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Response properties of periodontal mechanosensitive neurons in the superior alveolar nerve of the rat

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Abstract : Slowly-adapting periodontal mechanosensitive (PM) neuron activities were recorded from the superior alveolar nerve of the rat, and their response properties to mechanical stimulation of the upper incisor tooth were studied. When the tooth was stimulated in 12 directions on the horizontal plane, the great majority (96%) of PM units were directionally selective. The response field in more than half of the PM units (55%) was of the medium type (90°~180°, response angle), and that of 34% was of the broad type (180°~360°). Eighty-six percent of the PM units fired at relatively regular interspike intervals in response to tooth stimulation applied in the optimal direction, while the remaining 14% responded at irregular intervals. When the tooth was stimulated with a force of 0.10 N, the rate of spike interval fluctuations [percentage of the standard deviation (SD) of the interval distribution as compared to the mean interval (mean), SD/mean×100] was 5.6% for the regular interval units and 44.8% for the irregular ones. Compared with the response properties of PM neurons that have been reported in the cat superior alveolar nerve and rat trigeminal ganglion, the present findings suggest that the difference in the response angle range between the rat and cat afferents is due to the morphological difference between the incisor tooth of the rat and the canine tooth of the cat, and that about half of rat PM afferents branch in the area that extends from the trigeminal ganglion to the rostral part of the orbit.

抄録：ラットの上顎切歯を機械的に刺激し、上歯槽神経から遅順応性歯根膜機械応答ニューロンの反応を記録した。歯を水平面上の12方向から刺激した時、歯根膜ユニットの大部分は刺激に対し方向特異性を示した。応答野では、ユニットの過半数(55%)は反応角度が90°から180°までの中間型のものであり、34%は反応角度が180°から360°までの広作動型のものであった。歯を最適刺激方向から刺激した時、ユニットの86%は歯の刺激に比較的に規則的なスパイク間隔で応答し、残り14%は不規則な応答を示した。スパイク間隔の変動率はスパイク間隔が規則的なタイプで5.6%、不規則なタイプで44.8%であった。以上の結果と既に報告されているネコ上歯槽神経やラット三叉神経節の歯根膜ニューロンの反応特性とを比較すると、ラットとネコの歯根膜神経における反応角度の違いはラット切歯とネコ犬歯の形態的な違いによるものであり、ラットの求心性神経の約半数は三叉神経節から眼窩吻側部の間で分枝しているのではないかと推定される。

Introduction

The response properties of periodontal mechanosen-

sitive (PM) primary afferents have been mainly studied in the cat and dog¹⁻⁸⁾. The great majority of PM afferents are single-tooth units sensitive to mechanical stimulation of the canine tooth. They show slowly-

adapting responses with directional selectivity to stimulation.

Recently, Tabata and Hayashi⁹⁾ reported that the response characteristics of the PM neurons in the rat trigeminal ganglion differ from those of cat PM afferents. The response field—the range of response angles when the tooth is stimulated in several directions horizontally—of the rat ganglion neurons is broader than that of the PM afferents of cat superior alveolar nerve⁴⁾. Interspike intervals of the responses in more than half (62%) of rat trigeminal ganglion PM afferents were irregular⁹⁾; whereas, those in the great majority (93%) of cat inferior alveolar nerve PM afferents were relatively regular⁸⁾.

We investigated whether this difference in results is due to differences in these animal species or in the recording sites of the primary afferent nerve. Single spike discharges of slowly-adapting PM afferents were recorded from the superior alveolar nerve of the rat, and their response properties, the response field, optimal stimulus direction, threshold intensity, and spike intervals of response to tooth stimulation were examined. The response properties obtained were compared to those of the rat trigeminal ganglion neurons and cat PM afferents in order to determine the reason for the differences.

Materials and Methods

Twelve adult rats (0.3~0.4 kg) were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). After cannulation of the femoral vein, they were again anesthetized with pentobarbital sodium (5~10 mg/kg/h, i.v.). Their ECGs were monitored throughout the experiments. An adequate state of anesthesia was ensured by monitoring the heart rate, spontaneous muscle activities, and unexpected nociceptive reflexes. Body temperature was maintained at 37°~39°C with a thermostatically controlled heating pad.

The rat's head was fixed in a stereotaxic apparatus, and the left eye ball was removed from the orbit. The anterior branch of the superior alveolar nerve running along the ventromedial side of the infraorbital nerve was exposed and transected proximally. A fine strand was isolated from the nerve under a surgical micro-

scope. The unitary discharges of a functionally single nerve fiber in a liquid paraffin pool were recorded with a silver wire electrode. The unitary discharges were displayed on an oscilloscope (Nihonkoden VC-10) and monitored aurally. The data were recorded on magnetic tape (TEAC R-80) for off-line analysis.

The method used for the physiological characterization of the PM units is described in detail elsewhere^{9~12)}. Briefly, the left upper incisor tooth was stimulated manually in 12 directions on the horizontal plane and in 7 directions on the sagittal plane with calibrated von Frey hairs at a force of 0.05 N or 0.10 N to obtain the response field (range of response angles), optimal stimulus direction, and interspike intervals of response. The manual stimuli were applied to the tooth very carefully to make the stimulus intensity and the response of units reproducible and were repeated two to four times in each direction. The right upper incisor tooth was cut off 1 mm from the gingiva so that the left incisor tooth could be easily stimulated in various directions. A rough estimate of the threshold intensity was made with six calibrated von Frey hairs at forces of 0.01 N, 0.02 N, 0.05 N, 0.10 N, 0.25 N and 0.50 N.

Results

One hundred thirty-nine slowly-adapting PM units were recorded from the left superior alveolar nerve of the rat. All responded to mechanical stimulation of the ipsi-lateral upper incisor tooth. Thirty-two units (23%) showed spontaneous discharges.

Response field and optimal stimulus direction

The response field of the PM units was obtained from the values of all the responses relative to the maximum response when the upper incisor tooth was stimulated in 12 directions on the horizontal plane and in 7 directions on the sagittal plane. Karita and Tabata⁴⁾ and Tabata and Karita¹²⁾ have divided the response fields obtained on the horizontal plane into 5 types on the basis of the response angle: narrow (response angle range, <90°), medium (90° to 180°), broad (180° to 360°), eccentric (=360°), and non-eccentric (=360°). Neurons of the eccentric type were

Fig. 1 Neuronal discharges and response fields of a medium type unit when the upper incisor tooth of a rat was stimulated mechanically in 12 directions on the horizontal plane (A) and in 7 directions on the sagittal plane (B). The distance from the center of the circle of the response field represents normalized values of all the responses relative to the maximum response. The magnitudes of the responses were obtained from the number of spike discharges during a 1s period after the onset of stimulation. The maximum response of this PM unit occurred when the tooth was stimulated in the rostro-ventral direction on the sagittal plane. The bold horizontal bars beneath the records indicate mechanical stimulation (0.10N). Med, mediolateral; Caud, caudorostral; Lat, lateromedial; Rost, rostrocaudal; Vent, Ventrodorsal; Dor, dorsal.

activated weakly by mechanical stimulation even when opposite to the optimal stimulus direction. Non-eccentric type neurons responded very similarly to tooth stimulation in all the stimulus directions. The first three response types were classified as directionally selective and the last two types as directionally non-selective.

An example of the medium type response field is shown in Figure 1. The optimal stimulus direction on the horizontal plane was oriented rostro-medially (Fig. 1A). The magnitude of the response of the directionally selective units decreased as the stimulus angle diverged from the optimal direction. The maximum response, however, was obtained by stimulation in the rostro-ventral direction on the sagittal plane (Fig. 1B). When the tooth was stimulated horizontally, 96% (134/139) of the PM units were directional-

ly selective to tooth stimulation. Table 1 shows the incidence of each type of response field with directional selectivity to tooth stimulation. More than half (55%, 76/139) of the units were of the medium type, and 34% (47/139) were of the broad type.

The optimal stimulus direction determined by stimulation on the horizontal plane was oriented predominantly in the rostro-medial or caudo-lateral direction (Fig. 2A). When stimuli were applied sagittally, PM units having the optimal stimulus direction in the rostro-medial and caudo-lateral directions on horizontal stimulation respectively showed the most vigorous discharges on tooth stimulation in the rostro-ventral and caudo-ventral directions (Fig. 2B). In three-dimensional space, the optimal stimulus direction for the PM units of the rat superior alveolar nerve was the rostro-medial, ventral direction or the

Fig. 2 Angular distribution of the optimal stimulus direction of directionally selective PM units when the left upper incisor tooth of the rat was stimulated mechanically on the horizontal (A) and on the saggital (B) planes. Numerals show the number of PM units. Med, mediolateral; Caud, caudorostral; Lat, lateromedial; Rost, rostrocaudal; Vent, Ventrodorsal; Dor, dorsal.

Table 1 Response fields (RFs) of incisor tooth-sensitive PM units in the superior alveolar nerve of the rat.

Type of RF	DS	Response angle	No. of PM units
Narrow	+	$\theta \leq 90^\circ$	11 (7.9%)
Medium	+	$90^\circ < \theta \leq 180^\circ$	76 (54.7%)
Broad	+	$180^\circ < \theta \leq 360^\circ$	47 (33.8%)
Eccentric	-	360°	4 (2.9%)
Non-eccentric	-	360°	1 (0.7%)
Total			139

Directionally selective (DS) units are shown as positive (+) and directionally non-selective ones as negative (-).

caudo-lateral, ventral direction.

Regularity of the spike intervals

The interspike intervals of response were analyzed in 139 PM units when the incisor tooth was stimulated in the optimal direction. Eighty-six percent (119/139) of the units responded at relatively regular spike intervals. The remaining 14% responded at irregular ones. Figure 3 shows spike density histograms and spike discharges of PM units of the regular (A) and irregular (B) interval types when the incisor was stimulated with a force of 0.10 N. The intervals of 21 spikes occurring 0.5 s after the stimulus was given were measured and analysed for 80 units (60 units, regular interval and 20 units, irregular interval type). The rate of spike interval fluctuation was defined as the percentage obtained from a comparison of the

standard deviation (SD) of the interval distribution with the mean interval (mean) ($SD/\text{mean} \times 100$). Figure 4 shows the distributions of the rates of spike interval fluctuation. For PM units of the regular interval type the distribution was less than 10%, whereas for irregular units it was more than 15%. Irregular interval type units with a spike interval fluctuation rate ranging from 15 to 25% frequently showed responses that lack spikes in contrast to the spike sequence of regular interval type units.

The mean spike interval of the responses evoked by tooth stimulation at a force of 0.10 N was 10.5 ± 4.5 ms (mean $\pm$ SD, $n=60$) in PM units of the regular interval type and 17.0 ± 10.7 ms ($n=20$) in units of the irregular type. The mean spike interval of the former was significantly shorter than that of the latter (Welch's analysis, $p < 0.02$). The mean rate of spike interval fluctuations in the responses was $5.6 \pm 1.9\%$ ($n=60$) for the regular interval units and $44.8 \pm 23.6\%$ ($n=20$) for the irregular ones.

Threshold intensity of mechanical stimulation

Distributions of the threshold intensity of PM units of the regular and irregular interval types are shown in Figure 5. PM units could be divided into 5 groups on the basis of the ranges of the von Frey hair thresholds: < 0.01 N, < 0.02 N, < 0.05 N, < 0.10 N and < 0.25 N. The great majority (88%, 122/139) of the PM units responded to tooth stimulation at a force range of less than 0.01 N. No significant difference was found in the distribution of the threshold intensity between the regular and irregular interval units (χ^2 -test, $p < 0.05$).

Fig. 3 Spike density histograms and spike discharges of the PM units of the rat. Bottom traces are the spike discharges during the periods 0.5 to 0.8 s and 2.0 to 2.3 s after the onset of stimulation, as shown in the respective spike density histograms. (A) PM unit of the regular spike interval type. The mean spike intervals and rates of spike interval fluctuation were 11.5 ± 1.1 ms and 9.7% during the period of 0.5 to 0.8 s and 12.3 ± 0.8 ms and 6.5% during the period of 2.0 to 2.3 s. (B) PM unit of the irregular spike interval type. The mean spike intervals and rates of spike interval fluctuation were 8.3 ± 6.0 ms and 72.3% during the period of 0.5 to 0.8 s and 14.5 ± 8.6 ms and 59.3% during the period of 2.0 to 2.3 s. Mechanical stimulation (0.10 N) was applied to the incisor tooth in the optimal direction for each unit.

Discussion

The respective incidences of the medium (90° to 180° , response angle) and broad (180° to 360° , response angle) units were 55% and 34% in the response field of the PM units in the rat superior alveolar nerve. In rat trigeminal ganglion neurons, however, they are 38% and 41%⁹⁾. The incidence of medium type units for the superior alveolar nerve is significantly higher than that for the trigeminal ganglion neurons (χ^2 -test, $p < 0.02$). This suggests that some PM neurons branch in the area that covers the rostral part of the orbit to the trigeminal ganglion.

In cat canine tooth-innervating PM units recorded from the superior alveolar nerve in the orbit, the predominant type of response field (84%) is the medium one⁴⁾. The incidence of the medium units in

the cat superior alveolar nerve was considerably higher than that (55%) in the rat superior alveolar nerve. This difference in response fields may be due to the morphological difference between the incisor tooth of the rat and the canine tooth of the cat.

Pressure in the labiolingual (rostral) direction given to the rat upper incisor tooth induces inhibition of masseter muscle activity, whereas pressure given in the opposite direction enhances it¹³⁾. The optimal stimulus direction for the rat upper incisor tooth was oriented in the rostro-medial, ventral and the caudo-lateral, ventral directions in three-dimensional space (Fig. 2). The rostro-medial, ventral direction closely corresponds to the labiolingual direction on the horizontal plane and is the direction from which the rat takes food into its mouth. The caudo-lateral, ventral direction is close to being coincident with the lingual direction, and it is the direction from which

Fig. 4 Distribution of the rates of interspike interval fluctuation ; percentages of the standard deviation (SD) as compared to the mean interval (mean) ($SD/\text{mean} \times 100$). Vertical broken line : demarcation between the regular and irregular spike interval units.

the upper incisor tooth contacts the lower tooth on occlusion. Thus, periodontal sensory information about the stimulus direction functions in the control of the activities of the masticatory muscles and the biting force when food is chewed.

The periodontal mechanosensitive nerve that innervates the upper incisor tooth of the rat enters the lingual periodontal ligament through slits located in the mid-region of the alveolar socket¹⁴. PM receptors are distributed densely in the periodontal ligament of the incisor tooth on the lingual side of the root, but are not distributed on the labial side^{14,15}. Morphological observations suggest that PM receptors are Ruffini endings^{3,14-20}. PM receptors fire when the ligament in which they are located comes into tension²¹⁻²³. Because the optimal stimulus direction is oriented in the rostro-medial or caudo-lateral direction, PM receptors should be under tension upon tooth stimulation in one, if not both, of these directions. We therefore assume that the incisor tooth rotated about its fulcrum near the mid-region of the tooth root when the crown was stimulated mechanically.

In rat trigeminal ganglion neurons, 62% of the PM units fired at irregular spike intervals upon tooth stimulation⁹. In the superior alveolar nerve of the rat, however, only 14% of the units are of the irregular spike interval type. Because Ruffini endings generate

Fig. 5 Distribution of the threshold intensities of PM units when the maxillary incisor tooth of the rat was stimulated in the optimal direction. Open column : regular spike interval unit. Hatched column : irregular spike interval unit.

spikes at regular intervals²⁴, Tabata and Hayashi⁹ and Tabata *et al.*⁸ considered that the irregular spike interval type response is caused by interference at the branching point between the firings from plural Ruffini endings. Therefore, about half of the PM afferents may ramify in the area that extends from the trigeminal ganglion to the rostral part of the orbit.

When the incisor tooth of the rat was pressed with a force of 0.10 N, the mean spike interval of the PM units in the superior alveolar nerve was 10.5 ms for the regular interval units and 17.0 ms for the irregular ones. In the trigeminal ganglion neurons, it was 15.1 ms for the regular interval units and 29.2 ms for the irregular ones when the tooth was stimulated with a force of 0.05 N⁹. This difference in the mean intervals is considered to be due to the difference in the stimulus intensities (0.10 N and 0.05 N) used in the two studies.

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