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博 士 学 位 論 文

Conditioning Stimulation of the Central Amygdaloid Nucleus  
Inhibits the Jaw-Opening Reflex in the Cat

(扁桃体中心核条件刺激による開口反射抑制作用)

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Conditioning Stimulation of the Central Amygdaloid Nucleus  
Inhibits the Jaw-Opening Reflex in the Cat

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Running head: INHIBITION OF JOR BY AMYGDALOID NUCLEUS

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**Abstract** To elucidate a function of the central amygdaloid nucleus (ACE) in the trigeminal system, the ACE conditioning effect on the jaw-opening reflex (JOR) regarded as a nociceptive reflex investigated in the cat anesthetized with pentobarbital sodium. The JOR to molar tooth pulp stimulation with an intensity 1.2-1.5 times the threshold was recorded in the ipsilateral digastric muscle. As conditioning stimulation, a train of 33 rectangular pulses (0.5 ms in duration) at 330Hz with an intensity of 300  $\mu$ A was applied to the ipsilateral ACE. The conditioning stimulation inhibited a JOR that had a latency of  $7.90 \pm 0.86$  ms ( $n=36$ ). The inhibition was maximum ( $83.1 \pm 11.2\%$ ) at a conditioning-test (C-T) interval of 110 ms and continued for C-T intervals of up to 1000 ms. Likewise, microinjection of 0.5M monosodium glutamate (10  $\mu$ l) into the ACE inhibited the JOR for approximately 10 min. Additionally, the ACE conditioning stimulation inhibited the JOR induced by the stimulation of the sensory trigeminal nuclear complex in a similar manner, but not the jaw-opening response induced by the stimulation of the trigeminal motor nucleus (Mo V). Also, the conditioning stimulation neither influenced the evoked potentials induced by the tooth pulp stimulation at the main sensory and rostral nuclei nor the jaw-closing reflex induced by the stimulation of the mesencephalic trigeminal nucleus. These results suggest that the excitation of the cell bodies in the ACE exerts an inhibitory modulation on the JOR with no effect on the non-nociceptive reflex such as the jaw-closing reflex at the level of Mo V.

*Key words:* central amygdaloid nucleus, tooth pulp, jaw-opening reflex, glutamate, nociception.

## INTRODUCTION

The amygdaloid complex, one of the components of the limbic system, contains high levels of opioid and enkephalin receptors that modulate nociception in the central nervous system (1,2). And it is known that the nociceptive threshold is increased by a microinjection of neurotensin and an enkephalinase inhibitor into the amygdaloid central nucleus (ACE), and that the antinociceptive effects are antagonized by administration of naloxone in the rat (3,4). A neurohistochemical study showed that a large number of neurotensin and enkephalin fibers were present in the ACE, and enkephalin-containing neurons were only found in the ACE (5). Moreover, the ACE has direct efferent connections with the periaqueductal gray and nucleus raphe magnus (6,7,8) which are concerned in the descending control of nociceptive transmission (9,10). Therefore, it is reasonable to expect that the ACE may have a modulatory effect on nociception.

On the other hand, the amygdaloid complex has been demonstrated to participate in the control of jaw movements. Electrical stimulation of the lateral amygdaloid nucleus that is heavily connected with the ACE induces a rhythmical jaw movement accompanied by jaw-closing movements in the cat and rabbit (11,12), and modulates the hypothalamically elicited attack response in the cat (13,14). Stimulation of the ACE facilitates the mylohyoid-digastric motoneurons and inhibits the masseteric ones in the rat (15,16,17). Gary Bobo and Bonvallet (1975) indicated that stimulation of the lateral division of the ACE facilitates the

masseteric reflex and stimulation of the medial one inhibits the reflex in the cat (18).

The jaw opening reflex (JOR) induced by electrical stimulation of the tooth pulp has been used as a reflex measure of pain sensitivity (19,20). Recently, we found that the JOR was inhibited by the ACE conditioning stimulation (21). It is conceivable that the inhibition is a consequence of the antinociceptive effects of the ACE and/or its modulatory effects on the jaw movements. The purpose of this study is to elucidate which of those effects are associated with the inhibition of the JOR by the ACE. The present study implies that the inhibition is manifested through a mechanism that involves the motor components of the JOR.

#### METHODS

*Preparation of animals.* The experiment was carried out on 36 adult cats weighing 2.0 to 4.4 kg. For surgery, the animals were anesthetized with ketamine hydrochloride (25 mg/kg, i. m.) and treated with atropine sulfate (0.2 mg/kg, i. p.). The femoral vein was then cannulated for additional administration of pentobarbital sodium (5-10 mg/kg, i. v.). Depth of anesthesia was checked repeatedly throughout the experiment by touching the pinna or whiskers; if a reflex was elicited, a supplementary dose of the pentobarbital sodium was administered. For electrical stimulation of the tooth pulp, small cavities were drilled into the dentine

of the bilateral upper and lower molar teeth, not exposing the pulp, and a stimulating electrode (small screw) with a lead wire (0.15 mm in diameter) was implanted into each cavity. The head of the screw and the surrounding area were covered with polycarboxylate cement and dental acrylic resin. After the animal was stereotaxically placed, holes were made in the parietal and supraoccipital bone for inserting the electrodes. Then, the cerebellum was aspirated for stimulation and recording from the brainstem. The wound edges and pressure points were carefully infiltrated by a xylocaine ointment. All exposed brain surfaces were covered with paraffin oil or agar for protection.

*Recording and stimulation.* The test stimulation (a single rectangular pulse, 0.5 ms in duration) was bipolarly delivered to the tooth pulp, and the intensity was maintained at 1.2-1.5 times the threshold for the JOR (50-400  $\mu$ A). A concentric bipolar electrode (Unique Medical, S-121) for the conditioning stimulation was stereotaxically placed within the amygdaloid complex (anterior 11.0-13.0, lateral 8.0-12.0, vertical -1.0 to -15.0) according to Berman and Jones' atlas (22). The stimulation was a train of 33 rectangular pulses (0.5 ms in duration) at a frequency of 330Hz at an interval of 8-10 s with an intensity of 50-400  $\mu$ A. The stimulating side of the amygdala was usually ipsilateral to the EMG recording side.

To observe the effect of the conditioning stimulation on the JOR induced by the stimulation of the brainstem, the same electrode as used for the amygdala stimulation was stereotaxically inserted into the sensory trigeminal nuclear complex and motor nucleus (posterior 18.3-4.0, lateral 3.5-4.5, vertical -7.0 to

-3.5) at an angle of 35-45°. A single rectangular pulse (0.5 ms in duration, 20-100  $\mu$ A) was bipolarly delivered to these portions. On the other hand, evoked-potentials induced by the tooth pulp stimulation were recorded in the sensory trigeminal nuclear complex and the effects of the conditioning stimulation on the potentials were observed. In addition, the jaw-closing reflex was elicited by electrical stimulation (single pulse, 0.5 ms in duration, 300-600  $\mu$ A) of the mesencephalic trigeminal nucleus (posterior 2.0-2.5, lateral 2.5-3.0, vertical -0.5 to 0).

EMG activities from the ipsilateral digastric (JOR) and masseteric (jaw-closing reflex) muscles were recorded bipolarly by a needle electrode and fed into an amplifier (Nihon Kohden, AVB-10). Low and high cut frequencies were 15Hz and 1KHz, respectively. The signals were then averaged ten times by a computer (Nihon Kohden, QC-111J) and these data were drawn by an X-Y recorder (Rikadenki, RW-201).

Monosodium glutamate (0.5M in distilled water) was microinjected into the ACE in 4 animals. After determining the most effective sites for JOR inhibition by electrical stimulation of the ACE, the stimulating electrode was withdrawn and a 31-gauge injection needle attached to the syringe was inserted into the same site. Then, 10  $\mu$ l glutamate was injected slowly over 2 min by a microinjector (Narishige IM-1).

*Histological analysis.* The stimulating and recording sites in the amygdala and brainstem were marked by depositing iron ions from the electrodes with an anodal current (200  $\mu$ A, 25-35 s). At the termination of the experiments, animals were sacrificed with an

overdose of pentobarbital and then perfused with saline followed by 10% formalin containing 2% potassium ferrocyanide. Frozen coronal sections of 50  $\mu\text{m}$  thickness were taken and then stained with cresyl violet. The marked sites were determined by histological examination of the serial sections.

## RESULTS

Aspects of the digastric EMG activity (JOR) related to the stimulus intensity. With increasing stimulus intensity, the EMG amplitude increased and the latency shortened. Therefore, 1.2-1.5 times the JOR threshold used as the tooth pulp stimulation. The mean ( $\pm$ S.D.) intensities of the maxillary (n=21) and mandibular (n=15) tooth pulp stimulation were  $200.5 \pm 144.0$  and  $273.3 \pm 172.4$   $\mu\text{A}$ , respectively. Stimulation with these intensities had a mean ( $\pm$ S.D.) latency of  $7.90 \pm 0.86$  ms (n=36; range 6.11-9.28 msec). No statistically significant differences were found between the latencies for the maxillary and mandibular tooth pulps.

The JOR evoked by tooth pulp stimulation was inhibited by conditioning stimulation of the lateral amygdaloid nucleus (LA), the periamygdaloid area (PAA), the central amygdaloid nucleus (ACE), the internal capsule (IC), and the entopeduncular nucleus. Conditioning stimulation of the other amygdaloid nuclei, such as the medial and basolateral nuclei, did not produce any influence on the JOR. The stimulation of LA, PAA, and IC induced the jaw-closing movement as the intensity was increased, but the ACE

stimulation alone did not induce the jaw movements. The effect of entopeduncular conditioning stimulation has been reported elsewhere (23).

#### *Effect of ACE conditioning stimulation*

##### *1. JOR to tooth pulp stimulation*

The conditioning stimulation of ACE reduced the amplitude of the digastric EMG response (JOR) elicited by tooth pulp stimulation (Fig. 1). The most effective inhibitory sites were found in the medial division of the ACE as verified by histological examination. The magnitudes of inhibition varied considerably according to the intensity and the duration of the conditioning stimulation. For this reason, the parameters of the ACE conditioning stimulation were determined first. Stimulus frequency was determined to 330Hz following ASANUMA AND SAKATA's method (24). In proportion to increases in the intensity of the conditioning stimulation, the magnitude of the inhibition became larger. Conditioning stimulation with an intensity of 300  $\mu$ A reduced the amplitude of the JOR to 24.1% of the control value (n=12). The magnitude of the inhibition showed little change when the intensity was increased to 400  $\mu$ A (Fig. 2A). Likewise, when the duration of the conditioning stimulation at 330Hz was prolonged at a fixed intensity (300  $\mu$ A), the inhibition became more pronounced as shown in Fig. 2B. However, the magnitude of the inhibition was relatively constant despite increasing the duration to more than 100 ms (n=6). Therefore, we determined that the parameters of the ACE conditioning stimulation were 300  $\mu$ A in

intensity and 100 ms in duration.

Fig. 3 indicates the time course of the ACE conditioning stimulation on the JOR in 14 trials in 11 animals, and the right figurine is a typical example. The percentage of the control JOR to tooth pulp stimulation is plotted against the C-T interval (the interval between the onset of the conditioning and test stimuli). The inhibitory effect reached its peak (16.9% of control) at a C-T interval of 110 ms and then gradually recovered to 90.1% of the control value at a C-T interval of 700 ms. The inhibition at a C-T interval of even 1000 ms was statistically significant (*t*-test,  $p < 0.05$ ).

To investigate which side of the ACE stimulation was more effective, JORs were recorded bilaterally in 5 experiments. JORs were evoked by ipsilateral tooth pulp stimulation to the EMG recording side. As compared with the side contralateral to the JOR recording side, the inhibitory effect of the ipsilateral ACE stimulation with the same intensity was twice as large ( $70.0 \pm 13.2$  vs.  $34.9 \pm 9.1\%$ ) as shown in Fig. 4. Thus, ACE conditioning stimulation exerts an inhibitory effect predominantly on the ipsilateral side rather than the contralateral one.

## 2. JOR to brainstem stimulation

To investigate whether the inhibitory process was exerted at the levels of the trigeminal motor nucleus or of the sensory nuclear complex, the effects of ACE conditioning stimulation were examined on the JOR evoked by the stimulation of each nucleus. The JOR due to the brainstem stimulation was evoked with a stimulus intensity that was just above threshold (50-140  $\mu$ A). Stimulations of caudal (0-5 mm below the obex and 3.0-4.5 mm lateral

from the midline) and rostral (5-9 mm above the obex and 4.5 mm lateral from the midline) nuclei evoked the JOR with a mean ( $\pm$ SD) latency of  $6.69\pm 0.48$  ms ( $n=5$ ) and  $5.24\pm 0.443$  ms ( $n=5$ ), respectively. As can be seen in Fig. 5A, the ACE conditioning stimulation inhibited to same degree the JOR induced by stimulation of these nuclei (right 2 columns) as the JOR evoked by the tooth pulp stimulation (left column). Effects of the ACE stimulation on the jaw-opening response induced by direct electrical stimulation of the ventromedial region of the motor nucleus (Mo V) were examined in the same animals. The jaw-opening response had a mean ( $\pm$ SD) latency of  $1.96\pm 0.96$  ms ( $n=6$ ). ACE conditioning stimulation did not diminish the jaw-opening responses. Likewise, Fig. 6A indicates that ACE conditioning stimulation inhibited the JORs induced by the stimulation of tooth pulp and rostral and caudal nuclei. However, the conditioning stimulation did not influence the jaw-opening response induced by the stimulation applied to Mo V at all (98.8% of the control) as shown in left open column.

### 3. Brainstem field potential to tooth pulp stimulation

Tooth pulp stimulation with an intensity at 1.2-1.5 times the threshold evoked field potentials in the main sensory and rostral nuclei but not in the caudal nucleus. The mean peak times ( $\pm$ SD) of these potentials at 7 mm and 5 mm rostral to the obex (rostral nucleus) were  $4.07\pm 0.56$  ms ( $n=6$ ) and  $4.48\pm 0.85$  ms ( $n=5$ ), respectively; that for the main sensory nucleus was  $4.41\pm 0.35$  ms ( $n=5$ ). These evoked potentials were not influenced at all by the ACE conditioning stimulation that totally inhibited the JOR evoked by

tooth pulp stimulation as shown in Fig. 5B. Hatched columns in Fig. 6B indicate that the electrodes for the conditioning stimulation were placed within the ACE in each experiment, because JORs induced by the tooth pulp stimulation were inhibited by the conditioning stimulation. The JOR was reduced to 13.2-16.4% of the control value by the ACE stimulation. However, the ACE conditioning stimulation did not diminish the field potential evoked by the tooth pulp stimulation as shown by the open columns in Fig. 6B. The amplitudes of field potentials under the conditioning stimulation were 100.0, 97.3, and 105.9% at 9, 7, and 5 mm rostral to the obex, respectively.

#### *4. Jaw-closing reflex*

In 4 animals, the effects of ACE conditioning stimulation were examined on the jaw-closing reflex induced by the electrical stimulation of the mesencephalic trigeminal nucleus (Mes V). The reflex was recorded from the masseteric muscle. The Mes V stimulation with an intensity of just above the threshold induced the reflex which had a mean latency ( $\pm$ SD) of  $4.24 \pm 0.43$  ms. The amplitude of the reflex, which fluctuated around the control level, was not altered by the ACE stimulation that inhibited the JOR induced by the tooth pulp stimulation as indicated in Fig. 7.

#### *Effect of glutamate microinjection*

To determine whether the inhibition of the JOR induced by the ACE electrical stimulation was caused by the excitation of the passing fibers of the cell bodies in ACE, glutamate was injected into the ACE. Injection of 10  $\mu$ l of monosodium glutamate into the ACE caused a decrease in the ipsilateral digastric EMG (JOR)

to tooth pulp stimulation in 4 animals. This decrease took about 1 min to reach a maximum (19.0-59.3% of control) and lasted for about 10 min as shown in Fig. 8. There was considerable variation between preparations in the magnitude and duration of the glutamate effect. As a control, the same dose of saline (vehicle) was microinjected into the ACE in 2 animals. Such injection was observed to cause no manifest change in the amplitude of JOR. Finally, histological analysis revealed that for all 4 animals the needles were placed in the ACE.

#### DISCUSSION

*Inhibitory effect of ACE on JOR* ACE conditioning stimulation reduced the EMG amplitude in the digastric muscle, but did not evoke the jaw-closing movement even if the stimulus intensity was increased. Gary Bobo and Bonvallet concluded that the facilitation or inhibition of the masseteric reflex elicited by ACE stimulation was induced by excitation of the surrounding pathway (18). Our findings that the inhibition of the JOR lasting for ten minutes was elicited by the glutamate microinjection into the ACE excludes the possibility of fiber excitation. It is highly probable that the inhibition of JOR by electrical stimulation of the ACE was induced by the excitation of cell bodies rather than the passing fibers in or around the ACE.

The effects of the ACE conditioning stimulation on the jaw reflexes has also been observed by other workers. It was reported

in the rat that ACE stimulation induced predominantly contralateral activation of the mylohyoid-digastric motoneuron (15,17). These results are incompatible with our present findings. Differences in the anesthetic, the species of animals, or the sites stimulated might account for the discrepancy. And it is possible that the effects depend on the excitation of the fibers in ACE and/or the neighboring area, because they employed a high intensity electrical stimulation (0.5-2.0 mA) and did not conduct an experiment using chemical stimulation such as the glutamate microinjection. Their results are similar to the findings of Gary Bobo and Bonvallet's experiment that stimulated the fibers (18). Furthermore, as the ACE inhibitory effect was observed even when the duration of the conditioning stimulation was shortened to 20 ms (Fig. 2B), it is difficult to understand why ACE stimulation facilitated the jaw-opener motoneuron at a C-T interval of 10-20 ms as indicated in their study. On the other hand, it has been reported that ACE conditioning stimulation inhibited the jaw-closing reflex (18). In our study, however, the reflex induced by the stimulation of Mes V was not modulated by ACE stimulation. It is possible that this discrepancy also depends on whether the electrical stimulation excites the cell body.

*JOR inhibition at brainstem* We investigated the sites of JOR inhibition at the level of the medulla. ACE conditioning stimulation inhibited considerably the JOR induced by the stimulation of the trigeminal sensory nuclear complex but not the JOR induced by the stimulation of Mo V. Also, the field-potentials evoked by tooth pulp stimulation in the main sensory and rostral nuclei were not influenced by the ACE stimulation. These findings provide exper-

imental evidence that the site of the inhibitory effect exerted by ACE on JOR is at the level of Mo V. That is, the JOR inhibitory effect of ACE acts on the motor system rather than on the sensory system. On the other hand, it is thought that the ACE participates in antinociception from the observations that the microinjection of an enkephalinase inhibitor or neurotensin into ACE elicited antinociceptive effects (3,4). The present results suggest that the antinociceptive effect of ACE acts at the level above the medulla and spinal cord. The assumption is consistent with the observation that microinjection of an enkephalinase inhibitor into ACE resulted in an increase in the hot-plate latency with no effect upon the tail-flick response (4).

*Pathway from ACE to Mo V* The direct projection from the ACE to the sensory trigeminal nuclear complex or Mo V has not been observed. Takeuchi *et al.* (1988) revealed in an HRP study that the supratrigeminal region received projections from the ipsilateral ACE and projected to the contralateral Mo V (25). This connection is supported by an electrophysiological study (26). On the contrary, present study demonstrated that the inhibitory effect of ipsilateral ACE stimulation was almost twice that of the contralateral one. These data, therefore, rule out the possibility that the ACE modulatory effect is relayed through the supratrigeminal region.

The ACE sends efferents to the ventromedial hypothalamic nucleus and lateral hypothalamic area (8) which connect reciprocally with the periaqueductal gray (PAG) (27,28,29) and directly to the PAG (7). It was reported that the typical effect of hypo-

thalamic conditioning stimulation on the JOR was inhibition without an inhibition of fore and hind limb flexor reflexes (30). This result resembles the present findings. However, Landgren and Olsson (1980) reported that conditioning stimulation of the same area elicited a facilitating effect on the jaw-closing reflex induced by the Mes V stimulation in addition to the inhibitory effect on the JOR (31). Comparing these results with our observations, the duration of the inhibitory effect was about one fourth of ours and we could not observe a facilitatory effect on the jaw-closing reflex. In addition, direct projections to the Mo V do not arise within the PAG and axon terminals from the PAG are distributed in the surrounding area of Mo V that exerts the inhibitory effect on the jaw-opener motoneurons (32). It is, therefore, probable that the hypothalamus and PAG do not contribute to the ACE inhibition of JOR.

The medullary parvocellular reticular formation (PcRF) that has reciprocal connections with Mes V and receives afferent projections from the ACE projects to Mo V (33,34) recently reported that stimulation of the PcRF induced IPSPs in the jaw-opener motoneurons in the cat, and that this area in PcRF corresponded to 3.5-6.7 mm caudal to the area that evokes monosynaptic EPSPs in digastric motoneurons (35). It seems likely, therefore, that the ACE-Mo V pathway incorporating the PcRF contributes to the ACE inhibition of JOR.

*Functional considerations* The ACE is involved in the autonomic and behavioral emotional responses to conditioned fear (36,37). Although the functional significance of this inhibition is not known, the ACE inhibitory effect on the JOR that was observed in

the present study could not be considered as a part of these behavioral responses (freezing, arrest or startle). The reason is that the jaw-closing (non-nociceptive) reflex induced by stimulation of the Mes V was not inhibited by the ACE stimulation.

Bernard et al. (1990) recently reported that a large majority of ACE neurons in the rat were affected by nociceptive stimuli applied to several parts of the body, and confirmed that the nociceptive input to the ACE was relayed in the parabrachial nucleus (PB) (38,39). It has been recognized that the origin of somatosensory input to PB is lamina I of the spinal and trigeminal dorsal horn (40). ACE also receives afferents from the ventromedial hypothalamus, the lateral hypothalamic area, the parafascicular thalamic nucleus, and posterior thalamic group (41,42). Moreover lateral and basolateral amygdaloid nuclei which receive fibers from the somatosensory cortex project to the ACE (6,43,44). Accordingly, it is likely that the ACE receives integrated information from various levels of the neuraxis and thereby modulated the nociceptive reflex without any effect on the non-nociceptive reflex such as the jaw-closing reflex at the level of the final common path. In addition, ACE modulated the nociception at the level above the medulla and spinal cord as suggested by Al-Rodhan et al (4). That is, it is possible that the ACE decreases the reactions to noxious stimulation by means of the inhibition of both sensory and motor systems.

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## LEGENDS

Fig. 1. Coronal brain section indicating the site of conditioning stimulation (arrow) in the central amygdaloid nucleus (ACE). The lower part shows the inhibitory effect of the ACE conditioning stimulation (C-T interval of 110 msec, 300  $\mu$ A) on the digastric EMG response to tooth pulp stimulation (lower molar, 300  $\mu$ A). Abbreviations: BL, basolateral amygdaloid nucleus; BM, basomedial amygdaloid nucleus; IC, internal capsule; LA, lateral amygdaloid nucleus; OT, optic tract; PAA, periamygdaloid area; PU, putamen.

Fig. 2. Relationship between the parameters (intensity in A and duration in B) of ACE conditioning stimulation and the magnitude of JOR inhibition. In A, the C-T interval and duration of conditioning stimulation of 330Hz were 110 ms and 100 ms, respectively. In B, the test stimulus was applied at 10 ms after the cessation of the conditioning stimulation of 300  $\mu$ A (330Hz). All points indicate the mean percent in amplitude of the EMG response with the conditioning stimulation vs. control. The vertical bars at each point indicate the standard deviation.

Fig. 3. Effect of ACE conditioning stimulation on the JOR at various C-T intervals. The ordinate shows the mean percent change in amplitude of EMG response with conditioning stimulation vs. control, and the abscissa shows the C-T interval in msec. A significant inhibition was obtained even at a C-T interval of 1000 msec (*t*-test,  $p < 0.01$ ). Inset on the right is a typical

example of the inhibitory effect. EMG responses were evoked by the stimulation of the upper molar (120  $\mu$ A). Each numerical value indicates the C-T interval. Calibration: 0.4 mV, 10 ms.

Fig. 4. Inhibitory effects of the ipsilateral and contralateral ACE stimulations to the EMG recording site (n=5). The ordinate shows the rate of inhibition (%). A significant difference was obtained by the Wilcoxon test ( $p < 0.025$ ).

Fig. 5. A: Effect of the ACE conditioning stimulation (at a C-T interval of 110 ms and with an intensity of 300  $\mu$ A) on the JORs evoked by the tooth pulp and brainstem stimulation. This data was obtained from the same animal. The JORs induced by the stimulation of either the rostral or caudal nuclei were inhibited in a similar manner as the JOR by tooth pulp stimulation, whereas the jaw-opening response induced by the stimulation of the motor nucleus was not influenced. Arrows indicate the time when the stimulus was applied. B: Effect of the ACE conditioning stimulation (at a C-T interval of 110 ms and with an intensity of 300  $\mu$ A) on the field potentials evoked by tooth pulp stimulation in the main sensory and rostral nuclei. This data was obtained from the same animal. The conditioning stimulation that inhibited the JOR induced by the tooth pulp stimulation did not suppress the field potentials which were recorded in these nuclei.

Fig. 6. Effect of the ACE conditioning stimulation. A: On the JORs induced by the brainstem stimulation (open columns). B: On

the field potentials evoked by tooth pulp stimulation (open columns). In A and B, hatched columns show the effect of the conditioning stimulation on the JOR induced by the tooth pulp stimulation. The ordinate shows the percent of amplitude response (JOR in A; JOR and field potential in B) with the conditioning vs. control. The abscissa shows the stimulus sites in A, and the recording sites in B ; the numbers indicate the distance from the obex to rostral direction. The vertical bar in each column is the standard deviation. \* shows significant difference ( $p < 0.01$ ) determined by *t*-test.

Fig. 7. Effect of the ACE conditioning stimulation on the JOR (opening) induced by the stimulation of tooth pulp and jaw-closing reflex (closing) induced by the stimulation of mesencephalic trigeminal nucleus. In A, data were obtained from the same animal. In B, data were obtained from 5 animals. The ordinate shows the percent of amplitude response with the conditioning vs. control. The vertical bar in each column is the standard deviation. \* shows significant difference ( $p < 0.01$ ) determined by *t*-test.

Fig. 8. Effect of glutamate microinjection (10  $\mu$ l) into ACE on the JOR induced by tooth pulp stimulation. The photomicrograph indicates the injection site (arrow) in ACE. The first deflection of each potential is the stimulus artifact. EN, entopeduncular nucleus; the other abbreviations are the same as in Fig. 1.

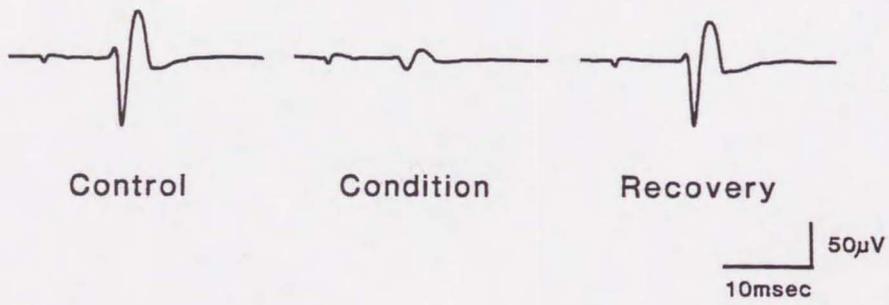
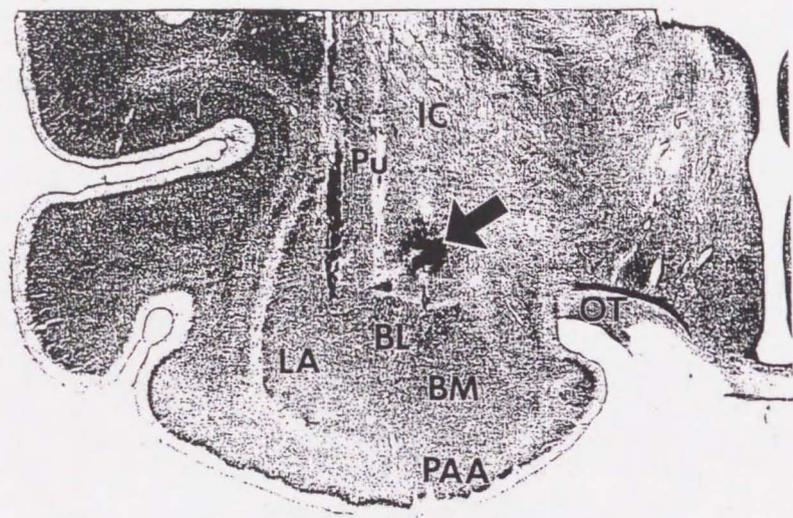


Fig. 1.

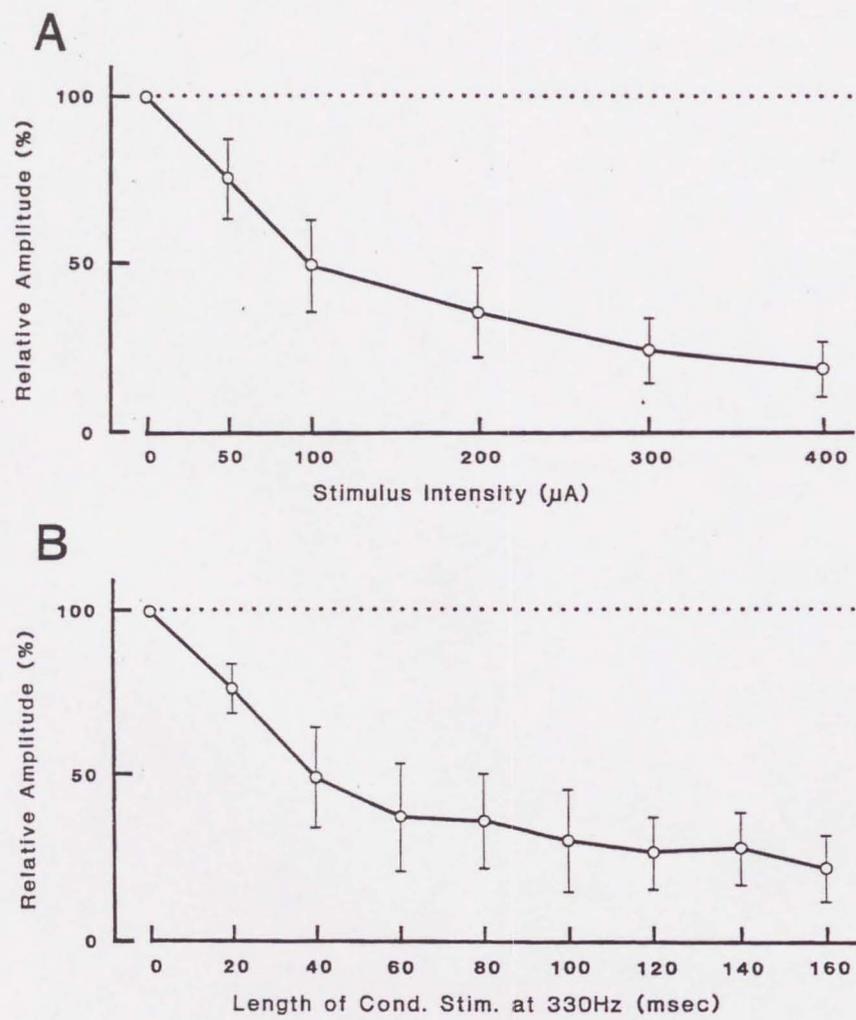


Fig. 2.

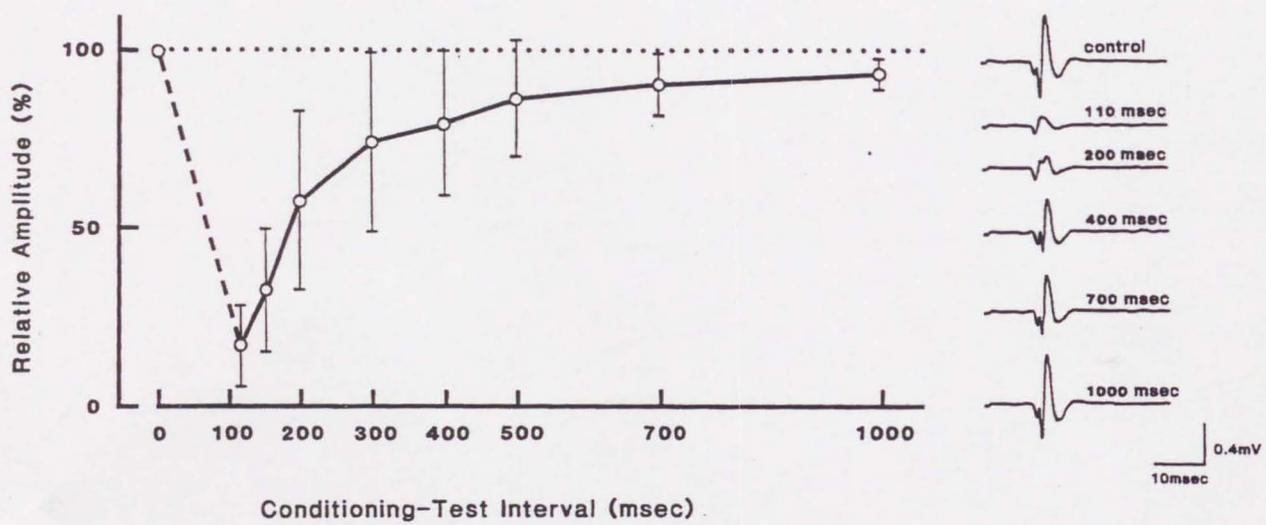


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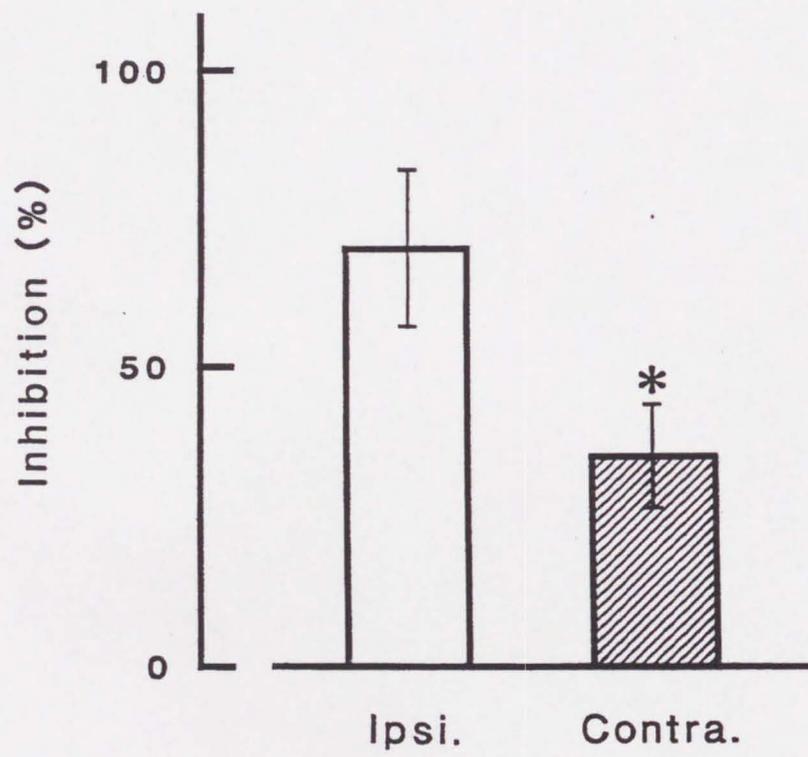


Fig. 4.

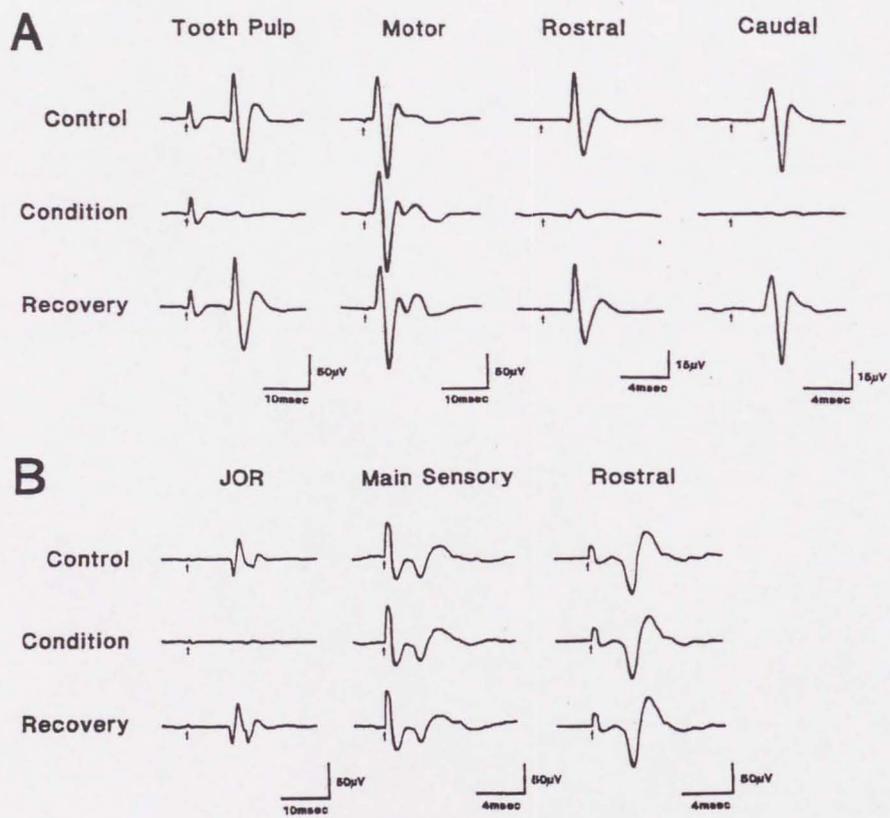


Fig. 5.

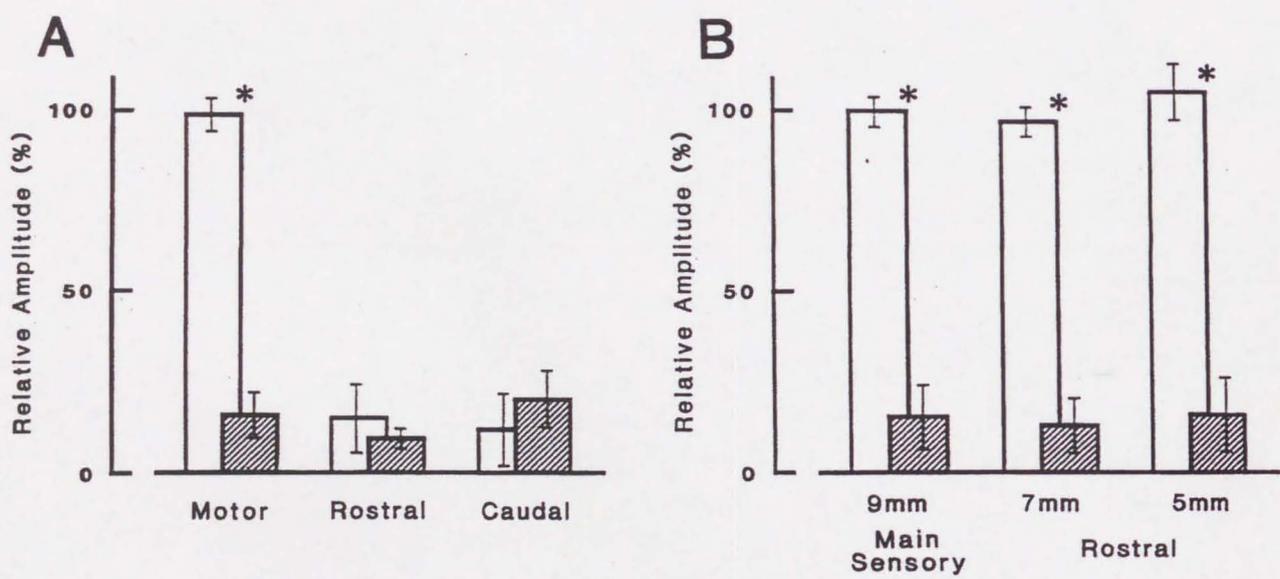


Fig. 6.

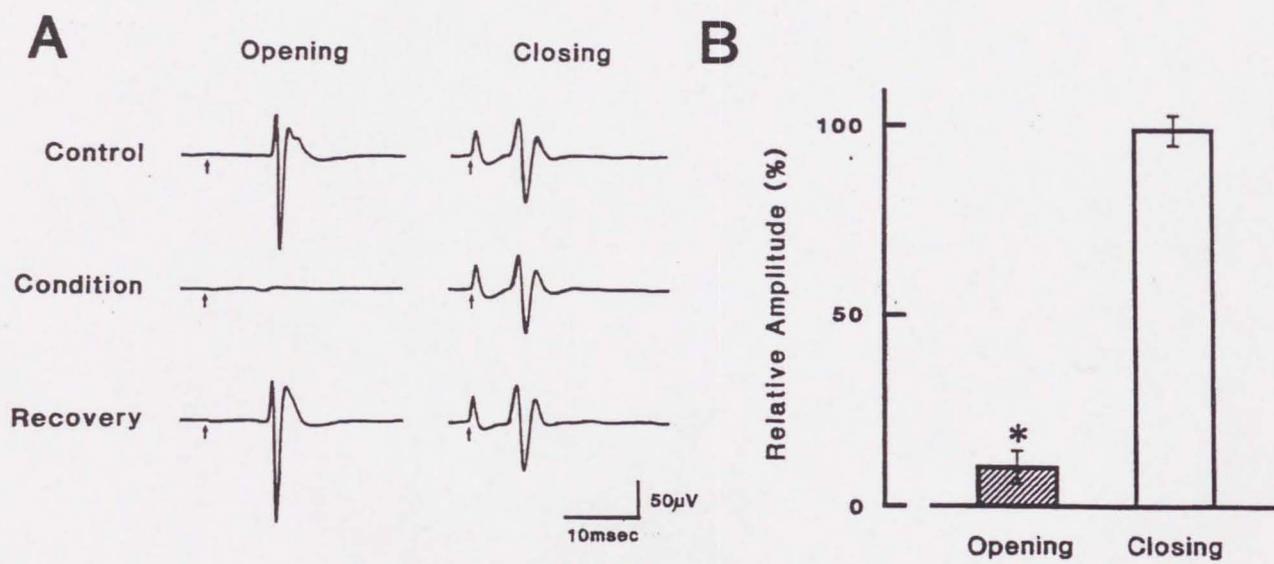
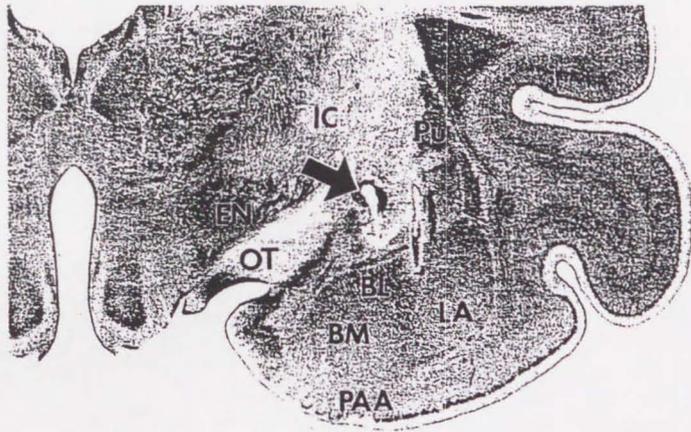


Fig. 7.



Glutamate Microinjection

Control



Injection

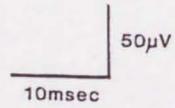
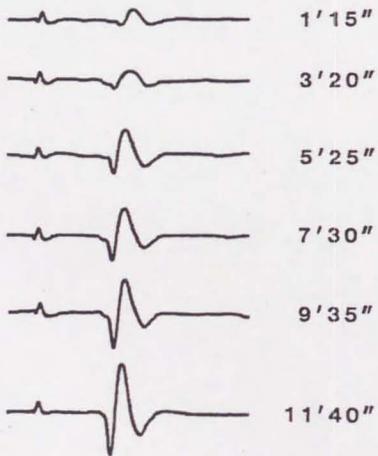


Fig. 8.



Inches 1 2 3 4 5 6 7 8

Centimetres 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

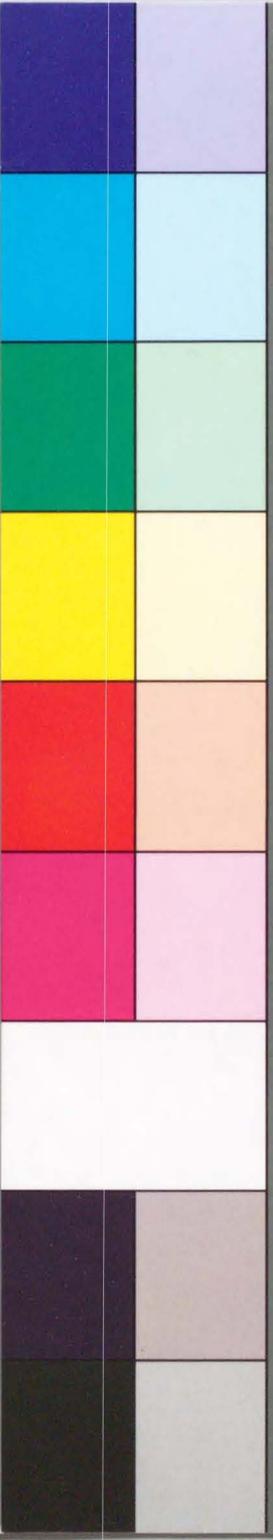
# KODAK Color Control Patches

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# Kodak

LICENSED PRODUCT

Blue Cyan Green Yellow Red Magenta White 3/Color Black



# Kodak Gray Scale



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A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19

