp load application.
Fig. 4. Relationship between Load and RMS obtained in response to the ramp-and-hold load under Vib⁻ and Vib⁺.

A: The Load-RMS relationships under Vib⁻ and Vib⁺ (black and gray curves) were approximated with double regression lines, respectively. The dotted and interrupted lines represent the 1st and 2nd regression lines, respectively. A thin and a thick curve were obtained by calculating RMS of EMG signals with a time window of 0.25 sec and that of 0.5 sec, respectively. Two pairs of black and gray lines are obtained by piecewise linear regressions on the two Load-RMS curves.

B: The time course of the difference between the RMSs under Vib⁻ and Vib⁺ (ΔRMS; dotted curve) was similar to that of the bite pressure (B-P) induced under Vib⁺ (solid curve).

Fig. 5. Pooled data analysis of the 1st and 2nd regression coefficients obtained from the Load-RMS relationships in response to the ramp-and-hold load under Vib⁻ and Vib⁺.

A: The 1st regression coefficient was significantly larger under Vib⁺ than under Vib⁻.

B: The ratio of the 2nd regression coefficient to the 1st regression coefficient was significantly larger under Vib⁻ than under Vib⁺.

Fig. 6. Masseteric muscle activities in response to the positive- and negative-ramp loads.

Sample records of R-M and L-M EMG activities, their RMS, B-P and M-P, measured in response to the positive- and negative-ramp load under Vib⁻ (A) and Vib⁺ (B), respectively. Arrows indicate the stretch reflex that occurred at the onset of the ramp load application. Arrowheads and asterisks indicate the increased RMS and the subsequent emergence of B-P, respectively, following unloading. Abbreviated titles for respective sample traces are the same as those applied in Fig. 2.
Fig. 7. Relationship between load and RMS obtained in response to the positive- and negative-ramp loads under Vib− and Vib+.

A: The Load-RMS relationships obtained in response to the positive- (a) and negative-ramp loads (b), respectively, under Vib− and Vib+ (black and gray curves), which were approximated with double regression lines, respectively. The dotted and interrupted lines represent the 1st and 2nd regression lines, respectively. A thick curve was obtained by calculating RMS of EMG signals with a time window of 0.5 sec. Two pairs of black and gray lines are obtained by piecewise linear regressions on the two Load-RMS curves.

B: The time course of the difference between the RMSs under Vib− and Vib+ (ΔRMS; dotted curve) was roughly similar to that of the bite pressure (B-P) induced during the positive- (a) and negative-ramp loads (b) under Vib+ (solid curve).

Fig. 8. Pooled data analysis of the 1st and 2nd regression coefficients obtained in response to the positive- and negative-ramp loads under Vib− and Vib+.

Aa: The 1st regression coefficient in response to the 1st positive-ramp load was significantly larger under Vib+ than under Vib−.

Ab: The ratio of the 2nd regression coefficient in response to the 1st positive-ramp load to the 1st regression coefficient was significantly larger under Vib− than under Vib+.

Ba: The 1st regression coefficient in response to the 1st negative-ramp load was significantly smaller under Vib+ than under Vib−.

Bb: NormRMS at zero load represents the normalized value of y-intercepts of the 1st regression lines obtained during the 1st negative-ramp load was significantly larger under Vib+ than under Vib−.
C: No significant differences between the baseline RMSs obtained before and after the ramp load application under Vib− and between the baseline RMSs obtained before the ramp load under Vib− and Vib+. Note a significant difference between the baseline RMSs obtained before and after the ramp load under Vib+.

Fig. 9. An internal model that shows calibration between the activity of spindle Ia/II afferents and that of periodontal mechanoreceptor (PMR) afferents during the isometric contraction in the jaw-closed position.

The relationships between PMR and spindle Ia/II afferent activities under Vib− and Vib+ (solid and dotted curves, respectively). Vibration shifts the calibration under Vib− in the right direction by $S_0$, provided that the vibration induces $S_0$ activity in spindle Ia/II afferents. A: Under Vib−, when increasing the load from 0 to a load causing $P_1$ activity of PMR afferents, $P_1$ is precisely counteracted by isometric force induced by $S_1$ activity of spindle Ia/II afferents. Under Vib+, the subject overestimates $P_1$ load to be counteracted by $(S_0 + S_1)$ activity, which causes isometric force that can counteract $P_2$ load. B: Under Vib+, when decreasing the load from $P_2$ to $P_1$, subjects try to decrease spindle Ia/II afferent activity from $S_2$ to $(S_0 + S_1)$ by referring to the illusory dotted curve, resulting in little decrease in isometric force because $(S_0 + S_1)$ activity produces isometric force that cause $P_2$ activity of PMR afferents. C: A diagram of possible neural circuit for internal model of calibration between spindle Ia/II and PMR afferent activities. A load causes $y$ activity of PMR afferents, which is calibrated in the internal model to find $x$ activity of spindle Ia/II afferents that can be seen when isometric force just counteract the applied load. Calculated outputs from the
cerebellum can indirectly activate $\gamma$-MNs to cause $x$ activity of spindle Ia/II afferents through the RF and thalamo-cortical-CPG pathway. Vibration of muscle spindles may cause subthreshold EPSPs in $\alpha$-MNs but activates cerebellar neurons, resulting in an illusion of jaw opening and causing a rightward shift of the calibration curve. Cx, cortex. CPG, central pattern generator. MTN, mesencephalic trigeminal nucleus. RF, reticular formation. TMN, trigeminal motor nucleus.

Fig. 10. Relationship between the 1st regression coefficient and the sensitivity to vibration
Sensitivity to vibration that was defined as the ratio of the 1st regression coefficient under Vib$^+$ to that under Vib$^-$ was plotted against the 1st regression coefficient under Vib$^-$. 
A 1st positive-ramp load

B 1st negative-ramp load

C

RMS (μV)

<table>
<thead>
<tr>
<th>Before</th>
<th>After</th>
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<tr>
<td>Vib(-)</td>
<td>Vib(+)</td>
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1st reg. coef. (x 10^-2 N^-1)

Vib(-) Vib(+)

Reg. coef. ratio, 2nd / 1st

Vib(-) Vib(+)

NormRMS at zero load

Vib(-) Vib(+)

* Indicates significant difference.
Sensitivity to vibration

$r = -0.63$

$p < 0.003$

1st reg. coef. $(x \times 10^{-2} \mu V/N)$